ENVIRONMENTAL BIOTECHNOLOGY

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ENVIRONMENTAL BIOTECHNOLOGY

(Role of Plants, Microbes & Earthworms in Environmental Management & Sustainable Development)

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PLANT BIOTECHNOLOGY

Rediscovering the Environmental, Social and Economic Virtues of Some 'Wonder Plants' Through Researches into Environmental Biotechnology

All green plants (terrestrial and aquatic) work as a 'natural pollutant sink' on earth intercepting dust and pollutants from air. This fact has been long recognised especially for the greenhouse gas carbon dioxide (CO_2) which is sequestered by green plants in the process of photosynthesis. Once the pollutants are released into the atmosphere, only the plants are the hope, which can mop up the pollutants by adsorbing them on their leaf surfaces or absorbing and metabolising (degrading) them into their metabolic system. Plants play an important role in mitigation of highly polluted atmosphere and extreme climates in urban and semi-urban areas. In a study made in China, on the role of urban forest and vegetation in air pollution abatement & reduction, found that there was 1262.4 tons of pollutants reduction by the forest cover in Beijing. Planting of trees and shrubs was recommended as a way to combat dust pollution in Russian cities and there was 2-3 times reduction in dust fall by planting a 8 m wide green belt between the roads and buildings.

During photosynthesis, plants absorb CO_2 and simultaneously other gases like oxides of sulfur & nitrogen (SO_2 & NO_x), ozone (O_3) and airborne ammonia (NH_3) through their stomata. Plants are also an efficient sink for polyaromated hydrocarbons (PAHs) in the air. Once inside the leaf, gases diffuse into the spaces between the cells of the leaf to be absorbed by water films or chemically altered by the plant enzymes in the metabolic process. Plants also reduce air pollution by intercepting suspended particulate matters (SPM) and aerosols and retaining them on the leaf surface by process of 'dry deposition'. Leaf surfaces are most efficient at removing pollutants that are water-soluble including SO_2, NO_2 and O_3.

Some plants are, however, relatively more 'tolerant' to air pollutants and can bio-accumulate them in their cells and tissues. Others are 'sensitive' which gets injured and damaged and respond to their injury by way of some visible morphological as well as physiological and anatomical changes. The more sensitive ones may altogether 'disappear' in response to pollutants in the air. Such plants work as 'bio-indicator' of air pollutants and work as an 'early warning device' for providing information on deteriorating air quality in a region'. In U.K., lichens (symbiotic organism made of algae & fungi) vanished from most industrial areas during the 19th century. As the air became cleaner after the legislation for control of sulfur dioxide (SO_2) pollution came into effect, the lichens have now returned to their habitat.

A new plant based biotechnology called 'phytoremediation' (Greek: *phyton* = plant; Latin: *remediare* = remedy) is emerging for environmental cleanup and restoration that uses plants to remove pollutants from the soil and water and render them harmless. It takes advantage of the natural abilities of plants to take up, bioaccumulate, store or degrade organic or inorganic substances. They are cost-effective, aesthetically pleasing, passive, solar-energy driven and pollution abating nature's biotechnology meeting the same objectives of fossil-fuel driven and polluting conventional technology. Plants involved in phytoremediation are adapted to thrive in very harsh environmental conditions of soil and water; absorb, tolerate, transfer, assimilate, degrade and stabilize highly toxic materials (heavy metals, radionuclides and organics such as solvents, crude oil, pesticides, explosives, chlorinated compounds and polyaromatic hydrocarbons) from the polluted soil and water. The organic pollutants may ideally be degraded to simpler compounds like carbon dioxide (CO_2) and water (H_2O), thus reducing the environmental toxicity significantly. Possibly due to their static (non-mobile) nature, plants had to evolve their survival modes even in odd environments including lands contaminated with xenobiotic substances.

Several aquatic plants have been reported for long to detoxify environmental pollutants in the water bodies and the aquatic ecosystems. Remediation of water contaminated even with 'chlorinated alkanes' have been shown with aquatic plants. Plant enzymes and the symbiotic microbes on their roots plays important role in biodegradation of complex organics.

After the Chernobyl disaster in Ukraine (erstwhile USSR) in 1986, the estimated cost of removal of radionuclides from contaminated pond waters was calculated to be US \$ 80 per thousand gallons by physical process. The phytoremediation technology using sunflower ranged from about US \$ 2–\$ 6 per thousand gallons of water. The US Army Environmental Centre is developing cost-effective phytoremediation technologies by constructed wetlands to cleanup groundwater contaminated with residues of explosives like TNT, RDX, DNT and Octahydro-1,3,5,7- tetranitro-1,3,5,7-tetraazocine (HMX). Over the past 20 years the constructed wetland technology using aquatic plant species specially the reeds (*Phragmites* Spp.) and the cattails (*Typha* Spp.) has been widely accepted in Europe and America as a low-cost, environmentally sustainable option to the conventional wastewater treatment facilities for both municipal and industrial wastewaters.

In the last few years, several commercial companies practicing phytoremediation biotechnology for environmental cleanup have come up in US and Europe. Important among them are Phytotech (USA), Phytoworks (USA), Earthcare (USA), Ecolotree (USA), BioPlanta (Germany), Piccoplant (Germany), Plantechno (Italy), Slater (UK) and Aquaphyte Remediation (Canada). A number of large industrial companies, mainly the oil and chemical industry, are also either employing phytoremediation technology or supporting it through appropriate funding.

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PHYTOREMEDIATION OF POLLUTED AIR

Green Plants as Natural Pollutant Sink on Earth, Bioindicators & Early Warning Devices of Pollutants in Air : Using the Tolerant Plant Species for Environmental Clean up : Significant Achievement of Researches into EBT

KEY WORDS

Plants as Living Biofilter & Natural Pollutant Sinks; Plants as Bioindicators of Pollutants in Air & An Early Warning Devices; Transgenic (Genetically Engineered) Plants Combating Air Pollution; Phytoremediation of Indoor Air Pollution.

BACKGROUND INFORMATION

All green plants (the terrestrial and aquatic vegetation on earth) work as a 'natural pollutant sink' on earth intercepting dust and pollutants from air. This fact has been long recognised especially for the greenhouse gas carbon dioxide (CO_2) which is sequestered by green plants in the process of photosynthesis. Once the pollutants are released into the atmosphere, only the plants are the hope, which can mop up the pollutants by adsorbing them on their leaf surfaces or absorbing and metabolising (degrading) them into their metabolic system. Plants play an important role in mitigation of highly polluted atmosphere and extreme climates in urban and semi-urban areas. Yang et al. (2005) (mentioned in Singh & Verma (2006) in a study on the role of urban forest and vegetation in air pollution abatement & reduction, found that there was 1262.4 tons of pollutants reduction by the forest cover in Beijing. Planting of trees and shrubs was recommended as a way to combat dust pollution in Russian cities and there was 2-3 times reduction in dust fall by planting a 8 m wide green belt between the roads and buildings.

During photosynthesis, plants absorb CO_2 and simultaneously other gases like oxides of sulfur & nitrogen (SO_2 & NO_x), ozone (O) and airborne ammonia (NH_3) through their stomata. (Bergmann, 1995; Yunus & Iqbal, 1996). Simonich and Hites (1994) reported that plants are also an efficient sink for polyaromated hydrocarbons (PAHs) in the air. Once inside the leaf, gases diffuse into the spaces between the cells of the leaf to be absorbed by water films or chemically altered by the plant enzymes in the metabolic process. Plants also reduce air pollution by intercepting suspended particulate matters (SPM) and aerosols and retaining them on the leaf surface by process of 'dry deposition'. Leaf surfaces are most efficient at removing pollutants that are water-soluble including SO_2, NO_2 and O_3.

Pollutant removal rates are the highest when the vegetative surfaces are wet or damp (such as after the rains or morning dews in winter). Such conditions can increase pollution removal rates ten-fold because the entire tree surface (barks, leaves & branches) is available as pollutant sink.

CHEMICALS IN THE AIR

Millions of tons of carbon dioxide, sulfur dioxide and nitrogen oxides are released into the atmosphere every day by our industries and automobiles. Ambient air today contain a frightening mix of toxic chemicals like carbon monoxide (CO), oxides of sulfur (SOx) and nitrogen (NOx), reactive hydrocarbons (HC) also known as volatile organic carbons (VOCs), total suspended particulate matter (TSP), heavy metal lead and organic compounds resulting from the automobiles. Five major air pollutants account for 98 % of pollution: carbon monoxide (52 %), sulfur oxides (14 %), VOCs (14 %), particulate matters (4 %) and nitrogen oxides (14 %). The remainder consists of lead, which is down 90 % since 1983. Most gaseous air pollutants are totally transparent except nitrogen oxide (NO_2), which is brown.

The oxides of nitrogen (NO_x) and the volatile organic carbons (VOCs) undergo photochemical reaction in sunlight to produce more deadly secondary pollutants called 'tropospheric ozone'(O_3) and the 'periacetyl nitrate' (CH_3COONO_2). Ozone and PAN are two deadly components of this 'photochemical urban smog' which plagues several cities of world today. Smog contains chemical cocktail of deadly pollutants from the automobiles- about 10,000 to 30,000 ppm of carbon monoxide (CO), 100 to 400 ppm of nitric oxide (NO_2), 600 to 3000 ppm of hydrocarbons (HCs) or the volatile organic compounds (VOCs), 50 to 150 ppm of ozone (O_3) and 50 to 250 ppm of periacetyl nitrate (PAN), sulfur dioxide (SO_2), and the suspended particulate matters (SPM). Aldehydes are major products in these reactions. Formaldehyde and acrolein account for about 50 % and 5 %, respectively, of the total aldehyde in urban air. Acrolein is a hazardous air pollutant (HAP).

PLANTS AS NATURAL POLLUTANT SINK

The National Botanical Research Institute (NBRI), Lucknow, has identified over 50 plants (trees, herbs and shrubs) as 'natural pollutant sink' (Anonymous, 1986). Some of the very prominent ones are :

1. *Allium cepa*
2. *Agremone mexicana*

3. *Azadirachta India*
4. *Avena sativa*
5. *Bougainvallea spp.*
6. *Coleus blumei*
7. *Cucumis sativus*
8. *Ficus bengalhensis*
9. *Ficus religiosa*
10. *Gingko biloba*
11. *Mangifera indica*
12. *Nerium odorum*
13. *Phaseolus vulgaris*
14. *Phoenix sylvestris*
15. *Sachharaum officinarum*
16. *Saraca indica*
17. *Simmondsia chinesis*
18. *Triticum aestivum*
19. *Zea mays*

There are unconfirmed reports about some trees e.g. *Ginkgo biloba* (very ancient and a living fossil), *Ficus religiosa* and the *Ficus bengalhensis* as having metabolic properties to assimilate carbon monoxide (CO) along with CO_2 in photosynthesis. (Verbal communication by Late Prof. GS Nathawat of University of Rajasthan, Jaipur, India, as having appeared in *Canadian Journal of Botany* in the 1980s).

> No wonder then, these trees are mentioned in religious epics as 'divine trees' with tremendous ability to purify the air in the surroundings. *Gingko* has been traditionally planted in Buddhist monastries and the other two trees *F. religiosa & F. bengalhensis* are trees of preference in all hindu temples. Hindu scriptures even mention the *Ficus religiosa* tree as the abode of Lord Krishna and hence worshipped.

IMPACT OF POLLUTANTS ON PLANTS AND PLANTS AS BIO-INDICATOR OF TOXIC POLLUTANTS IN AIR : AN EARLY WARNING DEVICE FOR BIO-MONITORING OF DETERIORATING AIR QUALITY

Air pollutants like nitrogen oxide (NO_2), sulfur oxide (SO_2), tropospheric ozone (O_3) and the suspended particulate matters (SPMs) have pernicious effects of varying magnitudes on important crops like wheat, mustard, mung and spinach plants at higher concentrations and on prolonged exposures. Some plants are, however, relatively more 'tolerant' to air pollutants and can bio-accumulate them in their cells and tissues. Others are 'sensitive' which gets injured and damaged and respond to their injury by way of some visible morphological as well as physiological and anatomical changes. The more sensitive ones may altogether 'disappear' in response to pollutants in the air. Such plants work as 'bio-indicator' of air pollutants and work as an 'early warning device' for providing information on deteriorating air quality in a region'

A number of air pollutants can onset early visible damage on plants and they can often provide very first evidence of air pollution. The visible plant damages that work as bioindicator of toxic pollutants in the air include 'mottled foliage', 'burning at leaf tips or margins', 'twig dieback', 'stunted growth' 'premature leaf and flower drop', 'abortion or early drop of blossoms', 'delayed maturity' and 'reduced yield or quality of fruits & seeds'. This provides an inexpensive way to bio-monitor the presence of air pollutants and quality of air in a region.

1. Impact of Sulphur Dioxide (SO_2) on Plants & the Bioindicator Species

Common sources of SO_2 are coal power plants, oil refineries, copper & iron smelters & fossil fuel furnaces. SO_2 is unique, at low concentration it is in fact beneficial to plants, while injurious at high concentrations. The injury is both visible and invisible. Many plants are known to be injured by SO_2 under natural & experimental exposure conditions. It has potential to reduce both yield and nutritional quality of crops and more perhaps to the mango crops.

Gaseous SO_2 is highly soluble in water and is ionised to form the hydrogen (H^+), sulfite (SO_3^{2-}) and bisulfite (HSO_3^-) ions depending upon the pH of the plant tissues. Free radicals produced during SO_3^{2-} oxidation, have been known to destroy many physiologically important compounds like amino acids, plant hormone IAA, chorophyll and b carotene in plants. Laboratory experiments have shown significant reductions in non-structural carbohydrates and proteins, and the nitrogen contents of seeds, fruits and vegetables when exposed to SO_2. (Singh & Verma, 2006).

The exposure of succulent, broad-leaved plants to SO_2 usually result in dry, papery blotches, colored tan, straw or even white and turn to interveinal browning on necrosis. More sensitive plants are alfalfa (*Medicago sativa*), beans, beets, buckwheat (*Fagopyrum esculentum*), soybean (*Glycine max*) and sunflower (*Helianthus anus*). At the National Monitoring Network in The Netherlands alfalfa and buckwheat are used for bio-monitoring SO_2 in ambient air. (Posthumus, 1983, 1985).

Lichens as Bio-indicators of Sulfur in the Environment : A Case Study from UK

Lichens are symbiotic organism resulting from the symbiosis of algae and fungi. They can grow on old walls and on the tree trunks of old trees in parks. Many species of lichens are extremely sensitive to sulfur dioxide (SO_2), and die while concentrations are still quite low. Rich flora of lichens are found in UK in pollution zones of 35 units and under, and disappears above that. Only 'crustose lichens' if any, can survive in heavy pollution zone of 150 units. Crustose lichens and few 'foliose lichens' survive in 70–125 units of pollution. 'Beared lichens' are occasionally found in low pollution zone of 40–50 units.

> In U.K. such lichens vanished from most industrial areas during the 19th century. As the air became cleaner after the legislation for control of sulfur dioxide pollution came into effect, the lichens have now returned to their habitat.

2. Impact of Nitrogen Oxides (NO_2) on Plants

NO_2 is less disruptive to plants and rather work as 'nutrient' in small concentrations but are phytotoxic at higher levels and prolonged exposures. Low NO_2 has been found to induce the production of chlorophyll pigments while it is reduced at higher concentrations. In sunflower, 300 ppb of NO_2 exerted a nutritional effect on plant growing on nitrogen-deficient soils, while 2000 ppb was phytotoxic. Study also revealed that although NO_2 was stimulant to growth at low concentrations, it was damaging to plants in combination with SO_2 at the same concentration.

3. Impact of Suspended Particulate Matters (SPM) on Plants

Suspended particulate matters (SPM) may cause ultrastructural and physiological disturbances in plants. Wax crystals which are the barriers between the plants and the environment fuse and flatten with age, but in the presence of SPM, erosion rate of wax increases. At worst, the epistomatal chamber of the leaf surface may be plugged totally by the withered and fused wax inhibiting transpiration, photosynthesis & respiration with grave consequences for the plants. Dust deposition on leaf cuticle due to particulate penetration into the epicuticular wax may reduce light incidence and reduce net photosynthesis. Dust deposition also can lead to clogging of stomata and inhibiting all physiological functions.

4. Impact of Tropospheric Ozone (O_3) on Plants & the Bioindicator Species

Ozone is a secondary pollutants resulting from photochemical reaction between hydrocarbons (HC) and NO_x from the automobiles in sunlight. It is probably most phytotoxic air pollutant to plants disrupting photosynthesis and other metabolic functions.

Long-term exposure to near ambient ozone may lead to appearance of 'chlorotic symptoms' , 'stippling' and 'speckling' (characterized by numerous tiny dots) on the upper leaf surface. Reduction in crop yield due to disrupted photosynthesis are other symptoms without any visible injury.

> Some excellent bioindicator plant species that has been used widely to detect ozone (O_3) in the lower atmosphere are tobacco (*Nicotiana tabacum*) cultivars Bel-W3 which are super sensitive to ozone. The classical ozone symptoms in tobacco cultivar Bel-W3 appear as sharply defined dot-like lesions on the adaxial side of the leaves resulting from the death of group of palisade cells. The cultivar Bel-B is highly ozone tolerant and have been used to biomonitor and control ozone in lower atmosphere. Susceptible tobacco plants are injured when the ozone concentration exceed 0.04 ppm. (Upadhaya & Kobayashi, 2006). Morning glory (*Ipomea* spp.) in Japan and the clover plant in Sweden have also been reported as bioindicator plants for ozone. Reduction in growth of radish (*Raphanus sativus*) has been observed as an indicator of ozone in Japan and Egypt. (Izuta et al. 1993).

5. Impact of Periacetyl Nitrate (PAN) on Plants & the Bioindicator Species

After ozone, PAN is the most phyto-toxic air pollutant. It is also a secondary pollutant in the lower atmosphere resulting by the same photochemical reaction between HC & NO_x which results into ozone formation in sunlight.

PAN is a deadly pollutant for human beings (affecting nervous system) and in plants cause premature senescence and leaf fall. Other symptoms are appearance of bands, blotches, bronzed and silvery areas. Even a minimum exposure for one hour to 0.01 to 0.05 ppm of PAN induces symptoms in susceptible plants.

The bioindicator plants for PAN in the environment are lettuce (*Lactuca sativa*), blue grass (*Poa annua*) and the Swiss chard (*Beta chilensis*). Petunias are also very sensitive to PAN and may be used as indicator plants for the chemical in the ambient air.

6. Impact of Ethylene on Plants & the Bioindicator Species

Ethylene is also many of the unburnt hydrocarbon products given out from the automobile exhaust. It also results from incomplete combustion of gas, oil and coal. It is a by-product of polyethylene manufacture.

Ethylene can modify the activities of plant growth hormones thus affecting the normal development of plant organs and tissues, without causing leaf tissues damage and necrosis. Injury to broad-leaved plants occurs as the downward curling of leaves and shoots (epinasty), followed by stunting of growth.

Posthumus (1983) studied the use of petunia plant (*Petunia axilliaris*) as the bioindicator plant for ethylene in The Netherlands. *Petunia hybrida* was studied as bioindicator plant for ethylene in Sweden in 1989. Potted petunia plants were placed at distances of 10, 20, 40, 80 and 120 m from a motorway with approximately 30,000 vehicles plying everyday. The results showed that petunia flowers were significantly smaller on plants closer to the motorway than those at distance. The abortion rate of flowers closer to the motorway was more frequent. (Upadhaya & Kobayashi, 2006).

7. Impact of Fluorides on Plants & the Bioindicator Species

Fluorides are generated from the glass, aluminum, pottery, brick and ceramic industries; from phosphate fertilizer plants, refineries and metal ore smelters. Typical symptoms of fluoride gas or particulate injury are 'yellowish mottle', wavy 'reddish or tan scorching' at the margins or tips of the broad leaved plants or 'tip burn' in grasses and conifers.

Gladiolus (*Gladiolus hortulanus*) is the most widely used plant as a bioindicator and for biomonitoring fluorides.

MECHANISM OF PHYTOREMEDIATION OF AIR POLLUTANTS BY TOLERANT SPECIES

Several tolerant plant species (trees, shrubs & herbs) have been identified through botanical surveys at the National Botanical Research Institute (NBRI) in Lucknow. Tolerant plant species can bio-accumulate pollutants in their cells and tissues. The response of various antioxidants to automobile exhaust pollutants was studied and it was found that *Amaranthus spinosus* & *Cephalandra indica* were equipped with a very good scavenging system to combat air pollution (Singh & Verma, 2006).

Farooq et al. (1988) exposed 12 common Indian tree species to varying concentrations of SO_2 to determine their tolerance levels and an order of sensitivity was determined like this—

1.*Tamarindis indica* > 2. *Pithecolobiam dulce* > 3. *Mangifera indica* > 4. *Ficus rumphie* > 5. *Holoptelea integrifolia* > 6. *Bombax ceiba* > 7. *Ficus bengalansis* > 8. *Azadirachta indica* > 9. *Ficus religiosa* > 10. *Syzygium cumuni* > 11. *Psidium guajava* > 12. *Ficus racemosa.*

Freer *et al.* (2004) studied relative pollutant dry deposition velocities and pollutant capture efficiencies of some species widely used in Europe urban and sub-urban areas. This included oak (*Quercus* spp.), alder (*Alnus* spp.), ash (*Fraxinus* spp.), sycamore (*Acer* spp.), Douglas fir (*Pseudotsuga* spp.), weeping fig (*Ficus* spp.) and Eucalyptus (*Eucalyptus globulus*). Species with more complex stem structure and smaller leaves had greater deposition velocities.

Stomatal Closure Protect Plants From Pollution

Most of the plants when exposed to higher concentrations of air pollutants, tend to restrict the entry of air pollutants by closing their stomata. This is a natural reaction and adaptation in plants to save themselves against toxic situations in the air. Mansfield & Free-Smith (1984) found quick stomatal closure in silver birch in the presence of SO_2 in air. Tomato and peanut also close their stomata quickly on exposure to SO_2 thus restricting their entry.

Ascorbic Acid Provides Defense Mechanism in Plants Against Pollution

High amount of ascorbic acid (Vitamin-C) in plant cells has been implicated in providing resistance to plants against pollutants in air. It also plays important role in protection of chlorophyll from hydrogen peroxide (H_2O_2) induced damage. Ascorbic acid activates the defense mechanism in plants. Due to its multiple role in metabolism and defense of plants, ascorbic acid is used as very reliable parameter to denote tolerance levels in plants against all kinds of stress including pollution stress. Pollutants often increase their phytotoxicity by impinging a decrease in the ascorbic acid contents of plants, which results in increased susceptibility of plants to pollution. Ascorbic acid also has the potential to mitigate the SO_2 induced injury in plants. (Singh & Verma, 2006).

(1) Phytoassimilation of Sulphur Dioxide (SO_2) by Plants : SO_2,from the atmosphere, finds its entry mainly through the stomata into the leaf cells. Plants can utilize the absorbed SO_2 in a 'reductive sulfur cycle' to form sulfur containing amino acids needed for their growth & development. SO_2 is converted into SO_3^{2-} and SO_4^{2-} inside the leaf tissues which reacts with organic acids to form amino acids - cysteine, methionine and glutathione. The intermediate compounds formed in the sulphate reduction pathway are APS (Adenyl-5-phosphosulphate), CS^- (Career protein), $CSSO_3$ (Career protein with bound sulphite) and CSS^- (Career protein with bound sulphide). In a study,increased SO_4^{2-} concentrations were found in needles of spruce (*Picea abies*) and Scotspine in Norway when exposed to SO_2 gas. (Singh & Verma, 2006).

Of all plants, the mango trees (*Magngifera indica*) has shown higher tolerance and accumulation capacity to SO_2 in air .It can bioaccumulate high amount of sulfur rich compounds.(Singh & Verma, 2006).

(2) Phytoassimilation of Nitrogen Oxides (NO_2) by Plants : Direct evidence for the foliar absorption of NO_2 has been obtained by using N^{15} isotopes of nitrogen. Maximum absorption of NO_2 was by three cultivars of hybrid polpar (*Populus* spp.) i.e. 0.3 mg $N/dm^2/d$. Sunflower and maize showed concentrations range between 200-1000 ppb.(Okano et al. 1986). NO_2 after entering into the leaves through stomata, dissolves into the intracellular fluid to form nitrous acid which further dissociates into toxic 'nitrites' and H^+ ions. Normally the nitrites gets reduced to ammonia (NH_3) by the enzyme 'nitrite reductase' (NR) and consequently assimilated into amino acid 'glutamate' and proteins, thus alleviating the toxicity while also benefiting the plants. Experiments with N^{15} shows that about 65 % of the absorbed NO_2 is incorporated into organic nitrogen during a 3 hour exposure period in beans.

(3) Phytofiltration of SPM by Plants : Tree take up more pollutants including the particulate pollutants (PM_{10}), than shorter vegetation. The exposed surface of trees, such as the bark and leaves forms a 'natural sink' for the particulate pollutants, as they provide sites for the gravity or wind-blown settlement of particulates. A study indicated 27 % reduction in dust particles in London (Hyde Park) by green cover of 2.5 km^2 . Planting of trees and shrubs was recommended as a way to combat dust pollution in Russian cities and there was 2-3 times reduction in dust fall by planting a 8 m wide green belt between the roads and buildings. (Singh & Verma, 2006). Dochinger (1980) examined the ability of plants to abate particulate pollutants and reported a reduction of up to 42 % in overall dust fall by a canopy of coniferous plants in the urban areas of Ohio, U.S. Varshney & Mitra (1993) assessed the particulate abatement capacity (PAC) of three commonly grown hedge species and found their PAC in the following order—

Duranta plumieri > *Bougainvillea spectabilis* > *Nerium indicum*

They reported that the row of roadside hedges trapped nearly 40 % of SPM, most of which arise from the traffic movement

PHYTOREMEDIATION OF INDOOR AIR POLLUTION

Deteriorating quality of the indoor breathing air by in-house air pollutants is a major concern today as people spend nearly 80 to 90 % of their time in their house or other indoor premises. Studies indicate that pollution levels in the homes are up to 20 times worse than the air outside. The main sources could be wall paints, carpets, furniture & fixtures, gas stoves in kitchen. The pollutants more widely present in the indoor environment are—

1. Carbon monoxide (CO)
2. Nitrogen oxides (NO_x)
3. Formaldehyde
4. Volatile Organic Compounds (VOCs) e.g. toluene, xylene, ethylebenzine & chloropyrifos
5. Undesirable products of burning tobacco & wood

> Over 300 indoor plants have been identified that has the wonderful property of absorbing and removing indoor air pollutants such as benzene, formaldehyde, trichloroethylene and all other VOCs. They can also extract particulate matters from the air. (Wolverton & Wolverton, 1993; Wolverton, 1997; Orwell et al. 2004). Plant roots can also absorb pollutants and render them harmless in soils.

In a study sponsored by the National Aeronautics and Space Research Agency (NASA), spider plants (*Chlorphytum elatum*) were placed in a closed chambers with 120 ppm of CO in one and 50 ppm of NO_2 in other. After 24 hours the spider plants removed 96 % of the CO and 99 % of the NO_2. Experiments with pothos plants (*Eppiremnum aureum*) showed that 75 % of the CO was removed after 24 hours. (Wolverton, 1985).

Following broad-leaved indoor plants have been identified to remove formaldehyde & other contaminants including the VOCs from the indoor air-

1. Heart-leaf philodendron (*Philodendron scandens*)
2. Elephant-ear philodendron (*Philodendron domesticum*)
3. Green spider plant (*Chlorphytum elatum*)
4. Golden pothos (*Eppiremnum aureum*)
5. Peperomia (*Peperomia obtusifolia*)
6. Peace lily (*Spathiphyllum clevelandii*)
7. Snake plant (*Sansevieria traifasciata*)
8. Chinese evergreen (*Aglonema modestum*)

GREEN PLANTS : THE POTENTIAL CARBON SINK ON EARTH REDUCING GLOBAL WARMING

Plants on land (forest) or in oceans (phytoplanktons) remove vast amounts of CO_2 from atmosphere. They are natural 'carbon sinks' on earth. Plants lock up the carbon dioxide from the atmosphere as plant material (mainly complex carbohydrates) in the process of photosynthesis for a specified period of time possibly up to 100 years. At present, terrestrial ecosystems (forest and vegetation on land) absorb about 25-30 % of the CO_2 emitted by human activities, thus providing a valuable free environmental service to slow the rate of global warming and climate change. Each year 16 % of atmospheric carbon dioxide is cycled through green plants, and forest contain up to 85 % of all carbon that is bound up in living organisms. Considerable amount of this remains in the ground as soil organic matter (SOM).

$$6H_2O + 6CO_2 \text{ (green plants-sunlight} \rightarrow \text{photosynthesis)} \rightarrow C_6H_{12}O_6 + 6O_2$$

> Temperate forests sequester about 2.7 tons of carbon per hectare a year for the first 80 years of their lives. In temperate areas about 400 million hectares (more than the current forested area of the U.S.) of growing forests will be required to sequester 1 billion of the 3-4 billion tons of carbon accumulates in the atmosphere every year. In the tropics, where less carbon is sequestered per hectare (tropical forests are estimated to fix between 1 to 2 tones of CO_2 for each square kilometer of land area every year) locking up 1 billion tons of carbon a year would require about 600 million hectares of growing forest, the equivalent of about 75 % of the area of the Amazon basin. (World Development Report, 1992).

The Novel Concept of Carbon Credits

Governments in Australia and European nations are developing a strategy which require industries and motorists emitting carbon dioxide (CO_2) to buy *'carbon credits'* from organizations/farmers which grow trees or manage existing vegetation. Sequestered or absorbed carbon is locked for long period, in order to provide full offset for the

CO_2 emitted by our industries and automobiles. The growth of a typical tree tends to be slow in the early years as the trees establish themselves. Trees grow and sequester carbon fastest when they are about 10 to 20 years old. Study has shown that a 500 MW coal-fired power plant operating over a 35 year lifetime would require a tree plantation program over 1400 km^2, and maintained for several hundred years. Forestry programs are estimated to be able to provide a storage capacity of 1.2 gigaton (Gt) carbon per year. Tree plantations have secondary environmental benefits like improving biodiversity corridors, maintaining watershed hydrology and oxygenation of atmosphere.

ROLE OF ENVIRONMENTAL BIOTECHNOLOGY & PLANT GENETIC ENGINEERING IN DEVELOPMENT OF TRANSGENIC PLANTS TO COMBAT AIR POLLUTION

Genetic engineering has produced some 'wonder plants' to combat air pollution. A number of such plants such as *Arabidopsis umbellate, Pittosporum tobire* and *Raphiolepis umbellate* are now available to work as 'natural sink' for the air pollutants. (Singh & Verma, 2006). Key enzymes and their genes involved in metabolising NO_2 such as 'nitrate reductase' (NR), 'nitrite reductase' (NiR), glutamine synthetase (GS) and those involved in metabolism of SO_2 such as 'sulfite oxidase' and 'sulfate oxidase' have been identified. Transfer and 'over-expression' of these genes may help in development of transgenic NO_2 and SO_2 - philic plants which can take up high amounts of these pollutants from the atmosphere and metabolise them. Takahashi et al, (2001) found that enrichment of genes coding for synthesis of 'nitrate reductase' enzymes improved assimilation of nitrogen dioxides (NO_2) in *Arabidopsis* plants.

CONCLUDING REMARKS

Air pollutants like sulfur and nitrogen oxides, ozone and suspended particulate matters (SPMs) can also be ameliorated by plants. Despite of the adverse effects of pollutants on plant life there are some tolerant 'wonder species' which can absorb, adsorb, detoxify, bio-accumulate & metabolise the pollutants. They act as a 'living filter and natural sink' for the air pollutants. Researches into biodiversity and environmental biotechnology has helped in identifying and producing more natural pollution fighters. Phytoremediation of polluted air, water & soil can be implemented by several naturally occurring plants whose numbers are growing after studies identify and more and more such plants.

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2

PHYTOREMEDIATION OF POLLUTED LAND & SOIL

A Low-cost Nature's Biotechnology for Environmental Clean Up of Chemically Contaminated Lands by Tolerant Plant Species : A Great Achievement of Researches into EBT

KEY WORDS

Phytoextraction; Phytovolatalization; Phytostabilization; Phytostimulation; Rhizofiltration; Phytotransformation; Phytodegradation; Excluder Plants; Hyper-accumulator Plants Species; Transgenic (Genetically Engineered) Plants Combating Soil Pollution; Symbiotic Engineering.

BACKGROUND INFORMATION

Contaminated soils and waters pose major environmental, agricultural and human health problems worldwide. Phytoremediation (Greek: *phyton* = plant; Latin: *remediare* = remedy) is emerging 'green bioengineering technology' for environmental cleanup that uses plants to remove pollutants from the soil or to render them harmless. It takes advantage of the natural abilities of plants to take up, bioaccumulate, store or degrade organic or inorganic substances. They are cost-effective, aesthetically pleasing, passive, solar-energy driven and pollution abating nature's biotechnology meeting the same objectives of fossil-fuel driven and polluting conventional technology. Plants involved in phytoremediation are adapted to thrive in very harsh environmental conditions of soil and water; absorb, tolerate, transfer, assimilate, degrade and stabilize highly toxic materials (heavy metals, radionuclides and organics such as solvents, crude oil, pesticides, explosives, chlorinated compounds and polyaromatic hydrocarbons) from the polluted soil and water. The organic pollutants may ideally be degraded to simpler compounds like carbon dioxide (CO_2) and water (H_2O), thus reducing the environmental toxicity significantly. Possibly due to their static (non-mobile) nature, plants had to evolve their survival modes even in odd environments including lands contaminated with xenobiotic substances.

Traditionally, remediation of heavy metal contaminated soils involves 'off-site' management by excavating and subsequent disposal by burial in secured landfills. This method of remediation is very costly affair and merely shifts the contamination problem elsewhere. Additionally, this involves great risk of environmental hazard while the contaminated soils are being transported and 'migration of contaminants' from landfills into adjacent lands and water bodies by leaching. Soil washing for removing inorganic contaminants from soil is another alternative to landfill burial, but this technique produce a 'residue' with very high metal contents which requires further treatment or burial.

Large scale phytoremediation of the contaminated sites has been achieved for heavy metals, organic xenobiotics, and radionuclides. Phytoremediation has been carried out commercially or has been demonstrated as pilot scale studies on nearly 200 contaminated sites (involving all categories of organic and inorganic chemical and radiological contaminants) in the U.S. The plant biomass eventually becomes valuable biological source for the community or for the plant based industries. The roots, shoots and leaves may be collected (harvested) and incinerated to decompose the contaminants.

CONTAMINANTS SUITABLE FOR REMOVAL BY PHYTOREMEDIATION

The following recalcitrant organic & inorganic pollutants & radioactive contaminants are best suited for phytoremediation technology-

1. Benzene
2. Toluene
3. Ethylbenzene
4. Xylene
5. Chlorinated Solvents (TCE & PCE)
6. Chlorinated Pesticides
7. Organophosphates
8. Insecticides
9. Polyaromatic Hydrocarbons (PAHs)
10. Polychlorinated Biphenyls (PCBs)
11. Nitrotoluene ammunition wastes
12. Petroleum Hydrocarbons
13. Nutrients like nitrate, phosphate and ammonium
14. Toxic heavy metals (Cadmium, Lead, Nickel, Chromium, Mercury, Arsenic etc.)
15. Hazardous Air Pollutants e.g. Oxides of Sulfur & Nitrogen
16. Radionuclides (Cesium, Strontium, Uranium etc.)

COMMERCIAL ORGANIZATIONS INVOLVED IN PHYTOREMEDIATION & BUSINESS POTENTIAL

In the last few years, several commercial companies practicing phytoremediation biotechnology for environmental cleanup have come up in US and Europe. Important among them are Phytotech (USA), Phytoworks (USA), Earthcare (USA), Ecolotree (USA), BioPlanta (Germany), Piccoplant (Germany), Plantechno (Italy), Slater (UK) and Aquaphyte Remediation (Canada). A number of large industrial companies, mainly the oil and chemical industry, are also either employing phytoremediation technology or supporting it through appropriate funding.

TABLE 1

Phytoremediation Markets in the U.S. in 2005

Phytoremediation Works Carried Out	Value in Million US Dollars
1. Removal of Heavy Metals from Contaminated Soil	70 - 100
2. Removal of Heavy Metals from Contaminated Groundwater	1 - 3
3. Removal of Heavy Metals from Wastewater	1 - 2
4. Removal of Radionuclides	40 - 80
5. Removal of Organics from Contaminated Groundwater	35 - 70
6. Others	65 - 115
Total	214 - 370 Million Dollars

Source: Eapen *et al.* (2006)

SOURCES & STATE OF HEAVY METALS IN SOIL AND THEIR BIOAVAILABILITY FOR PHYTOREMEDIATION

Developmental activities and their byproducts are the sources of metal contamination of our soils & water bodies. Main sources are—

1. Metalliferous Mining & Smelting : Arsenic (As), cadmium (Cd), lead (Pb) & mercury (Hg);
2. Industries (Metal & Electroplating, Saw Mills etc.) : Chromium (Cr), cobalt (Co), copper (Cu), Zinc (Zn), nickel (Ni), As, Cd & Hg;
3. Atmospheric Deposition from Industries & Automobiles: Uranium (U), As, Cd, Cr, Cu, Pb & Hg;
4. Agricultural activities: Selenium (Se), As, Cd, Cu, Cr, Pb, Zn & U
5. Waste disposal (MSW landfills & wastewater treatment plants): As, Cd, Cr, Cu, Pb, Hg, & Zn
6. Wastewater from electroplating, paint and cement industries discharge heavy metals like cadmium (Cd), copper (Cu), lead (Pb), mercury (Hg) nickel (Ni), zinc (Zn) and arsenic (As).

Heavy metals are defined as metals having density more than 5 g/cm^3. The group of heavy metals are about 65. Some heavy metals, such as cobalt (Co), chromium (Cr), copper (Cu), nickel (Ni) & zinc (Zn) are essential and serve as micronutrients for plants like calcium (Ca), potassium (K), magnesium (Mg), manganese (Mn), iron (Fe) and sodium (Na). They are used for redox-processes, as components of various enzymes and for regulation of osmotic pressure in cells. Other metals have no biological role e.g. cadmium (Cd), lead (Pb), mercury (Hg), aluminum (Al), gold (Au) and silver (Ag). Some of them e.g. Cd^{2+}, Ag^{2+}, Hg^{2+} tend to bind the SH groups of enzymes and inhibit their activity (Turpeinen, 2002).

> Heavy metals are one very significant category of the industrial pollutants which are unique being selectively toxic, persistent and non-biodegradable. At high concentrations, both essential and non-essential metals can damage cell membrane, alter enzyme specificity, disrupt cellular function, and even damage the structure of DNA. They have been linked to birth defects, cancer, skin lesions, retardation leading to disabilities, kidney & liver damage and several other health problems. Remediation of metals presents a different set of problems when compared to organics. Organic compounds can be degraded while metals normally need to be physically removed by excavation or immobilized by plant roots.

METAL CONCENTRATIONS IN SOIL & THEIR BIOAVAILABILITY FOR UPTAKE BY PLANTS

Metal concentration in soil can range from < 1 mg / kg to as high as 100,000 mg / kg (Eapen *et al.* 2006). Metals are often tightly bound to soil particles. Cations of heavy metals are often bound to soil particles because of soil cations exchange capacity. Binding mechanisms of heavy metals is complex and vary with composition of soil, soil acidity and redox conditions. The binding affinity of cations reduces cation movement in vascular plants, particularly in the negatively charged xylem cells. The slow desorption of heavy metals in soil has been a major impediment to the successful phytoremediation program of the metal contaminated sites.

Metal bioavailability is often low in soil systems. Metals are more bioavailable at acidic pH values. Generally, only a fraction of soil metal is readily available (bio-available) for the plant uptake. In the soil, the organic matter and the clay mineral content are important factors that can reduce metal bioavailability. Clays, with high cation exchange capacities, such as montmorillonite, appear to reduce metal bioavailability & toxicity. In a study used to investigate the effect of cadmium (Cd) on microbial biodegradation of toxic organic compound 'phenanthrene', a total of 394 mg cadmium (Cd) per kg of soil was added, but only 3 mg cadmium (Cd) / L were actually bioavailable. Similarly only 1 % of the total zinc (Zn) used in a study was present in the aqueous phase. In another study, 20 mg / L of soluble metal initially amended was below the detection limit i.e. 0.03-0.04 mg/L. At 100 mg / L of total metal amended, only 1mg cadmium (Cd) / L, and less than 0.12 mg copper (Cu) / L and chromium (Cr) /L were found in the aqueous phase. (Sandrin & Hoffman, 2006). Plant roots also increase metal bioavailability by extruding protons to acidify the soil and mobilize the metals. (Zhao *et al.*, 2001). By decreasing the pH below 5.5, metal bioavailability for plant roots can be enhanced. But that may also inhibit plant growth.

The Soluble & Mobile Metals

The bulk of the metal in soil is commonly found as 'insoluble' compounds unavailable for transport into the plant roots from the aqueous phase. Metals, which are taken up by plants are those which exist as soluble components in the soil solution or are easily desorbed or solubilized by root exudates. Solubility of metals is dependent on soil characteristics and is strongly influenced by pH of the soil and the degree of complexation with soluble ligands.

However, some metals, such as zinc (Zn) and cadmium (Cd) are considered as 'easily mobile' heavy metals as they occur primarily as 'soluble or exchangeable' form and readily bioavailable. Copper (Cu), chromium (Cr) and molybdenum (Mo) are mainly bound in silicates and thus are 'slightly mobile' and available. Others such as lead (Pb), occur as 'insoluble precipitate' (phosphates, carbonate & hydroxy-oxides) in soil which is significantly much less mobile and largely unavailable for plant uptake.

TABLE 2

Range of Heavy Metals in Contaminated Soil

Metals	Contamination Range (mg/kg	Regulatory Limit (mg/kg)
Lead (Pb	1000 - 6,900,000	600
Arsenic (As	100 - 102,000	20
Cadmium (Cd)	100 - 345,000	100
Chromium (Cr)	5.1 - 3,950,000	100
Copper (Cu)	30 - 550,000	600
Mercury (Hg)	0.1 - 1,800,0000	270
Zinc (Zn)	150 - 5,000,000	1,500

Source: Eapen et, al; In SN Singh & RD Tripathi (ed.) '*Environmental Bioremediation Technologies*' ; Springer (2006).

PLANT SPECIES INVOLVED IN PHYTOREMEDIATION

Several plants are being identified and trialed to be used in phytoremediation task. The most versatile plant species that has been identified after rigorous laboratory and field experiments are—

TABLE 3

Important Plant Species Identified for Phytoremediation Works

1.	Sunflower (*Helianthus anus*)
2.	Vetiver grass (*Vetiveria zizanioides*)
3.	Indian Mustard (*Brassica juncea*)
4.	Poplar tree (*Populus* Spp.)
5.	Brake fern (*Pteris vittata*)

Contd. ...

Contd. ...

6. Barmuda grass (*Cynodon dactylon*)
7. Bahia grass (*Paspalaum notatum*)
8. Cumbungi (*Typha angustifolia*)
9. Redroot pigweed (*Amaranthus retroflexus*)
10. Kochia (*Kochia scoparia*)
11. Foxtail barley (*Hordeum jubatum*)
12. Switch grass (*Panicum variegatum*)
13. Musk thistle (*Carduus nutans*)
14. White raddish (*Raphanus sativus*)
15. Catnip (*Nepeta cataria*)
16. Big bluestem (*Andropogan gerardii*)
17. Alpine pennycress (*Thlaspi* Spp.)
18. Canada wild rye (*Elymus candensis*)
19. Nightshade (*Solanum nigrum*)
20. Wheat grass (*Agropyron cristatum*)
21. Alfa-alfa (*Medicago sativa*)
22. Tall Fescue (*Festuca anundinacea*)
23. Lambsquarters (*Chenopodium berlandieri*)
24. Reed grass (*Phragmites australis*)
25. Tall wheat grass (*Thynopyron elongatum*)
26. Rhodes grass (*Chloris guyana*)
27. Flatpea (*Lathyrus sylvestris*)
28. Carrot (*Daucus carota*)
29. Willows (*Salix viminalis*)
30. Periwinkle (*Cathranthus roseus*)

A number of them are still wild, while others have been domesticated for their food value. They are highly salt and toxicity tolerant, have extensive root binding system and were tried in the rehabilitation works. Number of them readily absorb, volatilise and / or metabolise compounds such as tetrachloroethane, trichloroethylene, metachlor, atrazine, nitrotoluenes, anilines, dioxins and various petroleum hydrocarbons. Ideal species for the job are members of the grass family Graminea and Cyperacea and the members of families Brassicaceae (in particular the genera *Brassica, Alyssum* and *Thlaspi*), and Salicaceae (in particular willow and poplar trees). Grasses such as the vetiver, clover and rye grass, Bermuda grass, tall fescue etc. have been particularly effective in the remediation of soils contaminated by heavy metals and crude oil (Kim, 1996).

Hybrid poplar, willows, sunflower, alpine pennycress, clover, Indian mustard, redroot pigweed and ferns have been plants of choice for many commercial phytoremediation applications.

The Hyper-accumulator Species

There are species which can bioaccumulate very high concentrations of metals in their stem and leaves. These are 'hyper-accumulators' species. More than 400 plant species ranging from herbs to trees have now been identified from the families of Euphorbiacea, Asteraceae, Brassicaceae and Rubiaceae which are hyperaccumulators of metals (Eapen *et al.*, 2006). Most have been found in the contaminated areas of temperate Europe and the USA, New Zealand and Australia. They can accumulate 100 - 500 times higher levels of metal concentrations in their above-ground parts. (Chaney *et al.* 1997). Plants are called hyperaccumulators when they can accumulate more than 0.1% Pb, Co, Cr or more than 1 % Mn, Ni, or Zn in plant shoots when grown in their natural habitats (Baker & Brooks, 1989). The degree of accumulation of metals like Ni, Zn, and Cu observed in hyperaccumulator species often reaches 1 - 5 % of their dry weight. (Raskin *et al.*, 1997). Hyper-accumulator plants tends to be contaminant specific. No plant species has been found that demonstrate a wide spectrum of hyper-accumulation (Watanabe, 1997).

> These hyper-accumulator plants possess 'genes' that regulate the amount of metals taken up from the soil by roots and deposited other locations within the plants. There are number of sites in the plant that could be controlled by different genes contributing to the hyper-accumulation genetic traits. These genes govern processes that can increase the 'solubility of metals' in the soil surrounding the roots as well as the 'transport proteins' that move metals into the root cells.

TABLE 4

Some Known Hyperaccumulator Species of Different Metals

Metals and Plant Species		Concentration of Metal Accumulated (mg/kg)
A. Nickel (Ni)		
Thlaspi spp.	(Brassicaceae)	2000 - 31,000
Alyssium spp.	(Do)	1280 - 29,400
Berkheya codii	(Asteraceae)	11,600
Pentacalia spp.	(Do)	16,600
Psychotria corinota	(Rubiaceae)	25,540
B. Zinc		
Thlaspi caerulescene	(Brassicaceae)	43,710
Thlaspi rotundifolium	(Do)	18,500
Dichopetalum gelonioides	(Do)	30,000

Contd. ...

Contd. ...

Metals and Plant Species		Concentration of Metal Accumulated (mg/kg)
C. Cadmium		
Thlaspi caerulescene	(Brassicaceae)	2,130
D. Lead		
Minuartia verna	(Caryophyllaceae)	20,000
Agrostis tenius	(Poaceae)	13,490
Vetiveria zizaniodes		> 1500
E. Cobalt		
Crotolaria cobalticola	(Fabaceae)	30,100
Haumaniastum robertii	(Lamiaceae)	10,232
F. Copper		
Ipomea alpina	(Convolvulaceae)	12,300
G. Manganese		
Maystenus sebertiana	(Celastraceae)	22,500
Maystenus bureaviana	(Celastraceae)	19,230
Macadania neurophylla	(Proteaceae)	55,200
H. Selenium		
Astragalus racemosus	(Leguminosae)	1,49,200
Lecithis ollaria	(Lecithidiaceae)	18,200
I. Arsenic		
Pteris vittata	(Fern)	

Source : Eapen *et al.*; In SN Singh & RD Tripathi (ed.) '*Environmental Bioremediation Technologies*'; Springer (2006).

It may be possible to revegetate closed hazardous landfills, industrial sites, and dis-used mine areas with hyper-accumulator species. Recent researches have indicated that the range of soil types suitable to hyper-accumulators can be widened by the incorporation of essential nutrients (e.g. N and P), and also chelating agents like EDTA which increase the plant availability of metals, particularly those in clay-textured soil.

MECHANISM OF PHYTOREMEDIATION OF CONTAMINATED SOILS

The plants act as 'accumulators' and 'excluders'. Accumulators survive despite concentrating contaminants in their aerial tissues. They biodegrade or biotransform the contaminants into harmless forms in their tissues. Adaptations to tolerate toxic compounds in plants appears to be processes like immobilization, exclusion, chelation & compartmentalization. These mechanisms not only control the uptake and accumulation of essential and non-essential heavy metals, but also detoxify them.

Phytoremediation of organic and inorganic contaminants involves either physical removal of compounds (phyto-extraction) or their bioconversion (phyto-degradation or phyto-transformation) into biologically inert forms. The conversion of metals into inert forms can be enhanced by raising the pH (e.g. through liming), or by addition of organic matter (e.g. sewage sludge, compost etc.), inorganic anions (e.g. phosphates) and metallic oxides and hydroxides (e.g. iron oxides). The plants themselves can play a role here by altering soil redox conditions and releasing anions and /or lignins (Salt *et al.* 1995).

Phytoremediation technology works mainly through—

1. Phytoextraction (Phytoaccumulation of Contaminants)

Plant roots uptake (extract) metal or radioactive contaminants from the soil, polluted water and the wastewater, translocate and accumulate them in their roots. Plant roots absorb both organics and inorganics. Considerable amount of the contaminants may be translocated above through the xylem and accumulated in the shoots and leaves. The radionuclide uptake by plant roots need not necessarily result in translocation to shoots. The majority of cesium-137 (Cs^{137}) taken up by plants tends to be localized in roots. However, 80 % of the strontium-90 (Sr^{90}) taken up by the plants, is usually localized in the shoots. (Eapen *et al.*, 2006).

> Phytoextraction exploits vascular plant's natural ability to take up variety of chemical elements (macro & micronutrients) through the root system, deliver them to the vascular tissues, and to transport and compartmentalize in different organs.

Major limiting factor for phytoextraction are lower availability of metals in soil and poor translocation from roots to shoots. The bioavailability of a given compound for phytoextraction depends upon the lipophilicity and the soil or water conditions e.g. pH and clay content. Addition of soil amendments increased the metal availability in solutions to more than 10-fold for Cs^{137} and 100-fold for lead (Pb) and uranium (U). (Huang *et al.*. 1998).

This technique has been effectively used by Phytotech Inc. (USA) for removal of lead (Pb) and cadmium (Cd) from contaminated soil. Excessive selenium (Se) in farm soils is also successfully remediated by this technology. (Eapen *et al.*, 2006). Above-ground biomass, loaded with metals or radionuclides is harvested, processed for volume reduction and further element concentrations & safely recycled to reclaim metals of economic importance (industrial uses), or disposed off as 'hazardous waste' in secured landfills or incinerated in case of radionuclides.

TABLE 5

Plants Identified with Potential for Phytoextraction of Metals

Plant Species		Metals Extracted
1.	Indian mustard (*Brassica juncea*)	Cd, Cr, Cu, Ni, Zn & Pb
2.	Sunflower (*Helianthus annus*)	Do
3	Rapeseed	Do
4.	Amaranthus (*Amaranthus retroflexus*)	Do
5.	Chenopodium (*Chenopodium album*)	Do

2. Phytostabilization (Immobilization of Contaminants)

Phytostabilization is stabilizing process for contaminated soils and sediments in place using plants, thus preventing the lateral or vertical migration of toxic metals by leachating. Certain plant species immobilize contaminants in the soil and groundwater through absorption by and adsorption on to roots or precipitation within the root zone (rhizosphere). Plants capable of tolerating high level of metal contaminants and having efficient growth rate with dense root systems (to bind / sorb contaminants) & canopies are preferred. Trees which transpire large amounts of water for hydraulic control (e.g. poplar) and grasses with fibrous roots (e.g. vetiver grass) to bind & hold soil are best suited for phytostabilization. Generally, plants suitable for 'soil conservation' is good for phytostabilization of soil contaminants.

This technique is best applicable in phytostabilizing metal contaminants of waste landfill sites (e.g. Pb, Cd, Zn, As, Cu, Cr, Se, and U) where the best option is to immobilize them *in situ*. Addition of manure, digested sewage sludge, straw etc. to inorganic waste sites may help in binding of metals. Supplementing with limes (CaO) and limestones ($CaCO_3$) may help neutralizing acid soils that help in binding of cationic metals with inorganic wastes.

> Phytostablization is particularly suitable for stabilizing radionuclide-contaminated sites, where one of the best alternative is to hold contaminants in place to prevent 'secondary contamination' and exposure to humans and animals. Plants roots also help to minimize water percolation through soil, thus reducing radionuclide leaching significantly. The technology is very suitable for controlling tailings in arsenic, zinc, cadmium and uranium mining areas.

Success Story

Mine tailings at Superfund site at South Dakota in the US containing up to 1000 mg/kg of arsenic (As) and lower concentrations of cadmium (Cd); smelter in Kansas, U.S, with up to 200,000 mg/kg of zinc (Zn) could be phytostabilized by decreasing vertical migration of leachate to groundwater using hybrid poplar (*Populus* spp.) trees (Hse, 1996).

3. Phytovolatilization (Evapo-transpiration of Detoxified Contaminants)

Phytovolatilization exploits a plant's ability to transpire large amount of water from their leaf pores (stomata). Plants absorb and transpire the impurities from soil through their aerial organs (leaves). This system is a combination of hydraulic control and enhanced evapotranspiration.

> Phytovolatilization is a mechanism by which plants convert a contaminant into volatile form and transpire the detoxified vapor through their aerial organs. Rate of transpiration is a key factor for this technology. For a 5 year old poplar tree (*Populus* spp.) it was estimated to be 26 gpd (gallon per day) . A single willow tree (Salix spp.) has been found to transpire 5000 gpd and an individual cottonwood tree transpire 50 to 35 gpd.

Some contaminants like selenium (Se), mercury (Hg) and volatile organic compounds (VOCs), can be released through the leaves into the atmosphere. (Cunningham and Ow, 1996). It is also being used for remediation of tritium (H^3) contaminated water. Some transgenic plants e.g. *Arabidopsis thaliana* have been found to convert organic and inorganic mercury salts to the volatile and elemental form. (Watanabe, 1997).

4. Rhizofiltration

Rhizofiltration is the use of plant roots to sorb, concentrate or precipitate metal contaminants from aqueous medium. Although the technology is more suitable for decontamination of polluted water or removal of organic and inorganic impurities from wastewater by the use of aquatic plants, terrestrial species can also be used by growing hydroponically or on floating platforms in treatment ponds. An ideal plant for rhizofiltration should quickly produce significant amount of root biomass with large surface area when grown hydroponically.

5. Phytostimulation (Microbe Stimulated Phytoremediation)

Soil microbes have been found suitable to enhance the bioavailability of metals for phytoremediation and increase the phytoremediation potential of plants by complimenting the process in many ways. Soil microbes may degrade organic pollutants into simpler organic compounds and supply them as nutrient to plants for enhanced phytoremediation of the polluted site.

> Microbial activity in the rhizosphere of plants is several-fold higher than in the bulk soil. The population of microflora present in the rhizosphere is much higher than present in the vegetation-less soil. It is due to the presence of nutrients from the root exudates of plants. (Suthersan, 1997).
>
> A typical microbial population present in the rhizosphere, per gram of air-dried soil comprises - bacteria 5 x 10^6, actinomycetes 9 x 10^5, and fungi 2 x 10^3 (Schnoor *et al.*,1995).

The rhizosphere in plants is divided into two general areas, the inner rhizosphere at the root surface and the outer rhizosphere immediately adjacent to soil. The microbial population is larger in the inner zone where the root exudates are concentrated. A large variety of exudates are secreted from roots in the form of sugars, amino acids and essential vitamins. Root exudates may also include acetates, esters and benzene derivatives. Enzymes are also present in the rhizosphere and this may act as substrates for the microbial population. Enzymes secreted by plant roots or the microbial community in the rhizosphere comprise esterases different oxido-reductases (phenoloxidases and peroxidases). Plant peroxidases are exuded by some members of Fabacea, Graminae and Solanaceae. White radish (*Raphanus sativus*) and horse radish (*Armoracia rusticana*) secrete 'peroxidase' while the aquatic green algae *Nitella* & *Chara* secrete 'laccase'. (Dias *et al.*, 2006).

Chemolithotrophic bacteria have been shown to enhance metal availability. Several strains of *Bacillus* and *Pseudomonas* have been reported to increase cadmium accumulation by *Brassica juncea.* Naturally occurring *Rhizobacteria* were found to promote selenium (Se) and mercury (Hg) bio-accumulation in plants growing in wetlands. (Singh et. al., 2006).These microbes grow much better if organic manures are added to the soil.

Uptake of hydrophobic xenobiotics of larger size can be facilitated by primary microbial biodegradations in the rhizosphere. The hydrophobic persistent organic pollutants like 'polychlorinated dibenzo-p-dioxins and furans (PCDD/Fs) and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) with present log K_{ow} value above 4 have been reported to be taken up by roots and transported to shoots in *Cucurbita pepo*. (Singh et. al., 2006).

Plants which have been identified to promote rhizosphere degradation of petroleum compounds are—

1. Big bluestem (*Andropogon gerardii*),
2. Indian grass (*Sorghastrum nutans*),
3. Switch grass (*Panicum virgatum*),
4. Canada wild rye (*Elymus canadensis*),
5. Western wheat grass (*Agropyron smithic*) and some more.

Aprill & Sims (1990) studied the degradation of 4 PAHs viz. benz(a)anthracene, chrysene, benzo(a)pyrene and dibenz (a,h)anthracene in the root zone of mixture of 8 prairie grasses and found that the disappearance of the PAHs were consistently greater in the vegetated columns of soil than in the unvegetated controls.

Plants which have been identified to enhance microbial degradation of PAH compounds in the rhizosphere are—

1. Alfalfa (*Meticago sativa*),
2. Fescue (*Festuca anundinacea*),
3. Big bluestem (*Andropogon gerardii*), and
4. Sudan grass (*Sorghum vulgare sudanense*).

Wheat grass (*Agropyron cristatum*) has been found to enhance microbial degradation of 'pentachlorophenol' (PCP) and *Phragmites australis* the microbial degradation of phenols in the soil. Mackova *et al.* (1997), reported effective degradation of PCBs (Polychlorinated Biphenyls) by cells of *Solanum nigrum* that were infected with bacterial strains of *Agrobacterium tumefaciens* and *A. rhizogenes*. Hairy root cultures of *S. nigrum* proved capable of transforming PCBs under controlled conditions. Plant cells proved capable of transforming PCBs even after growth had stopped. The red mulberry (*Morus rubra*) appears to be another good plant for enhancing microbial degradation of PCBs.

Root exudates in the form of compounds structurally analogous to the chemicals utilized by microorganisms, would enhance the activity of the bacteria present in the rhizosphere accelerating the degradation process. Certain phenolic compounds in the root exudates has been found to support growth of PCB-degrading bacteria and serve as structural analogs for PCB degradation. (Zhao *et al.*, 2001).

Sometimes inoculation of plant soil by microbes enhance the phyto-degradation of organic contaminants present in the soil. Degradation of 'pentachlorophenol' (PCP) was accelerated by the proso millet after the soil in which it was growing was inoculated with *Pseudomonas* strain SR 3.

6. Mycorrhiza Assisted Phytoremediation

It is generally considered that the majority of plants growing under natural conditions have symbiotic relationship with mycorrhizal fungi roots, which results in increase in root surface area and enhanced absorption of nutrients. Mycorrhizal fungi have been reported in plants growing on heavy metal contaminated soils/sites indicating its heavy metal tolerance and a potential role in heavy metal phytoremediation. Mycorrhizal species *Glomus, Gigaspora* and *Entrophospora,* have been reported to be associated with most of the plants growing in the heavy metal polluted soils indicating its obvious role in assisting the plants in tolerating and removal of metals by phytoremediation. (Khan *et al.,* 2000).

CELLULAR MECHANISM OF UPTAKE & DETOXIFICATION OF HEAVY METALS BY PLANTS : ROLE OF METAL CHELATORS & TRANSPORTER PROTEINS

Chelation of heavy metals in the cytosol by high affinity ligands followed by subsequent compartmentalization of the ligand-metal complex is thought to be a potentially very important mechanism of heavy metal detoxification and tolerance by plants. It is a process in which the metal cations binds to a chemical compound, resulting in an 'uncharged metal complexes' that become easily bioavailable for uptake & transport in plants. Several natural and synthetic chelators are known. Among the natural chelators are organic acids, amino acids, phytochelatins (PC) and metallothionein (MT) and among the synthetic ones are ethylene diamine tetra acetic acid (ETDA) & ethylene glycol tetra acetic acid (EGTA). The use of natural & synthetic chelates has been shown to dramatically stimulate lead (Pb) accumulation in plants. They prevent Pb precipitation and keep the metal as soluble chelate - Pb complex bioavailable for uptake by plant roots & its transport to shoots. (Sriprang, 2006). Addition of synthetic chelates like EDTA has been found to be very effective in facilitating the plant uptake of Cd, Cu, Zn & Ni.(Raskin *et al.* 1997). Malic & Citric acids are two organic acids in plant roots that have been shown to make uncharged complexes with heavy metals.

Uptake and Translocation of Metals from Soil to Roots and to the Shoots : Role of Transporter Proteins

Accumulator plants must have the ability to uptake metals from the soil to roots and translocate them to the shoots at high rates. Because of their charge, metal ions cannot move freely across the cellular membrane, which are lipophilic structures. Membrane proteins mediate ion transport into cells and they are known as 'transporter proteins' (TP). They contain binding domains, which binds to specific ions from extracellular space through the hydrophobic environment of the membrane into cell. (Sriprang & Murooka, 2006). Transporter proteins are usually specific and one TP usually binds with an individual heavy metal only. Thus, Zinc (Zn) transporter protein (ZnTP1) can only uptake and bind with Zn but not with Cd.

Storage of Metals in Plant Cells

The Vacuolar Compartmentalization for Tolerance : The vacuole in plant cells appears to be the main storage site for metals. Compartmentalization of metals in the vacuole appears to be the part of the tolerance mechanism of some metal hyper-accumulator plants. The nickel (Ni) hyper-accumulator *T. goesingens* enhances its Ni tolerance by compartmentalizing most of the intracellular leaf nickel components into the vacuole. (Sriprang & Murooka, 2006).

Role of Phytochelatins (PCs)

PCs are metal-binding peptides in plants made of three amino acids (Glu, Cys & Gly) and are know to play an essential role in the heavy metal detoxification by chelating heavy metals in the cytosol & sequestering PC- metal complexes in the plant cell vacuoles via transport across the tonoplast. Production of PC is activated / stimulated by heavy metals and the best activator is cadmium (Cd) followed by Ag, Bi, Pb, Zn, Cu, Hg and Au cation. It has been studied that Cd stimulates synthesis of PCs, which rapidly form a low molecular weight Cd-PC complex. The Cd-PC complex is transported into the vacuole by a Cd/H antiport and an ATP - dependent PC-transporter protein. A gene (*Hmt1*), which codes for PC-transporter protein in yeast was isolated. The *Hmt1* gene encodes for a member of family of ATP-binding cassette (ABC) membrane transport proteins that is located in the vacuolar membrane. The gene (*Hmt1*) product i.e. a protein (Hmtl) is responsible for transporting the Cd-PC complex into the vacuole. (Sriprang & Murooka, 2006).

PCs contain strongly nucleophilic sulfydryl group and thus can react with many toxic specie within the plant cell such as the free radicals, active oxygen species, and cytotoxic electrophilic organic xenobiotics and obviously heavy metals.

Role of Metallothioneins (MTs)

MTs are super cysteine-rich metal-chelating proteins in plants meant to sequester toxic heavy metals such as cadmium (Cd). MTs bind readily with Zn, Hg, Cu, Cd & Ag.

FATE OF CHEMICALS ABSORBED IN PLANT ROOTS & SHOOT CELLS

Contaminants once absorbed inside the plant roots are either degraded into simple and harmless compounds or transformed into some inert harmless materials. This they do with the aid of 'enzymes' (described as biological catalysts) or symbiotic microbes inhabiting in their roots.

Phytodegradation

Certain plant species breakdown the contaminants after absorbing them. This they do through enzyme-catalyzed metabolic process within their root or shoot cells. Others breakdown the contaminants in the substrate itself by secreting enzymes and chemical compounds. The enzymes secreted are usually dehydrogenases, dehalogenases, oxygenases peroxidases, phosphatases, laccases and reductases. Plants can also degrade 'aromatic rings' in complex organics with the aid of enzymes. Phenols have been found to be degraded by radish (*Raphanus sativus*) and potato (*Solanum tuberosum*) that contain 'peroxidase' enzymes.

The biodegraded constituents are converted into insoluble and inert materials that are stored in the lignin or released as exudates (Watanabe, 1997). Enzymes cytochrome P450, peroxygenases & peroxidases are involved in plant oxidation of xenobiotics. Other enzymes like Glutathione s-transferase, carbo-oxylesterases, o-glucosyltransferases and o-malonyltransferases are associated with xenobiotic metabolism in plant cells & transportation of intermediates.

Several plants biodegrade contaminants with the aid of microbes which live in symbiotic association on their roots.

Phytotransformation

Several inorganic and organic contaminants once absorbed inside the root, may become biochemically bound to cellular tissues (biotransformed), in forms that are biologically inert or less active (Watanabe, 1997). In many cases, plants have the ability to metabolize organic pollutants by phytotransformation and conjugation reactions followed by compartmentalizing products in their tissues. Poplar trees (*Populus* Spp. have been found to transform 'trichloroethylene' in soil and groundwater.

TABLE 6

Plant Enzymes Implicated in Phytodegradation & Phytotransformation of Organics & Inorganic Compounds

	Enzymes	Contaminants Degraded / Transformed into Less Toxic Forms
1.	Phosphatase	Organophosphates
2.	Aromic Dehalogenase	Chlorinated aromatic compounds (e.g. DDT, PCBs etc.)
3.	O-demythlase	Metaoalchor, Alachlor
4.	Cytochrome 450, Peroxidases & Peroxygenases	PCBs
5.	Glutathione s-transferase, carbo-oxylesterases o-glucosyltransferases, o-malonyltransferases	Xenobiotics
6.	β-cyanoanaline synthase	Cyanide

Sources : (Sandermann, 1994; Macek *et al.*, 2000; Prasad, 2006).

Phytodetoxification of Toxic Cyanide (CN) by Phytotransformation

Plants possess the necessary mechanism to detoxify the cyanides from mining wastes resulting from gold & silver mining. Cyanide (ammonium thiocyanate) is the leach reagent of choice for gold (Au) and silver (Ag) extraction. During several metabolic functions, plants are confronted with cyanide as a byproduct of metabolism. This occurs particularly during synthesis of 'ethylene' in mature tissues, where 'hydrogen cyanide' (HCN) is formed as byproduct.

Consequently vascular plants have evolved effective strategies for detoxifying the toxic cyanide with the aid of enzymes. Those identified with cyanide detoxification are *Salix* spp. & *Sorghum* spp. Plants only survive cyanide exposure up to the doses they can eliminate. The cyanide detoxifying enzyme system is 'beta-cyanoalanine synthase' which connects free cyanide and cysteine to cyanoalanine. The final metabolic product is 'aspargine', a non-toxic essential amino acids in plants.(Manning, 1988; Trapp *et al.*, 2003).

Summary of the Metal Uptake & Transport in Plants

1. Metal ions are mobilized by secretion of chelators in soil and by acidification of the rhizosphere (soil in the vicinity of root system);
2. Uptake of hydrated metal ions or metal-chelator complexes mediated by the transporter proteins residing in the plasma membrane & energized by ATP (cellular energy molecules- adenosine triphosphate produced by the cell mitochondria);
3. Metals are chelated again in the cell cytosol by various binding natural chelators and ligands (PCs & MTs);
4. PCs quickly form complex with Cd (because cadmium is the best activator of PCs and PCs have greater affinity for Cd) and the PC-Cd complex is transported into the cell vacuole. Other metals also follow the same process and are transported into the cell vacuole.

SOME WONDER PLANTS FOR PHYTOREMEDIATION OF CONTAMINATED LANDS : POTENTIAL FOR COMMERCIALIZATION

1. The Vetiver Grass (*Vetiveria zizanioides*)

The 'wonder grass' vetiver is native of India, and has been used worldwide for phytoremediation. It grows very rapidly and becomes effective for environmental restoration works in only 4-5 months as compared to 2-3 years taken by trees and shrubs for the same job. It can tolerate very high acidity and alkalinity conditions (pH from 3.0 to 10.5); high soil salinity (EC = 8 dScm), sodicity (ESP = 33 %) and magnesium; very high levels of heavy metals Al, Mn, Mg, As, Cd, Cr, Ni, Cu, Pb, Hg, Se, Zn and the herbicides and pesticides in soils.

Vetiver roots can absorb and accumulate several times of some of the heavy metals present in the soil and water. (Truong and Baker, 1998). Studies further indicated that very little (1 to 5 %) of the arsenic (As), cadmium (Cd), chromium (Cr) and mercury (Hg) and very moderate amount (16 to 33 %) of copper (Cu), lead (Pb), nickel (Ni) and selenium (Se) absorbed were translocated to the shoots above. Hence its green shoots can be safely used as feed or grazed by animals or harvested for mulch. Vetiver can be disposed off safely elsewhere, thus gradually reducing the contamination levels. Vetiver also has high capacity to absorb and remove agro-chemicals like carbofuran, monocrotophos and anachlor from soil thus preventing them from contaminating and accumulating in the crop plants. (More about VGBT has been discussed in Chapter 5 of this section).

Success Story

At the Scott Lumber Company site in Missouri, U.S., 16,000 tonnes of soils contaminated with polyaromatic hydrocarbons (PAHs) were biologically treated with VGT. The PAH concentration was effectively reduced by 70 %.(Pinthong *et al.*, 1998).

2. The Brake Fern (*Pteris vittata*)

It is hardy, versatile and fast-growing perennial plant easy to propagate. It can grow on contaminated soils up 1,500 ppm of arsenic and is extremely efficient in extracting arsenic (As) from both soil and water and translocating it into its above-ground biomass mainly the fronds. It can reduce the concentration of 200 ppm of arsenic in water to nearly 100-fold (10 ppm) within 24 hours and can hold up to 22 grams of arsenic per kg of plant matter.

Brake fern has great potential to remediate arsenic contaminated soils and is very cost-effective. Arsenic concentration in fern fronds growing in soil spiked with 1,500 ppm arsenic increased from 29.4 ppm to 15,861 ppm in two weeks. In the same period, ferns growing on soil containing just 6 ppm arsenic accumulated 755 of arsenic in their fronds, a 126-fold enrichment. Arsenic concentration in roots were less than 303 ppm, while in the fronds reached 7,234 ppm. Up to 93 % of the arsenic was concentrated in the fronds. (Ma *et al*, 2001).

Chinese brake fern has been shown to grow in soils of 50–4030 mg/kg arsenic and even in tailings containing arsenic as high as 23,400 mg/kg of soil. (Chen *et al*, 2002). More wild fern species are coming to light with phytoremediation properties.

Success Story

In 2001, the Edenspace System Corporation in the US used the Chinese brake fern to treat 1.5 acre site contaminated with arsenic (As) in New Jersey, North Carolina. The fern phytoextracted more than 200 fold of arsenic (As) in above ground part. (Singh, *et al.*, 2006)

3. Hybrid Poplar (*Populus* x *Canadensis*)

Hybrid poplar trees has assumed great significance in phytoremediation of several kinds of toxic organic pollutants including TCE (trichloroethylene), a potential carcinogen commonly found in groundwater as well as in contaminated sites.(Kassel *et al.* 2002). While several organic pollutants are metabolized or degraded to less toxic compounds and bioaccumulated in the plant, others like volatile chlorinated organic compounds e.g. BTEX (benzene, toluene, ethylbenzene & xylene) are released to the atmosphere. Poplar trees has also been used in phytoremediation of soil and groundwater contaminated PAHs (polycyclic aromated hydrocarbons), cyanide and pesticides. (Singh *et al.* 2006).

Normally 1000-2000 hybrid poplar per acre are planted for phytoremediation of contaminated land. Poplar trees grow very fast. They can grow 9 to 15 feet / year. The average life of hybrid poplar is about 30 years, and every 4-6 years, the above ground biomass can be cut and removed and new shoots grow from the cut stem.

Success Stories

1. In Milwaukee, Wisconsin, USA, the Ecolotree Inc. used the hybrid poplar trees to phytoremediate soil and groundwater contamination with petroleum related organics, PAHs and chlorinated organics released by accidental spills in 2000. The poplar trees were buried up to 10 feet below the surface and a sub-surface aeration system was provided to encourage deep rooting into groundwater.

2. In Illinois, USA, in 1999, the Ecolotree Inc. used the hybrid poplar to treat soil contaminated with chemical fertilizer & pesticides. Some 440 trees, of about 12 to 18 feet tall bare root poplar were planted into 6 feet deep trenches.

3. The Occidental Petroleum Corporation, LA & University of Washington, USA, used hybrid polar to treat several sites in the US contaminated with 'trichloroethanol'.

4. Hybrid polar trees were successfully used by other commercial companies like Phytokinetics Inc. in the US to treat groundwater contaminated with chlorinated volatile organics including dichloro-benzidine at several superfund sites.

5. Technical University of Denmark, used poplar trees to phytoremediate soils contaminated by gasoline and diesel compounds at old gas filling station at Axelved Denmark, and cyanide, PAHs, oil and BTEX (benzene, toluene, ethylbenzene & xylene) contaminated soil at former municipal gas work site in Denmark.

6. The Polish Academy of Sciences, used the poplar trees to remove pesticides stored in bunkers at a resort Niedwiady, Poland. (Singh, *et al.*, 2006)

4. Sunflower (*Helianthus annus*)

Sunflower is economically important as an 'oil yielding' plant and the oil is considered to be very healthy rich in 'polyunsaturated' lipids good for heart. It has gained much importance as phytoremediator for environmental cleanup of both chemically & radiologically contaminated soil & water bodies (by growing hydroponically). Sunflower plants, when tested in batch experiment significantly reduced the concentrations of heavy metals cadmium (Cd), chromium (Cr), copper (Cu), manganese (Mn), nickel (Ni) and lead (Pb) within an hour of treatment. (Eapen *et al.*, 2006).

Success Stories

1. Daimler Chysler, the car manufacturing company in the US had 4,300 cubic pounds of soil contaminated with lead (Pb) ranging from 75 - 3,450 mg/kg of soil at its Detroit Forge Site in 1998. Sunflower plants were used for phytoremediation. Successive crops of sunflower (with Indian mustard plant) were planted in 24 inches deep ex-situ treatment cell on an impermeable concrete base. In single season of crop growth the lead contents in the soil was brought down to 900 mg/kg of soil and subsequently it was removed completely after successive crop growth. The total cost of phytoremediation treatment by sunflower was US $ 50.00 per cubic yard, which saved more than US $ 1.1 million compared to the estimated cost of physio-chemical treatment by soil excavation and disposal in landfills. (Singh, *et al.*, 2006).

2. Sunflower & the Indian mustard plant (*Brassica juncea*) was used to phytoremediate the lead (Pb) contaminated soil at industrial facility in Connecticut, USA. A combined phytoextraction and phytostabilization treatment for three successive years (1997-2000) costed less than US $ 40 per cubic yard of treated soil. (Singh, *et al.*, 2006).

3. The Edenspace System Corporation in the US used the sunflower and the Indian mustard to treat various sites in the USA contaminated with heavy metals. The plants took up heavy metals more than 3.5 % of their dry weight. The company also used this plant to remediate uranium (U) contaminated soils (47 mg/kg of soil) at US Army Sites at Aberdeen, Maryland. The sunflower plants bioaccumulated uranium at the rate of 764 mg/kg - 1669 mg/kg of soil. (Singh, *et al.*, 2006).

5. Indian Mustard (*Brassica juncea*)

The Indian mustard is an oil yielding plant. Mustard oil is used in the Indian culture for cooking as well as for medicinal purposes. It has large root system with good biomass and can also be grown hydroponically. It has gained much significance as 'phytoremediator' for environmental cleanup of both chemically & radiologically contaminated soil & water bodies (by growing hydroponically). The Indian mustard concentrate toxic heavy metals (Pb, Cu and Ni) from hydroponics solution to a level up to several percent of their dried shoot biomass (Sriprang, 2006). Several superior 'transgenic species' of *B. juncea* have been produced for improved phytoremediation function. (Described below).

Success Stories

1. The Edenspace System Corporation of USA, used the Indian mustard plant to treat the radionuclide strontium ($Sr^{89/90}$) contaminated soil at Fort Greely in Alaska, USA. The plants bioaccumulated more than 10-15 fold of strontium ($Sr^{89/90}$) higher than in soil. They also used the Indian mustard with sunflower to treat various sites in the USA contaminated with heavy metals. They accumulated more than 3.5 % of heavy metals of their dry weight. They also used the Indian mustard to remove cesium - 137 (Cs^{137}) from the contaminated pond waters after the Chernobyl Nuclear Power Plant disaster in Ukraine in 1986. (Singh, *et al.*, 2006).

2. The Brookhaver National Lab, New Jersey, USA, used Indian mustard to remove radionuclides cesium - 137 (Cs^{137}) and strontium - 90 (Sr^{90}) by phytoextraction from contaminated soil. (Singh, *et al.*, 2006).

3. The Phytotech, Florida, USA used the Indian mustard plant to remediate lead (Pb) and cadmium (Cd) contaminated soil at the Czechowice Oil Refinery, Katowice, in Poland. (Singh, *et al.*, 2006).

4. As described above the Indian mustard plant was used with sunflower (*Helianthus annus*) to phytoremediate the lead (Pb) contaminated soil at industrial facility in Connecticut, USA. (Singh, *et al.*, 2006).

6. Alpine pennycress (*Thlaspi caerulescens*)

It is a small weedy member of the broccoli & cabbage family (Brassicaceae) and can thrive on soils having very high levels of cadmium (Cd) and zinc (Zn). It is a 'hyper-accumulator' plant and can bio-accumulate up to 30,000 ppm (mg/L) Zn and 1,500 ppm Cd in its shoots while exhibiting few or no toxicity symptoms. A normal plant can be poisoned with as little as 1000 ppm of zinc or 20 to 50 ppm of cadmium in its shoots.

7. The Bermuda grass (*Cyanodon dactylon*)

It is very common grass species and can colonize and thrive in soils containing up to several thousands ppm (parts per million) of arsenic (As) (Ashley and Lottermoser, 1999). It also absorb and concentrate arsenic in its above ground parts.

8. Nightshade (*Solanum nigrum*)

Mackova *et al.* (1997), reported effective degradation of PCBs (Polychlorinated Biphenyls) by cells of *Solanum nigrum* that were infected with bacterial strains of *Agrobacterium tumefaciens* and *A. rhizogenes*. Hairy root cultures of *S. nigrum* proved capable of transforming PCBs under controlled conditions. Plant cells proved capable of transforming PCBs even after growth had stopped.

9. Drum-Stick Tree (*Moringa oleifera*)

Some plant extract have metal binding properties and can help remove several contaminants from aqueous solutions. Researches have indicated that the seeds of some trees possess strong anti-coagulant properties like 'alum' and remedify contaminated waters. Others have strong anti-bacterial, anti-protozoal and anti-viral properties and are significantly effective in inactivating bacteria, protozoas and viruses and prevent their re-growth. These trees are drum-stick tree (*Moringa oleifera*) and the other is *Strychnos potatorum.*

10. Strychnos Tree (*Strychnos potatorum*)

A chemical compound from the seeds of *S. potatorum* readily binds with toxic heavy metals like cadmium and mercury and radioactive materials like uranium, thorium and other radioactive isotopes present in the nuclear wastes. *S. potatorum* is highly effective in removing industrial pollutants from the effluents. (Evans, 1991,UNEP Report).

11. The Willow Tree, Alfalfa & Cottonwood

In 1999 - 2001, the native willow tree, alfalfa, cottonwood and several grasses were used to treat 140 acre of land (site) contaminated by wood preservative waste which included PCPs (pentachlorophenol) and PAHs (polyaromated hydrocarbons) at Union Pacific Railroad, Wyomin, USA . (Singh, *et al.*, 2006)

ROLE OF ENVIRONMENTAL BIOTECHNOLOGY & GENETIC ENGINEERING IN IMPROVING EFFICIENCY OF PHYTOREMEDIATION : SOME ACHIEVEMENTS

Several genes that are involved in metal uptake, translocation, sequestration and bioaccumulation has now been identified. Transfer of these genes into candidate plant will result in developing 'transgenic plants' with enhanced ability for metal uptake and accumulation for removal from environment. Environmental biotechnology provides us the tools to accelerate the phytoremediation process through either 'over-expression' of those genes responsible for the sequestration of heavy metals & radionuclides in plants or through 'gene transfer'. Many appropriate genes of foreign origin have been transferred in plants like *Arabidopsis thaliana, Nicotiana tabaccum, Brassica juncea, Brassica oleraceae* var *botrytis, Lycopersicon esculentum* etc. to enhance the phytoremediation efficiency of these plants.

Transgenic plants so far have been developed for the hyper-accumulation of toxic heavy metals like Hg, As, Pb, Cd, Co, Ni, Zn, Cu etc. from soil, and from air gaseous pollutants like NO_2 ,SO_2 and organic pollutants e.g. 2,4,6-trintrotoleune and organomercurials etc. Higher plants have evolved many genes (and their products-enzymes), which have potentials to metabolize or degrade different kinds of xenobiotic compounds. (Bizily *et al.*, 2000; Pan *et. al.*, 2005).

Many hyper-accumulating plants are rare, with small population occurring in remote places or have restricted distribution. They often have slow growth rate and produce small biomass. Such hyper-accumulator species may provide suitable genes involved in metal uptake, translocation and sequestration for enhancing phytoremediation.

If genes from highly metal tolerant & hyper-accumulator plants are transferred to high biomass yielding & fast growing cultivars, this can do miracle. Some fast-growing hyper-accumulator species with large biomass has also been identified. These are nickel (Ni) hyper-accumulators *Alyssum bertolonii* and *Berkheya coddii*. They produce 9 & 22 tons/ ha of shoot dry matter respectively, in small-scale field experiments. The arsenic (As) hyper-accumulator *Pteris vittata* can also produce large biomass under favorable conditions.

Important Achievements

1. With the aim of creating 'new transgenic phytoremediator plants' that can tolerate and hyper-accumulate high levels of toxic metals, various *MT* (metallotheonein) genes were introduced into plants such as in *Nicotiana* spp., *Brassica* spp. and *Arabidopsis thalina*. Transgenic plants, that express *MT* genes, have shown to be highly tolerant to cadmium (Cd) and other metals in soil. However, the metal uptake was not markedly altered.

Transfer of *human MT-2* gene to tobacco (*Nicotiana tabaccum*) resulted in transgenic plant with enhanced Cd tolerance and accumulation. Transfer of *pea MT* gene in *Arabidopsis thaliana* resulted in enhanced copper (Cu) accumulation in the transgenic *A. thaliana*. Transfer of *yeast CUP1* gene in cauliflower (*Brassica capitata*) resulted in 16-fold higher accumulation of cadmium (Cd) in the transgenic cauliflower. (Sriprang & Murooka, 2006; Eapen *et al.* 2006).

2. Certain metals such as mercury (Hg) and selenium (Se) can be phytovolatalised usually through plant-microbe interactions (Cunningham and Owe, 1996). Genes for synthesizing the enzyme 'bacterial mercuric ion reductase' has been engineered into *Arabidopsis thaliana* and the resulting transformant transgenic plant is capable of tolerating and volatalising mercuric ions. The toxic cation is absorbed by the root and reduced to volatile Hg (O) by the introduced mercuric ion reductase. (Rugh *et al.* 1996).

3. Transgenic yellow poplar expressing a modified *MerA* gene and *MerB* gene has been produced for the phytoremediation of mercury (Hg) which has become global problem in aquatic environments resulting from its industrial use in blaching operations as a catalyst, as a pigment in paints and in mining of gold. The genes *MerA* and *MerB* were isolated from mercury resistant bacteria which synthesizes the enzymes 'mercuric iron reductase' (MerA) and 'organomercurial lyase' (MerB) respectively. The transgenic poplar MerA expressing *MerA* gene, released 10 times more elemental mercury (Hg) than the untransformed plantlets. Transgenic plants MerB expressing *MerB* gene were significantly more tolerant to methylmercury and other organomercurials compared to the untransformed plants. The MerB plants (with *MerB* genes) effectively converted the highly toxic methylmercury to elemental mercury (Hg^{2+}), which is about 100 times less toxic in plants. They were released from the plants by phytovolatalization.

The MerA and MerB double-transgenic poplar plant showed the highest tolerance to organic mercury (up to 10 μM) compared to the wild type plants (0.25 μM). (Rugh *et al.* 1998; Bizily *et al.* 2000).

4. Transfer and over-expression of two genes for production of enzymes γ-glutamyl cysteine synthetase and glutathione synthetase in transgenic Indian mustard (*Brassica

spp.) resulted in accumulation of higher levels of GSH and PC. They exhibited enhanced cadmium (Cd) tolerance and accumulation and also extracted more Cd, Cr, Cu, Pb, and Zn than wild plants (Zhu, *et al.* 1999).

5. Transgenic Indian mustard (*Brassica juncea*) was produced by introducing foreign genes.

(a) A gene *APSl* encoding ATP-sulfurylase from *Arabidopsis thaliana* and its over-expression in *B. juncea* led to 2-3 fold higher accumulation of selenium (Se) in shoots and 1.5 fold higher accumulation in roots as compared to the wild type plants. (Pilon-Smits *et al.*, 1999).

(b) Transfer and over-expression of the gene *gshII* encoding the enzyme 'glutathione synthetase' (GS) from *E.coli* into *B.juncea* increased the cadmium (Cd) accumulation efficiency of the transformed transgenic plant to 3-fold high. (Zhu *et al.*, 1999).

6. Transgenic plant of *Arabidopsis thaliana* have been produced by transfer of foreign genes with great value in phytoremedation technology.

(a) The transfer of mercuric ion reductase gene (*merA*) from *E.coli* made the transgenic plant more tolerant to $HgCl_2$ and volatalized elemental mercury.

(b) The transfer of mercuric ion reductase genes (*merB*) from *E.coli* made the plant more tolerant to methyl mercury and other organomercurials. The transgenic plant could grow on 10-fold higher methyl mercury than the wild type. (Rugh *et al.*, 1996).

(c) Transfer of both genes (merA & merB) from *E.coli* to *A.thaliana* made the plant to grow on 50-fold methyl mercury. (Bizily *et al.*,1999 & 2000).

(d) Transfer of bacterial genes encoding arsenate reductase (*ars c*) and g-glutamyl cysteine synthetase (g-*ECS*) together from *E.coli* led to higher arsenic (As) tolerance and 4-17 fold higher arsenic hyperaccumulation in shoots. (Dhankher, *et al.*, 2002).

(e) Transfer and over-expression of gene *NiR cDNA* synthesizing the enzyme 'nitrate reductase' into *A. thaliana* led to 40 % increase in assimilation of nitrogen dioxide (NO_2).

7. Transgenic tobacco (*Nicotiana tabaccum*) plant was produced by transferring bacterial nitroreductase gene (pNITRED3). This increased the tolerance to the2,4,6-trinitrotoluene (TNT) (recalcitrant military explosive) and also its 'detoxification' by the transgenic tobacco.(Hannink *et al*,. 2001).

8. *Nicotiana glauca* is widely distributed, fast growing, high biomass producing and a herbivore-repulsive plant. Gisbert *et al.* (2003) used this plant to over-express wheat gene encoding PCS. The transgenic plant showed greatly increased tolerance to metals, such as lead (Pb) and cadmium (Cd), developing seedling roots 160 % longer than the wild types. Seedlings grown in the mined soils containing high levels of Pb (1572 ppm), accumulated double concentration of this heavy metal than the wild type.

9. Somatic cell hybrid produced between *Brassica juncea* (a high biomass Pb accumulator plant), and *Thlaspi caerulescens* (a known Zn and Ni hyperaccumulator), showed increased tolerance to Pb, Ni and Zn and the total amount of lead (Pb) phyto-extracted was much greater because of the high biomass produced. (Dushenkov *et al.* 2002).

10. Transporter protein and intracellular high-affinity binding sites mediate the uptake of the metals and other substances across the plasma membrane. Many 'metal transporter genes' have been cloned recently. (Datta & Sarkar, 2004). Maser *et al.*, 2001 (quoted in Singh *et al.*, 2006) have cloned genes of ZIP (Zn regulated transporter / Fe-regulated transporter like proteins) family e.g. *ZNT1* & *ZNT2*, from *Thlaspi careulescens*, which are highly expressed in roots of the accumulator plants.

Future Prospects

1. If a plant could be genetically altered to produce higher levels of endogenous organic acids such citric or malic acids, the PCs and MTs which works to bind metal cations to form their complexes and facilitate their bioavailability, uptake and transport, this can significantly improve phytoremediation process by phytoextraction.

2. It has been found that the amount of free histidine (which chelates & form complexes with nickel & facilitate their uptake and transport) increases in plant roots with their exposure to nickel in soil. By genetically modifying histidine metabolism, it might be possible to increase the capacity of nickel (Ni) bioaccumulation by plants.

3. Hyper-accumulating plants often accumulate a specific element only, thus limiting its applicability to phytoremediate sites which have multiple metal contamination. If the genes from various species known to produce selected enzyme that help bioaccumulation of specific metal is transferred to a particular species this plant can become a 'super-hyper-accumulator' capable of phytoremediating sites with multiple metal contamination.

4. Transporter proteins, isolated from hyper-accumulating species, such as Zn - Transporter Protein (ZnTP1) can only uptake zinc (Zn), but not the toxic metal cadmium (Cd). They are specific. Molecular study for alteration of such genes (*ZnT1*) for transport of other metals may be useful for enhancing phytoextraction.

5. The expression of the iron-transporter gene (*IRT1*) producing the iron-transporter protein (IRTP1) mediate the uptake of Na^{+} and Cd^{2+} in yeast cells. Therefore, the transfer of such gene (*IRT1*) to plants may enhance the uptake of metal ions by the plant roots.

'SYMBIOTIC ENGINEERING' : NOVEL BIOTECHNOLOGY FOR HEAVY METAL BIOREMEDIATION BY LEGUME-BACTERIA SYMBIOSIS

A novel bioremediation system for heavy metals using the symbiotic relationship between leguminous plant and genetically engineered rhizobia bacteria has been suggested by Sriprang *et al.* (2002). It has been observed that rhizobium grow slowly for long times in soil, but if they infect a compatible legume they grow rapidly. Successful infection by even a single bacterium can lead to the formation of a 'nitrogen-fixing nodule' on the root of legume plant containing over 10^8 bacterial progeny. This special feature of symbiotic relationship gives clue for biotechnological transfer and expression of *MT* (metallothionein) genes that sequester heavy metals from contaminated soil. Once symbiosis with *MT* genes is established with legumes, the heavy metals will be accumulated in the nodules. This would be an alternative and more cost-effective method to remove heavy metals from soil.

Sriprang *et al.* (2002) developed this novel 'plant-bacterial bioremediation' technology for heavy metal removal by the introduction of the chimeric *MTL4* gene to *Meso-rhizobium haukii* sub-species *rengei* strain B3 which is a genetically engineered bacterium capable of establishing symbiotic relationship with a legume *Astralagus sinicus* (used as a green manure in rice fields in Japan & China), by eliciting the formation of nitrogen-fixing root nodules. The rhizobia undergo differentiation into a distinct cell type called as 'bacteriod', which is capable of fixing atmospheric nitrogen. Thus the legume *A. sinicus* would be used as a green manure to improve fertility in rice fields as well as to scavenge heavy metals from the soil. This was also a first report that a 'foreign gene' was expressed in bacteroids in the nodules.

CONCLUDING REMARKS

Phytoremediation technologies are based on the principles of bioengineering & environmental biotechnology that a 'living plant' can be considered as a 'solar driven pump', which can extract, concentrate, degrade, volatalize or vaporize, soluble toxic substances from the soil, water or air, through their natural water & mineral uptake, transport, partitioning, assimilation & transpiration mechanisms and systems. It is also cost-effective, economically viable, ecologically compatible and socially acceptable and safer methods for management of polluted lands & soil as compared to the physical and chemical methods (as there is no risk from exposure to the toxic substances at waste and spill sites and remediation of polluted soil in world may involve a cost up to US $ 300 billions by other methods). (Raskin *et al.*, 1997).

It is a cheap alternative to the SUCK, MUCK and TRUCK cleaning approach to contaminated sites. The technology capitalizes on the synergistic relationship among plants, microbes, water & soil that have evolved naturally over millions of years.

The technology can be implemented *in-situ* or *ex-situ* to cleanup diverse organic contaminants, e.g. petroleum hydrocarbons, gas condensates, solvents, crude oil, pesticides, herbicides, explosives, chlorinated compounds and polyaromatic hydrocarbons as well as typical inorganic toxicants like heavy metals, metalloids, radionuclides etc.

By planting 'hyper-accumulator' plants in metal contaminated soils & industrial waste sites, several heavy metals can be removed by harvesting the above-ground parts. The precious metals can be recovered back from the plant parts (leaves & roots) through solvent extraction method for reuse in industries thus preventing the mining of these metals and the consequent environmental problems. Growing hyper-accumulator plants in contaminated soils can reduce soil erosion, increase soil organic matter and restore fertility. The technology is 60 to 80% less costly as compared to the conventional physical & chemical methods of soil & water decontamination.

Furthermore, the physio-chemical methods used for soil remediation render the land useless as a medium for plant growth as they also remove all biological soil organisms such as the useful microbes e.g. nitrogen fixing bacteria & mycorrhizal fungi, and also the earthworms—all necessary for maintaining soil fertility.

In addition to pulling out the toxic contaminants from the soil to matabolize, concentrate (bioaccumulate) or evaporate, the phytoremediation techniques involve extensive pull out and evaporation of water from the plant covered sites. This high consumption and recycling of water can also prevent wash out of polluants and slow down the possible migration of toxic compounds through the soil and into the groundwater.

Researches into environmental biotechnology promises to enhance the phytoremediation efficiency of known phytoremediator plants. A genetic combination of 'fast growing', 'high biomass yielding' & ' high tolerance & hyper-accumulation' of toxic metals in plant shoot is best suited for the purpose of phytoremediation. It should also be easily cultivated and harvested.

These days several commercial organizations are employing phytoremediation technology for environmental clean up and restoration. Phytotech Inc. of US is using Indian mustard (*Brassica* spp.) to remove lead (Pb) from soil and sunflower (*Helianthus anus*) to remove uranium (U) and cesium (Cs) from aqueous waste stream. Phytoworks Inc. of US is focusing on remediation of organics and mercury (Hg) by using transgenic plants metabolizing mercury. Earthcare Inc. is working on phytoremediation of organic contaminants.

The novel bioremediation system called 'symbiotic engineering' involving advantages of both the bacterium rhizobium and the leguminous plants using many useful genes like *AtPCS, MTL4* (metallothionein gene) and *IRTI* (iron regulated transporter gene) may provide another valuable bioremediation tool.

However, there are some limitations of all phytoremediation technologies. These are -

1. The contaminated sites / water bodies may be too toxic for plants to survive and work;
2. In case of phytoremediation, if the contaminants are below the root zone, it may not be extracted or stabilised;
3. Time required for phytoremediation of contaminated sites may be too long, often going in years.

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Useful Website

www.mobot.org/jwcross/phytoremediation/ (Using Plants to Clean Up Soils)

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3

PHYTOREMEDIATION OF POLLUTED WATER

A Low-cost Nature's Biotechnology for Environmental Clean Up of Chemically & Radioactively Contaminated Water by Aquatic Plants : An Achievement of Researches into EBT

KEY WORDS

Organic Contaminants; Inorganic Contaminants; Radiological Contaminants; Aquatic Macrophytes; Hydroponically Grown Terrestrial Plants for Phytoremediation; Rhizofiltration; Phytovolatalization; Phytostimulation; Constructed Wetlands Technology for Phytoremediation

BACKGROUND INFORMATION

The 21st century has been called as the 'Century of Water' and the year 2000 was declared as the 'International Year of Freshwater' by UNEP. Water - the 'elixir of life', is the thread that knits together the web of life on earth. It purifies and keeps our bodies healthy, provides us with food, is home to millions of creatures, regulates the global climate, dilute pollutants, and sustains every nation's economic wealth as the essential resource for our industries, agriculture and transportation.

Chemical pollution of water is a growing concern all over the world. Chemicals are released into the global aquatic systems in the form of liquid, dust, fumes or gas. Such releases can be planned (part of the development process e.g. industrial smokestack emissions, automobile exhaust emissions which settles down in rivers and lakes, discharge of domestic and industrial wastewater into rivers and streams, etc.) or unplanned (accidental). Chemicals can also enter the water system during transport (e.g. from the site of manufacture to the site of use), during their intended use (e.g. pesticide spray) or through disposal in landfills and waterways.

The notable environmental contaminants of water bodies are 'radionuclides' as well as 'organic' and 'inorganic' pollutants. Several inorganic contaminants in fact also constitute the 'micro & macro nutrients' for aquatic organisms in traces. Inorganic pollutants include nitrate (N), phosphate (P), per chlorate, cyanide (CN), boron (B), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), zinc (Zn), arsenic (As), cobalt (Co), chromium (Cr), nickel (Ni), selenium (Se), vandalium (V), fluoride (F) and strontium (Sn) etc.

Inorganic elements such as boron, copper, iron, manganese, molybdenum and zinc are essential as plant nutrients in traces but become pollutants when present in excess. Inorganic elements such as arsenic, cobalt, iron, manganese, zinc, chromium, nickel, selenium, vandalium, fluoride and strontium are essential as nutrients to aquatic animals in traces but become pollutants when present in excess. There are some most toxic trace elements which are NOT required by any organisms such as lead (Pb), cadmium (Cd) and mercury (Hg).

Several aquatic plants have been reported for long to detoxify environmental pollutants in the water bodies and the aquatic ecosystems. Remediation of water contaminated even with 'chlorinated alkanes' have been shown with aquatic plants. Plant enzymes and the symbiotic microbes on their roots plays important role in biodegradation of complex organics.

CHEMICAL CONTAMINANTS IN NATURAL WATER SOURCES RESULTING FROM DEVELOPMENTAL ACTIVITIES

Natural water, whether surface or groundwater already carry some impurities due to natural causes specially if the catchment areas are not properly managed. Over the years, and with the explosion of human population the natural water sources (rivers, lakes and streams) in the environment have been contaminated by various human developmental activities.

In the wake of rapid industrial development and pollution, some new chemical and biological contaminants have entered into the natural water pools of earth. Both municipal and industrial wastewater contain a wide variety of chemicals in much higher concentrations than in the natural water. Several of them pass into natural water courses as they cannot be removed from the wastewater by conventional treatment technologies.

The effect a chemical pollutant has in water depends on the nature of the pollutant and on factors such as acidity (pH), temperature, water hardness, presence of organic materials such as algae and weeds, and the oxygen content of the water. Very low concentrations of heavy metals and other chemicals in water can have an enormous impact. The toxicity of certain heavy metals tends to increase as the pH of the water decreases.

The toxicity of chemicals in water depends upon two important factors - '*bioaccumulation*' and '*biomagnification*'. Bioaccumulation is the accumulation of a chemical by an organism to a concentration that exceeds that of the surrounding environment. Glaring example is pesticide aldrin whose concentration in the tissues of experimental snails has been found to be nearly 5000 times the concentration of aldrin in the water in which the snails live (WHO Report, 1996). The same is the fate of DDT and mercury (Hg) in water. Several times of their concentration have been found in fishes.

The more a chemical is persistent, the greater the potential for bioaccumulation. The octanol / water partition coefficient of a chemical is a good indicator of its bioaccumulation potential. The higher the coefficient, the more a chemical will tend to bioaccumulate. Chemicals in the environment can also become increasingly concentrated in the tissues of animals including human beings higher up in the food-chain. This is called biomagnification. Several thousand fishermen died, others suffered severe brain damage after eating fish from the minamata bay contaminated by methyl mercury in Japan in 1950.

TABLE 1

Maximum Permissible Contaminant Levels for Drinking Water Prescribed by WHO

Contaminants Permissible	Level Permitted (in ppm)
1. Lead	0.15
2. Arsenic	0.05
3. Copper	1.3
4. Iron	0.3
5. Zinc	5.0
6. Selenium	0.1
7. Chromium (Total)	0.1
8. Manganese (Desired)	0.5
9. Fluoride	4.0
10. Chloride	250
11. Sulfate	500
12. Nitrate (NO_3)	10
13. pH (Desired)	6.5-8.5
14. Total Solids (Desired)	500
15. Cyanide	0.2
16. Alkyl Benzene Sulfonate	0.5
17. Carbon Chloroform Extract	0.2
18. Radium 226 & 228 combined	5 pCi/litre
19. Asbestos	7 million fibers/litre
20. Gross Beta Radiation	4 mrem/year

Source : EPA, U.S.A. (1998)

Following are some of the important chemical contaminants encountered in the natural water sources which have to be removed completely to provide safe drinking water to society-

(a) Organic and Inorganic Solids in Natural Water

Solids in water consists of organic or inorganic particles. Solids can either be suspended or dissolved. Suspended solids includes all organic and inorganic materials suspended in water. They provide adsorption sites for biological and chemical agents, and give microorganisms protection against chlorine disinfectants. Heavy metals, particularly cadmium (Cd), chromium (Cr), lead (Pb), mercury (Hg), selenium (Se) and arsenic (As) may be adsorbed on the suspended solids (SS) in raw water. Surface water often contain inorganic solids, such as clay, silt, and other soil constituents from erosion. Organic solids like plant fibers and bacteria are also common.

(b) Synthetic Organics in Surface and Groundwater

With the development of gas chromatography and mass spectroscopy, it has become possible to identify hundreds of organic compounds in surface and groundwater. Some are biodegradable, others non-biodegradable. The biodegradable organic matters in water or wastewater is expressed as BOD (biochemical oxygen demand). Higher the BOD, more is the concentration of organics in water / wastewater. There are some natural organic matters (NOM) in water, but the majority are synthetic, common of which are pesticides and industrial solvents.

> Major industrial organic solvents identified in groundwater are trichloroethylene (TCE), perchloroethylene (PCE), trichloromethane (TCM), dichloromethane (DCM) and trichloroethane (TCA). Others are synthesized when NOM in water reacts with chlorine during disinfection process.

Organic compounds in the industrial wastewater comprise a broad range of industrial solvents, disinfectants and other pesticides, gasoline, resins, polychlorinated biphenyls (PCBs), dioxin, phenol and chemical reagents. Phenol is an acutely toxic liquid chemical waste in wastewater with a benzene ring (C_6H_5OH). It is mainly used or produced in integrated steel mills, synthetic textile mills, and in resin (plastic) manufacturing. PCBs and dioxins are also the most dangerous chemicals known to man.

> NDMA (*N-nitrosodimethylamine*), a principal ingredient in rocket fuel and MTBE (methyl tertiary butyl ether), a highly soluble gasoline additive are new discoveries in surface and ground water. New, highly complex, organic compound 'acrylonitrile' (produced by the chemical industry for the use in textile industry as a raw material for the manufacture of certain new synthetic fibers) discovered in streams are extremely toxic.

Other organic compounds found in stream waters are ciprofloxacin, amoxicillin, doxycycline, norfloxacin, tetracycline, sulfamethaxazole and trimethoprim–all antibiotics; anthracene, benzopyrene, naphthalene, phenanthrene- all polyaromatic hydrocarbons (PAH); chlordane, coarbaryl, diazinon, dieldrin, chlorpyrifos, methyl parathion, lindane—all pesticides.

Nearly all organic compounds found in water are toxic, carcinogenic (inducing cancer) and mutagenic (causing birth defects). Organic matter in water may become the host for several opportunistic microorganisms. As microbes metabolize organic matters, they consume oxygen and can lead to oxygen depletion and deterioration in natural water quality.

(c) The Chemical Disinfection Byproducts in Drinking Water

Raw water has to be disinfected before community supply and chlorination is the most common disinfection process.

> When chlorine is added to water containing natural organic matters a variety of toxic organic compounds are formed. Although present in low concentrations many of them are known or suspected potential human carcinogens. Important among them are trihalomethanes (THMs), haloacetic acids (HAAs), trichlorophenol, and aldehydes.

Ozonation is alternative method of water disinfection by the use of ozone gas (O_3). Ozone also reacts with most organic matter in the water and the byproducts formed are organic peroxides, unsaturated aldehydes, and epoxides, which are of health concerns to humans.

(d) Chlorinated Organic Solvents in Water

Major chlorinated industrial solvents identified in groundwater are carbon tetrachloride (CTC), trichloroethylene (TCE), perchloroethylene (PCE), trichloromethane (TCM), dichloromethane (DCM), dichloroethane (DCA), trichloroethane (TCA) tetrachloroethylene, methyl chloride and vinyl chloride. They are released in their manufacture in the petrochemical industry or in the resulting solvent use in drycleaning industries. These substances have contaminated many deep-lying waters through industrial discharges and dumping into disused wells and leachates. They are highly carcinogenic and mutagenic and must be removed from water.

(e) Refractory Organics (ROs) in Water

Refractory organics are resistant to biodegradation. Constituents of woody plants like tannins and lignic acids, phenols and cellulose are found in natural water systems. Other non-biodegradable constituents in raw water are some polysaccharides with exceptionally strong bonds and benzene with its ringed structure discharged from the petroleum refineries. These organics tend to resist conventional methods of water and wastewater treatment technologies.

(f) Complex Hydrocarbons in Water

Hydrocarbons like petrol, kerosene, oils and lubricants have been found in surface and ground waters. They are discharged from oil refineries, gasworks and industrial effluents and fumes. The polycyclic aromatic hydrocarbons (PAH) such as benzopyrene, benzofluoranthene, benzoperylene, benzofluoranthene and indenopyrene are highly carcinogenic and must be removed from water.

(g) Agricultural Pesticides in Water

Several agricultural pesticides have been found in surface water resulting from the run-off from the agricultural fields. Important pesticides discovered in raw water are aldrin and dieldrin, DDT (all isomers), chlordane (all isomers), heptachlor and hexachloro-epoxy, methoxychlor, dichlorophenoxyacetic acid, simazin and attrazine They are highly carcinogenic and mutagenic.

> Nearly 300 UK water supplies were found to be contaminated with pesticides above the WHO limits. (UNEP Report, 2004).

(h) Explosives Contaminated Groundwater

There are numerous defense disposal sites across the USA with groundwater contaminated by residues of explosives such as TNT (Trinitrotoluene), RDX (Royal Demolition Explosive) and HMX (High Melting Explosives) DNT.

(i) Surfactants (Surface-Active Agents) in Water

Surfactants are large non-biodegradable organic molecules that are slightly soluble in water, and cause foaming in surface waters and water treatment plants. Surfactant tend to collect at the air-water interface and create a very stable foam. Typical example are the synthetic detergent 'alkyl-benzene-sulphonates' (ABS) introduced in 1950.

(j) Volatile Organic Compounds (VOCs) in Water

Many industrial wastewater contain VOCs that may be flammable, toxic, and odorous. Typical example are vinyl chloride, 1,1,1-trichloroethane, trichloroethylene, benzene, ethylbenzene, methylene chloride, and toluene, di-*n*-butyl phthalate, naphthalene, *p*-chloro-*m*-cresol, and phenol; pesticides e.g. DDT, dieldrin, and heptachlor; and the polychlorinated biphenyls (PCBs).

> VOCs are of great environmental and health concern because once such chemical is in the vapour state they are much more mobile and also contribute to a general increase in the reactive hydrocarbons in the atmosphere, leading to the formation of 'photochemical smog' and 'tropospheric ozone'. VOCs are also potential carcinogens.

Several VOCs pass through the conventional wastewater treatment plants and contaminate receiving surface waters (rivers and streams). Vinyl chloride, trichloroethane, trichloroethylene, benzene, ethylbenzene, methylene chloride, and toluene have been found in surface waters and groundwater.

(k) Persistent Organic Pollutants (POPs) in Water

> Certain chemicals in the water persist in the environment for exceptionally long period of time. They resist photolytic, chemical and biological degradation. They are semi-volatile, highly toxic and transported to the remotest corner of Earth, even up to the Artic.

Typical examples of POPs are high-molecular weight chlorinated aromatic hydrocarbons, pesticides like aldrin, dieldrin, endrin, heptachlor, chlordane, mirex, hexachlorobenzene, indane, and DDT; PCBs, phthalates, polychlorinated dioxins and furans, and the toxaphene. DDT can remain in the environment for decades. Dioxins are one of the most stable and dangerous chemical compound ever know to the civilization.

(l) Endocrine Disrupting Chemicals in Water

A wide array of industrial chemicals (some 45) specially the synthetic chemicals and the POPs in water sources can disrupt the genetically based messages through disruption of 'endocrine function' causing reproductive and developmental abnormalities, neurological and immunological problems and cancer.

(m) Inorganic Compounds in Water

Natural water sources have been found to contain several inorganics in the form of nutrients e.g. nitrogen, phosphorus, calcium, iron, potassium, manganese, cobalt, boron and sulfur. Fluorides, chlorides, arsenic, metals including heavy metals, are other important inorganics found in natural water whose concentration is increasing.

Nitrogen (N) and phosphorus (P) are alarmingly increasing in surface waters. Excess amount of phosphorus and nitrogen contribute to 'algal blooms' and *eutrophication* (oxygen depletion and deterioration) of surface waters. Complex inorganic phosphates, such as P_2O_5, at levels as low as 0.5 ppm, can interfere with normal coagulation and sedimentation processes in water-purification plants.

Nitrate in Water

Nitrate (NO_3) pollution of drinking water is a growing concern as human health hazard worldwide with increased use of chemical nitrogen urea (NH_2CONH_2) in modern agriculture, fertilizing lawns and gardens, parks and golf courses, from where they easily run-off into the local water courses or seep into underground aquifers. Intensification of livestock production is also releasing nitrogen rich liquid manure into the environment. Nitrogen is also originating from waste disposal, industrial effluents, including paper & munitions manufacture. Nitrifying bacteria (*Urobacteria, Nitrosomonas & Nitrobacter*) in the soil convert the chemical & other forms of nitrogen into 'nitrate' which is the cause of concern. Being highly soluble nitrate readily leaches through soil and move into groundwater. It is undetectable in water without testing as it is colorless, odorless, and tasteless.

The denitrifying bacteria (*Bacillus, Chromobacter, Pseudomonas, Spirillium* etc.) in soil help in converting the dangerous 'nitrates' to harmless gaseous nitrogen (N_2) but there is associated risk of generation of greenhouse gases 'nitrous oxide' (N_2O) and carbon dioxide (CO_2) in the process. N_2O also plays role in destruction of stratospheric ozone.

In the recent years concern for nitrates in potable water has been growing alarmingly as nitrate is a potential health hazard. WHO recommends 10 mg/L of nitrate-nitrogen in drinking water as the safe limit. Nitrate concentration 10 - 45 mg/L or more is considered to be 'carcinogenic' (due to formation of 'nitrosamines in guts) and causative factor for the 'blue-baby syndrome' (methemoglobinemia) in which the nitrate in gut is converted into 'nitrite', which then combines with blood hemoglobin to form 'methamoglobin' thuis decreasing the ability of the blood to carry the vital oxygen. Nitrate rich water is also reported to affect the human CNS (central nervous system) and CVS (cardiovascular system).

Fluoride in Water

With the rising geological explorations and weathering of fluoride bearing rocks (fluorapatites), excessive fluoride have started entering into groundwater. The aluminum and phosphate fertilizer industries are also contributing significant amount of fluoride into the human environment.

Excessive discharge of groundwater (which is occurring in most part of world) leads to increase in concentration of fluoride in water. The WHO permissible limits of fluoride in water is 0.7 to 1.2 ppm above which it may have toxic effects.

Arsenic (As) in Water Inorganic arsenic of geological origin is found in groundwater in several parts of world including in India and Australia. It is highly persistent in the environment and can pass into human food chain. Fish bio-accumulate arsenic as organic compounds.

In well -oxygenated water and sediments, nearly all arsenic is present in the stable form of arsenate. Some chemical forms of arsenic adhere strongly to clay and organic matter. There is potential for arsenic to be released from water and sediments. Since many arsenic compounds adhere strongly to soils, water percolating down does not usually move arsenic through more than a short distance in soil into the deep groundwater aquifers.

> High levels of arsenic in drinking water is proving carcinogenic and also mutagenic (causing birth defects). The WHO established 10 ppb (parts per billion) as a provisional guideline for safe limit of arsenic in water in 1993. Thousands of US water-supply systems exceed the WHO limit of arsenic in water and it is 5 times higher. This is linked with high risk of prostrate cancer.

SOURCES & STATE OF HEAVY METALS IN WATER BODIES

Developmental activities and their byproducts are the sources of metal contamination of our soils & water bodies. Main sources are—

1. Metalliferous Mining & Smelting : Arsenic (As), cadmium (Cd), lead (Pb) & mercury (Hg);
2. Industries (Metal & Electroplating, Saw Mills etc.) : Chromium (Cr), cobalt (Co), copper (Cu), Zinc (Zn), nickel (Ni), As, Cd & Hg;
3. Atmospheric Deposition from Industries & Automobiles: Uranium (U), As, Cd, Cr, Cu, Pb & Hg;
4. Agricultural activities: Selenium (Se), As, Cd, Cu, Cr, Pb, Zn & U
5. Waste disposal (MSW landfills & wastewater treatment plants): As, Cd, Cr, Cu, Pb, Hg, & Zn

Some heavy metals, such as cobalt (Co), chromium (Cr), copper (Cu), nickel (Ni) & zinc (Zn) are essential and serve as micronutrients for plants like calcium (Ca), potassium (K), magnesium (Mg), manganese (Mn), iron (Fe) and sodium (Na). They are used for redox-processes, as components of various enzymes and for regulation of osmotic pressure in cells. Other metals have no biological role e.g. cadmium (Cd), lead

(Pb), mercury (Hg), aluminum (Al), gold (Au) and silver (Ag). They are non-essential and potentially toxic to soil microbes. Some of them e.g. Cd^{2+}, Ag^{2+}, Hg^{2+} tend to bind the SH groups of enzymes and inhibit their activity (Turpeinen, 2002). At high concentrations, both essential and non-essential metals can damage cell membrane, alter enzyme specificity, disrupt cellular function, and even damage the structure of DNA.

AQUATIC PLANTS INVOLVED IN PHYTOREMEDIATION

Several aquatic plants from green algae (non-seeded plants) to angiosperms (seeded plants) are being identified and experimented to be used in phytoremediation of chemically contaminated (by toxic organics and heavy metals) water and wastewater. The floating hydrophyte water hyacinth (*Eichhornia cressipes*) can remove heavy metals by 20-100 %. In just 24 hours the weed can extract more than 75 % of lead (Pb) from contaminated water. It also absorbs cadmium (Ca), nickel (Ni), chromium (Cr), zinc (Zn), copper (Cu), iron (Fe) and pesticides and several toxic chemicals from the sewage. Another freshwater free floating species *Ceratophyllum demersum* could serve as a 'biofilter of toxic metals'. It can accumulate arsenic (As) with a 20,000 - fold concentration factor. (Weis & Weis, 2004). Another versatile species is *Myriophyllum aquaticum*. It has been successfully tested for phytoremediation of soils contaminated by trinitrotoluene (TNT) as well as trichloroethylene (TCE) and PCP. It can also phytotransform perchlorate. (Susaral *et al.*, 1999).

> Phytoremediation has also been successfully tested for remediation of radionuclides such as uranium (U^{238}) from wastewater in Ohio, U.S and remediation of cesium (Cs^{137}) and strontium (Sr^{90}) from a pond near Chernobyl, Ukraine. The nuclear accidents at Chernobyl in Ukraine (1986) spewed hazardous radioactive materials in the lakes & ponds located around the NPP. Some green algae like *Chara, Nitella* and *Ulothrix* have been identified as performing great phytoremediation functions. If they could be induced to grow in uranium mining effluents, they would provide a simple, long-term solution to remove uranium (U) and other radionuclides from contaminated water. (Prasad, 2006).

The most versatile plant species, both aquatic and terrestrial (grown hydroponically) that has been identified for phytoremediation (of both chemically and radioactively contaminated water) after rigorous laboratory and field experiments are -

TABLE 2

Aquatic Plants Involved in Various Phytoremediation Functions

Important Aquatic Species	Phytoremediation Functions
Azolla filiculoides (Water fern)	Metal hyper-accumulation
Apium graveolens (Celery)	Removes sulphonated anthraquinones from textile wastewater
Bacopa monnieri (Water hyssop)	Metal accumulation
Carex gracillus	Degrades trinitrotoluene (TNT) & uptake uranium (U)

Contd. ...

Contd. ...

Important Aquatic Species	Phytoremediation Functions
Canna flaccida (Water lily)	Removal of heavy metals and nitrates
Ceratophyllum demersum (Coontail)	Degrades complex organics, remove radionuclides and hyper-accumulates arsenic (As)
Eichhornia cressipes (Water hyacinth)	Metal hyper-accumulation, removal of radionuclides & removal of nitrates
Eirophorum angustifolium	Phyto-stabilization of submerged metal rich mine tailings
Elodea canadenesis	Removal of nitrates from water
Eleocharis tuberosa (Water chestnut)	Transformation of TNT
Glyceria fluitans	Phyto-stablization of mine tailings & treatment of acid mine drainage
Hydrilla verticillata (Pond scumb)	TNT transformation and metal accumulation
Hydrocotyle umbellate (Pennywort)	Accumulation of toxic metals
Ipomea aquatica (Water spinach)	Metal accumulation
Lemna minor (Duckweeds)	Heavy metal accumulation, concentration of technetium-99, nitrate removal
Myriophyllum aquaticum (Parrot feather)	Degradation of perchlorate & organophosphates Transformation of halogenated organics (TCE, PCP) Halocarbon metabolism
Nymphaea violacea (Water lily)	Uptake of radionuclides - uranium & thorium
Nelumbo nucifera (Indian lotus)	TNT transformation
Potamogeton Spp. (Pond weed)	Degradation of explosives & heavy metals uptake
Polygonum punctatum	Uptake of readionuclide cesium-137 (Cs^{137})
Rumex hydrolapatum	Removes sulphonated anthraquinones from textile wastewater
Salvinia rotundifolia (Floating moss)	TNT transformation
Typha Spp. (Cattails)	Degrades TNT and perchlorate, removes nitrates
Tamarix Spp. (Salt cedar)	Hydraulic control of arsenic (As) in water
Vallisneria americana (Tape grass)	Transformation of trichloroethane (TCE) & metal uptake
Wolffia Spp. (Duckweeds)	Absorption of organic & inorganic pollutants
Zizania aquatica (Wild rice)	Uptake of radionuclide iodine -129 (I^{129})

Source : Prasad (2006) & also from other authors cited in references.

TABLE 3

Terrestrial Plants Grown Hydroponically for Phytoremediation of Polluted Water

Important Species	Phytoremediation Functions
Brassica juncea (Indian mustard)	Heavy metal removal
Hellianthus annus (Sunflower)	Removal of radionuclides & heavy metals
Vetiveria zizionides (Vetiver grass)	Heavy metal removal (Hyper-accumulator)

TABLE 4

Aquatic Plants Involved in Bio-monitoring & Bio-accumulation of Heavy Metals

Important Aquatic Plant Species	Metals
Azolla Spp. (Water fern)	Cr, Ni, Zn, Fe, Cu, Cd & Pb
Bacopa monnieri (Water hyssop)	Hg, Cr, Cu & Cd
Carex Spp.	Cu, Pb, Zn, Co, Ni, Cr, Cd, Fe, Mn & Mo
Ceratophyllum demersum (Coontail)	As, Cd, Cu, Cr, Pb, Hg, Fe, Mn, Zn, Ni & Co
Eichhornia cressipes (Water hyacinth)	As, Cd, Co, Cr, Cu, Al, Ni, Pb, Zn, Hg, P, Pt, Pd, Os, Ru, Ir & Rh
Elodea candensis	Cu, Cd, Pb, Zn, Cr & Ni
Hydrilla verticillata	Hg, Fe, Ni & Pb
Lemna minor (Duckweeds)	Mn, Pb, Ba, B, Cd, Cu, Cr, Ni, Se, Zn & Fe
Ludwigia natans	Hg & Methyl Mercury (Hg)
Myriophyllum Spp. (Parrot feather)	Cd, Cu, Zn, Pb, Fe, Hg, Ni & Cr
Nymphaea Spp. (Water lily)	Ni, Cr, Co, Zn, Mn, Pb, Cd, Cu, Hg, & Fe
Potamogeton Spp. (Pond weed)	Ni, Cr, Co, Zn, Mn, Pb, Cd, Cu, Hg, As, Se & Fe
Pistia stratoites (Water lettuce)	Cu, Al, Cr, P & Hg
Ranunculus aquatilis	Mn, Pb, Cd, Fe & Pb
Salvinia Spp. (Water fern)	Cd, Fe, Pb, Cr & Mn
Scapania uliginosa	B, Ba, Cd, Co, Cr, Cu, Li, Mn, Mo, Ni, Pb, Sr & V
Typha latifolia (Cattails)	Ni, Cr, Co, Zn, Mn, Pb, Cd, Cu, Hg & Fe
Vallisneria americana (Tape grass)	Cd, Cr, Cu, Ni, Pb & Zn

Source : Prasad (2006).

MECHANISM OF PHYTOREMEDIATION IN AQUATIC MEDIUM

Phytoremediation technology in aquatic medium works mainly through—

1. Rhizofiltration

It is based on a combination of principle of phytoextraction and phytostabilization (Discussed in Chapter 1) specially suited to remove metals and radionuclides from polluted water. Contaminants are absorbed and concentrated by plant roots, then precipitated as their carbonates and phosphates (Salt *et al.* 1995).

Rhizofiltration also works in the efficient removal of radionuclides and toxic organics such as tetrachloroethane, trichloroethylene, metachlor, atrazine, nitrotoluenes, anilines, dioxins and various petroleum hydrocarbons (Rice *et al.* 1997).

2. Phytovolatilisation

Plants absorb and transpire the impurities from soil and water through their aerial organs. Some contaminants like selenium (Se), mercury (Hg) and volatile organic compounds (VOCs), can be released through the leaves into the atmosphere. (Cunningham and Ow, 1996).

3. Phytostimulation : Plant-Assisted Microbial Degradation (Rhizosphere Biodegradation)

Many organic compounds are degraded by microorganisms located in the rhizospheres (on the roots) of aquatic plants. Certain plant roots release substances that are nutrients for microorganisms, bacteria and fungi, that provide favorable habitats for soil microbes to act (Cunningham and Ow, 1996). A typical microbial population present in the rhizosphere, per gram of air-dried soil comprises - bacteria 5×10^6, actinomycetes 9×10^5, and fungi 2×10^3 (Schnoor *et al.*, 1995).

A large variety of exudates are secreted from roots in the form of sugars, amino acids, essential vitamins & enzymes. Root exudates may also include acetates, esters and benzene derivatives. Enzymes are also present in the rhizosphere and this may act as substrates for the microbial population. This results in increased biological activity of the microbes in the area immediately surrounding the root zone (rhizosphere).

By encouraging a microbiologically active rhizosphere, the plants facilitate accelerated digestion (biodegradation) of wide variety of organic contaminants in the upper soil layers and / or wastewater / polluted water (Anderson *et al.* 1993). The water hyacinths (*Eichhornia cressipes*) works on the same biological principle. It harbours several microbes in its root zone which perform the task of biodegradation of heavy metals in polluted water and also helps in absorption and adsorption of chemical impurities. Interestingly, gram-negative bacteria appear to have some important metabolic capabilities for degrading xenobiotic chemicals not found in gram-positive bacteria.

Enzymes secreted by plant roots or the microbial community in the rhizosphere comprise esterases different oxido-reductases (phenoloxidases and peroxidases). Plant peroxidases are exuded by some members of Fabacea, Graminae and Solanaceae. White radish (*Raphanus sativus*) and horse radish (*Armoracia rusticana*) secrete 'peroxidase' while the parrot feather (*Myriophyllum aquaticum*) and the aquatic green algae *Nitella* & *Chara* secrete 'laccase'. (Dias *et al.*, 2006). Aquatic plants grown in 'constructed wetlands' (described below in this chapter) provides greater surface area for microbial association and growth with enhanced phytoremediation activities.

FATE OF CONTAMINANTS ONCE ACCUMULATED INTO AQUATIC PLANTS

1. Phytodegradation

Certain plant species breakdown the contaminants after absorbing them. This they do through enzyme-catalyzed metabolic process within their root or shoot cells. Others breakdown the contaminants in the substrate itself by secreting enzymes and chemical compounds. The enzymes secreted are usually dehydrogenases, oxygenases and reductases. The biodegraded constituents are converted into insoluble and inert materials that are stored in the lignin or released as exudates (Watanabe, 1997). Some plants biodegrade contaminants with the aid of microbes which live in symbiotic association on their roots;

2. Phytotransformation

Several inorganic and organic contaminants once absorbed inside the root, may become biochemically bound to cellular tissues (biotransformed), in forms that are biologically inert or less active (Watanabe, 1997). In many cases, plants have the ability to metabolize organic pollutants by phytotransformation and conjugation reactions followed by compartmentalizing products in their tissues.

TABLE 5

Plant Enzymes Implicated in Phytodegradation & Phytotransformation of Organics & Inorganic Compounds

Enzymes	Contaminants Degraded / Transformed into Less Toxic Forms
1. Phosphatase	Organophosphates
2. Aromic Dehalogenase (e.g. DDT, PCBs etc.)	Chlorinated aromatic compounds
3. O-demythlase	Metaoalchor, Alachlor
4. Cytochrome 450, Peroxidases & Peroxygenases	PCBs
5. Glutathione s-transferase, carbo-oxylesterases, o-glucosyltransferases, o-malonyltransferases	Xenobiotics
6. β-cyanoanaline synthase	Cyanide

Sources : (Sandermann, 1994; Macek *et al.*, 2000; Prasad, 2006).

REMOVAL OF NITRATES FROM GROUNDWATER CONTAMINATION : A BIOTECHNOLOGICAL INNOVATION

Three aquatic plants *Cladophora* spp. (green algae), *Elodea Canadensis* & *Scirpus pungens* have been shown to effectively remove nitrates (NO_3) from nutrient enriched water bodies. Plants such as bulrush, arrowhead, cattail, sweet flag, water hyacinth, duck weeds, bamboo and poplar have been shown to clean the water polluted with nitrates and make it safe for human and animal use. (Dwivedi, 2006).

A novel biotechnological method for nitrate removal from drinking water has been developed in Germany where 'nitrate' (NO_3) is converted into 'nitrogen' (N_2) gas with the aid of plant enzymes. Nitrate containing groundwater is passed through a column where three enzymes - nitrate reductase, nitrite reductase and nitrous oxide reductase are co-immobilized along with electron-carrying dye which are energized by electric current.

This provides the reducing power to drive the conversion of nitrate to nitrogen gas and water. Prototype columns for field tests have been made to process & treat 500 L of nitrate contaminated groundwater per minute. Nitrates are completely removed in a single pass and no contaminant residues are left in water. (Mobetec GmbH, German Patent Application, 1990) (Dwivedi, 2006).

The technology is also being used to cleanup huge nitrates wastes containing organic nitro-compounds accumulated in defense laboratories involved in explosive manufacture such as TNT (Trinitrotoluene), RDX (Royal Demolition Explosive) and HMX (High Melting Explosives) in the U.S.

USE OF DRY BIOMASS OF AQUATIC PLANTS FOR TREATMENT OF TEXTILE EFFLUENTS

Dry biomass of aquatic plants like the water hyacinths (*Eichornia cressipes*) and the giant duck-weeds (*Spirodela polyrrhiza*) has been shown to remove chemical dyes and heavy metals from the textile effluents. Dried roots of water hyacinths removed 'methylene blue' and basic blue dyes efficiently (Low *et al.*, 1995). Similarly, the dried giant duck weeds can remove methylene blue at broad pH range of 3 - 11. (Warunasantigul *et al.*, 2003).

They have significantly lower operational costs than the convention treatment by 'activated carbons' (activated sludge methods) and they also absorb and remove the heavy metals from the effluents. Moreover, about 90 % of the reactive dyes persists after being subjected to activated sludge treatment.

SOME WONDER AQUATIC PLANTS FOR PHYTOREMEDIATION OF POLLUTED WATERS : POTENTIAL FOR COMMERCIALIZATION

1. The Water Hyacinths (*Eichhornia cressipes*)

Water hyacinths are green floating hydrophytes with large flat leaves and a long spongy petiole partly submerged in water and anchoring in the soil with fibrous roots. It is globally discredited as noxious aquatic weed of ponds, lakes and wetlands but they are endowed with some unique environmental properties for cleaning the polluted water bodies.

Water hyacinths harbor large number of microorganisms in symbiotic relationships on their roots which feed off the minerals and organic chemicals (contaminants) from the effluents. The microbe digest wastes and pollutants and produce sugars and amino acids which are absorbed by the host plant roots. In turn the host plant supply oxygen and nutrients to the microbes for rapid biochemical action and also restore oxygen in the vicinity of the pond and regulate carbon dioxide levels by photosynthesis.

Water hyacinth can remove heavy metals by 20-100 %. In just 24 hours the weed can extract more than 75 % of lead (Pb) from contaminated water. It also absorbs cadmium (Ca), nickel (Ni), chromium (Cr), zinc (Zn), copper (Cu), iron (Fe) and pesticides and several toxic chemicals from the sewage. In just 7 days of exposure it can lower BOD by 97% and remove over 90% of nitrates and phosphates. It can also remove radioactive substances.

Water hyacinth could take up significant amount of Sr^{90} rapidly (Jayaraman & Prabhakar, 1982). It also removes algae and fecal bacteria, the suspended matters and removes odor causing compounds. In fact, the weed fosters the growth of a zooplankton *Daphnia* that feed on the harmful pathogens.

TABLE 6

Phytoremediation of Municipal Wastewater by Water Hyacinth (In mg/L)

Parameters	Raw Municipal Sewage	Treated Sewage	
		After 15 days	After 23 days
1. TSS	320	135	70.5
2. BOD	310	70	9.6
3. Total Nitrogen	65.1	40.1	3.4
4. Total Phosphorus	10.6	4.6	1.1
5. Cadmium	1.6	1.0	0.3
6. Coliform Bacteria	140×10^4	21×10^4	1×10^4

Source : Tripathi & Shukla (1991), In WWF (India) Report, 1991.

Success Stories

(1) The Boston University, the University of Florida and the Texas State Department of Health Resources in the US is treating sewage with water hyacinths (*Eichhornia)* on experimental basis and has obtained excellent results. The City of San Deigo in California, US, is spending $ 3.5 millions in cultivating water hyacinth for wastewater treatment. The plants are grown hydroponically in a filter of rocks through flows the waste water.(Report of the US Magazine SPAN in India, 1991).(UNEP Report, 1991).

(2) In India, the Central Leather Research Institute, Madras, has successfully used water hyacinths to clean tannery effluents. Water hyacinth has also been used to augment sewage treatment in Calcutta's Salt Lake Swamp. The entire sewage of the city estimated to be around 680 million liters per day is discharged into these lakes. The treated sewage is then utilized for fish farming and irrigation of rice and vegetables in the adjoining farms. (Report of the WWF (India) 1991).

(3) Several exploratory research, pilot plant and full scale studies have been conducted in India at the University of Roorkee, Banaras Hindu University (BHU), Varanasi and at the University of Rajasthan, Jaipur (by author), with very successful results.

Environmental Cost- Benefit Analysis of Phytoremediation by Water Hyacinth

(1) Can save tremendous amount of energy (electricity) and prevent the greenhouse gas emitted and the chemicals used in the conventional mechanical treatment of wastewater.

(2) The weed biomass of water hyacinths generated as by-product is used to produce biogas and biofertiliser. The ash content of water hyacinth contains 30% potash and 13% lime making it an excellent fertilizer.

(3) Each kilogram of water hyacinth by dry weight yields about 370 litres of biogas with average methane content between 69-91%. The calorific value of the gas when used as fuel is about 580 Btu/ft^3

(4) One hectare of sewage pond in U.S. were found to yield between 20-40 tonnes of water hyacinth per day producing approximately 70,000 cum of biogas.

(5) Water hyacinth has found new uses in the paper and board industries and can be moulded into cement boards and used as a substitute for the dangerous asbestos.

2. The Duckweeds (*Lemna* spp.)

Duckweeds (*Lemna* Spp.) are tiny floating aquatic plants common in lakes, ponds and the freshwater wetlands. They form a green mat over the water surface. They are also endowed with unique environmental restoration properties. They can 'absorb' and 'adsorb' all the dissolved gases and substances, including the heavy metals, from the wastewater. Within 2 to 3 weeks the quality of wastewater improves significantly in terms of BOD and DO values, heavy metals and suspended solids and becomes useful for irrigation, industrial uses and aquaculture.

Duckweeds purifies the wastewater rich in phosphorus, nitrate and potassium until the water is crystal clear with phosphorus and nitrogen contents coming down to 0.5 mg/litre within 20 days. (UNEP Report, 1991).It prevents unwanted algal growth and 'eutrophication' by cutting sunlight below.

In an investigation of toxicity removal and fate of phenol in water treated by *Lemna gibba*, almost 90 % of the applied phenol disappeared from the contaminated water over a 16 days growth period. (Barber *et al.*)

Success Stories

(1) In Bangladesh, the duckweeds are being used under an UNDP sponsored project to treat and purify sewage and the treated sewage ponds are used for fish farming. The duckweed biomass are periodically harvested and used to make cattle feeds. (Shane Cave, UNEP Report, 1991).

(2) An American engineer in California, Viet Ngo, has successfully used *Lemna* to clean sewage and wastewater and.to convert then into useful material.(UNEP Report, 1991).

Environmental Cost-Benefit Analysis of Phytoremediation by Duckweeds

(1) Can save tremendous amount of energy (electricity) and prevent greenhouse gas emitted and chemicals used in the conventional mechanical treatment of wastewater.

(2) The weed biomass of duckweeds generated as by-product is used to produce cattle feed and also nutritive feed material for pisiculture (fish farming).

SOME WONDER TERRESTRIAL PLANTS CAPABLE OF GROWING HYDROPONICALLY FOR PHYTOREMEDIATION OF POLLUTED WATERS: POTENTIAL FOR COMMERCIALIZATION

Terrestrial plants like vetiver (*Vetiveria zizanioides*), sunflower (*Helianthus annus*) and Indian mustard (*Brassica junceae*) have large root systems and greater biomass and are especially suitable for growing hydroponically. Species that do not readily transfer contaminants from the roots to stem are preferred, since the accumulated metals and radionuclides can be removed by simply harvesting the roots.

1. The Indian Mustard (*Brassica junceae*)

The Indian mustard plant has large root system with good biomass and can also be grown hydroponically. It has gained much significance as 'phytoremediator' for environmental cleanup of both chemically & radiologically contaminated soil & water bodies. The Indian mustard concentrate toxic heavy metals (Pb, Cu and Ni) from hydroponics solution to a level up to several percent of their dried shoot biomass (Sriprang, 2006). Several superior 'transgenic species' of *B. juncea* have been produced for improved phytoremediation function.

Success Story

In a study, the Indian mustard grown hydroponically was capable of removing lead (Pb) from aqueous solutions in the range of 4 to 500 mg/L (Dushenkov, *et al.* 1995). It could effectively remove other metals like Cd, Cr, Cu, Ni and Zn from aqueous medium. (Eapen *et al.*, 2006).

2. Sunflower (*Helianthus anus*)

Sunflower is a terrestrial plant which can be grown hydroponically in ponds and constructed wetlands for phytoremediation of polluted water. It is reported to absorb radionucleides from water if grown hydroponically. Fortunately, the radioactive materials were bio-accumulated in the roots, shoots and the leaves which was harvested to remove the contaminants and landfilled. The flowers and seeds did not contain any contaminants and hence could be used economically.

Success Stories

(1) The technology was successfully employed by Phytotech Inc. (USA) using hydroponically grown sunflower plant (*Helianthus anus*) to remove radionuclides from uranium contaminated water at a DOE pilot project in Ohio, US. It was shown that concentrations of cesium (Cs), strontium (Sr) and uranium (U) from water were significantly reduced within few hours.

(2) A phytoremediation study using sunflower plants (*Helianthus anus*) was conducted in the radionuclide contaminated ponds in the vicinity of Chernobyl, Ukraine after the nuclear accident in 1986. It was shown that the sunflower plants grown hydroponically in the pond could take up 90 % of the cesium-137 (Cs^{137}) (from 80 Bq/L of Cs^{137}) in just 12 days. It was estimated that 55 kg of dry sunflower biomass could remove the entire radioactivity from the pond in Chernobyl having 9.2×10^6 Bq cesium-137 (Cs^{137}). (Dushenkov, *et al.* 1999).

(3) Phytotech Inc. (USA) and the International Institute of Cell Biology at Kieve, Ukraine conducted study on phytoremediation potential of hydroponically grown sunflower (*Helianthus anus*) and found that they could also effectively remove strontium-90 (Sr^{90}) from ponds in Chernobyl with bioaccumulation concentration of 600 μg/L for both roots and shoots. Study revealed that hydroponically grown sunflower plants

reduced Sr^{90} concentrations from 200 μg/L to 35 μg/L within 48 hours and it was further reduced to 1 μg/L.(Dushenkov, 1999).

(4) Phytotech Inc. also used sunflower to remove uranium (U) from the contaminated ponds near Chernobyl after the nuclear disaster. Uranium concentration was reduced 10-fold within an hour. Sunflower roots concentrated uranium (U) from solution by up to 10,000 fold. (Eapen, *et al.*, 2006).

Environmental Cost Benefit Analysis of Phytoremediation by Hydroponically Grown Sunflower

The estimated cost of removal of radionuclides from contaminated water by sunflower ranged from about US \$ 2 - \$ 6 per thousand gallons of water (adjusting the value of the 'sunflower oil' which is edible oil free of contaminants). It is calculated to be US \$ 80 per thousand gallons by physical process. (Terry, 2003).

3. The Vetiver Grass (*Vetiveria zizaniodes*)

Vetiver has finely structured network of 'deep and spongy root system' often reaching 3- 4 meters in the very first year of growth and can be grown hydroponically. It can tolerate very high acidity and alkalinity conditions (pH from 3.0 to 10.5); very high levels of heavy metals Al, Mn, Mg, As, Cd, Cr, Ni, Cu, Pb, Hg, Se, Zn and the herbicides and pesticides in soils & water. Vetiver can withstand prolonged submergence in water, it also behaves as a wetland plant. It can efficiently absorb dissolved nitrogen (N), phosphorus (P), mercury (Hg), cadmium (Cd), lead (Pb) and all other heavy metals from the polluted streams, ponds and lakes and its efficiency increase with age. Works done in China have confirmed that vetiver can effectively remove dissolved nutrients, specially the N and P from wastewater and reduce the growth of blue green algae (which cause eutrophication) within two days under experimental conditions. Phosphorus (P) is removed up to 99 % after 3 weeks and nitrogen (N) 74 % after 5 weeks. (Zheng, *et al.*, 1998). Works done in Thailand showed that vetiver can also effectively remove substantial quantities of cadmium (Cd), mercury (Hg), chromium (Cr), arsenic (As) and lead (Pb) from municipal wastewater. (More has been discussed in Chapter 5).

> Vetiver can easily thrive in wetlands and can be used in the 'constructed wetlands' for removal of nitrogen (N) and phosphorus (P) and heavy metals from the polluted storm water, municipal and industrial wastewater, and effluents from abattoirs, feedlots, piggeries and other intensive livestock industries. An innovative idea is to grow vetiver hydroponically on floating platforms which could be moved from one place to the other, and to the worst affected parts of the lakes and ponds. The advantage of the platform technology is that the top portions of the grass can be harvested easily for stock feed or mulch and the roots can also be removed for oil production.

THE CONSTRUCTED WETLANDS TECHNOLOGY USING KNOWLEDGE OF ENVIRONMENTAL BIOTECHNOLOGY TO REMEDIATE MUNICIPAL & INDUSTRIAL WASTEWATER FOR REUSE

Based on the understandings of 'chemical breakdown' and 'nutrient removal' properties of aquatic organisms (plants, animals & microbes) in the natural wetland systems, ecologists and environmental biotechnologists are advocating to construct 'artificial wetlands' and utilize them for the treatment of municipal and industrial wastewater, urban stormwater runoff, agricultural wastewater runoff, acid mine drainage and leachates from metal mines and waste landfills.

Over the past 20 years the constructed wetland technology using aquatic plant species and also terrestrial plants with massive root systems capable of growing hydroponically on floating platforms in polluted waters such as the vetiver grass has been widely accepted in Europe and America as a low-cost, environmentally sustainable option to the conventional wastewater treatment facilities which is based on the use of chemicals and high input of energy with consequent emission of greenhouse gases. (Greenway, 2004 & 2006).

Wetland systems treats waste & polluted water by physical, chemical and biotic processes, in a close association of appropriated plants, microorganisms, macro-organisms (vertebrates & invertebrates) and substrates. Macrophytes (rooted emergent plants) enhance physical filtration, prevent clogging in vertical flow systems, mediate oxygen transfer to the rhizosphere and favour microbial colonization. In sub-surface systems, there is an oxygen gradient, with high partial pressures near the plant roots, to be replaced progressively by anaerobic and anoxic environments. The mixture of aerobic, anoxic and anaerobic zones stimulates different microbial communities that can degrade even complex organic pollutants like 'azo dyes' almost to mineralization.

Selection of Aquatic Plant Species for the Constructed Wetlands

Relatively few plants can thrive in high-nutrient, high-BOD, high-sediment waters of constructed wetlands. Among those plants are cattails *(Typha angustifolia & Typha latifolia)*, reed grass *(Phragmites australis)*, the bulrushes (*Schoenoplectus* Spp.) and they have been extensively used in the constructed wetlands to treat even toxic wastewaters. Cattails can degrade explosives including the TNT and the perchlorates. In Australia *Phragmites* has been used extensively. Their root / rhizome systems extend vertically deeper into the sediment and therefore facilitate a large aerobic microenvironment for the biochemical process to occur rapidly. It degrades trinitrotoluene (TNT) and volatalize methyl iodide. Recently *Baumea articulata, Carex fasicularis, Phylidrum languinosum* and *Schoenoplectus mucronta* have been experimented with.

Greenway (2004) has identified 66 native species of macrophytes (8 free-floating, 8 creepers, 5 submerged, 5 floating-leaved attached, and 40 emergent species) growing in surface-flow constructed wetlands receiving secondary-treated effluents in Queensland, Australia. Many species were found growing in effluent containing up to 20 mg /L of NH_4 - N, 16 mg/L of NO_x-N, and 9 mg/L of PO_4-P.

Animal Communities Inhabiting the Constructed Wetlands

After the establishment of plant communities, a wide variety of invertebrate and vertebrate animal species come to inhabit the wetlands in a natural process of colonization (or some of them may have to be introduced like plants). Invertebrates include the micro-crustaceans (copepods, ostracods, claderans) rotifers and nematodes. The macro-invertebrates include the annelids (earthworms), molluscs (clams and pond snails), crustaceans (shrimps and crayfish), and insects (dragonflies, damselflies, water beetles, water boatman etc.). Invertebrate species are critical in providing the 'food base' for the vertebrate life of the constructed wetlands which includes the fishes, amphibians (frogs & tadpoles) and reptiles, water-birds and mammals.

Aquatic animals play a significant role as the GRAZERS (herbivores) and PREDATORS (insectivores / carnivores). Grazers consume green plants from large emergent reeds to minute phytoplankton while the predators consume smaller animals to minute zooplanktons. The most important open-water grazers are zooplankton, including rotifers (Rotifera). Grazers often eat the periphyton mats coating water plants. Direct grazing on plants is rare. Water fleas such as *Daphnia* graze significant numbers of bacteria and phytoplankton, and in turn are prey for invertebrate and vertebrate consumers.

Tadpoles feed on detritus and periphyton in the littoral zone. The vertebrate species especially the insectivorous frogs and reptiles play a crucial role in the biological control of the 'mosquitoes' through predator-prey interaction. Some invertebrate like the dragonflies also eat mosquitoes and serve as food for the larger birds and insectivores.

Microbial Communities Inhabiting the Constructed Wetlands

They include photosynthesizing (autotrophic) unicellular and filamentous green algae and blue-green algae (cyanobacteria), heterotrophic (bacteria, fungi and protozoa) and chemo-lithotrophic microorganisms such as the nitrifying bacteria (*Nitrosomonas* Spp.) and the denitrifying bacteria (*Nitrobacter* Spp.). Microorganisms occur in the water column as 'plankton' and attached to surfaces of submerged plants as 'epiphytes' and 'biofilms'. Microbes also occur in the sediments as 'microbenthos'. Anaerobic bacteria occur in low oxygen environments in the sediments.

Certain microbes help to maintain aerobic conditions in the water. These are the autotrophic microorganisms that infuse oxygen into the water by photosynthesis and also absorb nutrients. Among the microbes the nitrifying and the denitrifying bacteria plays the critical roles in improving the water quality. Nitrification is particularly important in the constructed wetlands because the nitrates (NO_3- N) made available can be used by the aquatic plants for growth. Microbes also remove inorganic phosphate from the water column or sediment porewater and convert this to organic microbial biomass.

THE ROLE OF PLANTS, ANIMALS AND THE MICROBES FOR REMOVAL OF CONTAMINANTS IN CONSTRUCTED WETLANDS

Constructed wetlands provides an array of physical, chemical and biological processes to facilitate the removal, recycling, transformation or immobilization of sediments, metals and nutrients. Most of these processes are facilitated by wetland plants, associated biofilms and the microorganisms. Wetlands plants and microbes collectively work to efficiently break down the toxic chemicals in the polluted water, bio-accumulate and bio-transform them.

Aquatic plants grown in 'constructed wetlands' provides greater surface area for microbial association and growth with enhanced phytoremediation activities. Most wetland plants in the solid matrix, can establish associations with bacteria and /or fungi. Plants plays minor role as compared to the microbes.

> Microorganisms play critical role in the operation of constructed wetlands. They help reduce BOD, remove and recycle nutrients, heavy metals, hydrocarbons, some pesticides and herbicides from the constructed wetlands by breaking them down in the system. They degrade biodegradable organic materials which descends to the bottom releasing the nutrients contained in them. Microbes also help transform the toxic chemicals into 'non-toxic' forms which is then available to the plants to take up as nutrient material. Heterotrophic microbes feed on the detritus (dead organic matter) and decompose them to release the locked nitrogen and phosphorus. Bacteria feed on the animal matter while the fungi on plant matter.

In the operation of the wetland systems there is continuous interactions between the biotic and the abiotic components and within the biotic components involving the two processes-physical and biogeochemical. In these processes the nutrients from the water (carbon, nitrogen, phosphorus, sulfur and other materials) are either accumulated or removed, recycled or finally stored in the sediments, thereby improving the quality of water column above. Plants and microorganisms remove and recycle nutrients either from the water column or the sediments and incorporate them in their tissues. Most important, bacteria and plants in wetlands can transform inflowing ammonia, nitrate, and phosphate into organic forms, which are later released downstream as detritus. The sediments, biotic components and the detritus are the major storehouse of nutrients.

Rooted macrophytes remove nutrients directly from the sediments, whereas the floating plants remove nutrients from the water column. Submerged macrophytes such as *Potamogeton* and *Ceratophyllum* usually absorb dissolved inorganic nutrients directly from the water column through their leaf surfaces. *Triglochin procera* is also an effective nutrient remover. The floating hydrophytes duckweeds (*Lemna* Spp.) and water hyacinths *(Eichhornia cressipes)* have also been found to be highly effective nutrient and metal remover. Wastewater treatment by duckweeds and the water hyacinth are becoming a preferred technology in India and several other developed and developing countries including the U.S.

a. Removal of Heavy Metals

Metals are often easily sequestered by the wetland soils or the biota or both. Wetland plants along with the microbes work to break down the sediments and chemicals in wastewater and absorb heavy metals from them. Metal oxidizing bacteria are in the aerobic zones of the wetland. Soil microbes can accumulate metals in tissues in concentrations up to 50 times higher than the surrounding soil. Microorganisms do not actually degrade inorganic metals, but changes their oxidation state. This can lead to an increase in solubility (and subsequent removal by leaching), or precipitation and reduction in bioavailability. The principal mechanism controlling micro-remediation of inorganic metals are oxidation, reduction, methylation, demethylation, metal-organic complexion, and ligand degradation. To date arsenic (As), chromium (Cr), mercury (Hg) and selenium (Se) have responded well to microbial removal. (Greenway, 2004 & 2006).

> Some wetland plants have been found to accumulate heavy metals in their tissues at 100,000 times the concentration in the surrounding water. The submerged aquatic macrophytes have very thin cuticle and therefore, readily take up metals from contaminated water through entire body surface. Further, they redistribute metals from sediments to water and finally take up in the plant tissues and hence maintain circulation. Benthic rooted macrophytes (both submerged & emergent) plays an important role in metal bioavailability from sediments through rhizospehere exchanges and other career chelates. They readily take up metals in their reduced forms from sediments and oxidize them in the plant tissues making them immobile and hence bioconcentrate them to a great extent.

Some wetland plants have been found with inherent metal tolerance and bioaccumulation. Important among them are water hyacinth (*Eichhornia crassipes*), cattail (*Typha latifolia)* and reeds *(Phragmites australis)* and *Glyceria fluitans*. They can be easily established on 'submerged mine tailings'. (Prasad, 2006). They have been used to treat effluents from mining areas that contain high concentrations of heavy metals such as cadmium (Cd), zinc (Zn), mercury (Hg), nickel (Ni), copper (Cu), and vanadium (Va). They transform the toxic heavy metals to non-toxic forms and incorporate them into their body tissues. Metals are mostly stored in the roots and rhizomes of emergent plant species which can be harvested to remove the metals. Metals are removed by adsorption, precipitation, filtration and sedimentation.

Duckweed (*Lemna minor*) and water hyacinth (*Eichhornia cressipes*) have been found to be particularly very effective in removing heavy metals from the contaminated water and render them 'biologically unavailable' to other organisms. As discussed above water hyacinth can remove heavy metals by 20-100 %. In just 24 hours the weed can extract more than 75 % of lead from contaminated water. It also absorbs cadmium, nickel, chromium, zinc, copper, iron and pesticides and several toxic chemicals from the sewage. (Tripathi & Shukla, 1991; Sinha & Sinha, 2000; Sinha *et al.*, 2006).

b. Removal of Hydrocarbons

Microorganisms break down the complex hydrocarbons in the wastewater by using the three general mechanisms—aerobic and anaerobic respiration and fermentation. Sediments in the wetlands are mostly anaerobic and provide conditions for breaking down complex organics and hydrocarbons (greases, fats, solvents and fuels) and also sequester metals, reducing their bioavailability. The complex 'hydrocarbons' are converted into simpler molecules of 'carbon dioxide' and 'methane' by anaerobic microbes. Anaerobic microbes can degrade the halogens (reductive dehalogenation) and nitrosamine, reduction of epoxides to olefins, reduction of nitro groups and ring fission of aromatic structures. (Nicholas, 1996; Eweis *et al.*, 1998).

c. Removal of Pesticides

Several aquatic microbes have been identified in constructed wetlands which can break down the chlorinated substances like the herbicides and pesticides. *Flavaobacterium* and *Pseudomonas* can destroy the organophosphates, the fungus *Zylerion xylestrix* can destroy the group of pesticides and herbicides aldrin, dieldrin, parathion and malathion. Majority of the organochlorines appears to be biotransformed, forming cunjugates with the soil humic matter in the wetlands. (Nicholas, 1996; Eweis *et al.*, 1998).

d. Removal of Pathogens

The biggest challenge in treatment of wastewater is not only the toxic chemicals and high nutrient content but also the sewage-born PATHOGENS. Pathogenic microbes generally pass unaffected through the conventional wastewater treatment plants. Disinfection by chlorination is proving to be a curse in disguise as it results into formation of unwanted by-products—the 'trihalomethanes' which are carcinogenic. Constructed wetlands, however, provide suitable conditions for pathogen removal by 'NATURAL BIOCONTROL and DISINFECTION' of treated wastewater by predator microbes. Pathogens may also be adsorbed to finer particles and sediment. Pathogen removal occurs in several ways-

1. Natural UV radiation
2. Chemical oxidation
3. Attack by lytic bacteria and bacteriophages (viruses)
4. Filtration, sedimentation, absorption, and
5. Natural die-off.

Both sub-surface flow wetland (SSF) and surface flow wetland (FWS) can achieve up to 90 % removal of fecal coliform bacteria from primary treated sewage and 99 % removal from secondary treated sewage. Study by QDNR (2000) have shown that constructed wetlands can remove 95 % of pathogens and indicator organisms. Fecal-coliform removal is also very high, producing effluent with < 1000 cfu/100 mL and as low as 100 cfu/ 100 mL, acceptable for crop and golf course irrigation and agriculture use.

In order to maximize pathogen removal, a combination of densely vegetated and open water zones should be used. The densely vegetated zones maximize filtration and sedimentation of particles to which pathogens will be adsorbed, while the open water zones will maximize UV disinfection. Healthy populations of natural-wetland microbes (bacteria and viruses) should be encouraged to promote predation, lysis and competition with pathogenic human microbes. Water fleas like *Daphnia* can predate upon bacteria and phytoplankton.

INDUSTRIAL APPLICATIONS OF PHYTOREMEDIATION BY CONSTRUCTED WETLANDS BASED ON KNOWLEDGE OF ENVIRONMENTAL BIOTECHNOLOGY

Industrial applications of constructed wetlands based on the understanding and knowledge of environmental biotechnology are increasing. They include—

1. Treatment of food processing wastewater e.g. fruit and vegetables, sugar production, poultry and meat processing, breweries and distilleries;
2. Treatment of textile wastewater & paper and pulp mill wastewater;
3. Treatment of wastewater from petrochemicals e.g. polishing of secondarily treated refinery wastewater and wash-down runoff from petrochemical industries;

4. Treatment of mining wastewater e.g. acid coal mine drainage with high concentrations of dissolved iron, manganese, aluminum and sulfate; metal mine drainage from lead, zinc, silver, copper, nickel and uranium mines & cyanide from gold & silver mining.

Some Success Stories of Treatment of Industrial Wastewater by Phytoremediation in Constructed Wetlands

Reports about full scale applications of constructed wetlands in removal of xenobiotics or recalcitrant compounds from industrial effluents are scarce. There are few reports—

1. Treatment of Chemical Industry Effluents

Dias (2000), reported about 'Vertical Flow Reed (*Phragamites* Spp) Bed' installed at the chemical industry Quimigal, S.A. in Portugal. This constructed wetland has total planted area of 10,000 sqm, which efficiently removes toxic nitro-aromatic compounds, such 'aniline', 'nitro-phenols' and 'nitrobenzene'.

2. Treatment of Textile Wastewater

Industrial textile dyes are important component in the textile wastewater. Textile dyes have been designed and synthesized to be highly persistent and resistant to washing and action of chemical solvents and sunlight. There are currently more than 10,000 different textile dyes commercially available in the world markets. Azo, indigoid and anthraquinone are the major chromophores used in the textile industries. They are complexed with heavy metals copper (Cu), cobalt (Co) and chromium (Cr) and are of considerable public health concern. Half-life of some of the textile dyes like the 'reactive blue' 19 is 46 years at 25^0 C and pH 7. Reactive dyes typically have poor fixation rates to fabrics, and dye concentrations up to 1,500 mg/L could be found in the liquor that is discharged into the sewers.

One of the first experiment made was in the 'Horizontal Bed Reed (*Phragamites* Spp.) of 150 sqm in Australia (Davis & Cottingham, 1994). In Georgia, U.S., Coats American is currently using constructed wetlands as the final step in the textile wastewater treatment operations. There is remission of a large portion of residual dye content, an appreciable decrease in the chemical oxygen demand (COD), and apparent lower chronic toxicity from the textile effluent. (Dias, *et al.*, 2006). In addition to color removal the wetlands plants also removed the heavy metals from the dyestuff by bio-accumulation

3. Phytoremediation of Cyanide Contaminated Gold Mine Wastewater in Constructed Wetlands

Cyanide is the leach reagent of choice for gold (Au) and silver (Ag) extraction during gold & silver mining and consequently results in the mined wastewaters. A diluted sodium cyanide (0.05 %) solution is sprayed on gold-containing crushed ore, placed in heaps. The cyanide readily forms a water-soluble complex with the gold from which the precious metal is recovered. Currently there are about 875 gold and silver mines throughout the world, of which about 460 use cyanide as leaching reagent, thus using 347,000 tons of sodium cyanide every year contaminating huge volume of wastewater. (Prasad, 2006).

Salix spp. & *Sorghum* spp have been successfully used in constructed wetlands to detoxify the cyanide contaminated wastewaters. These plants possess the cyanide detoxifying enzyme system called 'beta-cyanoalanine synthase' which phytotransform cyanide into 'aspargine', a non-toxic essential amino acids in plants. (Prasad, 2006).

CONCLUDING REMARKS

Phytoremediation technologies based on the principles of bioengineering & environmental biotechnology are very cost-effective, economically viable, ecologically compatible and socially acceptable methods for management of polluted waters as compared to the physical and chemical methods. Current groundwater cleanup technologies, such as 'granular activated carbon' and 'advanced oxidation methods' incur heavy expenditure and are cost-prohibitive.

After the Chernobyl disaster in Ukraine in 1986, the estimated cost of removal of radionuclides from contaminated pond waters was calculated to be US $ 80 per thousand gallons by physical process. The phytoremediation technology using sunflower ranged from about US $ 2 - $ 6 per thousand gallons of water.

The US Army Environmental Centre is developing cost-effective phytoremediation technologies by constructed wetlands to cleanup groundwater contaminated with residues of explosives like TNT, RDX, DNT and Octahydro-1,3,5,7- tetranitro-1,3,5,7-tetraazocine (HMX).

Over the past 20 years the constructed wetland technology using aquatic plant species specially the reeds (*Phragmites* Spp.) and the cattails (*Typha* Spp.) has been widely accepted in Europe and America as a low-cost, environmentally sustainable option to the conventional wastewater treatment facilities for both municipal and industrial wastewaters.

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4

PLANTS COMBATING RADIOLOGICAL POLLUTION

Developing a Low-cost Sustainable Alternative to the Costly Physio-Chemical Technology for Environmental Clean Up by EBT

KEY WORDS

Radionuclides; Radioisotopes; Sunflower Plants; Indian Mustard Plant; Beet Plant; Amaranth Plant; Water Hyacinth; Half-Life of Radioactive Substances; Phytoremediation of Cesium-137; Phytoremediation of Strontium-90; Phytoremediation of Uranium-234, 235 & 238; Phytoremediation of Plutonium-238, 239, 240 & 241.

BACKGROUND INFORMATION

Spread of radioactivity in the human environment is the result of our urge to generate 'nuclear power' without emission of carbon dioxide (CO_2) which is the main culprit greenhouse gas (GHG) causing global warming. Uranium mining, nuclear power plants and phosphate mining generate radioactive waste. Several nuclear accidents in the past especially that of Chernobyl in Ukraine (1986) have spewed hazardous radioactive materials in our environment.

Some radioactive elements such as uranium (U^{238}), thorium (Th^{230}), polonium-210 (Po^{210}) and lead (Pb^{310}) are associated with granites and deposits of phosphate and lignite. Granites and phosphates are being mined and used in several developmental programs unleashing radioactive materials in the environment. Since fossil fuels also contains natural radioactive materials, coal, oil and natural gas plants emit small amounts of radioactive pollutants into the air which is higher than that routinely emitted by nuclear plants.

CAUSES OF RADIOLOGICAL POLLUTION OF ENVIRONMENT

Radioactive contamination of soil & water can be due to—

1. Nuclear power generation

(a) Mining & milling of radioactive materials (nuclear fuel) release Rn^{222}, uranium-238 (U^{238}) and the daughters [thorium-232 (Th^{232}), radium-226 (Ra^{226}), polonium-210 (Po^{210}) & lead-310 (Pb^{310})];

(b) Nuclear fuel reprocessing release plutonium (Pu^{238}, Pu^{239}, Pu^{240}, Pu^{241}).

(c) Routine emissions from operation of nuclear power plants release carbon-14 (C^{14}), tritium (H^3), strontium - 90 (Sr^{90}), cesium- 137 (Cs^{137}) & iodine-131 (I^{131}). For the most part they bind to aerosols and are carried down to earth by rainwater;

2. Nuclear power plant (NPP) accidents

This can release cocktail of strontium-90 (Sr^{90}), cesium-137 (Cs^{137}), iodine-131 (I^{131}), zirconium-95 (Zr^{95}), plutonioum-238 & 239 ($Pu^{238-239}$) & polonium-210 (Po^{210});

> In 1986, the Chernobyl Nuclear Disaster in Ukraine (erstwhile USSR) was worst in developmental history of NPP. It released about 30 radionucleides with a total radioactivity of about 2900 PBq. Clouds of radioactive debris hurled in the sky for several days and the dust were carried away by the wind to other nations. Deadly radioactive elements fell to Earth in nuclear rain contaminating the soil and surface water and passed into the food chain of grazing animals. Milk and butter were found to be contaminated and large number of cattle had to be slaughtered and incinerated.
>
> In two weeks the nuclear fallout covered 20 countries of the northern hemisphere. 135 tones of uranium, plutonium and other radioactive elements lie buried and entombed at the site of disaster which will remain alive for at least 100,000 years. (UNEP Reports, 1990-2000).

3. Fallout from nuclear weapon production & testing

Release mainly I^{131}, Sr^{90}, Cs^{137}, zirconium-95 (Zr^{95}), ruthenium-106 (Ru^{106}), plutonioum-238 & 239 ($Pu^{238-239}$) and polonium-210 (Po^{210}).

4. Oil drilling

It brings out the naturally occurring radionuclides to the earth's surface e.g. U^{238}, Ra^{226}, Rn^{222} & Th^{232}

5. Research laboratories and hospitals

They may also contribute radioisotopic wastes like iodine -131 (I^{131}) and phosphorus-32 (P^{32}) in the municipal wastewater.

HALF-LIFE OF RADIOACTIVE SUBSTANCES

Every radioactive substance has a '*half-life*' (the length of time it takes for half of its radioactivity to decay and die away). Some radioactive materials with short half-lives becomes safe relatively quickly. For example iodine -131 has an 8-day half-life. After 50 days, its activity drops by over 90 %. Half-life of strontium-90 is 19.9 years, cesium-137 is 33 years, radium-222 is 1600 years & that of uranium-238 is 4.5 million years.

TABLE 1

Half-life of Some Radioisotopes Commonly Used in Developmental Activities

Mn^{56}	2.6 hours
Cu^{64}	12.8 hours
I^{131}	8 days
Fe^{59}	45 days
Zr^{95}	65 days
Cs^{137}	33 years
Sr^{90}	19.9 years
Ra^{226}	1600 years
U^{238}	4.5 million years

Source: *From Publications of UNEP and WHO (1992-2006).*

PHYTOREMEDIATION OF SOIL / WATER CONTAMINATED BY RADIONUCLIDES : POTENTIAL FOR COMMERCIALIZATION

Radionuclides from soil are more difficult to be removed by phytoremediation. Not every site is conducive to phytoremediation as a result of excessively high contaminant concentration, which may be unsuitable for plant growth. Phytoremediation is still not commercially used but it has been successfully tested for remediation of uranium (U^{235} & U^{238}) from wastewater in Ohio, U.S and remediation of cesium (Cs^{137}) and strontium (Sr^{90}) from a pond near Chernobyl, Ukraine.

> The phytoremediation technology was successfully employed by Phytotech Inc. (USA) using hydroponically grown sunflower plant (*Helianthus anus*) to remove radionuclides from uranium contaminated water at a DOE (Department of Energy) pilot project in Ohio, US. It was also used to remove radionuclides from the contaminated ponds near Chernobyl in Ukraine after the Chernobyl nuclear disaster in 1986. It was shown that concentrations of cesium (Cs), strontium (Sr) and uranium (U) from water were significantly reduced within few hours. Uranium concentration was reduced to 10-fold within an hour. Concentration of cesium (Cs) was reduced after 6 hours and within 24 hours, almost all the Cs was removed. Concentration of strontium (Sr) was reduced to 35 μg/L within 48 hours and at the end of 4 days it was further reduced to 1 μg/L.

1. Phytoremediation of Cesium - 137 (Cs^{137})

Cesium-137 (half-life 32 years) is one of the most important constituents of nuclear fallouts and is also a consequence of spills and accidents at NPPP (Nuclear Processing & Power Plants). Cesium binds tightly to soil, and after the Chernobyl disaster (1986) 60-90 % of Cs^{137} was found to be bound in soils unavailable for plant uptake.

Following plants have been identified which can tolerate and bio-accumulate cesium-137 significantly—

1. Sunflower (*Helianthus anus*),

2. Indian mustard (*Brassica juncea*),
3. Water hyacinth (*Eichornia crassipes*)
4. Corn (*Zea mays*),
5. Beet (*Beta vulgaris*),
6. Red pigweed (*Amaranthus retroflexus*),
7. Quinoa (*Chenopodium quinoa*) and
8. Russian thistle (*Salsola kali*)

Amaranthus retroflexus was shown to bioaccumulate high concentrations of Cs^{137} from soil. Indian mustard (*Brassica juncea*) and corn (*Zea mays*) can also remove high amounts of Cs^{137} from soil. (Arthur, 1982; Jayraman & Prabhakar, 1982). Some studies showed that complete removal of Cs^{137} by phytoremediation may take from 4 to 7 years. (Eapen *et al.*, 2006). However, Dushenkov, *et al.* (1999) found a drastic reduction in Cs^{137} in solutions in which sunflower plants (*Helianthus anus*) were grown hydroponically.

Success Story

A phytoremediation study using sunflower plants (*Helianthus anus*) was conducted in the radionuclide contaminated ponds in the vicinity of Chernobyl, Ukraine after the nuclear accident in 1986. It was shown that the sunflower plants grown hydroponically in the pond could take up 90 % of the Cs^{137} (from 80 Bq/L of Cs^{137}) in just 12 days. It was estimated that 55 kg of dry sunflower biomass could remove the entire radioactivity from the pond in Chernobyl having 9.2 x 10^6 Bq cesium-137 (Cs^{137}). (Dushenkov, *et al.* 1999).

2. Phytoremediation of Strontium-90 (Sr^{90})

Strontium-90 (half-life 28 years) is very mobile and available for plant uptake. Following plants have been identified which can tolerate and bioaccumulate strontium-90 significantly and substantially—

1. Sunflower (*Helianthus anus*),
2. Water hyacinth (*Eichornia crassipes*),
3. Russian thistle (*Salsola kali*) and
4. Atriplex (*Atriplex* spp.)
5. Indian Mustard (*Brassica juncea*)

Study revealed that hydroponically grown sunflower plants reduced Sr^{90} concentrations from 200 μg/L to 35 μg/L within 48 hours and it was further reduced to 1 μg/L.(Dushenkov, 1999). Water hyacinth could take up significant amount of Sr^{90} rapidly (Jayaraman & Prabhakar, 1982).

Success Stories

1. Phytotech Inc. (USA) and the International Institute of Cell Biology at Kieve, Ukraine conducted study on phytoremediation potential of hydroponically grown sunflower (*Helianthus anus*) and found that they could effectively remove strontium-90 (Sr^{90}) from ponds in Chernobyl with bioaccumulation concentration of 600 μg/L for

both roots and shoots. It was estimated that 55 kg of dry sunflower biomass could remove the entire radioactivity from the pond in Chernobyl having 1.4×10^8 Bq strontium-90 (Sr^{90}). (Dushenkov, *et al.* 1999).

2. The Edenspace System Corporation of USA, used the Indian mustard plant (*Brassica juncea*) to treat the radionuclide strontium ($Sr^{89/90}$) contaminated soil at Fort Greely in Alaska, USA. The plants bioaccumulated more than 10-15 fold of strontium ($Sr^{89/90}$) higher than in soil. (Singh, *et al.*, 2006).

3. Phytoremediation of Uranium—234, 235 & 238 ($U^{234, 235, 238}$)

Operation of nuclear reactors, nuclear weapon research, nuclear fuel production and nuclear waste processing for disposal has resulted into widespread contamination of surface soil and ground water by uranium isotopes in several parts of earth. Under acidic conditions of soil, uranyl (UO_2^{2+} is prominent uranium contaminant, while hydroxide complexes such as UO_2OH^+ and phosphate complexes form under natural soil conditions. Uranyl cations are taken up more readily by plants compared to Uranium complexes. Natural chelating agents like citric acid and other organic acid exudates from plant roots have been shown to help in uranium (U) uptake. (Cornish, *et al.*, 1995).

Following plants have been identified which can tolerate and bioaccumulate uranium (U) significantly and substantially—

1. Sunflower (*Helianthus anus*)
2. Indian mustard (*Brassica junceae*)
3. Beet (*Beta vulgaris*)
4. Amaranth (*Amaranthus* spp.)

Huang *et al.* (1998) found that addition of 20 m mol / kg citric acid increased the uptake of uranium and its bioaccumulation in shoots of *Brassica spp.* and *Amaranth* spp. Beet (*Beta vulgaris*) showed the greatest bioaccumulation of uranium (U), and addition of citric acid increased uranium (U) accumulation by a factor of 14. (Eapen *et al.*, 2006).

Success Stories

1. Phytotech Inc. (USA) used hydroponically grown sunflower plant (*Helianthus anus*) to remove radionuclides from uranium contaminated water at a DOE pilot project in Ohio, US. (Eapen, *et al.*, 2006).

2. Sunflower was also used by Phytotech Inc. to remove uranium from the contaminated ponds near Chernobyl after the nuclear disaster in 1986. Uranium concentration was reduced 10-fold within an hour. Sunflower roots concentrated uranium (U) from solution by up to 10,000 fold. (Eapen, *et al.*, 2006).

3. A commercial scale pilot plant for rhizofiltration system was set up at Ashtabula site in Ohio, US. It contained wastewater with 20-870 µm/L of uranium. About 95 % of the uranium contaminant was removed in just 24 hours. (Eapen *et al.*, 2006).

4. The Edenspace System Corporation in the US used the sunflower to remediate uranium (U) contaminated soils (47 mg/kg of soil) at US Army Sites at Aberdeen,

Maryland. The sunflower plants bioaccumulated uranium at the rate of 764 mg/kg - 1669 mg/kg of soil. (Singh, *et al.*, 2006).

4. Phytoremediation of Plutonium ($Pu^{238, 239, 240 \& 241}$)

Plutonium isotopes are present in soil as a result of nuclear weapon testing, nuclear fuel reprocessing.

The seaweed *Saragassum* species have been shown to have very high affinity for plutonium with a concentration factor of 21,000 over the marine water. (Eapen *et al.*, 2006).

CONCLUDING REMARKS

Conventionally physico-chemical methods are used for treatment of radioactive contaminated sites at a high cost. This includes excavation and removal of top soil, soil washing, leaching with chelating agents, flocculation and reverse osmosis-ultrafiltration for contaminated water.

Radionuclides from soil are more difficult to be removed by phytoremediation. Not every site is conducive to phytoremediation as a result of excessively high contaminant concentration, which may be unsuitable for plant growth. Phytoremediation is still not commercially used but it has been successfully tested for remediation of uranium (U^{235} & U^{238}) from wastewater in Ohio, U.S and remediation of cesium (Cs^{137}) and strontium (Sr^{90}) from a pond near Chernobyl, Ukraine.

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5

VETIVER GRASS BIOTECHNOLOGY (VGBT)

Using the 'Wonder Grass' for Environmental Restoration - Land Stabilization & Rehabilitation, Pollution Abatement & Erosion Control : A Great Achievement of Studies into EBT

KEY WORDS

Vetiver Grass - Resistance to Pest, Disease, Drought & Fire; Vetiver Root - Strength of Mild Steel; Vetiver Root - A Biological Sieve; Stabilization of Mining Overburdens; Decontamination of Polluted Soils; Rehabilitation of Waste Landfills; Wastewater / Stormwater Treatment by VGBT in Constructed Wetlands; Heavy Extraction of Nutrients & Heavy Metals from Water Bodies.

BACKGROUND INFORMATION

Number of green plants- trees, herbs, grasses and shrubs, both aquatic and terrestrial, have been discovered to have endowed with the wonderful properties of environmental restoration besides decontamination of polluted soil and water. These include stabilization of engineered slopes and embankments on highways, railways, bridges and dams, and prevention of soil erosion, stabilization and rehabilitation of mining overburdens, rehabilitation of waste landfills by leachate retention and purification, removal of nutrients & heavy metals and prevention of eutrophication in streams & lakes and wastewater / storm water treatment.

VGBT by the use of vetiver grass (*Vetiveria zizanioides*) is a 'biological' or 'soft engineering' method that is responsive to serious environmental mitigation needs over a broad range of ecological conditions for wide applications that normally require 'hard engineering' solutions.

> In Malaysia, shear tests done on vetiver roots showed that the tensile strength of the roots were at 75 Mpa (one third of the strength of mild steel reinforcement) is as strong as, or even stronger than that of many hardwood species which have been proven positive for 'root reinforcement' in steep slopes. (Truong, 2001).

THE WONDER GRASS-VETIVER : A DIVINE PLANT

The 'wonder grass' vetiver (*Vetiveria zizanioides*) is native of India, and has been used for land protection, soil and moisture conservation for centuries. Two genotypes of *V. zizanioides* viz. the wild and fertile north Indian and the sterile south Indian genotype exist and are being mostly used in Asia. The sterile one is preferred globally because it do not pose the threat of becoming a weed. Worldwide use of vetiver started in the 1980s following its promotion by the $US 100 million World Bank Watershed Management Project in India.

Major works in environmental management are being done in India, China, Thailand and Australia. A global network with 4000 members over 100 countries, and a regional network have been established in Latin America, Europe, China, the Pacific Rim and the Oceania. U.S., France, Italy, Spain, Soviet Russia, China, India, Sri Lanka, Malaysia, Fiji and Thailand are using the grass extensively for protection of their lands and water bodies.

Australia has also taken great initiative towards the use of this wonder grass for various environmental purposes including decontamination and rehabilitation of contaminated lands (sites) and water bodies, stabilization of mining overburdens, sediment control and soil conservation. All the researches and its environmental applications conducted in Australia is based on the genotype 'Monto'. (Truong and Loch 2000).

Environmental Properties of Vetiver

Vetiver grows very rapidly and becomes effective for environmental restoration works in only 4-5 months as compared to 2-3 years taken by trees and shrubs for the same job. It has stiff and erect stem and finely structured network of 'deep and spongy root system' often reaching 3- 4 meters in the very first year of growth. When buried under sediment, vetiver root will establish from the nodes thus continuing to grow with the new soil level. New shoots emerge from the base helping it to withstand heavy traffic and heavy grazing pressure.

> Vetiver is highly resistant to pest, diseases and fire and tolerant to prolonged drought, flood, frost and submergence. It is difficult to burn vetiver even in dry and frosted conditions. It can re-grow very quickly after being affected by adverse environmental conditions.

It can withstand extreme temperatures from –15 ·C to 48 ·C in Australia and even higher in India and South Africa (over 55 ·C). It can grow in regions where annual rainfall vary from 200 mm to 3000 mm. In Sri Lanka it has been shown to survive where rainfall is as much as 5000 mm per annum. It can tolerate very high acidity and alkalinity conditions (pH from 3.0 to 10.5); high soil salinity (EC = 8 dScm), sodicity

(ESP = 33 %) and magnesium; very high levels of heavy metals Al, Mn, Mg, As, Cd, Cr, Ni, Cu, Pb, Hg, Se, Zn and the herbicides and pesticides in soils.

TABLE 1

Tolerance and Toxicity Levels of Vetiver and Other Plants to Heavy Metals in Soil

Heavy Metals	Other Plants (mg / kg)	Vetiver (mg / kg)
Arsenic (As)	20	100-250
Cadmium (Cd)	1.5	20- 60
Nickel (Ni)	<60	100 -200
Selenium (Se)	2-14	> 74
Zinc (Zn)	200	> 750
Manganese (Mn)	500	578
Copper (Cu)	35-60	50-100
Chromium (Cr)	50	200-600
Lead (Pb)	300	> 1500
Mercury (Hg)	1	>6

Source : Truong & Baker (1998): *Vetiver Grass System for Environmental Protection*

THE MECHANISM OF VETIVER ACTION

Vetiver works as a 'biological sieve' in preventing the movement of soil (and the attached pollutants), by conserving and 'cleaning' water, and by strengthening, through its root system, the soil profiles, thus preventing water induced slippage and collapse and subsequent damage to life and property. It has been found to –

1. Reduce soil loss by 90 % and rainfall run-off by 70 % thus improving groundwater recharge;
2. Remove excess agrochemicals from the farm soil and increase crop yield by as much as 40 % ;
3. Improve tree seedling growth (15 %) and survival rate (95 %);
4. Rehabilitate wastelands (gullies, mined areas, degraded lands) and improve polluted sites (landfills).
5. It can even prevent or at least significantly reduce natural disasters caused by hurricanes, landslides and massive floods. (Grimshaw, 2000).

ENVIRONMENTAL RESTORATION PLANS & STRATEGIES BY VGBT

1. Erosion Control and Sediment Trapping

Vetiver is a 'living wall'. The massive root systems of vetiver binds the soil firmly and make it very difficult to be dislodged and eroded under high velocity of wind or water flows. Stems also stand up to relatively deep water flow and when planted close together, form dense hedges which reduces water flow velocity and work as an effective 'sediment filter' (for both coarse and fine sediment) trapping the silt from the run-off water behind the hedge. Chemical pollutants in run-off water are often adsorbed by these sediments. Vetiver filter strips are extensively used in Queensland Australia, to trap sediments in both agricultural and industrial lands. At working quarries, vetiver hedges are planted across waterways and drainage lines. This significantly reduced erosion and trapped the silts thus lessening the sediments in the dam water.

In Louisiana, US, the vetiver grass was very successfully used for 'gully erosion' control. Three scenic streams were getting filled with silt. Check dam was built to control the problem but it failed. Vetiver was planted near the check dams, on the sides and slopes. Within 8 weeks the hedges grew to 2m and trapped the silt and mud that was going into the stream.(Truong and Baker, 1998).

2. Decontamination of Polluted Soils

Vetiver roots can absorb and accumulate several times of some of the heavy metals present in the soil and water. (Truong and Baker, 1998 a). Studies further indicated that very little (1 to 5 %) of the arsenic (As), cadmium (Cd), chromium (Cr) and mercury (Hg) and very moderate amount (16 to 33 %) of copper (Cu), lead (Pb), nickel (Ni) and selenium (Se) absorbed were translocated to the shoots above. Hence its green shoots can be safely used as feed or grazed by animals or harvested for mulch. Vetiver can be disposed off safely elsewhere, thus gradually reducing the contamination levels.

TABLE 2

Absorption and Distribution of Heavy Metals in Vetiver Shoot and Root

Metals	Soil (mg/kg)	Shoot (mg/kg)	Root (mg/kg)
Arsenic (As)	959.00	9.6	185.00
Cadmium (Cd)	1.60	0.31	14.20
Chromium (Cr)	600.00	18.00	1750.00
Copper (Cu)	50.00	13.00	68.00
Lead (Pb)	1500.00	72.30	74.50
Mercury (Hg)	6.17	0.12	10.80
Nickel (Ni)	300.00	448.00	1040.00
Selenium (Se)	23.60	8.40	12.70
Zinc (Zn)	750.00	880.00	1030.00

Source: Truong, Paul (1999): *Vetiver Grass Technology for Mine Rehabilitation*

Farm Soil Decontamination

With the heavy use of agro-chemicals in the wake of green-revolution, most farmlands in world today are badly polluted. Vetiver has high capacity to absorb and remove agro-chemicals like carbofuran, monocrotophos and anachlor from soil thus preventing them from contaminating and accumulating in the crop plants. At the Scott Lumber Company site in Missouri, U.S., 16,000 tonnes of soils contaminated with polyaromatic hydrocarbons (PAHs) were biologically treated with VGT. The PAH concentration was effectively reduced by 70 %.(Pinthong *et al.*, 1998).

3. Stabilization and Rehabilitation of Mining Overburdens

Some Success Stories from Australia : Vetiver Grass Biotechnology (VGBT) is now being successfully used to stabilize mining overburdens. It is currently being used to stabilize a very large dam wall of a bauxite mine in Northern Territory and a bentonite mine, coal and gold mines in Queensland. It is also being used for a large-scale application to control dust storm and wind erosion on a 300 ha tailings dam.

(a) Bentonite Mine Tailings : Commercial Minerals Limited, operates a large bentonite mine and processing plant in Queensland, Australia. The mine spoils were extremely erodible as they had high sodium content, high sulphate, very little moisture and extremely low in nutritional value. The major ecological concern of the mining operation was the run-off of sediment laden stormwater from the disturbed areas to the surrounding catchment areas. Vetiver grass was grown as hedges on the highly sodic bentonite spoils to arrest the run-off and also for erosion and sediment control. Mulching and fertilization was done and within 10 months of planting excellent results were seen. Shoot growth was on an average 3 cm per week over the first three weeks and root growth was also extensive. The hedges supported 100 % soil moisture within a 3.4m arc along the rows.

When the hedges were complete (with no gaps) it trapped up to 200 mm deep sediment. This sediment now hosts several annual and perennial native species. Samples of runoff water was collected upstream and downstream of the vetiver hedges which indicated that vetiver was able to remove most of the solids and pollutants from the clay contaminated stormwater. Heavy rains inundated the vetiver rows and some plants remained submerged for over 2 weeks and yet in healthy conditions.(Truong et. al, 1998).

(b) Coal Mine Tailings : The overburden of open cut coal mine in central Queensland, Australia is generally highly erodible. These soils are usually highly sodic (ESP 33 %), saline, acidic (pH 3.5) and alkaline (pH 9.5), and extremely low in nitrogen (1.3 mg/kg) and phosphorus (13 mg/kg) and high in soluble sulphur (6.1 mg/kg), magnesium (2400 mg/kg), calcium (1200 mg/kg) and sodium (2760 mg/kg). Plant available copper, zinc, magnesium, and iron are also high. Soil with exchangeable sodium percentage (ESP) higher than 15 is considered to be strongly sodic. Moreover, the sodicity of coal tailings is further exacerbated by the very high levels of magnesium compared to calcium.

To rehabilitate an old coal mine tailings dam with a surface area of 23 ha and capacity of 3.5 million cubic meter, vetiver grass was grown on these mining spoils with 20% slopes. Mulching and fertilization was done with DAP application. Within 2-3 months vetiver established firmly and stabilized the slope of spoil dump . The microenvironment also became receptive for the growth of native species. (Radloff et.al, 1995; Truong and Baker, 1996; Truong, 1999).

(c) Gold Mine Tailings : Fresh gold mine tailings in Australia are typically alkaline (pH 8-9), low in plant nutrients, and very high in free sulphate (830 mg/kg), sodium and total sulphur (1-4 %) and high in arsenic. Vetiver established on such spoils even without fertilizers but growth was improved with application of 500 kg/ha of DAP.

> Due to high sulphur content, old gold mine tailings are often extremely acidic (pH 2.5 - 3.5), high in heavy metals and low in plant nutrients. Arsenic is 1120 mg/kg, chromium 55 mg/kg, copper 156 mg/kg, manganese 2000 mg/kg, lead 353 mg/kg, strontium 335 mg/kg, and zinc 283 mg/kg. These tailings are source of contaminants, both above ground and underground to the local environment. Field trials were conducted on two 8 years old gold mine, one with soft surface (pH 3.6; sulphate 0.37%) and the other with hard crust (pH 2.7; sulphate 0.85). Excellent growth of vetiver was observed when supplied with DAP at 300 kg/ha. (Truong, 1999).

(d) Bauxite Mines Tailings : Bauxite mine tailings are highly caustic (alkaline) with pH as high as 12. Vetiver is successfully growing on these aluminum tailings in Northern Territory of Australia and has stabilised a very large dam wall of bauxite spoils. (Truong, 1999).

Some Success Stories from China : Environmental rehabilitation works were carried out with *Vetiveria zizanioides, Cynodon dactylon, Paspalaum notatum* and *Imperata cylndraca* var. *major* at the Lechang lead (Pb) and zinc (Zn) mines in Guangdong Province which covered an area of 1.5 square kilometer producing approximately 30,000 tons of tailings annually, with a dumping area of 60,000 square meter. The tailings contained very high content of heavy metals lead (Pb), cadmium (Cd), zinc (Zn) and copper (Cu). It had very low levels of nutrients nitrogen (N), potassium (K) and phosphorus (P) and organic matter. The tailings were amended with 10 cm of domestic refuse + complex fertilizer (NKP). *V. zizanioides* was the best species to revegetate the mine tailings.

Some Success Stories from South Africa : In South Africa vetiver has been very successfully used to stabilise / rehabilitate 'slime dams' (tailings) at de Beers Diamond Mines where surface temperature was 55 ·C. (Knoll, 1997).

4. Rehabilitation of Waste Landfills : Leachate Retention and Purification

Success story from Australia : Municipal and industrial waste landfills and industrial waste sites are usually contaminated with heavy metals such as arsenic (As), cadmium (Cd), chromium (Cr), nickel (Ni), copper (Cu), lead (Pb) and mercury (Hg) which are highly toxic to both plants and humans. Works done in Queensland have shown that vetiver can stabilise the highly erodible slopes and drainage lines and also suck up the leachate substantially from the contaminated landfill sites. Leachate from a landfill near Judy Holt Park, at Wellington point in Australia was polluting a nearby watercourse. A biological barrier of vetiver was laid and today the area is ecologically restored with no sign of toxic leachate and the native species have come up in the area. Vetiver is successfully being used for checking landfill seepage problems by the Redland Shire Council in Brisbane. It is proving its worth in Brisbane valley preventing run-off into local waterways from the effluent of landfills and acid sulphate soils that might otherwise leach into the Lake Somerset. The massive root system is removing extensive nitrogen and phosphorus build up from the effluent at Church Youth Camp, just 200 meters from the lake (Truong and Baker,1998 b).

Success story from Thailand :

At a major landfill in Bangkok, where 5000 tons of garbage was dumped everyday, a section was marked for vetiver plantation in July 1999. After four months, it was found that vetiver was able survive fairly well despite the presence of leachate and toxicity normally present at all waste dump sites. Work done in China showed that vetiver can also purify and cleanse the urban garbage leachate. Small-scale planting of vetiver was carried out on a garbage dump in Guangzhou city and it was found that the grass could not only survive well but also eliminate some of the foul odor from the dump site. Of all, the ammoniac nitrogen was the best cleansed, and its purification rate was between 83 - 92 % indicating that vetiver can strongly absorb ammoniac nitrogen dissolved in water. Phosphorus was removed by 74 %.(Xia, 1998).

5. Removal of Nutrients and Heavy Metals and Prevention of Eutrophication in Streams and Lakes

Because vetiver grass can withstand prolonged submergence in water, it also behaves as a wetland plant. It can efficiently absorb dissolved nitrogen (N), phosphorus (P), mercury (Hg), cadmium (Cd), lead (Pb) and all other heavy metals from the polluted streams, ponds and lakes and its efficiency increase with age. Works done in China have confirmed that vetiver can effectively remove dissolved nutrients, specially the N and P from wastewater and reduce the growth of blue green algae (which cause eutrophication) within two days under experimental conditions. Phosphorus (P) is removed up to 99 % after 3 weeks and nitrogen (N) 74 % after 5 weeks. (Zheng, *et al.*, 1998).

Vetiver has the potential of removing up to 102 tonnes of nitrogen and 54 tonnes of phosphorus / year / hectare of vetiver. This can be achieved by both planting vetiver on the edges of the streams or on the shallow parts of the lakes where usually high concentrations of soluble N and P occur. An innovative idea is to grow vetiver hydroponically on floating platforms which could be moved from one place to the other, and to the worst affected parts of the lakes and ponds. The advantage of the platform technology is that the top portions of the grass can be harvested easily for stock feed or mulch and the roots can also be removed for oil production.

6. Wastewater / Storm Water Treatment by VGBT in Constructed Wetlands

Constructed wetland technology (CWT) using aquatic and wetland plants in artificially created wetlands for municipal wastewater / storm water treatment and purification are also considered as a part of phytoremediation technology. Vetiver can easily thrive in wetlands and can be used in the constructed wetlands for removal of nitrogen (N) and phosphorus (P) and heavy metals from the polluted storm water, municipal and industrial wastewater, and effluents from abattoirs, feedlots, piggeries and other intensive livestock industries.

Works done in Thailand showed that VGT can also effectively remove substantial quantities of cadmium (Cd), mercury (Hg), chromium (Cr), arsenic (As) and lead (Pb) from municipal wastewater. Chinese study also revealed successful use of vetiver as a wetland plant to remediate animal waste from a piggery. (Hengchaovanich, 2003). Vetiver roots can accumulate several times of some of the heavy metals present in the wastewater. (Truong and Baker, 1998 a).

ENVIRONMENTAL-ECONOMICS OF VGBT

EE works highly in favor of VGBT. It can reduce point source erosion from highways and building sites at much reduced costs, often less than 90 % of the cost of the 'hard engineering' solutions. The cost-benefit analysis of VGBT done in China (developing country), where labor cost is cheaper, indicates that this soft engineering solution cost approximately 10 % of the corresponding hard engineering solution for environmental problems. In Australia (a developed nation), where the labor cost is higher, the VGT would cost between 27 to 40 % of the hard engineering solution. (Hengchaovanich, 2003). In the U.S., VGT costs around one-tenth to one-third of conventional engineering technology and its use is likely to increase by more than 10 fold in future.

> The root of vetiver produces an essential oil called 'vetiver oil' which is used in perfumery industry. The south Indian genotype is specially useful in oil production. The Department of Natural Resources in Australia is producing world class perfume 'Guerlain'. Vetiver oil is also an 'insect repellant'.Vetiver grass also has herbicide / weedicide properties. Methanol extracts of ground stem and root was found to be very effective in preventing the germination of a number of monocot and dicot weed species. (Techapinyawat *et al.*, 1996).

CONCLUDING REMARKS

VGBT is a low cost technology as compared to conventional (engineering) methods for combating land pollution and soil erosion. It is also virtually maintenance free, the grasses regrow very quickly and its efficiency improves with age. VGBT is also aesthetically pleasing, passive, solar-energy driven and pollution abating nature's (green) technology meeting the same objectives of fossil-fuel driven and polluting conventional technology. They thrive in very harsh environmental conditions of soil and water; absorb, tolerate, transfer, assimilate, degrade and stabilise highly toxic materials (heavy metals and organics such as solvents, crude oil, pesticides, explosives and polyaromatic hydrocarbons) from the polluted soil and water; and firmly holds the soil in place by their extensive root network to prevent any erosion.

> The US Corps of Engineers Construction Engineering Research Laboratory have been using vetiver grass for bioengineering solutions in burrow pits, abandoned strip mines, stream banks and embankments and gully heads. It can stabilize engineering structures such as river banks, small dams, and levees which requires hard engineering solutions of stones, gabions, mattresses) to strengthen all these structures and thus help prevent catastrophic events due to structural failures.

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Useful Websites

http:// www.vetiver.org (The Vetiver Network Home Page)

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SECTION-II

MICROBIAL BIOTECHNOLOGY

Small is Beautiful : Rediscovering the Environmental, Social and Economic Virtues of Some Friendly Microbes Through Researches into Environmental Biotechnology

Microbes are miracle of nature. They constitute a very ancient and archaic group of living organisms appearing on earth almost 3000 million years ago. They inhabit a wide variety of habitats from soil, air and water to the bodies of plants, animals and human beings. Endowed by the wide genetic diversity the microbes have developed remarkable degree of biological specializations and ability to adapt themselves to survive in diverse environmental conditions and in very difficult conditions where no other life can survive. This has provided them with special abilities to combat several environmental contaminants and purify the environment.

Microbes have proved to be an exceptionally rich source of useful products for human use and benefits. Since ancient time mankind has exploited useful microbes for making cheese and curd, fermenting liquor, curing tobacco and preparing leavened bread. Microbes have played an important traditional role as a natural source for production of 'amino acids and vitamins', referred to as 'primary metabolites'. Many microbes have the capacity to synthesize a wide variety of 'secondary metabolites'. These compounds may be nonessential for them but useful for human beings. The quest for novel compounds in modern industrial development has focused on these products of 'secondary metabolism' of microbes and biotechnology has provided the useful tool. With the advent of 'genetic engineering' human genes can now be transferred into microbes to produce valuable secondary product like 'human insulin'.

Since 4000 years ago mankind have also been using microbes to decompose the 'food & farm wastes' (kitchen waste, cattle dung and the crop residues which contain 100 % organic matter) into earthly smelling brown to black powdery mass called 'compost' by simply dumping the wastes into pits and covering them with soil (source of microbial inoculums) and used them as soil conditioners to maintain its fertility and boost crop production. The traditional composting technology has now been significantly improved with our modern scientific knowledge in 'microbiology' and 'environmental biotechnology' to 'biodegrade' all kinds of organic wastes including the 'municipal solid wastes (MSW) 'containing sufficient organic components under a completely controlled environmental conditions. The best part is that it diverts massive organic wastes (a potential resource) from ending up in the landfills thus reducing the economic burden on the municipal councils. In the eroded landscapes of world, which is prone to suffer from massive land degradation, composting is one of the keys strategies to maintain proper soil health in both rural and urban situations. Compost not only improves soil structure, but also improves biological activities and increases water holding capacity in soils which is much needed in the world today as the farmlands are becoming both physiologically (due to depleting soil moisture) and biologically (due to depleting biological organisms) dry in the wake of high-tech mechanical and chemical agriculture which heralded the green revolution. This is being referred to as the 'rape of the soil' to augment food productivity.

Another feature of considerable environmental significance is that microbes are adapted to thrive in 'adverse conditions' of high acidity / alkalinity / toxicity and high temperature. They can develop 'biological resistance' against any toxic substance in the environment due to special 'jumping genes'. Hence while a number of them may be killed due to high toxicity, some resistant microbes survive and are cultured for further use. Under favorable conditions of growth e.g. pH, temperature and moisture and adequate supply of nutrients like vitamins, magnesium, manganese, copper, sulfur, potassium, phosphorus and nitrogen, microbes can degrade the complex hazardous organic chemicals into simpler and harmless ones. After the use of 'super bug' in cleaning up oil spills, there have been several successful stories of microbial technique in clean-up of contaminated lands and soils. Microbial bioremediation has developed from the laboratory to a fully commercialized technology over the last 30 years in many industrialized nations. Microbes have been mostly used to treat industrial waste streams, with the organisms either 'immobilized' on to different support matrixes or in a 'free-living' state, enclosed in treatment tanks or other kinds of reactor vessels. Microbial technology has gained popularity for 'site remediation' as it treats the soil contaminants in site itself, and significantly cuts down the excavation costs of soil. It is intrinsic process done *in-situ* and relies on the naturally occurring biological process carried out by native and indigenous microorganisms. It can be applied to sites with high water table and does not destroy the site that is to be treated (detoxified). Microbial remediation can handle all types of soil pollutants starting from rare metals to radionucleides. Native microbes in any contaminated site is 'acclimatized' and are capable of transforming the toxic metals to their oxides or hydroxides.

Microbes have proved to be an exceptionally rich source of useful biological products for human use and benefits since time immemorial, and there is every indication that they will continue to provide more useful products in the future too, as our knowledge in microbiology and environmental biotechnology advances. Environmentalists are viewing microbes as an 'eco-friendly nano-factories' for metal remediation through biotechnological applications employing microbes, such as yeast, bacteria, algae, diatoms and actinomycetes. Utilization of microbes for intracellular or extracellular biosysnthesis of 'nanoparticles' with different chemical composition, sizes and shapes, and controlled monodispersity can be a novel, economically viable and environmentally sustainable eco-friendly bio-nanotechnology that can reduce toxic chemicals in the production of nanoparticles and nano-scale new materials with much improved physical, chemical, electrical and biological properties than the conventional materials with potential for wider application in developmental activities.

Biomining is a new concept of using microbes in metal mining industries. In recent years bio-mining has been recognized as an economically viable and environmentally sustainable technology for the mining and processing of precious metal ores and concentrates containing high levels of 'sulfide minerals'. Microbes are now being used in the mineral processing industries to leach 'sulfide ores' to recover copper, nickel, zinc, molybednum and cobalt, and to pre-treat ores prior to extraction of gold. Bacterial processing has been part of copper mining industry for over 40 years. Bacteria *Thiobacillus* and *Leptospirillum*, eat forms of sulfur and iron instead of the normal organic carbon-based diet. Biomining by bio-oxidation of mineral ores can reduce capital costs by 12 to 20 %, operating costs by 10 %, and construction time by 25 %. Recovery rates are higher, and in the case of gold can lead to increase in production ranging from 2 to 13 %.

Microbiological Resource Centers (MIRCENS) were established in Brazil, Guatemala, Egypt, Kenya, Thailand and Senegal. Each MIRCENS maintains a regional 'gene bank' of microbial resources. The MIRCEN in Thailand is working on bioconversion of Cassava waste into liquid fuel bio-alcohol (ethanol), while the one in Guatemala is working on converting coffee waste into biogas (methane) & biofertilizer. The MIRCENS in Kenya, Senegal, Egypt and Brazil are exploring biofertilizers based on nitrogen-fixing bacteria, which it is estimated, could cut the world's chemical fertilizer bill by US $ 15 billion a year. The Cairo MIRCEN is also examining the use of microbes in 'pest & vector control' and in degrading persistent pesticides pollutants.

Microbe based technologies present an economically viable and environmentally sustainable alternatives for today's mining, mineral, site remediation, solid waste degradation and wastewater treatment industries. In the past few decades, new metal treatment and recovery techniques, based on 'biosorption' have been explored using both dead and living microbial biomass with remarkable efficiency.

6

MICROBES IN THE SERVICE OF MANKIND

Environmental Biotechnology Providing the Tool for Using Beneficial Microbes for Greater Economic & Environmental Benefits & The Emergence of Nanobiotechnology for the Production of Superior Biological Materials

KEY WORDS

Microbial Production of Drugs & Chemicals; Microbes - The Miniature Factories Producing Human Insulin; Microbial Production of Cleaner Energy; Microbial Treatment of Organic Wastes (Composting); Microbial Degradation of Toxic Wastes & Chemicals; Microbial Treatment of Wastewater; Microbes Degrading Bio-plastics; Biopiracy of Useful Microbes from India; Biomining - Using Microbes for Extracting Minerals Without Environmental Hazards; Microbial Synthesis of Nanoparticles - Birth of Nano-biotechnology.

BACKGROUND INFORMATION

Microorganisms constitute a very ancient and archaic group of living organisms appearing on earth almost 3000 million years ago. They inhabit a wide variety of habitats from soil, air and water to the bodies of plants, animals and human beings. Endowed by the wide genetic diversity the microbes have developed remarkable degree of biological specializations and ability to adapt themselves to survive in diverse environmental conditions and in very difficult conditions where no other life can survive. This has provided them with special abilities to combat several environmental contaminants and remediate the environment.

Microbes are miracle of nature. They have proved to be an exceptionally rich source of useful products for human use and benefits. Such products vary enormously in terms of both structural complexity and biological activity.

Since ancient time mankind has exploited useful microbes of nature for making cheese and curd, fermenting liquor, curing tobacco and preparing leavened bread. Microbes have played an important traditional role as a natural source for production of 'amino acids and vitamins', referred to as 'primary metabolites'. Many microbes have the capacity to synthesize a wide variety of 'secondary metabolites'. These compounds may be nonessential for them but useful for human beings. The quest for novel compounds in modern industrial development has focused on these products of 'secondary metabolism' of microbes and biotechnology has provided the useful tool. With the advent of 'genetic engineering' human genes can now be transferred into microbes to produce valuable secondary product like 'insulin'.

MICROBIAL DIVERSITY & GENETIC VERSATILITY FOR ENVIRONMENTAL ACTION

There are considerable diversity in the morphology of the microbes ranging from unicellular forms e.g. bacteria, actinomycetes, spirochaets & protozoa to multicellular forms e.g. blue-green algae (Cyanobacteria), some fungi and chlamydobacteria, possibly some cornybacteria, mycobacteria, cocci etc. to non-cellular forms e.g. viruses. To date, there are some 100,000 known species of fungi, 15,000 known species of bacteria, over 30,000 species of protozoa, and 1,500 or so known species of blue-green algae. The World Data Center on Microorganisms (WDCM) is housed in Tokyo, Japan, which maintains a global register on microbial strains, covering over 571 culture collections in 55 countries. UNEP helped strengthened the WDCM.

In recent decades large number of 'xenobiotics' have been released into the environment posing serious threats to human health and the environment. While many of these chemicals are rapidly degraded by microorganisms, some resist attack and remain recalcitrant. Given time, however, most microbes, in particular bacteria, are able to adapt to using these compounds as carbon and energy sources. The biochemical versatility is largely due to the plasticity of the microbial genomes. By modifying the existing genes, a novel metabolic capacity can be developed that allows xenobiotics to be metabolized and rendered harmless.

Microbes : Indispensable to Life on Earth

Microbial diversity is indispensable to the survival of all life forms on earth including the human beings. One cannot imagine the situation in which the miracle drug 'penicillin' was not developed from the fungus *P. notatum* by Alexander Fleming in the 1940s. This must have saved millions of lives since the World War II.

And, as the 'decomposer' of the global ecosystem they help in biodegradation of 'organic wastes' generated by man, plants & animals, and all other organisms during their lifetime, and then biodegrade their body parts after their 'death and decay' to retrieve and recycle the minerals from them back into the ecosystem. Without them, the earth would become a 'dustbin' of filth and garbage, animal and human excreta and their carcasses, leaf litter & plant debris, and the global ecological system would fail to operate & collapse. Besides, they help in renewing soil fertility, and production of variety of industrial raw materials, drugs and chemicals.

Recently, microorganisms have been found to play significant role in environmental sanitation & purification by biodegrading various kinds of toxic chemicals and hazardous wastes.

THE MICROBIOLOGICAL RESOURCE CENTERS (MIRCENS) OF WORLD

Microbiological Resource Centers (MIRCENS) were established in Brazil, Guatemala, Egypt, Kenya, Thailand and Senegal. Each MIRCENS maintains a regional 'gene bank' of microbial resources. The MIRCEN in Thailand is working on bioconversion of Cassava waste into liquid fuel bio-alcohol (ethanol), while the one in Guatemala is working on converting coffee waste into biogas (methane) & biofertilizer. The MIRCENS in Kenya, Senegal, Egypt and Brazil are exploring biofertilizers based on nitrogen-fixing bacteria, which it is estimated, could cut the world's chemical fertilizer bill by US $ 15 billion a year. The Cairo MIRCEN is also examining the use of microbes in 'pest & vector control' and in degrading persistent pesticides pollutants. (UNEP Reports, 1996-2006). (Details has been discussed in Chapters 19 & 20)

EARTHWORMS : THE STIMULATOR & PROLIFERATOR OF MICROBIAL POPULATION IN SOIL

Earthworms stimulate and accelerate population of aerobic decomposer microorganisms in the soil (Winding *et al.* 1997; Binet *et al.*, 1998). They hosts millions of decomposer (biodegrader) microbes in their gut (as they devour on them) and excrete them in soil along with nutrients nitrogen (N) and phosphorus (P) in their excreta (Singleton *et al.*, 2003). The nutrients N & P are further used by the microbes for multiplication and vigorous action.

Edward and Fletcher (1988) showed that the number of bacteria and 'actinomycetes' contained in the ingested material increased up to 1000 fold while passing through the gut. A population of worms numbering about 15,000 will in turn foster a microbial population of billions of millions. (Morgan & Burrows, 1982).

Singleton *et al.* (2003) studied the bacterial flora associated with the intestine and vermicasts of the earthworms and found species like *Pseudomonas, Mucor, Paenibacillus, Azoarcus, Burkholderia, Spiroplasm, Acaligenes,* and *Acidobacterium* which has potential to degrade several categories of organics. *Acaligenes* can even degrade PCBs and *Mucor* dieldrin. (More is discussed in Chapter 11-14)

INDUSTRIAL/COMMERCIAL USE OF MICROBES BY BIOTECHNOLOGY

Since ancient time mankind has exploited useful microbes of nature for industrial purposes, such as, for making cheese and curd (using bacterium *Lactobaccilus lacti*), fermenting liquor (wine & beer by using the fungus yeast), curing tobacco for and preparing leavened bread (using yeast).

1. Production of Drugs and Chemicals

For more than 50 years now, the primary emphasis of microbial screening for natural products has been the search for 'antibiotics' to treat human diseases. Although the most dramatic success has been achieved in the field of antibacterial therapy with antibiotics, such as the use of 'penicillins', 'cephalosporins' and 'tetracyclines', significant achievements have also been made in the treatment of cancer and fungal infections through microbial screening. More recently the trend has been to expand the horizons of natural products screening to search for compounds having pharmacological activities for other therapeutic uses, such as for 'antihypertensive agents', 'hypocholesteremic agents', and 'immunomodulators'.

Ever since Sir Alexander Fleming discovered the miracle drug and antibiotic 'penicillin' from the fungus *Penicillium notatum* several microbes have been examined for potential drugs and chemicals values and there is a long list of valuable drugs that have been obtained from the useful microbes in nature.

Microbes as Miniature Factories for Production of Human Insulin

The human hormone 'insulin' produced by beta-cells in pancreas plays critical role in carbohydrate metabolism and its deficiency leads to an impaired health condition called 'diabetes' (rise in blood glucose levels) in human beings. For long time 'bovine insulin' from pig pancreas has been used for human beings to treat diabetes.

With the advancement in our knowledge in biotechnology and genetic engineering now human genes producing insulin can be synthesised and transferred into bacteria to produce human insulin. *Escherichia coli* offers a means for rapid and economical production of 'recombinant proteins' which form the raw materials for hormones formation.

As bacteria is a single cell organism and contain a single gene (just one DNA molecule), it is possible to replace the bacterial gene with human insulin producing gene. When such genetically altered bacteria is grown in appropriate nutritive medium, it forms new bacterial colonies and their DNA codes for insulin formation as do the beta cells in human pancreas. The bacterial ribosomes in the cytoplasm are used as platform for insulin production. This insulin is exactly the human insulin only produced extracellular. The individual bacterial cells perform the role of pancreatic beta cells outside human body. Today human insulin produced in microbial cells is available and marketed all over the world.

2. Production of Clean Energy Sources (Bio-fuels) from Biomass

Biomass energy can be converted into cleaner bio-fuels (bio-gas & bio-alcohol) through appropriate microbial fermentation or 'biomethanation' technologies. Production of biogas and bio-alcohol from biomass enhance the utilization efficiency of biomass energy making it economically and environmentally more sustainable.

Microbial Technology for Commercial Production of Biogas

Biogas which is essentially methane (CH_4) is a cheap, clean and convenient source of energy generated from biological resources for multipurpose uses like heating, cooking, lighting and also electricity generation. Biogas can be produced from a variety of plant materials and even from the waste biomass of municipal solid waste (MSW) with high carbon/nitrogen (C / N) ratio. Each ton of organic waste biomass by dry weight yields about 36 cum of biogas and 350 kg of biomanure. The MIRCEN in Guatemala is working on converting coffee waste into biogas (methane)

Biotechnology of anaerobic digestion of biomass to produce methane consists of two distinct groups of anaerobic microorganisms—

1. The *'acetogens'*
2. The *'methanogens'*

The microorganisms involved in production of biogas methane are-

1. *Bacillus spp.*
2. *Cellulomonas spp.*
3. *Closteridium spp.*
4. *Eubacterium spp.*
5. *Methanobacterium omelianskii*
6. *M. formicicum*
7. *M. sarcina barkerii*
8. *M. suboxydans*
9. *Methanosarcina spp.*
10. *Methanospirillum spp.*
11. *Methanothrix spp.*
12. *Ruminococcus spp.*

They are present at several trophic levels. At the higher levels, organisms attack waste molecules through hydrolysis and fermentation, reducing them to simpler hydrocarbons methane (CH_4) and carbon dioxide (CO_2) in the last two steps of the destructive process. It is crucial that a delicate balance of population must be maintained between the two distinct groups of essential anaerobes- the *acetogens* and the *methanogens* in the final stages of reaction. The *acetogens* convert most of the carbohydrate to acetate, CO_2, and H_2 and some organic acids. The *methanogens* convert organic acids and CO_2 to CH_4 (Methane) in the presence of the available H_2.

Carbohydrates ⑦ *Acetogens* ⑦ Acetic Acid + Organic Acids + CO_2 + H_2

Organic Acids + CO_2 + H_2 ⑦ *Methanogens* ⑦ CH_4 (Methane) + Byproducts + Energy

The microbial reaction is allowed to occur in a reactor (biodigester) made of concrete or iron with a dome like upper chamber to collect the gas and an outlet for the discharge of the digested slurry into a tank. The biodigester brings the substrate and the microbial enzymes into intimate contact for sufficient period of time. Long microbial residence time (MRT) is necessary due to slow growth of *'methanogens'* and fermentation occurs in batches -continuous and semi-continuous. While a solid retention time (SRT) is necessary for efficient methane production, low hydraulic retention time (HRT) is desirable for system economy. 30 days is considered to be ideal retention time.

Methanogens is kinetically very sensitive to fluctuations in temperature and pH. Temperature between 25·-35· C and pH between 6.6 to 7.6 is considered optimum for fermentation and at lower temperature and pH the digestion is retarded and gas production falls. A temperature between 25·- 35· C and a retention time of 20-50 days is good for maximum output of gas. 35· C is the ideal temperature, and at lower temperature the output of gas is low. At 10· C and pH below 6.6, digestion and gas production stops. It is therefore, recommended to imbed the biodigester into earth and insulate it properly to maintain an uniform temperature throughout the year. It is also suggestive to use part of the gas to produce heat around the gas plant and maintain the required temperature. And, to produce one cubic meter of the gas from the cattle dung and farm wastes which is considered as the best 'feed material' the volume of the biodigester required is 2.5 cum. The by-product of digestion which comes out as slurry is a wonderful nitrogenous biomanure rich in NKP. The slurry is almost sterile because of the very high 'die-off' rate of the pathogens in the digestion chamber.

Microbial Technology for Commercial Production of Ethanol

Ethanol is assuming great value all over the world as an environmentally cleaner fuel and a good substitute of petroleum products as it is a highly inflammable liquid. Ethanol derived from plant sources have clear potential to be sustainable, low cost and high performance, are compatible with present and future automobiles and transportation systems, and provide near-zero net greenhouse gas emissions. Using biocatalysts cost can be significantly cut by orders of magnitude, making biofuel ethanol competitive with gasoline (petrol and diesel). Brazil is already using it as an 'auto-fuel' since 1975. Mixed with petrol, it increases the energy efficiency. The new bio-alcohol technology based on our knowledge in environmental biotechnology could dramatically increase production to as much as 150 billion litres of ethanol- the equivalent of one quarter of our current petrol use (UNEP Report, 2004).

All biomass, even the waste biomass rich in starch and cellulosic materials are good raw materials for ethanol production by enzymatic fermentation carried out by microorganisms 'yeast' and some bacteria. Bagasse is most appropriate raw material. 6000 kg of bagasse upon fermentation yields 1000 litres of ethanol of 95 % strength. The MIRCEN in Thailand is working on bioconversion of cassava waste into liquid fuel ethanol.

Microorganisms involved in production of ethanol are—

1. *Candida pseudotropicalis*
2. *C. utilis*
3. *Closteridium thermoacellum*
4. *Sacchromyces cerevisiae* (Yeast)
5. *Thermoanerbacter ethanolicus*
6. *Zymomonas mobilis*

> Biotechnology has engineered yeasts, enzymes and bacteria - capable of breaking down plant products (biomass) into complex sugars from which a wide variety of bio-based products including bio-alcohol ethanol.

3. Production of Hydrogen from Water Through Biotechnological Researches: The Cleanest Form of Fuel for a Pollution Free Sustainable Future

Two energy currencies hold the greatest promise for a renewable and clean (pollution free) energy future- electricity and hydrogen. Electricity is clean and fast, but cannot be effectively stored. Hydrogen can be readily stored and transported. It is this 'energy carrier' potential of hydrogen that is exciting and holds great promise for solving the future energy problem of the civilization. Hydrogen has the highest density of energy per unit of weight of any chemical fuel, it is essentially non-polluting, it is by far most abundant element in the universe, can be extracted from water and also from the fossil fuels, transforming the dirty and non-renewable fossil fuels of today, into a clean and renewable energy source of tomorrow. Hydrogen is versatile, can be used in diverse applications requiring electricity or gas, can be used for utility power generation in power plants, in all present generations of automobiles with internal combustion engines or in future generations of automobiles with fuel cell technology.

> Hydrogen can be produced from water (H_2O) which occupy two-third part of the earth. However, hydrogen obtained from water by electrolysis is not cost-effective, but if it can be done by 'photolysis' (as it happens during photosynthesis) where sun provides the energy for photosplition, it would become very cheap.
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> Researches done at University of California, Berkley, discovered that the unicellular green algae *Chalmydomonas reinhardtii* when starved of sulphur produces 'hydrogenase' enzymes which split water into hydrogen and oxygen - a biological version of electrolysis (termed as photolysis) where sun pays the electric bill. Attempts are being made to produce a 'mutant algae' which can produce huge amount of hydrogenases and split water rapidly to increase production of hydrogen by tenfold. Such solar-algal ponds would become future source of hydrogen fuel. Whoever develops and controls this solar powered hydrogen production technology from water will become the OPEC of the future.

Oh & Logan (2005) reported hydrogen and electricity production from a food processing wastewater using fermentation and microbial fuel cell technology.

Some more microorganisms that have been found to produce the enzyme 'hydrogenase' which split water into hydrogen and oxygen are–

1. *Closteridium butyricum*
2. *Rhodopseudomonas gelatinosa*
3. *R. palustris*
4. *R. rubrum*
5. *Rhodospirillum molischianum*

4. Treatment of Wastewater by Microbes

Important microbes identified for the treatment of industrial wastewater including the degradation of hazardous chemicals in them are–

Bacteria

1. *Alcaligenes spp.,*
2. *Achromobacter spp.*
3. *Beggiatoa spp.,*
4. *Candida tropicalis,*
5. *Cunninghmela elegans,*

6. *Closteridium spp.,*
7. *Chromobacter spp.*
8. *Coloriolus versicolor*
9. *Flavobacterium spp.,*
10. *Halsicomenobacter hydrossis,*
11. *Hypomicrobium spp.*
12. *Microthrix parvicella,*
13. *Micrococcus denitrificans*
14. *Myriophyllum spp.*
15. *Nocardia spp.,*
16. *Nostocoida limicola,*
17. *Phanerochaet chrysoporium & P. sordida,*
18. *Psuedomonas spp.* etc.
19. *Sphaerotilus natans,*
20. *Schoenoplectus spp.*
21. *Stemphylium loti* (Can also detoxify 'cyanide' in aqueous waste)
22. *Thiothrix spp.,*
23. *Zoogloea spp.*
24. *Zylerion xylestrix,*

Green Algae & Blue-green Algae

1. *Anbaena spp.*
2. *Chlorella vulgaris*
3. *Hydrodictyon spp.*
4. *Microcystis spp.*
5. *Oscillatoria spp.*
6. *Scendesmus spp.*
7. *Spirulina spp.*

(Details have been discussed in Chapter 8)

5. Microbial Degradation of Toxic Chemicals & Hazardous Wastes

1. *Acalegenes* spp. ⑦ Halogenated hydrocarbons, alkylbenzene sulphonate, PAHs & PCBs
2. *Artrobacter* spp. ⑦ Benzene, pentachlorophenol, phenoxyacetate & PAHs
3. *Azotobacter* spp. ⑦ Aromatic compounds
4. *Bacillus* spp. ⑦ Phenol, cresol, long chain alkanes, phenyl ureas & salicylates

5. *Candida tropicalis*	⑦	PCBs & formaldehydes
6. *Corynebacterium* spp.	⑦	Halogenated hydrocarbons & phenoxyacetates
7. *Cunninghmela elegans*	⑦	PCBs, PAHs
8. *Flavobacterium* spp.	⑦	Aromatic compounds
9. *Methosinus trichosporium*	⑦	Benzene, toluene, ethylbenzene & cresols;
10. *Myobacterium* spp.	⑦	Benzene, aromatic compounds, cycloparaffins
11. *Nocardia* spp.	⑦	Alkylbenzenes, napthalenes, phenoxyacetate & PAHs
12. *Pseudomonas* spp.	⑦	Organophosphates, parathion, melathione, benzene, alkylbenzoates, anthracene, toluene, xylene, phenylureas & PCBs
13. *Rhodotorula* spp.	⑦	Benzeldehyde
14. *Streptomyces* spp.	⑦	Phenoxyacetate, diazinon & halogenated hydrocarbons;
15. *Trichosporon cutaneum*	⑦	Phenol
16. *Xanthomonas* spp.	⑦	PAHs & hydrocarbons

(Details have been discussed in Chapter 7)

6. Microbial Degradation of Herbicides & Pesticides in Soil

Fungi

1. *Aspergillus japonicus*
2. *A. niger*
3. *Aureobasidium pollulans*
4. *Canadida tropicalis*
5. *Chrisoporium lingorum*
6. *Fusarium flocciferum*
7. *F. solani*
8. *Geotrichum candidum*

Bacteria

1. *Flavobacterium spp.*
2. *Phonerochaete chrysoporium*
3. *Pseudomonas putida*
4. *Sternum hirsutum*
5. *Trametes versicolor*

(Details have been discussed in Chapter 7)

7. Microbial Degradation of Organic Wastes

Fungi

1. *Agaricus* spp.

2. *Mucor* spp.
3. *Rhizopus* spp.

Bacteria

1. *Azatobacter* spp.
2. *Closteridium* spp.
3. *Nitrobacter* spp.

8. Microbes that Can Biodegrade Plastics

In Turkey, scientists have found that the soil microorganism *P. chrysosporidium* can biodegrade LDPE (Low Density Polyethylene) plastic bags containing 12% starch. Scientists in Japan have used biochemical machining and enzymes found in filamentous fungi to biodegrade or cut down the molecular chains present in biodegradable plastics.

BIO-MINING : USE OF MICROBES FOR SUSTAINABLE MINING & MINERAL EXTRACTION

Microbes are now being used in the mineral processing industries to leach 'sulfide ores' to recover copper, nickel, zinc, molybednum and cobalt, and to pre-treat ores prior to extraction of gold. Bacterial processing has been part of copper mining industry for over 40 years.

> In recent years bio-mining has been recognized as an economically viable and environmentally sustainable technology for the mining and processing of precious metal ores and concentrates containing high levels of 'sulfide minerals'. Rod shaped bacteria called *Thiobacillus* and *Leptospirillum*, eat forms of sulfur and iron instead of the normal organic carbon-based diet. Biomining by bio-oxidation of mineral ores can reduce capital costs by 12 to 20%, operating costs by 10%, and construction time by 25%. Recovery rates are higher, and in the case of gold can lead to increase in production ranging from 2 to 13%. Under controlled conditions, such as in agitated and aerated tanks and in specially constructed heap leach pads gold's bio-oxidation is rapid and highly effective. In 4 to 5 days, a sulphidic gold concentrate is bio-oxidised to achieve greater than 90% gold recovery.

New class of 'hyperthermophile microbes' has been discovered in the Bismarck Sea, north of Papua New Guinea. They are endowed with the natural ability to extract and process minerals from their ores at high temperatures. They grow fastest between 80 and 100 °C. About 60 species of hyperthermophiles have been discovered. These deep sea microbes can survive at surface and also be grown under laboratory conditions. They are all anaerobic, and gain their energy by using sulfur or nitrate to oxidize the reductive gases such as hydrogen. Biomining with the aid of microbes have significantly less impact on the environment.

These microbes are also finding uses in treatment of toxic tailings resulting from mining activities. When bacterial leaching is used with a solvent extraction electrowinning (SX-EW) plant, base metal operations are capable of producing metallic products without smelting and refining.

ROLE OF MICROBES IN PRODUCTION OF NANOPARTICLES—A NEW & ADVANCED SUPERIOR MATERIAL FOR MANKIND : BIRTH OF NANOBIOTECHNOLOGY ?

Microbes have acquired new role in biosynthesis of metal 'nanoparticles' (an entirely new material not known to mankind earlier) which form the backbone of the emerging new technology named as 'nanobiotechnology'—a highly promising discipline of science & technology that will help redesign the future of several scientific knowledge and concepts and have potential to change every aspect of human life. (Bhat, 2003).

Nanotechnology is a new science concerned with the materials and systems whose structure and components exhibit significantly improved physical, chemical and biological properties. It is an *'enabling technology'* that leads to generation of new capabilities, new superior products and new markets for new uses. The new products are applicable to information technology (IT), human health & medicine, sustainable energy development and environmental cleanup.

Noria Taniguchi first coined the term 'Nanotechnology' while measuring precise machining tolerances of materials in the range of 0.1 - 100 nanometer (nm). The technological achievements and the accruing economic benefits from the use of nanotechnology developed over the next 15-20 years has been estimated by the National Science Foundation to be approximately US $ 1 trillion. (Tolles & Rath, 2003). It will be possible to produce macroscopic 'living-like' organisms made of nanoparticles that would remediate toxic & hazardous heavy metals from contaminated lands and waters (environment).

Nanotechnology applies the technique and processes of 'micro-fabrication' to build devices for studying bio-systems and has a wide range of applications in variety of fields from space science to health science and deep oceanic research. Nano materials, besides providing new research avenues, form the basis of a new class of 'atomically engineered materials' (AEM) with exciting new properties. These new materials can be designed to get desired properties by manipulating and attaching atoms in different ways.

The Nanostructures (Nano Crystals or Nano Particles)

Nano-crystals cover a size range of 1-100 nm (nanometer) and are intermediate to the molecular size regime on one hand and the macroscopic bulk on the other. Laws relating to physical, chemical, biological, electrical, magnetic and other properties at the nano-scale are very different from those that apply to macro-matter in universe. Nano particles are not governed by gravity and inertia and their behaviour is completely different from the commonly accepted and familier properties of macro particles. The unique chemical, electrical, magnetic, optical and other properties of nano scale particles have led to their use in a broad range of industries, including environmental biotechnology, catalysis, data storage, energy storage, micro-electronics and others. There have been other concerted efforts to integrate 'microelectronics' with 'molecular biology' into a 'bio-electronics' technology with a number of potential commercial applications in information technology, medicine, agriculture and environmental science.

Many important nano-structures are composed of the group IV elements Silicon (Si) of Gelenium (Ge) of the periodic table, type III-V 'Semi-conducting Compounds', such as GaAs or type II-VI 'Semi-conducting Materials' such as CdS. The possibility of modifying 'existing materials' through nanotechnology has become a recipe for the preparation of 'advanced & superior materials'.

Widely used methods for the fabrication of nanostructures are lithography using radiation or by chemical means (often using toxic chemicals) which results into chemical contamination of the environment.

Metal Nanoparticles in Environmental Remediation

Metal nanoparticles is very important in several biotechnological applications, including in the fields of 'bio-mineralization', 'bio-remediation', 'bio-leaching', and 'microbial corrosion'. Material scientists are viewing the uses of microbes in toxic heavy metal bioremediation with interest for nano-fabrication of environmentally useful submicron scale particles. If we could build it in microbes, it is possible to use them as an effective 'nano-factories' for heavy metal remediation. Formation of inorganic particles within microorganisms might become a central discipline in biometric and bioengineering applications.

Joerger *et al.* (2001) reported that bacterium *Pseudomonas stutzeri* AG 259 isolated from silver mine, when placed in a concentrated aqueous solution of silver nitrate ($AgNO_3$) resulted in the reduction of the silver ions (Ag^+) and the formation of silver 'nanoparticles'. Ahmad *et al.* (2003) reported an alkalotolerant actinomycetes *Rhodococcus* Spp. capable of synthesizing 'gold nanoparticles'. Another novel alkalothermophilic actinomycetes *Thermomonospora* Spp. Isolated from self-heating compost pile exposed to $AuCl_2$, completely reduced it to $AuCl_4$ ions producing gold nanoparticles.

Mineralization (Catalytic Destruction) of Toxic Halocarbons by Metal Nanoparticles

Many hydrocarbons are toxic, mutagenic, and resistant to microbial degradation. However, they can be catalytically destroyed by metal nanoparticles produced by microbes. Studies indicate that the silver and gold nanoparticles cause catalytic destruction of the halocarbons forming silver chloride ($AgCl_2$) and amorphous carbon. The destruction is more efficient with silver nanoparticles in the size range of 2-150 nm. (Nair & Pradeep, 2003). Application of this 'catalytic destruction' method by metal nanoparticles in detection, extraction, and degradation of environmentally toxic halocarbons in general and pesticides in particular, will be very rewarding and novel technology for bioremediation.

Alivisatos (2001), reported the presence of inorganic crystals in magnetotactic (magnetic sensing) bacteria. The bacterium has within it a chain of about 20 magnetic crystals between 35 - 120 nm diameter. The crystals are made of either 'magnetite' or 'greigite' nanoparticles (both highly magnetic iron materials). The properties of magnetic separation of heavy metals and radionuclides in conjugation with bioaccumulation of metals by magnetotactic bacteria, can be used for mineral processing and decontamination of wastes. Magnetotactic bacteria immobilize heavy metals from surrounding solutions and this property of the bacterium can be exploited for their use in treatment of wastewater for metal removal.

THE MICROBIAL SYNTHESIS OF NANO PARTICLES & NANO MATERIALS : THE EMERGENCE OF NANOBIOTECHNOLOGY

Researchers in the field of nanotechnology and nano particle synthesis and assembly have turned to biological systems for biosynthesis, since they have potential to control the shape of nano particles, which is not possible in the conventional chemical synthesis and it is also very cost-effective & eco-friendly method. Nature has devised a wonderful system for biosynthesis of inorganic materials in nano and macro-length scales in microbes. Organisms synthesizing inorganic materials include magnetotactic bacteria, sillceous material synthesizing diatoms (marine algae) and S-layer bacteria which produce gypsum and calcium carbonate layers. (Joerger *et al.*, 2001). Biological synthesis of metal nano particles using microbes, such as bacteria, yeasts, algae, actinomycetes and fungi, is gaining momentum due to the eco-friendly nature of the micro-organisms which reduce the use of toxic chemicals. This is a 'green chemistry' approach towards the synthesis of nanoparticles.

TABLE 1

Micro-organisms Involved in Biosynthesis of Nanoparticles

Organisms		Nanoparticles	Mechanism	Size (nm)
1. *Pseudomonas stutzeri* AG 259	(Bacteria)	Silver	Intracellular	200
2. *Lactobacillus* Spp.	(Do)	Gold/Silver	Do	2-20
3. *Magnetotactic bacterium*	(Do)	Magneite/Greigite	Intra/Extracellular	35-120
4. *Thermomonospora* Spp.	(Actinomycetes)	Gold/Silver	Extracellular	2-20
5. *Rhodococcus* Spp.	(Do)	Gold	Intracellular	5-15
6. *Verticillium* Spp.	(Fungus)	Gold/Silver	Do	2-20
7. *Fusarium oxysporium*	(Do)	Gold/Silver	Extracellular	2-50
8. *Schizosaccharomyces pombe*	(Do)	Cadmium	Intracellular	2-20
9. *Torulla* Spp.		Lead	Do	2-20
10. Diatoms	(Marine Algae)	Siliceous	Intra/Extracellular	35-120

Source : Rajendran & Gunasekaran (2006)

BIOPIRACY OF USEFUL MICROBES FROM INDIA ?

Shocking evidence surfaced about the patenting of 'micro-organisms' collected from India by the American firms in the 1990s. Bag fill of soils were taken away from the Western Ghats region in Kerala. Millions of microbes of diverse species and variety were 'bio-pirated' in the garb of soil. These microorganisms have been stored at the American Type Culture Collection (ATCC) at Maryland, and have been employed by various companies and agencies for industrial purposes, primarily for the production of pharmaceutical products like antibiotics, for production of biofertilizers, and for bioremediation of contaminated lands & water. Among the American agencies which are involved in this 'bio-trade' are Bristol-Myers, Pfizer, Merck, Lepetit, and Lederle. According to Canadian research & action group RAFI (Rural Advancement Foundation International) at least 28 micro-organisms have already been patented and other 6 are under patent claims. ATCC alone have 381 fungal and 90 bacterial accessions from India. (Kothari, 1994).

ROLE OF ENVIRONMENTAL BIOTECHNOLOGY & GENETIC ENGINEERING IN IMPROVING MICROBIAL FUNCTION

The biochemical versatility in most microorganisms, especially the bacteria, is largely due to the plasticity of the 'microbial genomes'. By modifying the existing genes, a novel metabolic capacity can be developed that would even allow dangerous chemicals like 'xenobiotics' to be metabolized and rendered harmless.

> With the advancement in our knowledge in environmental biotechnology some bacterium have been genetically engineered (tailored) to eat pollutants, gobble them up and chemically alter them in their bodies. When pollutants are finished the bacteria die leaving clean soil and a biomass of dead bacteria containing harmless minerals. Scientists have fortified the aquatic bacteria *Asticcaculis excentrictis* by transferring two toxin producing genes from the *Bacillus sphaericus* which now can kill the mosquito larvae (spreading malaria) preventing them breed in open waters.

Many more new microbes have been genetically engineered which have potential environmental and economic benefits. Good examples are -

1. *Bacillus ferrooxidans* used for metal extraction;
2. *Bacillus thuringiensis* used to produce insect toxins for biological control of pests;
3. *Azospirillium brazilense* for production of plant hormones to promote plant growth; and
4. *Rhizobium meliloti* for enhanced biological nitrogen fixation in non-leguminous crop plants.

CONCLUDING REMARKS

Microbes have proved to be an exceptionally rich source of useful biological products for human use and benefits since time immemorial, and there is every indication that they will continue to provide more useful products in the future too, as our knowledge in microbiology and environmental biotechnology advances.

Microbe based technologies present an economically viable and environmentally sustainable alternatives for today's mining, mineral, solid waste and wastewater treatment industries. In the past few decades, new metal treatment and recovery techniques, based on 'biosorption' have been explored using both dead and living microbial biomass with remarkable efficiency. Microbial approach for metal detoxification offers high potential for selective removal of toxic metals.

In 2004, nearly 17 % of the world's primary energy and 19 % of its electricity was from the renewables including the biomass (biogas & bio-alcohol). It should top 50 % by 2020-25 as technology advances and is adequately supported by the strong political will of nations. (UNEP, 2006). The most promising is hydrogen fuel- an environmentally benign alternative to fossil fuels which can be stored in tanks, transported and used as fossil fuels without any harmful emission.

Utilization of microbes for intracellular or extracellular biosysnthesis of 'nanoparticles' with different chemical composition, sizes and shapes, and controlled monodispersity can be a novel, economically viable and environmentally sustainable eco-friendly bio-nanotechnology that can reduce toxic chemicals in the production of nanoparticles and nano-scale new materials with much improved physical, chemical, electrical and biological properties than the conventional materials with potential for wider application in developmental activities.

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7

MICROBIAL REMEDIATION OF CONTAMINATED LANDS & WATER

A Low-cost Nature's Biotechnology for Environmental Clean Up by Versatile Microbes : Significant Achievement of Researches into EBT

KEY WORDS

Aerobic & Anaerobic Biodegradation; Biotransformation; Microbial Detoxification of Metals; Microbial Destruction of Toxic Organics & Hazardous Wastes; Plant - Assisted Microbial Destruction of Toxic Compounds; Genetically Engineered Microbes; Symbiotic Engineering

BACKGROUND INFORMATION

Microbes are adapted to thrive in 'adverse conditions' of high acidity/alkalinity/ toxicity and high temperature. They can develop 'biological resistance' against any toxic substance in the environment due to special 'jumping genes'. Hence while a number of them may be killed due to high toxicity, some resistant microbes survive and are cultured for further use. Under favorable conditions of growth e.g. pH, temperature and moisture and adequate supply of nutrients like vitamins, magnesium, manganese, copper, sulfur, potassium, phosphorus and nitrogen, microbes can degrade the complex hazardous organic chemicals into simpler and harmless ones. After the use of 'super bug' in cleaning up oil spills, there has been several successful stories of microbial technique in clean-up of contaminated lands and soils. (USGS, 1997). The Microbiological Resource Centers (MIRCENS) at Cairo, Egypt is examining the use of microbes in degrading persistent pesticides pollutants. (UNEP Reports, 1996-2006).

Whilst microbial remediation (bioremediation) is a well established technology for the removal of organic soil contaminants, the use of microorganisms to transform inorganic contaminants like heavy metals is still being investigated. Environmentalists are viewing microbes as an 'eco-friendly nano-factories' for metal remediation through biotechnological applications employing microbes, such as yeast, bacteria, algae, diatoms and actinomycetes.

There are bacteria which can also ingest the most toxic 'cyanide' from water.

MICROBIAL BIOREMEDIATION OF HEAVY METALS

Metals play important role in the life processes of microbes. Some metals such as chromium (Cr), calcium (Ca), magnesium (Mg), manganese (Mn), copper (Cu), sodium (Na), nickel (Ni) and zinc (Zn) are essential as micronutrients for various metabolic functions and for redox functions. Other metals have no biological role e.g. cadmium (Cd), lead (Pb), mercury (Hg), aluminum (Al), gold (Au) and silver (Ag). They are non-essential and potentially toxic to soil microbes. Some of them e.g. Cd^{2+}, Ag^{2+}, Hg^{2+} tend to bind the SH groups of enzymes and inhibit their activity (Turpeinen, 2002).

Soil contamination by heavy metals may repress or even kill parts of the microbial community in soil. Interaction of metals with cellular proteins / enzymes are more commonly implicated in causing toxicity than interaction with membranes. Binding affects the structure and function of proteins and enzymes.

Metal Tolerance & Resistance by Microbes

It is generally assumed that the exposure to metals leads to the establishment of a tolerant / resistant microbial population. Microbial 'resistance' is defined as the ability of a micro-organism to survive toxic effects of metal exposure by means of a detoxification mechanism produced in direct response to the 'heavy metal species' concerned. Microbial 'tolerance' is defined as the ability of a micro-organism to survive metal toxicity by means of intrinsic properties and or environmental modification of toxicity.

Soil micro-organisms have been shown to bio-accumulate metals in tissues in concentrations up to 50 times higher than the surrounding soil

Micro-organisms employ a variety of mechanisms to resist and cope with toxic metals. The principal mechanism of resistance of inorganic metals by microbes are metal oxidation, metal reduction, methylation, demethylation, enzymatic reduction, metal-organic complexion, metal ligand degradation, intracellular and extracellular metal sequestration, metal efflux pumps, exclusion by permeability barrier and production of metal chelators such as metallothioneins and biosurfactants.

The Process and Mechanism of Microbial Remediation

Microbial remediation of toxic metals occurs in two ways—

(1) Direct reduction by the activity of the bacterial enzyme 'metal reductase'. It is applied for groundwater decontamination, using bioreactors (pump & treat) and also for soils after excavation (pulping or heaping and inoculation with appropriate microbial consortium). These techniques are *ex-situ* methods, and very expensive and has low metal extraction efficiencies.

(2) Indirect reduction by biologically produced hydrogen sulfide (H_2S) by sulfate reducing bacteria to reduce and precipitate the metals. This is an *in-situ* method, and an environmentally sound & inexpensive alternative to pump & treat (for contaminated groundwater) or excavate & treat (for contaminated soils). Microbial growth is induced in sub-surface zones by injecting substrates. The migrating metals are intercepted and immobilized by precipitation with biologically produced H_2S.

There are at least three major microbial processes that influence the bioremediation of metals and these are—

1. Biosorption & Bioaccumulation

Biosorption is sequestration of the positively charged heavy metal ions (cations) to the negatively charged microbial cell membranes and polysachharides secreted in most of the bacteria on the outer surfaces through slime and capsule formation. From the surface the metals are transported into the cell cytoplasm through the cell membrane with the aid of transporter proteins and get bioaccumulated.

2. Biologically Catalysed Immobilization

Inside the microbial cells, metal ions gets fixed to Iron (Fe)-Oxides and into organic colloids and becomes immobilised. This is achieved by enzymatic reduction by microbes (described below).

3. Biologically Catalysed Solubilization

Metal reducing bacteria enzymatically reduce and also under appropriate conditions, solubilize oxide minerals. Such dissolution reaction have been shown to release cadmium (Cd), nickel (Ni) and zinc (Zn) into solution during reduction of goethite (a form of Fe-oxide) by anaerobic bacterium *Closteridium* spp.

Microorganisms do not actually biodegrade inorganic metals, but changes (bio-transform) their oxidation state. This can lead to an increase in solubility (and subsequent removal by leaching), or precipitation and reduction in bioavailability. Metallic residues (heavy metals) may be converted into 'metal-organic combinations' that have less bioavailability (to pass into human food chain) than the 'metal-mineral combinations' of the heavy metals. Microbes transform the oxidation states of several toxic metals and increase their bioavailability in the rhizosphere (root zone) thus facilitating their absorption and removal by hyper-accumulating plants by phytoremediation.

Many divalent metal cations like Mn^{2+}, Fe^{2+} and Zn^{2+} are very similar in structure. Also, the structure of oxyanions, such as chromate, resembles that of sulphate. Evolution has endowed micro-organisms with effective mechanisms to distinguish between similar metal ions and between toxic and non-toxic metals. Microbes have solved this problem by developing two types of uptake mechanisms and systems for metal ions -

(1) Selective, substrate-specific uptake system that are slow and require considerable cellular energy (ATP) and is only produced by the cell in times of need;

(2) Substrate-non-specific rapid system, that transport metals using a chemiosmotic gradient across the cytoplasmic membrane of the bacteria rather than using ATP. (Nies, 1999).

Even highly evolved, substrate-specific uptake mechanisms may not prevent entry of toxic metals in cells. Once inside, metal cations can interact with various cellular components including cell membranes, proteins and nucleic acids. Incompletely filled d-orbitals allow metals to form complex compounds with organic ligands, such as the proteins, nucleic acids & cell wall materials of micro-organisms. The ability of microbial cell surfaces to form complex with metals lies in their net negative charge at normal growth pH. The outer membrane of Gram negative bacteria effectively complexes metals including magnesium (Mg), nickel (Ni), strontium (Sr), manganese (Mn), lead (Pb), iron (Fe), sodium (Na) and calcium (Ca). In Gram -eve bacteria the, the net -eve charge results from the phosphates and carboxyl groups of lipopolysaccharide molecules, while the -eve charge in Gram positive bacteria results largely from teichoic acid. A more negative cell surface charge may more effectively attract and bind toxic metal cations. Toxic metals readily binds to sulfhydryl group of proteins. (Nies, 1999; Sandrin & Hoffman, 2006).

The resemblance of some toxic heavy metals to essential metabolite (minerals) allows them to readily enter into the microbial cells. Thus, chromate (Cr) is often mistakenly taken up in place of sulphate (S), arsenate (As) is mistaken for phosphate (P), cadmium (Cd) is used as an enzyme co-factor instead of zinc (Zn) or calcium (Ca), nickel (Ni) and cobalt (Co) is mistaken for iron (Fe), and zinc (Zn) is very commonly mistaken for magnesium (Mg). (Nies, 1999; Sandrin & Hoffman, 2006).

MICROBIAL DETOXIFICATION OF METALS

One or more of the resistance mechanisms allows microbes to function in metal contaminated environments and detoxify them. Micro-organisms can detoxify metals by 'valence transformation', by 'extracellular chemical precipitation' or by 'volatilization'.

Generally, microbial transformations & detoxifications of metals occur either by redox conversions (reduction) of inorganic forms or conversions from inorganic to organic forms and *vice versa*. Most toxic heavy metals are less soluble and less toxic when in 'reduced state' than in 'oxidised state'. Reduction of metals can occur through dissimilatory metal reduction, where microbes utilize metals as terminal electron acceptor for anaerobic respiration.

To date arsenic (As), chromium (Cr), mercury (Hg), uranium (U) and selenium (Se) have responded well to detoxification by microbial reduction. *Oscillatoria* spp. (a blue-green algae), *Chlorella vulgaris* & *Chlamydomonas* spp. (green algae), *Arthrobacter, Agrobacter, Enterobacter* & *Pseudomonas aeruginosa* are some metal reducing microbes. (Ramasamy *et. al.*, 2006).

1. Microbial Reduction of Chromium (VI) to Cr (III)

Chromium is widely used in many industrial & developmental activities, such as in leather & tannery industries, electroplating, steel and automobile manufacturing, production of paint pigments and dyes, refractory and in wood preservation. Its world production is in the order of 10,000,000 tons per year. It is a hazardous contaminant and is a serious threat to human health as it readily spreads beyond the site of initial contamination through aquatic ecosystems and groundwater. (Viti & Giovannetti, 2006).

Chromium in environment is able to exist in several oxidation states, ranging from Cr (II) to Cr (VI), but in soils the most stable & common forms are trivalent Cr (III) and hexavalent Cr (VI) species. The trivalent & hexavalent forms can inter-convert. Cr (III) is essential for animal and human nutrition. Utmost consideration is given to Cr (VI) because it is water-soluble, highly toxic and mutagenic to most organisms and carcinogenic for humans. It is also involved in causing birth defects and the decrease of reproductive health.

A wide range of microbes have been found to have chromium (Cr) tolerance, resistance and reducing ability. The blue-green algae *Nostoc* have been reported to exist in a soil chronically polluted by chromium (about 5000 mg/kg of soil) from leather tannery. Other microbes tolerating / resisting Cr (IV) levels are *Arthrobacter crystallopoites* (500 mg/L), *Pseudomonas* spp. CRB 5 (520 mg/L), *Bacillus maroccanus* ChrA21 (1040 mg/L), *Corynebacterium hoagii* Chr B20 (1144 mg/L), *Bacillus cereus* ES04 (1500 mg/L). (Viti & Giovannetti, 2006). Anaerobic sulfate reducing and methanogenic bacteria possess inherent abilities to sorb more than 90 % of chromium to its cell biomass. Microbe reduce the highly soluble chromate ions to Cr (III), which under appropriate conditions precipitates as $Cr(OH)_3$. Organic matter (carbon sources) of the soil plays an important role in the reduction of Cr (VI) to Cr (III) by creating reducing conditions, such as increasing activities of soil microbes and by acting as an electron donor,& also by indirectly lowering the oxygen level of the soil (due to increased microbial respiration). Carbon sources, such as organic acids, manure, mollases, have been proposed to improve Cr (VI) reduction, that otherwise is very slow.

2. Microbial Reduction of Uranium (VI) to U (IV)

Microbe reduce uranyl carbonate, which is exceedingly soluble in carbonate-bearing groundwater, to highly insoluble U (IV) which precipitates from solution as the uranium oxide mineral uraninite. Recently scientists have succeeded in microbial binding of U (IV), which is then converted by the living cell to U (IV) and precipitated intracellularly. The *Geobacter* spp. have been found to work well to remove uranium (U) from groundwater. (Anderson *et al.*, 2003).

3. Microbial Reduction of Selenium (VI) to Elemental Se (0)

It causes precipitation of the selenium metal and reduced bioavailability. In addition, SeO_4 can be microbially methylated to volatile dimethyl selenide which escape from soil.

4. Role of Iron & Sulfate Reducing Bacteria in Reducing Toxic Metals

The iron (Fe) reducing bacterium e.g. *Geobacter* spp. and the sulfur reducing bacterium e.g. *Desulfuromonas* spp. are also capable of reducing the toxic metals. They have been found to colonise habitats with elevated metal concentrations. *G. metallireducens*, a strict anaerobe is able to reduce manganese (Mn), such as toxic Mn (IV) to Mn (II), and reduce uranium (U), such as toxic U (VI) to U (IV). The *G. sulfurreducens* & *G. metallireducens* are able to reduce chromium (Cr) such as highly toxic Cr (VI) to harmless Cr (III). The sulfur reducing bacterium is strictly anaerobic and gram-negative. It acquires its energy from sulfur (S) respiration and completely oxidises acetic acid (organic acid) with sulfur (S) to carbon dioxide (CO_2). Reduction of sulfur (S) produces hydrogen sulfide (H_2S) which reacts with heavy metal ions to form less toxic insoluble metal-sulfides. Furthermore, these bacteria are also able to enzymatically reduce and precipitate these heavy metals. (Bruschi and Goulhen, 2006). This approach of indirect metal reduction by biologically produced H_2S by sulfur reducing bacteria was developed in the 1980s and has been used commercially up to industrial scale. They lead to selective metal precipitation, such as copper (Cu) and zinc (Zn) sulfates and acidity removal.

Success Stories

(1) Pilot plants developed by Shell Research Ltd. and Budelco BV, France, using an undefined consortium of SRB (sulfate reducing bacteria), have been used successfully to remove zinc (Zn) and sulfate. The metals were precipitated as sulfides. Acetic acid, produced by the SRB was removed by the 'methanogenic bacteria' present in the consortium. This technology has been scaled up and now capable of treating 7000 cubic meter (cum) of contaminated soil per day. (Bruschi and Goulhen, 2006)

(2) In-situ bioremediation of uranium contaminated sites have been conducted successfully with *Desulfosphorosinus* spp. and *Closteridium* spp. (Bruschi & Goulhen, 2006).

(3) Treatment of other metals using anaerobic bioreactor with SRB community culture has been reported for 'phosphogypsum', a hazardous waste from fertilizer industries and for 'lead wastes' from car batteries. Lead waste is reduced to less hazardous PbS (Galena).

5. Microbial Methylation of Metals : Another Mechanism of Detoxification

Microbial methylation also plays an important role in metal detoxification, because methylated compounds are often volatile. Mercury, Hg (II) can be biomethylated by a number of bacteria e.g. *Pseudomonas* spp., *Bacillus* spp., *Closteridium* spp. and *Escherichia* spp. to gaseous methyl mercury. Biomethylation of arsenic (As) to gaseous arsines, selenium (Se) to volatile dimethyle selenide, and lead (Pb) to dimethyl lead has been observed in various contaminated soils. (Ramasamy et, al., 2006).

Acidophilic iron and sulfur oxidizing bacteria are able to leach high concentrations of arsenic (As), cadmium (Cd), copper (Cu), cobalt (Co) and zinc (Zn) from contaminated soils. Also heavy metals can be precipitated as 'insoluble sulfides' indirectly by the metabolic activity of sulfate reducing bacteria. (Ramasamy et, al., 2006).

MICROBIAL DESTRUCTION OF TOXIC ORGANICS

Several microbes have been identified in nature which can break down the hazardous organic substances in the environment (soil & water) including the xenobiotic compounds such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and the chlorinated substances like polychlorinated biphenyls (PCBs) in due course of time. Majority of the organochlorines appears to be biotransformed, forming cunjugates with the soil humic matter. Scientists at IIT, Madras have discovered a bacterial mixture which breaks down the deadly pesticide 'endosulphan' into simple inorganic chemicals under both aerobic and anaerobic conditions.

1. Bacterial Degradation of Toxic Organics

Naturally occurring aerobic bacteria have been found to decompose both natural and the synthetic hazardous organic materials to harmless CO_2 and water. However, anaerobic microbes are important for degrading the halogens (reductive dehalogenation) and nitrosamine, reduction of epoxides to olefins, reduction of nitro groups and ring fission of aromatic structures.

TABLE 1

Naturally Occurring Bacteria Capable of Destroying Some Hazardous Wastes and Chemicals by Biodegradation

Organisms	Chemicals Degraded
Flavobacterium spp.	Organophosphates
Cunniughamela elegans	PCBs (Polychlorinated Biphenyls) & PAHs
& *Candida tropicalis*	(Polycyclic Aromatic Hydrocarbons);
Alcaligenes spp. &	PCBs, halogenated hydrocarbons and alkylbenzene
Pseudomonas spp.	sulphonates, PCBs, organophosphates, benzene, anthracene, phenolic compounds, 2,4 D, DDT and 2,4,5-trichlorophenoxyacetic acid etc.
Actinomycetes	Raw rubber
Nocardia tartaricans	Chemical Detergents (Ethylbenzene)
Closteridium	Lindane
Arthrobacter & Bacillus	Endrin
Trichoderma & Pseudomonas	Malathion

Sources : Various Publications of UNEP, WWF and WHO (1992-2002)

2. Fungal Degradation of Toxic Organics

All fungus, but more specifically the wood-decaying members of Basidiomycetes called the white - rot fungus (WRF) are most efficient microbe that de-polymerize and even mineralize the 'lignin' contents which is hard to be degraded. The fungus produce a powerful 'extra-cellular ligninolytic enzyme system' which is a strong oxidant. Three main types of oxido-reductase activities can be found in this enzymatic system - the 'polyphenoloxidases', 'peroxidases' and auxiliary 'H_2O_2 generating oxidases'. More specifically, the key lignolytic enzymes synthesized by WRF are 'laccase' (polyphenoloxidase). Both, whole fungal cultures of WRF as well as the lignin-degrading enzymes were found to be useful to the bioremediation of a wide number of toxic pollutants including the recalcitrant 'xenobiotics' and more importantly the 'textile dyes' which is highly resistant to washing, chemical solvents, action of sunlight and microbial attack. Rate of degradation is affected by atmospheric oxygen concentration, nutrient nitrogen and the source of carbon.

Fungal Destruction of Chlorinated Aromatic Compounds

A very promising fungus which has the arsenal to degrade and destroy an assortment of persistent 'chlorinated aromatic compounds' have been identified. It is the white-rot fungus *(Phanerochaete chrysosporidium)*. In Turkey, scientists have found that *P. chrysosporidium* can biodegrade LDPE plastic bags containing 12 % starch. Scientists in Japan have used biochemical machining and enzymes found in filamentous fungi to biodegrade or cut down the molecular chains present in biodegradable plastics.

TABLE 2

Naturally Occurring Fungus Capable of Destroying Some Hazardous Wastes and Chemicals by Biodegradation

Organisms	Chemicals Degraded
Phanerochaete chrysoporium, P. sordida & Trametes hirsute	Halocarbons such as lindane, pentachlorophenol, DDT, DDE, PCBs, 4,5,6-trichlorophenol, 2,4,6-trichlorophenol, dichlorphenol, and chlordane
Zylerion xylestrix	Pesticides / Herbicides (Aldrin, dieldrin, parathion and malathion)
Mucor	Dieldrin
Yeast *(Saccharomyces)*	DDT

Sources: Various Publications of UNEP, WWF and WHO (1992-2002)

Use of Yeast for Removal of Textile Dyes from Polluted Waters

Industrial textile dyes have been designed and synthesized to be highly resistant to washing and action of chemical solvents and sunlight. There are currently more than 10,000 different textile dyes commercially available in the world markets. Azo, indigoid and anthraquinone are the major chromophores used in the textile industries. They are complexed with heavy metals copper (Cu), cobalt (Co) and chromium (Cr) and are of considerable public health concern. Conventional treatments like 'adsorbent by activated carbons' is very expensive and do not remove heavy metals.

Living yeast biomass specifically the strains of *Candida tropicalis* isolated from sewage has been shown to bio-absorb various textile dyes - the reactive black and red, and the remazol blue. Maximum bio-accumulation capacity range from 79 mg/g (per gram of yeast biomass) for reactive red to 112 mg/g for remazol blue. It has significantly lower operational costs and the heavy metals are also absorbed and removed. (Donmez, 2002).

Microbial Destruction of Polycyclic Aromatic Hydrocarbons (PAHs)

Among the most abundant environmental pollutants, the aromatic compounds are of major concern because of their persistence and toxicity. PAHs are obiquitous in nature found throughout the environment—in air, water and soil. PAHs are group of over 100 chemicals of which anthracene, benzo (a) pyrene, naphthalene, pyrene are common. They are formed by the incomplete combustion of fossil fuels (coal & oil) and wood. They are emitted from petroleum refineries, coal and oil power plants, coking plants, asphalt production plants, aluminum plants, industrial machinery manufacture and paper mills. They readily evaporate in air. People may be exposed to PAHs in the soil where coal, wood or petrol has been burnt. Food grown on such soils may contain PAH. However, several PAHs breaks down in sunlight in air over a period of days to weeks. Soil microbes can degrade them in weeks to months.

US EPA has identified 16 PAHs as 'priority pollutants' made of two or more fused benzene rings with potential for high 'bio-magnification', and therefore, toxic to living organisms. PAHs are able to 'bio-accumulate' in plants and animals including human beings. Benzo (a) pyrene, benzo (a) anthracene and chrysene are carcinogenic to humans, mutagenic in animal and bacterial cells, and also teratogenic, causing genetic defect in humans. Some PAHs can also induce 'immunodepressive effects'. These chemicals are thermodynamically stable, have very low aqueous solubility, are highly hydrophobic, and tend to be strongly associated with particles surface in the environment. Most of them are resistant to volatilization and photolysis and are therefore, very 'persistent' under natural conditions with 'half-lives' in soils ranging from 26 days for 'phenanthrene' (a volatile and degradable compound) to 6,250 days for 'benzo (a) anthracene' (a recalcitrant compound). (Sims & Overcash, 1983).

Bacteria capable of destroying PAHs are species of *Pseudomonas, Alcaligenes, Rhodococcus, Sphingomonas* and *Mycobacterium*. They utilize PAHs as their sole carbon and energy source. A diverse group of fungi, both ligininolytic and non-ligininolytic, are able to degrade PAHs. But they transform them co-metabolically to detoxified metabolites and do not use them as source of carbon and energy like bacteria. (Sutherland, 1992). Both prokaryotic and eukaryotic algae have been found to degrade PAHs in aquatic environments. The blue-green algae (Cyanobacteria) oxidize PAHs under photoautotrophic conditions to form hydroxylated intermediates.

MECHANISM OF MICROBIAL ACTION IN DEGRADATION OF TOXIC COMPOUNDS

This is achieved by biodegradation and biotransformation of complex toxic chemicals into harmless simpler biochemical products by heterotrophic microbes which cannot produce their own food and in order to survive extract 'growth nutrients' from the host material (which may be an organic or inorganic waste) by decomposing it. Microbes can also transform toxic substances into energy source. Microbes grow geometrically and in the process of decomposition produce a huge biomass of 'new cells'. Hence the biodegradation process become faster with time.

$$V_1 \text{ (organic material)} + V_2O_2 + V_3NH_3 + V_4PO_4^{3-} \text{ (microbes) } ⑦ \; V_5 \text{ (new cells)} + V_6CO_2 + V_7H_2O$$

Oxygen (O_2), ammonia (NH_3), and phosphate (PO_4^{3-}) are the nutrients needed by the microbes to oxidize the organic matter in the waste.

Biodegradation by microbes is system-specific. Unless a proper microbial consortium is both developed and maintained, a compound may not degrade in that system. Microorganisms adapt to degrade 'new synthetic compounds' either by utilizing catabolic enzymes they already possess or by acquiring new metabolic pathways. In any event the biological systems can lower the cost of downstream processes by reducing organic load. While a microbial community that is resistant to moderate levels of heavy metals can be developed, the accumulation of metal precipitates on the bacterial biomass may severely inhibit their activity. Hence, a pretreatment system to remove metal contaminants may be needed. If the toxin is non-biodegradable, microbial strains that are resistant to the toxins must be enriched.

Microorganisms break down the complex hydrocarbons in the hazardous waste by using the three general mechanisms—aerobic and anaerobic respiration and fermentation. Aerobic process requires adequate supply of oxygen and the biodegradation process is rapid and more complete and there is no problematic end products like methane and hydrogen sulfide.

(a) Anaerobic Degradation

It is a sequential, biologically destructive process in which the complex 'hydrocarbons' of hazardous wastes are converted into simpler molecules of 'carbon dioxide' and 'methane' by anaerobic microbes. The anaerobes use oxygen that is combined chemically with other elements, such as nitrates, carbonates, or sulphates. If nitrates are source of oxygen, nitrogen is formed and if sulfates, hydrogen sulfide is formed under anaerobic process. They also require complete absence of 'free oxygen' molecules. They require a narrow pH range than the aerobic systems. Anaerobic microbes are important for degrading the halogens (reductive dehalogenation) and nitrosamine, reduction of epoxides to olefins, reduction of nitro groups and ring fission of aromatic structures. Several microbes have been identified which can break down the chlorinated substances like PCBs. Majority of the organochlorines appears to be biotransformed, forming cunjugates with the soil humic matter.

A wide variety of industrial wastewaters can be successfully treated by anaerobic process. It is specially attractive for 'high strength' and 'warm temperature' wastewaters. As no mechanical aeration is required considerable amount of energy is saved. Anaerobic process may in fact be net energy producers instead of users. They also require a narrow pH range than the aerobic systems. For industrial wastewaters with much higher biodegradable chemical oxygen demand (COD) concentrations and elevated temperatures, anaerobic process may be more economical. Food processing and distillery industry wastewaters can have COD concentrations ranging from 3000 to 30,000 mg/L.

TABLE 3

Industrial Wastewaters Treated Successfully by Anaerobic Process

(1) Alcohol Distillation	(2) Breweries
(3) Chemical Manufacturing	(4) Pharmaceuticals
(5) Pulp and Paper	(6) Dairy and Cheese Processing
(7) Slaughterhouse and Meat Packing	(8) Softdrink Beverages
(9) Sugar Processing	(10) Fish and Seafood Processing
(11) Landfill Leachate	(12) Domestic Wastewater

Sources : Various Publications of UNEP, WWF and WHO (1992-2002)

The microbiology of anaerobic digestion consists of two distinct groups of anaerobic microorganisms- the *'acetogens'* and the *'methanogens'* present at several trophic levels. At the higher levels, organisms attack waste molecules through hydrolysis and fermentation, reducing them to simpler hydrocarbons methane (CH_4) and carbon dioxide (CO_2) in the last two steps of the destructive process. It is crucial that a delicate balance of population must be maintained between the two distinct groups of essential anaerobes- the *acetogens* and the *methanogens* in the final stages of reaction. The *acetogens* convert most of the hydrocarbons of waste to acetate, CO_2, and H_2 and some organic acids. The *methanogens* convert organic acids and CO_2 to CH_4 in the presence of the available H_2.

Hydrocarbons of Waste Organics (*Acetogens*)⑦ Acetate + Organic Acids + CO_2 + H_2

Organic Acids + CO_2 + H_2 (*Methanogens*) ⑦ CH_4 + Byproducts + Energy

Anaerobic microbes are important for degrading the halogens (reductive dehalogenation) and nitrosamine, reduction of epoxides to olefins, reduction of nitro groups and ring fission of aromatic structures. Several microbes have been identified which can break down the chlorinated substances like PCBs. Majority of the organochlorines appears to be biotransformed, forming cunjugates with the soil humic matter.

Advantages of Anaerobic Treatment

Anaerobic treatment has several attributes that are considered favorable in biological treatment—

1. Less energy is required as mechanical aeration is avoided. On the contrary a potential energy source methane (CH_4) is produced.
2. Significantly less land is required with smaller reactor volumes;
3. Extremely high destruction rates of wastes molecules can be achieved;
4. Solids (biological sludge) generation from the growth of biological cells (biomass production) is about 20 times less than the aerobic process, and therefore cost of disposal of solids are reduced;
5. Certain toxic organic wastes can only be destroyed by anaerobic digestion;
6. Many industrial wastewater lacks sufficient growth nutrients to support aerobic bacteria. Fewer nutrients are required to support the anaerobes;
7. There is complete elimination of 'off-gas' air pollution.

Disadvantages

1. Longer start-up time. While it is in days for aerobic process, it may be in months for anaerobic process;
2. May be more susceptible to upsets due to toxic substances;
3. It has potential for production of odors and corrosive gases;
4. It is not possible to remove biological nitrogen (N) and phosphorus (P);
5. May require alkalinity addition.

Anaerobic Digestion

Anaerobic digestion is a process of oxidizing organic matter in closed vessels in the absence of air. It is very effective in reducing the BOD of soluble organic liquid wastes, such as yeast, cotton kiering, slaughterhouse, dairy and white-water (from paper mills) wastes. BOD reductions of 60 to 92 % have been attained with all these wastes, at loadings of 0.003 to 0.191 pound of BOD per cubic foot of digester per day. Concentrations of organic matter ranged from 1565 to 17,000 ppm BOD.

TABLE 4

Some Hazardous Organic Chemicals Degradable by Anaerobic Digestion

1. Acetylsalicylic acid	13. Dimethoxy benzoic acid	25. Nitrobenzene
2. Acetaldehyde	14. Dimethylphthalate	26. Pentanol
3. Acetic anhydride	15. Ethyl acetate	27. Phenol
4. Acetone	16. Ferulic acid	28. Phthalic acid
5. Benzoic acid	17. Formaldehyde	29. Polyethylene glycol
6. Benzyl alcohol	18. Glycerol	30. Propanal
7. Butanol	19. Hexanoic acid	31. Proponol
8. Butylene glycerol	20. Methanol	32. Pyrogallol
9. Butyric acid	21. Methyl acetate	33. Sorbic acid
10. Catechol	22. Methyl acrylate	34. Valeric acid
11. Crotonic acid	23. Methyl ethyl ketone	35. Vannilic acid
12. Diethylphthalate	24. Methyl formate	36. Vinyl acetate

Sources: Various Publications of UNEP, WWF and WHO (1992-2002)

TABLE 5

Some Organic Compounds Transformed through Anaerobic Process
Toxic Chemicals Biological Transformation to Harmless Products

2,4-Dinitrophenol	⑦	Methane
2,4-Dimethyl phenol	⑦	Methane
Carbon tetrachloride	⑦	Degrade slowly
Aldrin	⑦	Dieldrin
DDT	⑦	Dechlorinated to DDE
Toxaphene	⑦	Reduced and dechlorinated
Halogenated Benzoates	⑦	Degraded after dechlorination to CO_2 and CH_4
Benzene	⑦	Degraded to CO_2 and CH_4
Isopropylbenzene	⑦	Degraded to CO_2 and CH_4
Ethylbenzene	⑦	Degraded to CO_2 and CH_4
Toluene	⑦	Degraded to CO_2 and CH_4

Sources: Various Publications of UNEP, WWF and WHO (1992-2002)

(b) Aerobic Degradation

Aerobic process requires adequate supply of molecular oxygen to decompose the organic matters and retrieve energy from it, which is needed by the bacteria to grow and multiply. The aerobic biodegradation process is rapid and more complete and there is no problematic end products like methane (CH_4) and hydrogen sulfide. Naturally occurring aerobic bacteria can decompose both natural and the synthetic hazardous organic materials to harmless CO_2 and water.

Organics + Molecular Oxygen (Aerobes) ⑦ CO_2 + H_2O + Other Products + Energy

Many hazardous organics are readily degraded aerobically, but because the depth of oxygen penetration in soils is limited, the rate and extent of biological detoxification is also limited. Mechanical aeration may sometimes be needed in biological treatment of landfill leachates and heavily contaminated wastewaters. The dissolved molecular oxygen concentration must be maintained at least at 2 pounds of oxygen per pound of solids destroyed. If the oxygen content is too low, portions of the solids may become anaerobic and adversely affect biological destruction.

MICROBIAL DESTRUCTION OF HAZARDOUS WASTES

Microremediation technology is now used to detoxify the hazardous chemical wastes before dumping in the land-fills and closing it finally. It has also been proposed for application *in-situ* by injecting naturally occurring microbes, cultured bacteria, nutrients and oxygen into the soil column or groundwater to form zones of microbial activity. The chlorinated and non-chlorinated pollutants (hazardous wastes) are broken down into carbon dioxide and water and a bacterial biomass is generated. Microremediation is typically able to reduce up to 90 % of some contaminants in field systems. The technology has been successfully used in Australia for treatment of PCBs at a site in NSW and for cleaning up 'chlorinated phenols' at a herbicide manufacturing plant near Melbourne. Animal manure were spread over the contaminated sites and in about an year full degradation of the contaminants occurred by mixed species of bacteria.

TABLE 6

Degradable Organic Hazardous Wastes and the Associated Microorganisms

Hazardous Organic Compounds	Microorganisms Involved in Degradation
Phenylmercuric acetate	*Pseudomonas, Arthrobacter, Citrobacter, Vibrio*
Raw rubber, hevea latex	*Actinomycetes*
Detergents	*Nocardia, Pseudomonas*
PCBs	Not identified
Malathion	*Trichoderma, Pseudomonas*
Endrin	*Arthrobacter, Bacillus*
Lindane	*Closteridium*
DDT	*Escherichia, Hydrogenomonas, Saccharomyces*
Dieldrin	*Mucor*

Sources : Various Publications of UNEP, WWF and WHO (1992-2002)

PLANT-ASSISTED MICROBIAL DEGRADATION

Many organic compounds are degraded by microorganisms located in the rhizospheres (in the root zone) of plants. Microbial activity in the rhizosphere of plants is several-fold higher than in the bulk soil. The population of microflora present in the rhizosphere is much higher than present in the vegetation-less soil. (Suthersan, 1997). Certain plant roots release substances that are nutrients for microorganisms, bacteria and fungi, that provide favorable habitats for soil microbes to act (Cunningham and Ow, 1996).

A typical microbial population present in the rhizosphere, per gram of air-dried soil comprises—

1. Bacteria: 5×10^6
2. Actinomycetes: 9×10^5
3. Fungi: 2×10^3

This results in increased biological activity of the microbes in the area immediately surrounding the root zone (rhizosphere). By encouraging a microbiologically active rhizosphere, the plants facilitate accelerated digestion (biodegradation) of wide variety of organic contaminants in the upper soil layers and / or wastewater / polluted water (Anderson *et al.* 1993). Interestingly, gram-negative bacteria appear to have some important metabolic capabilities for degrading xenobiotic chemicals not found in gram-positive bacteria.

A large number of bacteria and fungi are capable of degrading the hazardous chemical 'chlorobenzene' and mineralizing it. The end products are 2 & 4 - chlorophenol. Researches indicate that certain herbs stimulates microbial degradation of 'chlorinated organics' primarily via rhizospheric reductive dechlorination biodegrdation processes. Chlorobenzene can undergo microbial dechlorination and the benzene ring can be converted to catechol, followed by ring fission or oxidation of the side chain.

ROLE OF ENVIRONMENTAL BIOTECHNOLOGY & GENETIC ENGINEERING IN IMPROVING MICROBIAL DEGRADATION BY PRODUCING GENETICALLY ENGINEERED BACTERIA

With the advancement in our knowledge in environmental biotechnology some bacterium have been genetically engineered (tailored) to biodegrade those hazardous wastes and chemicals which otherwise cannot be degraded by the naturally occurring bacteria. In Germany, genetically engineered bacteria have been used to clean up polluted soils and oil spills in water. These bacteria eat pollutants, gobble them up and chemically alter them in their bodies. When pollutants are finished the bacteria die leaving clean soil and a biomass of dead bacteria containing harmless minerals.

TABLE 7

Genetically Engineered Bacteria Capable of Destroying Some Hazardous Chemicals

Bacterium	Chemicals Destroyed
1. *P. putida*	Camphor degradation
2. *P. oleovarans*	Alkane degradation
3. *P. cepacea*	2,4,5 - Trichlorophenoxyacetic acid degradation
4. *P. mendocina*	Trichloroethylene degradation
5. *P. diminuata*	Parathion (pesticide) degradation

Source: *Biotechnology and Biodegradation;* Kamely *et al.* (1990)

With genetic engineering it will be possible to enhance the reduction activities of hexavalent chromium {Cr (VI)} of the indigenous bacterial strains that express such activities at high levels under poor nutrient and stressful environmental conditions.

Development of Microbial Biosensors by Genetic Engineering

Genetic engineering has led to development of microbial 'biosensors'. These are 'genetically engineered bacteria'. They offer the potential to measure quickly, cheaply and accurately the degree of contamination of environmentally contaminated sites. More specifically, biosensors have been developed to detect heavy metals in contaminated sites. These microbial biosensors have used two distinct methods to detect heavy metal ions –

1. Protein (antibodies & enzymes) or
2. Whole cells (genetically modified or not)

Various biosensors have been designed to evaluate the heavy metal concentrations of metals like cadmium (Cd), mercury (Hg), nickel (Ni), copper (Cu), arsenic (As) and iron (Fe). (D' Souza, 2001; Verma & Singh, 2005; Bruschi & Goulhen, 2006).

FACTORS AFFECTING MICROBIAL REMEDIATION

Microbial remediation depends upon the presence of appropriate micro-organisms in the correct amounts and in combinations and in appropriate environmental conditions. Bio-stimulation & bio-augmentation are two essential factors influencing bioremediation by microbes.

Bio-stimulation

It is the addition of nutrients (usually source of carbon, nitrogen & phosphorus), oxygen or other electron donors or acceptors. These amendments serve to increase the number or activity or both, of naturally occurring micro-organisms available for bioremediation. Amendments can be added in either liquid or gaseous forms, *via* injection. Liquids can be injected into shallow or deep aquifers to stimulate the growth of micro-organisms involved in bioremediation. Anderson *et al.* (2003) removed uranium (U) from groundwater of an uranium-contaminated aquifer by biostimulating the *in-situ* activity of *Geobacter* spp..

Bio-augmentation

It is the addition of microorganisms that can bio-transform (usually toxic metals) or biodegrade (toxic organic compounds) a particular contaminant.

SUCCESS STORY OF MICROBIAL REMEDIATION FOR ENVIRONMENTAL CLEANUP IN UK

The town of Salem in New Hampshire, UK, faced a big environmental problem from the decommissioned wastewater treatment plant in 1987 (constructed in 1964) which showed symptoms of soil and groundwater contamination by 'chlorinated solvents' e.g. the chlorinated aliphatic hydrocarbon (CAH) at unacceptable levels. The potential impact of the CAHs could have been on the 30 bedrock water supply wells located within a 3 km radius of site. The major challenge for the cleanup was the large area (2-3 ha) of contaminated land and groundwater.

Bioremediation program was started in the year 2000 and included injections of 5 bio-stimulant (mixture of yeast & lactose) at the site. A total of more than 200,000 lb (90,720 kg) of bio-stimulant was injected during the program to enhance the anaerobic destruction of chlorinated solvents in groundwater. Bioremediation successfully destroyed the chlorinated solvents mass at an expenditure of just US $ 300,000.

It saved the municipalities an estimated US $ 2 million in cleanup cost by 'conventional treatment' approach (pump and treat) that would have required up to 20 extraction wells, a treatment building with air-stripper, treatment of the air stripper discharge gas, and at least 10 years of system operation and monitoring. (Schaffner, 2004).

THE LIMITATIONS OF THE MICROBIAL TECHNOLOGY

A limitation of the use of microbial technology for metal remediation is that although the metals are bound to microbes, they can be released back into the soil soon after decomposition of the microbes upon their death and decay and still be present in soil. The *in-situ* microbial remediation strategies may need to combine with phyto-remediation strategies by suitable hyper-accumulator plants that can effectively uptake the metals (made bioavailable by the microbes) from soil and bioaccumulate them in their roots & shoots, thus preventing their recycling in soil. Genetic engineering can resolve the problem by engineering 'bacteroids' (with metal binding peptides) in root nodules of plants. This has been termed as 'symbiotic engineering'.

CONCLUDING REMARKS

Microbial bioremediation has developed from the laboratory to a fully commercialized technology over the last 30 years in many industrialized nations. Microbes have been mostly used to treat industrial waste streams, with the organisms either 'immobilized' on to different support matrixes or in a 'free-living' state, enclosed in treatment tanks or other kinds of reactor vessels. Subsequently the metal-loaded microbial biomass is either disposed appropriately (in secured hazardous waste landfills or incinerated), or treated to 'recover' the metals for re-use in industries.

> Microbial technology has gained popularity for site remediation as it treats the soil contaminants in site itself, and significantly cuts down the excavation costs of soil. It is intrinsic process done *in-situ* and relies on the naturally occurring biological process carried out by native and indigenous microorganisms. It can be applied to sites with high water table and does not destroy the site that is to be treated (detoxified). Microbial remediation can handle all types of soil pollutants starting from rare metals to radionuclides. Native microbes in any contaminated site is 'acclimatized' and are capable of transforming the toxic metals to their oxides or hydroxides.

Bioremediation can be both '*in-situ*' and '*ex-situ*'. In the '*ex-situ*' bioremediation which is above ground treatment, soils have been excavated and washed or sediments have been extracted from subsurface and then decontaminated. This becomes more costly treatment. Recently, 'genetically engineered' microbes have been used for '*ex-situ*' bioremediation.

Bioremediation by microbes works either by transforming or by degrading contaminants to less toxic / hazardous chemicals or innocuous substances. In case of metal contaminated sites, the microbes interact with metals and transform them from one chemical form to another by changing their 'oxidation state' through addition or removal of electrons. In some microbial bioremediation strategies, the solubility of the transformed metal increases, thus increasing the mobility of the contaminants and allowing it to be more easily flushed out of the environment. In other strategies, the opposite occurs. The transformed metals precipitate out of the solution, leading to their immobilization. Both strategies of bio-transformation of metals either 'immobilize' the contaminants in place or 'accelerate' their removal.

Chemical treatments for the removal of heavy metals from contaminated sites/ materials are chemical extraction with acids and / or chelating agents for soil treatment and precipitation for groundwater cleaning. In industries, the metals contained in acid-drainage waters are most of the time precipitated using lime. Such treatments are expensive, and results into formation of large quantities of metal-hydroxides. Bioremediation of contaminated sites by microbes is very cost-effective and environmentally friendly as compared to the chemical treatment which requires excavation or pumping of contaminated materials from the polluted site, followed by the addition of reducing chemicals. It leads to considerable destruction of the site treated and the land may become useless for any future use.

Some risks and hazards of environmental biotechnology working with microbes are associated with three properties of microorganisms. They are :

1. The potential of a few strains to cause diseases thus behaving as pathogens);
2. The potential for undetected genotypic or phenotypic changes to alter a tested and approved microbial process; &
3. The ubiquity of organisms which can contaminate the system.

Less hazardous microorganisms that would fulfill the same objectives as the 'undesirable pathogen' can be discovered & selected from microbial diversity, or created by genetic engineering. Biotechnological researches for several years have employed selection techniques to screen wide genotypes of related organisms for non-pathogens as well as more complex methods by inducing mutations and selection of desirable strains. Genetic engineering is also used to remove the useful genetic components responsible for bioremediation properties from the otherwise pathogenic organisms and incorporate them into non-pathogenic organisms such as *Escherichia coli, Bacilus subtilis* and *Saccharomyces cerevisiae* and use them for bioremediation.

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MICROBES IN THE MANAGEMENT OF WASTEWATER

Collective Role of Bacteria, Fungi, Protozoa & Algae in the Treatment & Polishing of Municipal & Industrial Wastewater

KEY WORDS

Bio-geochemical Process; Aerated Lagoons; Trickling Filters; Activated Sludge Process; Rotating Biological Contactors; Dispersed Growth Aeration; Deamination; Ammonification; Flexible Fiber Biofilm Reactor.

BACKGROUND INFORMATION

All municipal and industrial wastewaters contain complex biodegradable organic materials and have to be treated microbiologically before discharging them into the environment (oceans, river ways, lakes and wetlands). Municipal wastewater and some industrial wastewater also contain toxic heavy metals. Microbiological methods use mixed communities of wide varieties of microorganisms (bacteria, protozoa, fungi, rotifers, and algae). Under favorable conditions of growth e.g. pH, temperature and moisture and adequate supply of nutrients like vitamins, magnesium, manganese, copper, sulfur, potassium, phosphorus and nitrogen the microbes degrade the complex organic chemicals (often toxic) into simpler and harmless ones.

Protozoa play very important role in aerobic microbiological treatment process by de·ouring free bacteria and consuming colloidal particulates from the wastewater thus aiding in effluent clarification and polishing. Protozoa require a longer SRT (solid retention time) than the aerobic heterotrophic bacteria, prefer dissolved oxygen concentration above 1.0 mg/L, and are sensitive to toxic materials. Hence, their presence is a good bioindicator of a trouble -free stable process operation. Algae absorb several dissolved minerals from the wastewater for their growth, carry on photosynthesis and enrich the medium with oxygen.

WASTEWATER : A POTENTIAL WATER RESOURCE FOR RECYCLING & REUSE

All wastewater, whether municipal (from domestic sources) or the industrial (process water and the cooling tower water) are now considered as 'potential water resource' from which 'useful water' for non-potable uses can be retrieved and recycled back into the human ecosystem. This is becoming a universal practice all over the world in the wake of declining potable water resources of earth due to increasing demand and the erratic rainfall resulting from the global warming. The rate of 'discharge' of groundwater resources is much higher than the rate of 're-charge' by rainfall, and as a result the groundwater table is constantly declining all over the world.

After proper treatment the municipal wastewater becomes fit enough for non-potable uses such as farm irrigation, garden use, industrial uses as cooling water etc. and if disinfected, it can be used for potable purposes including for drinking. This has become a practice in several U.S & Australian cities to treat the wastewater and discharge back into the rivers and dams for mixing and natural purification and again re-treat it before supplying to the community.

These days some wastewater is being used for 'hydrogen production' and electric generation. Oh & Logan (2005) reported hydrogen and electricity production from a food processing wastewater using fermentation and microbial fuel cell technology.

MICROBES INVOLVED IN WASTEWATER TREATMENT

Important microbes identified for the treatment of industrial wastewater including the degradation of hazardous chemicals in them are—

1. *Sphaerotilus natans,*
2. *Phanerochaet chrysoporium & P. sordida,*
3. *Zylerion xylestrix,*
4. *Candida tropicalis,*
5. *Cunninghmela elegans,*
6. *Halsicomenobacter hydrossis,*
7. *Microthrix parvicella,*
8. *Thiothrix spp.,*
9. *Nocardia spp.,*
10. *Nostocoida limicola,*
11. *Beggiatoa spp.,*
12. *Alcaligenes spp.,*
13. *Flavobacterium spp.,*

14. *Closteridium spp., &*
15. *Psuedomonas spp.* etc.

Temperature and pH are critical factors in microbial treatment of hazardous liquid wastes. pH range of 6-8 is generally good. Microbes can biodegrade and biotransform the complex carbonaceous organic matter into harmless simpler end products CO_2 and H_2O. Microbes grow geometrically and in the process of decomposition produce a huge biomass of 'new cells'. Hence the biodegradation process become faster with time.

V_1(organic material) + V_2O_2 + V_3NH_3 + $V_4PO_4^{3-}$ (microbes)⑦ V_5 (new cells) + V_6CO_2 + V_7H_2O

Oxygen (O_2), ammonia (NH_3), and phosphate (PO_4^{3-}) are the nutrients needed by the microbes to oxidize the organic matter in the waste. The microbes require a relatively constant source of wastewater and BOD to work efficiently. The biomass of new cells has to be removed constantly from the treated liquid by gravity settling (specific gravity of microbial biomass is slightly greater than water) because the biomass which itself is organic, will indicate as the BOD in the treated effluent. Microorganisms, however, are quite 'temperamental' and sensitive to changes in temperature, pH, oxygen tension, toxic compounds, mixing, and the character and quantity of food (organic matter) in the medium.

Microbial degradation is also system-specific. Unless a proper microbial consortium is both developed and maintained, a compound may not degrade in that system. Microorganisms adapt to degrade 'new synthetic compounds' either by utilizing catabolic enzymes they already possess or by acquiring new metabolic pathways. While a microbial community that is resistant to moderate levels of heavy metals can be developed, the accumulation of metal precipitates on the bacterial biomass may severely inhibit their activity.

Hence, a pretreatment system to remove metal contaminants may be needed. If the toxin is non-biodegradable, microbial strains that are resistant to the toxins must be enriched.

THE BIOGEOCHEMICAL PROCESSES

All microorganisms (algae, fungi, bacteria, cyanobacteria and protozoa) help remove and recycle nutrients, heavy metals, hydrocarbons, some pesticides and herbicides from the wastewater by breaking them down in the system. Microbes transform the toxic chemicals into 'non-toxic' forms. The heterotrophic microorganisms (heterotrophic bacteria, fungi and protozoans) feed on the detritus (dead organic matter) and decompose them to release the locked nitrogen and phosphorus. The autotrophic microorganisms (cyanobacteria, photosynthetic bacteria and unicellular algae) infuse oxygen into the water and also absorb nutrients. The nitrifying and the denitrifying bacteria play the critical roles in the purification of wastewater. Sediments are mostly anaerobic and provide conditions for breaking down complex organics (greases, fats, solvents and fuels) and also sequester metals, reducing their bioavailability. Biogeochemical processes also assist in hydrocarbon degradation by photochemical oxidation and sterilization of pathogens by natural UV radiation.

In the biogeochemical process the nutrients from the water (carbon, nitrogen, phosphorus, sulfur and other materials) are either accumulated or removed, recycled or finally stored in sediments, thereby improving the quality of water column above. Plants and microorganisms remove and recycle nutrients either from the water column or the sediments and incorporate them in their tissues.

1. Removal of Nitrogen

Aerial plant parts (above water) carry on photosynthesis and transfer oxygen to the submerged parts including the roots. Oxygen continuously 'leak' from the roots and help create an aerobic micro-environment in the sediments. This helps the aerobic nitrifying bacteria *(Nitrosomonas)* in the removal and transformation of nitrogen from the water and the sediments in the process of deamination, ammonification and nitrification. Deamination and ammonification is a decomposition process occurring both aerobically and anaerobically, wherein the dead organic matter (detritus) containing nitrogenous protein is degraded into amino acids and then into ammonia. Heterotrophic bacteria, fungi and the protozoans - all can decompose the detritus.

(i) Proteins (from detritus) ⑦ *Deamination* ⑦ Amino acids

(ii) Amino acids ⑦ *Ammonification* ⑦ Ammonia (NH_3)

(iii) NH_3 + H_2O ⑦ *Nitrification* ⑦ NH_4 + OH ⑦ NH_4 + OH

(iv) NH_4 ⑦ NO_2 ⑦ *Nitrification* ⑦ NO_3 (Nitrates absorbed by plant roots)

Denitrification occur under anaerobic conditions usually in the deeper sediments which lacks oxygen. Nitrates and nitrites are reduced to gaseous nitrous oxide and nitrogen by the denitrifying bacteria (*Nitrobacter* and *Pseudomonas denitrificans)* which diffuses into the water and ultimately escape into the atmosphere. Both nitrogen and sulfur may be entirely lost from the wetlands systems by denitrification to N_2 (nitrogen) or reduction to H_2S (hydrogen sulphide) gases which escape to the atmosphere.

NO_3 ⑦ Denitrification (*Pseudomonas denitrificans)*⑦ NO_2 ⑦ N_2O ⑦ N_2

SO_4 ⑦ Reduction (*Desulfovibrio)* ⑦ H_2S

2. Removal of Phosphorus

Under aerobic conditions, with plenty of oxygen around, the microbes also play a major role in mineralizing the organic phosphorus in dead biomass (detritus) and in sediments to soluble inorganic phosphorus. Phosphorus may be in the form of insoluble iron or aluminum phosphates on clay particles. Plants and microorganisms can also remove inorganic phosphates from the water column or sediment pore water and assimilate into organic phosphate. Phosphorus is immobilized through sorption and precipitation by ferric oxyhydroxide and the formation of ferric phosphates in moist aerobic conditions. Many hetertrophic bacteria are capable of assimilating and solubising the insoluble inorganic phosphates. Adsorption and desorption of phosphorus onto and from the sediments particles also occur.

SOME IMPORTANT MICROBIOLOGICAL TREATMENT TECHNOLOGIES

Some of the important microbiological treatment technologies used in wastewater treatment are discussed here.

1. Aerated Lagoons (Oxidation Ponds)

Lagooning in oxidation / stabilization ponds is an inexpensive procedure to remove and oxidize organic matter from the industrial wastewater in a natural self-purification phenomena involving both bacteria and algae (nature's balancing mechanism where metabolic product of one is used by other) completing the process in cyclic manner. The first phase is sedimentation in which the settleable solids are deposited in an area around the inlets to the ponds. Some suspended and colloidal matters are precipitated by soluble salts.

The sediments in the pond (sludge) is decomposed mostly by aerobic microbes utilizing the dissolved oxygen in the water (or even by anaerobic microbes in the deep bottom layers where oxygen is deficient) into inert residue and soluble organic materials such as CO_2, H_2O, nitrates, sulfates, and phosphates which the pond algae (usually *Chlorella* and *Scenedesmus* as they are hardy ones) use as the raw material for photosynthesis and give back O_2 to replenish the depleted oxygen supply of the pond water and keep the conditions aerobic so that the aerobic bacteria can function continuously at top efficiency.

The algae also remove dissolved minerals like, C, N, P, K, Mg, Ca, Zn, Cu, Mo, B, Va, Co, Fe, Mn, Si, from the wastewater. They need these minerals for their growth. Algae can be harvested and sold as animal feed supplements. A third type of microbe, the facultative anaerobe is capable of growth in both aerobic and aerobic conditions and aids in decomposing waste in the transition zone between aerobic and anaerobic conditions. It is always desirable to keep aerobic conditions as the aerobic bacteria cause most complete oxidation of organic matter and is most effective in the treatment of dairy, textile, and other highly soluble organic wastes. However, anaerobic fermentation has proved effective for treating citrus, slaughterhouse, and certain paper mills wastewater.

$C_{11}H_{29}O_7N$ + 14 O_2 (Dissolved) + H^+ (Aerobic Bacteria) ⑦ 11 CO_2 + 13 H_2O + NH_4^+

CO_2 + H_2O + Sunlight (photosynthesis by algae)⑦ O_2 (dissolve in water) + $C(H)_2O$

Most modern lagoons have maximum water depth of 4 feet. Near neutral pH and adequate oxygen concentration is maintained even by mechanical aeration. BOD removals range from 10% to 60% and even 90%.

2. Trickling Filtration Process (TFP)

Trickling filters are biological filters made of crushed stones which are coated with 'slime growths' (zoogleal forms) from the bacteria in the wastes. Crushed stones (traprock, granite, and limestone), 3 to 5 inches in diameter usually form the surface materials in the filter with the smallest stone at the top. Since smaller stones provide more surface per unit of volume, the contact material must be small in order to support a large surface of active microbial biofilm, but not so small that its pores becomes clogged by accumulated suspended matters. Other materials such as plastic rings are also proving very effective. They are also light weight, chemically resistant and have high specific surface.

The microbial community in the filter includes aerobic and facultative bacteria, fungi, algae and the protozoans. The facultative bacteria are predominating. Important species are-

1. *Achromobacter,*
2. *Flavaobacterium,*
3. *Pseudomonas,* &
4. *Alcaligenes.*

Within the slime layers, where adverse conditions prevail with respect to growth, the filamentous forms like *Sphaerotilus natans* and *Beggiatoa* spp. are found. In the lower reaches of the filter, the nitrifying bacteria are found.

Among the fungal species, *Fusazium, Mucor, Pencillium, Geotrichum, Sporatichum* and yeasts are common. They also help in waste stabilization, but mainly under low pH conditions. Sometimes the fungal growth is so rapid that it clogs the filter and restricts ventilation.

Algae grow only in the upper reaches of the filter where sunlight is available . Important species are *Chlorella, Ulothrix,* and *Phormidium.* The protozoas in the filter are predominantly of the ciliate group. Important species are *Vorticella, Opercularia,* and *Epistylis.*

The bacterial growth on the filter biofilm adsorb and oxidize the dissolved and colloidal organic matter from the wastewater brought in contact with them. The adsorbed substances are attacked by bacteria and enzymes and reduced to simpler compounds so that ammonia (NH_3) is liberated and oxidized by chemical and bacterial means. Part of the oxygen is supplied by spraying wastewater, blowing air into the filter, or allowing wastewater to drip into the filter. When the rate of contact is very high, such as 10 to 30 mgad (million gallons / acre / day) and continuous, the humus collected on the filter-bed surfaces is sloughed off continuously. A flocculent (humus like residue or sludge) containing many protozoa and fungi, also accumulates on the surface.

Function of the protozoa is to feed on the biological film and as a result, effluent is clarified and turbidity decreases. This also help maintain the biofilm in a higher growth rate.

Most modern trickling filters vary in height from 5 to 10 meter and are filled with a plastic packing material for microbial biofilm attachment. The plastic packing material is designed such that about 90 to 95 % of the volume in the tower consist of void space in which air is circulated to provide oxygen to the microbes in the biofilm. Excess biomass sloughs from the attached growth periodically.

Trickling filters have been found to reduce *Salmonella* bacterium by 84-99 %, enteric virus by 40-60 %, and cysts of *Entamoeba histolytica* by 88–89 %.

3. Activated-Sludge Process (ASP)

It is very suitable for large industrial plants wastes. In this process biologically active mass of microorganisms like ciliated and flagellated protozoa and the oxidizing aerobic bacteria are created in the young sludge. Protozoa are present in very rich proportion as high as 50,000 cells / ml of sewage water. Bacterial flora is gram-negative. They adsorb organic matter from the wastewater and oxidize it by enzymatic reaction into simple products like CO_2, H_2O, NO_3 and SO_4. Biological slimes containing these organisms develop naturally in aerated organic wastes which contain a considerable portion of matter in the colloidal and suspended state. For efficient removal of dissolved organic solids there must be high flocculent sludge (containing the zoogleal masses) to provide ample contact surface for accelerated biological activities. The desired concentration of 'active flocculent sludge' is maintained by recirculating a specific volume (about 20 %) of the secondary settled young sludge. Old, heavy sludge tends to get mineralized and deficient in oxygen which reduces the activity of flocculent sludge. Higher sludge quantities lead to greater BOD removal and create a need for more aeration and food (organic matter) for oxidizing bacteria. The average time of contact of the wastewater with active flocculent sludge and aeration range from 6 to 24 hours for various industrial wastes.

BOD removals are usually over 90 % when the loading are below 0.3 pound of BOD per pound of suspended solids in the waste under aeration. For optimum activity, the kinetics of activated-sludge requires young flocculent sludge in the logarithmic stage of growth, continuous loading of the organisms, and elimination of the anaerobic conditions at any point during the treatment. Presence of toxic metals and non-biodegradable organic matters in the industrial wastewater, lack of nutrients required for biological oxidation of the aerobic bacteria, high temperature and high or low pH value can interfere in the activate-sludge treatment. They have to be taken into consideration. Moreover, activated-sludge has a tendency to bulk with concentrated organic wastes, and it is difficult to develop an activated sludge from a soluble waste.

4. Dispersed-Growth Aeration (DGA)

The bacteria for oxidizing the organic matter is present in the supernatant liquor after the wastes have been aerated and settled. Two major types of bacteria are found -slime-enmeshed, dispersed, short, thick, round-ended rods and the other *Sphaerotilus*-like organisms often unsheathed. 5 million bacteria per milliliter of the supernatant are often found. A portion of this supernatant liquor is retained for seeding incoming wastes, while the settled sludge from the secondary settling tank is digested. Dispersed-growth aeration does require more air to achieve the same BOD reduction as the activated-sludge process (ASP). Aeration periods to achieve the same BOD removal are also 24 hours as compared to 6 in ASP. DGA is better adapted than ASP to treat concentrated soluble organic wastes like streptomycin and penicillin wastes from pharmaceutical industries in which there is tendency to bulk in the ASP. The percentage of BOD reduction decreases as the strength of the penicillin and streptomycin wastes are increased, but up to 80 % BOD reduction may be expected with wastes up to 3000 mg/L of BOD. Greater BOD reductions are possible when the BOD is less than 1000 mg/L and the waste is aerated for 24 hours. Optimum results are obtained with air rates of 2 to 3 cubic feet per gallon per hour. Stronger wastes require higher aeration rates. DGA is very suitable for the treatment of rag and jute paper-mill wastes.

5. Rotating Biological Contactors (RBC)

The RBC has gained wide acceptance in industrial and municipal wastewater treatment. It consists of a series of flat, closely spaced, parallel, circular and cylindrical discs made of PVC (Polyvinyl Chloride) or PS (Polystyrene) that are partially immersed (typically 40 %) in the wastewater being treated and rotated through it. The disc rotate slowly at about 1.0 to 1.6 revolutions per minute. Biological slime (biofilm) containing active microbial biomass covers the surface of the discs and adsorbs and absorbs colloidal and dissolved organic matter present in the wastewater. As the wastewater continuously flows down through the discs, excess slime generated by synthesis of solid waste materials in the wastewater is sloughed off gradually into the mixed liquor and subsequently separated by settling.

The rotating discs carry a film of wastewater into the air as it rotate out from the wastewater, and allows 'aeration' in the atmosphere. During this time it absorbs the 'oxygen' necessary for aerobic biological activity of the slime. Thus the rotating bio-disc system provide the following facilities towards wastewater treatment-

(1) Mechanical support for a captive, microbial population;

(2) Good aeration and oxygenation to the aerobic microbes for complete oxidation of waste organic matter, the rates of which can be adjusted by changing the rotational speed of the discs;

(3) Intimate contact between the biological slime and the wastewater, the intensity of which can be varied by changing the rotational speed.

Use of closely spaced parallel discs in the RBC is able to achieve a 'high concentration of active biological surface area' with 'high concentration of actively oxidizing organisms'. This, coupled with the ability to achieve 'high aeration rate for oxidation' (by adjusting the rotational speed of discs), enables the RBC process to have tremendous advantage over others, and give effective treatment to highly concentrated industrial wastes. At a loading of 11 lb BOD / day / 1000 ft^2 of surface area, 90 % BOD removal is obtained in 2000 mg/L BOD dairy waste. Because a buoyant plastic material is used for the discs and negligible head loss is encountered through the RBC system itself, the energy requirement of the system is very low. However, under overloaded situations, anaerobic conditions develop deep in the attached biofilm. *Beggiatoa,* a filamentous anaerobic bacteria forms a tenacious whitish biofilm over the discs that does not slough off under the normal RBC rotational sheer conditions.

6. Flexible Fiber Biofilm Reactor (FFBR)

Yu & Williams (2003) at Griffith University, Brisbane, are trying to develop a new aerobic microbial technology for treatment of food processing wastewater called the 'Flexible Fiber Biofilm Reactor'. The packing media used in the reactor are 'rayon fibers'. Such fibers allows very high specific surface area for the attachment of aerobic decomposer microbes leading to longer cell residence time. It is claimed to be very efficient with high rate of performance generating higher quality of effluents and less sludge is formed as compared to other aerobic processes. The hydraulic retention time (HRT) where the decomposer microbes have to be in contact with the wastewater constituents is also much shorter than other aerobic systems.

CONCLUDING REMARKS

The overall objectives of the microbiological treatment of wastewater are to—

(1) Oxidize dissolved and particulate biodegradable organic matters into acceptable end products. (Removal of dissolved organic matter from wastewater is one of the most important, and also most difficult task. Biological methods have proved most effective since bacteria are adept at devouring biodegradable organic matters in waste.)

(2) Capture and incorporate suspended and non-settleable colloidal solids into biological floc or biofilm;

Because some of the constituents and compounds found in industrial wastewater is toxic to microorganisms, pretreatment may be required before subjecting to biological treatment. It is most applicable to treat wastewater that contains a relatively constant source of biochemical oxygen demand (BOD) and very low concentrations (on the order of 1 mg/L) of toxic metals.

Microbial treatment is very cost-effective for some industrial wastewater which have high organic loads of contaminants such as those from the food-processing industries, the paper and pulp industries, and the chemical manufacturing industries. The most important issue of consideration is that if pathogenic microbes are used for waste treatment, they must be rendered inactive before being introduced into the environment. This also applies to genetically engineered organisms. Such cells can be inactivated by steam sterilization or chemical treatment.

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9

MICROBIAL COMPOSTING OF SOLID WASTES

Development of An Innovative Waste Dumpsite Composting Facility by EBT : A Cheaper Alternative to the Costly Landfills for Developing Countries

KEY WORDS

Composting—An Aerobic Process; Microbial Degradation of Organic Waste; Windrows Composting; Aerated Static Pile Composting; In-Vessel Composting; Compost Pits; Microbial Slurry Technology for Waste Dumpsite Composting; Composting of Hazardous Wastes.

BACKGROUND INFORMATION

Haug (1993) described composting as the '*biological decomposition and stabilization of organic substrates, under conditions that allow development of thermophilic temperatures as a result of biologically produced heat, to produce a final product that is stable, free of pathogens and plant seeds, and can be beneficially applied to land*'. Composting is an ongoing biological degradation process (aided by soil microbes and earthworms) in nature in which several kinds of organic materials are converted from 'unstable product' (which is likely to decompose further, creating objectionable odors, generating greenhouse gas methane and producing environmental insanitation) to an increasingly more 'stable product' whose value is upgraded as nutritive materials for the soil and that can remain in the environment without creating any environmental problem.

But in nature, where the environmental conditions are largely 'uncontrolled' it is a 'slow' process catalyzed by naturally occurring microorganisms (decomposer bacteria, fungi and the actinomycetes) in the soil and air.

Under the 'uncontrolled' conditions of composting by rotting and decaying there is considerable emission of greenhouse gases like methane (CH_4) , carbon dioxide (CO_2) and the oxides of nitrogen (NO_x) and the hydrogen sulfide (H_2S) giving foul odor and polluting the environment.

Taking lessons from the nature, a 'conventional composting technology' was developed by the civilization 4000 years ago to decompose the 'farm wastes' (cattle dung and the crop residues which contain 100 % organic matter) under somewhat controlled environmental conditions (dumping the wastes into pits and covering them with soil) to be used back in the farm as 'compost' for maintaining soil fertility and crop production. The nutrients going out in the form of 'farm waste' is 'recycled' back into the farm in the form of 'compost'. The compost consists mainly of microbial cells, microbial skeleton, by-products of microbial decomposition and un-decomposed particles of organic and inorganic origin.

IMPROVED COMPOSTING TECHNOLOGY BY MODERN KNOWLEDGE IN MICROBIOLOGY & ENVIRONMENTAL BIOTECHNOLOGY

The conventional composting technology used mainly for the decomposition of farm waste has now been significantly improved with our modern scientific knowledge in 'microbiology' and 'environmental biotechnology' to 'biodegrade' all kinds of organic wastes including the 'municipal solid wastes (MSW) 'containing sufficient organic components under a completely controlled environmental conditions. MSW contain 70-80 % by weight of organic materials. Yard waste which is part of the MSW may contain even higher percentage of organic matter and certain industrial wastes such as those from the 'food processing', 'agricultural' and 'paper-pulp industries' are mostly organic in composition. Controlling the environmental conditions i.e. the biological, physical and the chemical factors can significantly improve and enhance the composting (decomposition/ biodegradation) process without the emission of greenhouse gases, foul odor and pollution of the environment and without the loss of essential nutrients from the compost.

ENVIRONMENTAL FACTORS CONTROLLING COMPOSTING

1. Biological Factors

Better availability of the decomposer microorganisms e.g. bacteria, fungi and actinomycetes greatly influence decomposition. Other 'natural decomposers' in nature are earthworms. Certain species of earthworms e.g. *Perionyx excavatus, Eudrilus euginae* and *Elsinia fetida* have been found to be voracious waste eaters and decomposers. The Australian worm called 'tiger worms' are also voracious waste eaters. The earthworms contains millions of 'decomposer microbes' in their gut which perform the function. (Details has been discussed in Chapter 11)

Typical microbes involved in composting are following—

TABLE 1

Bacteria	Actinomycetes	Fungi
Aerobacter	*Nocardia brasiliensis*	*Rhizopus nigricans*
Bacillus megatheruin	*Thermomonospora viridis*	*R. arrhizus*
B. stearothermophilus	*T. curvata*	*Rhizoctonia spp.*
B. cereus	*Micromonospora parva*	*Geotrichum candidum*
B. mycoides	*M. vulgaris*	*Muco pusillus*
Pseudomonad spp.	*Thermomoactinomyces vulgaris*	*Mucor racemosus*
Flavobacterium spp.	*Actinoplanes spp.*	*Pencillium digitatum*
Sarcina spp.	*Pseudonocardia*	*Aspergillus tamarii*
Cellumonas folia	*Streptomyces violaaceoruber*	*Torulopsis spp.*
Chondrococcus exiguus	*S. thermoviolaceus*	*Absidia (ramosa)*
Mycococcus virescens	*S. rectus*	*Saccharomyces spp.*
M. fulvus	*S. thermofuscus*	*Pulluloria spp.*
Thibacillus thiooxidans	*S. thermovulgaris*	*Pythium spp.*
T. dentrificans	*Thermomonospora fusca*	*Hanisenula spp.*
Proteus spp.	*T. glaucus*	*Trichodermia koningi*
Micrococcus spp.	*Thermopolyspor polyspora*	*Aspergillus flavus*
		Fusarium moniliforme
Protozoa		*Penicillium duponti*
Chilmonas (Paramecium)		*Stysanus stemonitis*
Cyathomonas (Truncata)		*Glibotrys alaboviridis*
Lycogala epidendrum		*Humicola griseus*
Cercomonas (Crassicandra)		*H. insolens*
		Lypomyces spp.
Algae		*Absidis orchidis*
Hormidium (nitens)		*Candida parapsilosis*
Vaucheria (terrestris)		*Cladosporium herbarum*
Euglena mutabilis		*Rhodotorula rubra*
Protococcus vulgaris		*Zygorhynchus vuilleminii*
Dactylococcus (bicandatus)		*Trichosporon cutaneum*
Chlorococcum humicola		*Verticillium spp.*
Microcoleus vaginatus		*Synecephalastrum spp.*
Porphyridium (cruentum)		*Pichia spp.*
Kentrosphaera spp.		*Cyclindrocaron spp.*
		Chaetomium (thermophile)
		Sporotrichium hermophile
		Lenitinus conatus

Source : Various Publications of UNEP and WWF (1992-2002)

There are reports about mushroom fungi (*Lentinus conatus*) isolated from the deadwood of cashew completely degrading the cardboard sheets within a week. They can also degrade coir waste, paddy straw, sugarcane trash and baggase. The mushrooms are known to produce 'lignocellulolytic' enzymes to degrade cellulose and lignin. Biotechnological treatment and composting of 'liginocellulosic wastes' which is hard to be degraded by other microbes, might become economically viable and environmentally sustainable technology.

L. contatus exhibited very high lignocellulytic enzyme production even up to 50 (+0) C whereas all other mushroom fungi only produce up to 35 (+0) C. Drastic reduction in C/N ratio, amount of liginocellulose, and increased levels of macro and micro nutrients were recorded in paddy straw coir waste, sugarcane trash & baggase, a month after composting with *L .conatus*. Soil application of coir waste composted by *L.conatus* at 15.5 to 18.5 ton/ha and urea at 60 kg N/ha reduced the intensity of 'sheet blight disease' of rice and increased the yield significantly (Lakshmanan & Bommaraju, 2003).

2. Physical Factors

(i) Particle Size & Porosity : The particle size of the material being biodegraded is critical because decomposition begins at the surface of the waste particles. Generally, the smaller particles have a more surface area per unit of weight and therefore allow more microbial activity, leading to rapid decomposition. But if all particles pack so closely so as to allow very little air to circulate it would retard composting. The particle size should be such that there is enough surface area for rapid microbial activity and also enough porosity (void space) for air to circulate for microbial respiration.

(ii) Temperature : Temperature is single most important factor to consider when establishing a compost pile. There are two phases :

(1) Mesophilic Phase- initial rise of temperature from ambient to 45 ·C; and

(2) Thermophilic Phase- the continued rise of temperature to 70 ·C or higher. Microbial activity is best under an optimum range of temperature which is 50·C - 60·C. This is best for composting. At least a temperature of 55 ·C must be maintained for a period of no less than 5 days to destroy all the pathogenic organisms. Normally there is no need to provide external source of heat as the microbial activity generates sufficient heat during decomposition process. The heat is also retained in the compost pile as the moisture absorb heat and the air layer in the void spaces acts as 'insulator'.

3. Chemical Factors

(i) Availability of carbon as the source of energy : The decomposer microorganisms rely on the carbon skeleton of the organic matter for their energy. Carbon is oxidized to yield carbon dioxide (CO_2), water and humic byproducts. Some of the carbon is consumed by the microbes to form new microbial cells thereby multiplying in population. The humic byproducts resulting from the metabolic activities of one generation/type of microorganisms could be used as a food/energy source by another generation/type of microorganisms. This chain of succession of different types of microbes continues until there is little decomposable matter in the waste.

(ii) Availability of nutrients-nitrogen, phosphorus, potassium and trace elements: Microorganisms absorb nitrogen to help them use protein efficiently. Nitrogen is a 'critical factor' for composting because it is primarily lacking in some municipal solid waste (MSW).

> The ratio of carbon to nitrogen (C/N) is considered critical in determining the rate of decomposition. Generally 25 parts carbon to 1 part nitrogen by weight (C/N=25:1) is considered ideal for rapid decomposition and composting. When the C/N ratio is too high, (meaning insufficient nitrogen) it slows decomposition. This is because new microbial cells (population of new decomposer microbes) are not formed at sufficient rate in the absence of nitrogen. Nitrogen is essential for 'protein synthesis' which are building blocks of new microbial cells. When the ratio is low (meaning too much nitrogen), it leads to odor problem. As the decomposition proceeds and more and more carbon is lost to the atmosphere as CO_2, this ratio narrows. Typically the C/N ratio for the MSW range from 40 to 100 and yard wastes 20 to 80 as they have more carbon. Woody materials, dry leaves, straw, paper and garden mulch have high carbon values. In general, coarse, dried out materials contain little nitrogen, while green garden and kitchen wastes and cattle dung contains high levels of nitrogen. Hence proper blending of carbon and nitrogen containing waste materials is essential for composting.

(iii) Availability of moisture : Moisture is also a critical factor in composting. It helps in the biochemical reaction and also retain heat. Moisture content of 50-60 % of total weight of waste is considered to be ideal for composting. Moisture may be lost from the composting pile by biological oxidation, microbial consumption and also by simple evaporation. Moisture content below 40 % dramatically decrease microbial activity. Moisture should be just enough to prevent free flow of water by gravity and form leachate. Excessive moisture above 60 % also impedes 'oxygen transfer' from the voids containing air to the microbial cells creating 'anaerobic conditions' leading to 'rotting' and not real composting.

> It is of crucial importance that the composting mass is 'not compacted' so as to maintain sufficient 'air spaces' and the waste material must never be excessively moist.

(iv) Availability of oxygen :

> Composting is generally considered as an 'aerobic'(oxygen requiring) process. While decomposition do occur under 'anaerobic' (lack of oxygen) conditions too, it is very slow process (rotting and decay) and also release obnoxious gases with foul odor and the greenhouse gases methane (CH_4). Although a 5 % - 15 % oxygen concentration is considered adequate, higher concentration of oxygen will not have any negative effect.

(v) pH Value : pH value of the waste components is another critical factor in composting as it affects the nutrient availability to the microorganisms, the solubility of heavy metals and the overall metabolic activity of the microorganisms. A pH value of 6 - 8 is considered ideal for composting. Organic materials are naturally well buffered relative to pH changes. The organic nature of the waste materials being composted does not allow rapid down swings in pH during composting. But as composting progress, a slightly lower pH can be expected in the final products because the process produces carbon dioxide which when combined with water produces carbonic acid and lower the pH.

(vi) Presence of toxic chemicals in waste : Presence of toxic chemicals in the waste materials can seriously impede composting as they can kill the microbes. It is not unusual for some household hazardous chemicals like disinfectants and insecticides, used torch cells etc. to get mixed up with MSW. Some species of bacteria, fungi and the earthworms are, however, adapted to tolerate some toxic chemicals up to certain concentration.

METHODS OF COMPOSTING OF ORGANICS FROM THE MUNICIPAL SOLID WASTE (MSW)

Several methods of composting of organic wastes from the MSW has now been developed through technological innovations. They are—

1. Windrows Composting
2. Aerated Static Pile Composting
3. In-Vessel Composting Systems.
4. Composting Pits, &
5. Aerated Composting Bins

The first three are commercial composting methods while the other two can be practiced by every individual in homes and institutions in their backyards and the farmers in their farmyards.

1. Windrows Composting

Windrows are constructed typically 6 to 7 feet high and 14 to 16 feet wide at the base. The organic waste shredded and screened to approximately 1 to 3 inches and the moisture content is adjusted to 50 to 60 %. This shredded material is placed in the windrows and a temperature at or slightly above 55 ·C is maintained. Periodical turning and tearing of waste to facilitate constant 'aeration' of the waste pile is of crucial importance. Complete composting is achieved in 3 to 4 weeks time, within which the waste is turned up at least twice per week. Compost is allowed to cure for another 3 to 4 weeks without turning. During this time, the residual decomposable organic materials are further decomposed by fungi and actinomycetes.

2. Aerated Static Pile Composting

It is a modification of the windrows in which the mechanical turning of the waste pile (for aeration) is replaced by 'forced-aeration system'. This is done by mechanical pulling (negative pressure) or pushing (positive pressure) of air through the windrow. It was developed by the U.S. Department of Agriculture at Beltsville, Maryland. It is called as the 'Beltsville Process'. Originally developed for the aerobic composting of wastewater sludge, it can be used to compost a wide variety of organic wastes including hazardous waste. It involves embedding 'perforated ducts' in a bottom layer of wood chips or compost over which the organic waste is stacked. Typical pile heights are about 7 to 8 feet and a layer of screened compost is often placed on top of the newly formed pile for insulation and odor control. Each pile is provided with an individual blower to introduce air (oxygen) and control temperature within the pile. Composting is done for 3 to 4 weeks and is then cured for another 4 weeks.

3. In-Vessel Composting Systems

The system uses a vessel which can be vertical tower, horizontal rectangular and circular tanks, and circular rotating tanks which works as a 'reactor'. Mechanical systems are designed to mix the composting materials, control air-flow, oxygen concentrations and temperature and minimize odor. Basically, all in-vessel aeration systems are based upon agitation of the particles, or upon forced aeration. The detention time for in-vessel composting varies from 1 to 2 weeks, but there is a 4 to 12 weeks curing period after composting. In-vessel system has gained popularity in recent years because of faster throughput, odor control, lower labor cost, and smaller area requirements.

4. Compost Pits

This is most economical way to compost garden wastes such as grass clippings, coarse prunnings, herbs and seaweeds in homes, and the farm wastes such as crop residues, rejected / dropped fruits & vegetables, straws and cattle dung in the agriculture & horticulture farms. Depending upon the availability of land, a pit of 2 - 5 cum size is excavated in the earth preferably under a tree or shaded area (to avoid direct sun or rainfall) and the base is filled with loose organic soil. This forms the starting 'inoculums' of bio-degrader microbes (bacteria, actinomycetes & fungi). Every teaspoon of organic soil is reported to contain over millions of microbes. A compost pit of 1 cum size would take several months to fill (for a family of 2-3 persons) as the volume of composted waste goes on reducing significantly over the period creating more space for filling. Nearly 70 % of the waste biomass is lost into the environment (as organic gases) upon biodegradation and 30 % residual material with microbial skeleton is achieved as fine granulated brown to black compost.

> The pit is regularly filled with alternate layers of 'nitrogen-rich' waste materials (e.g. grass clippings, prunnings and herbs) with 'carbon-rich' waste materials (e.g. dry leaves, paper, sawdust and straw) to maintain a proper carbon / nitrogen (C/N) ratio. The layers are made 5-10 cm deep and organic soil is sprinkled between the layers to maintain a good population of degrader microbes. If the soil is mixed with previously composted materials, it forms better inoculums as compost is richer source of degrader microbes. The top layers are regularly turned with fork to improve aeration and add oxygen for the aerobic microbes. Water is also added regularly to keep the pile 'moist' but NOT 'wet'.

Kitchen wastes (especially the meat products & citrus peels), weed & bulbs, branches, roots and rose cuttings are avoided in pits. It takes about 8-10 weeks before the waste materials are degraded into fine, crumbly mix with earthly smell.

Compost pits can also be used for 'vermicomposting' by use of waste eater earthworms. Such pits have to be necessarily in a cool place and under shade with higher in moisture content, as the worms are very sensitive to heat. Vermicompost pits do not require frequent turning as the worms keep the waste pile aerated by burrowing actions. (More about vermicomposting has been described in Chapter 11).

5. Aerated Compost Bins

As awareness about composting of kitchen and garden wastes is growing, compost bins of various sizes and shapes with adequate provisions for aeration from side walls, open top with cover lid, and convenient to operate (by simply removing lid and filling waste), are available in market. Several compost bins have now been patented by companies. There are provisions to collect the excessive moisture at the bottom and can be used as 'liquid fertilizer'. The composted waste materials which settles down at bottom can be extracted out at intervals through the small windows in the walls.

Correct alternate layers of 'nitrogen rich waste' & 'carbon rich waste' (as in the compost pits) and sprinkling organic soil (as source of microbial inoculums) in between the layers as above is made here too. All kitchen wastes such as the cooked & uncooked food scraps (except the meat products) and the garden wastes (grass clippings & dry leaves) can be conveniently composted in aerated compost bins. Kitchen waste works as a rich source of 'nitrogen'. Turning is not required because of adequate provisions for aeration. However, constant turning of waste biomass, at least once or twice a week is desirable for faster degradation and composting. It takes about 8-10 weeks before compost can be started to be extracted from bottom windows.

MICROBIAL-SLURRY TECHNOLOGY FOR MSW COMPOSTING AT WASTE DUMP-SITE : THE INDIAN INNOVATION OF EXCEL INDUSTRIES, MUMBAI

An indigenous technology to compost the unseggregated municipal solid waste (MSW) biomass directly on the waste dump site (tip) and reuse the same dumping ground again and again after excavating the biodegraded mass (compost) from the ground was developed in India in the 1990's and is giving excellent results for managing the solid wastes generated by the community along with the recovery of a valuable resource in the form of compost. The non-biodegradable materials in the unseggregated wastes which do not decompose are finally separated mechanically and from them are retrieved some recyclable components for recycling. The balance residue in the form of inert materials is safely disposed in the adjacent land-fill site.

> The technology was developed by Ms. Excel Industries, Bombay, India. The Bombay Municipal Corporation is participating in the project by providing a part of dumpyard land on lease and required delivery of the garbage onto this waste dumpsite. The BMC takes 25% royalty on the sale of the compost which is marketed in the trade name of CELRICH Bio-Organic Soil Enricher. It is receiving very good response from the farmers of India. The largest plant with an installed capacity to bioprocess 500 tones /day of MSW is now operating in Bombay.

The Bioconversion Process and the Composting Mechanism

(1) The dump site is leveled and either cemented or paved with bricks on the bottom to prevent leachate and for easy movement of waste carrying vehicles. Long windrows, about 5 meters wide and 2-3 meters high (deep) are erected and the municipal solid waste is then stacked and heaped in the windrows.

(2) A 'microbial consortium' slurry containing active decomposer bacteria and enzymes are then added to the windrows to initiate rapid aerobic decomposition of waste biomass. The slurry is spread on the surface of the garbage and inside the heaps in the windrows with help of probes, so that it reaches deep and in every pocket of the heap.

(3) The microbial culture is known as 'Celrich Substrate DF BC-01. It is prepared after analyzing the composition of the waste and identifying the predominant materials such as celluloses, hemicelluloses, lignins, proteins and fats etc. The microbes produce enzymes such as cellulase, lipase, amylase, protease, pectinase and phospholipase to breakdown the long chain complexes of the substrates.

(4) About 1 kg of the consortium in the colloidal emulsion form is mixed with 20 litres of water to be used for spraying on about 3 cubic meter of solid waste and for one ton of waste 200 litres of water is needed. The water used can be recycled wastewater and not necessarily fresh water.

(5) The waste heaps are turned around once in 7 to 10 days for proper aeration and the inoculant slurry is sprayed in each turning to enhance decomposition and maintain the proper moisture level which is usually 45 - 55 %.

(6) The process is 'exothermic' and the windrows reach a temperature of 70·-75·C within 24-36 hours, killing the harmful pathogens and repelling all birds, stray animals, flies and mosquitos from the dump site.

(7) The entire process of aerobic decomposition of garbage is completed within 4-6 weeks and as the decomposition is complete the temperature comes down to normal.

(8) The decomposed waste biomass is then passed through rotary and vibratory screens to sieve the compost (which may be 25-30 % of the raw waste on dry weight basis) from the stones, plastics and other inert materials. The recyclable components from this left-over materials is separated and the rest are disposed off as rejects in the adjacent land-fill.

Advantages of the Microbial-Slurry Technology

(1) Such technology is truly sustainable and establish the concept of 'circular metabolism' of human ecosystems for development of sustainable urban ecosystems. Nutrients obtained from the farms (agricultural ecosystems) in the form of food for the city dwellers is returned back to the farms in the form of fertilizers. Thus both the human ecosystems, the urban as well as the agricultural ecosystems, complements and sustains each other.

(2) No prior segregation of waste is required. Segregation of wastes at source or before composting imposes the biggest obstacle in any composting technology because it is highly labour intensive job to segregate the often sticky biodegradable (compostable) matters from the non-biodegradable ones. It becomes much easier after composting. The soft decomposed powdery materials gets easily separated from the plastics, stones and pebbles.

(3) It recovers over 90 % of the organic matter. Recovery of compost depends upon the presence of organic matter in the garbage. About 20-25 % are recyclable materials and the rest about 20-25 % are inert materials which are safely disposed in adjacent land-fills.

(4) The same waste dump site can be reused after excavating the decomposed waste biomass and no additional land is needed for making new dump sites (tips).

(5) The need of actual 'land-fills' are also greatly reduced because very little is left to be disposed off after retrieving the compost and the recyclable materials.

(6) The problem of emission of tip gases such as methane, ammonia and hydrogen sulfide and of leachating and discharge of effluents is greatly reduced.

(7) The foul odor of the tip also disappear within 2-3 days of sanitization by the microbial culture giving great relief to the waste workers and the local residents.

(8) Microbial culture of active decomposer bacteria can be prepared from the 'sewage sludge' which contains active decomposer bacteria in millions/gram of the sludge.

(9) The technology can suit to all countries and all the existing waste 'tips' can be converted into composting plants and used to create windrows. The technology is also flexible for 150-700 MT of waste per day.

(10) There will be greater recovery of compost in developed countries as much higher amount of organic wastes reaches the dump sites in every city.

(11) The process can recycle all organic wastes from the households, restaurants and hotels, dairy, agriculture and agro-processing industries, brewing industries and slaughter houses .

(12) The compost produced is dark brown humus, rich in NKP, trace elements and contain active nitrogen fixing and phosphate solublising bacteria. Because of this and the high moisture retaining capacity (about 170 %) the compost will be good for dry land agriculture and very useful to ameliorate the poor soils.

TABLE 2

Environmental Cost-Benefit Analysis of the Microbial—Slurry Composting Technology

1.	City Population (In millions)	2	1.2	0.8	0.4
2.	Plant Capacity (MT/day)	700	500	300	150
3.	Land Required (In Hectare)	12	8	5	3
4.	Power Connection (KVA)	250	175	100	50
5.	Water Required (Kl/day)	280	200	120	60
6.	Manpower Required	70	60	40	20
7.	Waste Use (MT/YR)	2,55,500	1,82,500	1,09,500	54,000
8.	Compost Production (MT/YR)	63,000	45,000	24,000	11,000
1.	Capital Cost (AU $ in millions)	2.7	2.22	1.48	0.74
2.	Compost Value(AU $ in millions)	3.26	2.33	1.24	0.57

COMPOSTING OF ORGANIC HAZARDOUS WASTES : REDUCING TOXICITY AND RETRIEVING RESOURCES

Principles and methods involved in composting of organic hazardous wastes are the same as those in the composting of organic wastes of the MSW. However, the microbiology of HW-composting differs from that of MSW composting in the use of composting organisms (inoculum), at least initially. Certain types of organic hazardous waste molecules can be degraded by only one or a very few microbial species which may not be widely distributed or abundant in nature. In the absence of natural inoculum, 'microbial enrichment technique' is adopted to proliferate the required species in the waste substrate. This is done through either raising 'microbial culture' or by introducing material that would be likely to contain the desired organisms. Garden loam, composted sewage sludge and manures are excellent such material. In the ongoing composting process, 10–20% recirculation of the end product (compost) would be an ideal inoculum.

Factors Affecting Hazardous Waste Composting

1. Oxygen Availability : Aerobic condition is an essential factor for composting. However, an anaerobic phase is essential for successful composting of some hazardous waste like 'halogenated organic pesticides' and 'hydrocarbons'.

2. Porosity of Waste Material : It is very important that waste remains porous throughout the composting period. It ensures constant availability of oxygen in the waste interstices. A bulking agent like saw dust, paper or even municipal solid waste (MSW) is added to make the hazardous waste porous.

3. Particle Size of Waste Materials : The particle size of the material being biodegraded is critical because decomposition begins at the surface of the waste particles. Generally, the smaller particles have a more surface area per unit of weight and therefore allow more microbial activity leading to rapid decomposition. But if all particles pack so closely so as to allow very little air to circulate it would retard composting. The particle size should be such that there is enough surface area for rapid microbial activity and also enough void space for air to circulate for microbial respiration. For optimum results the size of waste should be between 25 and 75 mm.

4. Temperature : Microbial activity is best under an optimum range of temperature which is 50·C-55·C. If temperature goes beyond 66 ·C, biological activity is reduced significantly.

5. Moisture Content : Moisture content of 45-55% of total weight of waste is considered to be ideal for composting. Moisture content below 40% dramatically decrease microbial activity Moisture should be just enough to prevent free flow of water by gravity and form leachate. Excessive moisture above 60% also impedes 'oxygen transfer'.

6. Nutrients : Carbon, nitrogen, phosphorus, and an assortment of other elements are essential for composting. Nitrogen plays a critical role in composting. Inasmuch as most hazardous organic wastes are made of 'hydrocarbons', nitrogen probably is deficient. To increase nitrogen contents of the HW , organic wastes richer in nitrogen such as cattle dung or raw sewage sludge is added.

Significance of Hazardous Waste Composting

(1) The most significant use of composting of hazardous waste is in the disposal of 'biodegradable pesticides' and solid wastes that have been contaminated with organic hazardous materials. Liquid HW cannot be composted unless they are converted into a solid form through concentration or through absorption by a bulking agent. The use of bulking agent like saw dust has made composting a very promising means of disposing oily wastes.

(2) Operational and capital costs of composting are much lower than those for incineration of hazardous wastes, and the environmental impact is also much less than incineration.

(3) Composting of HW is also safer than 'landfarming' in terms of impact on land, water and air. Retention time is shorter and land requirements are far less.

(4) A combination of physical and chemical factors generated in composting (pH and temperature) hastens the destruction of less persistent pesticides such as 'malathion' and 'carbaryls'.

EMISSION OF GREENHOUSE GASES DURING AEROBIC COMPOSTING OF FOOD WASTE ?

Biodegradation of organic waste has long been known to generate methane (CH_4). Studies have also indicated high emissions of nitrous oxide (N_2O) in proportion to the amount of food waste used, and methane (CH_4) is also emitted if the composting piles contain cattle manure.

> N_2O emission is relatively high at the beginning of the composting process but declines after two days. N_2O is mainly formed under moderate oxygen (O_2) concentration. High emission of N_2O at the beginning might be due to the metabolism of the microbial community coming from food waste, as food waste have been found to generate N_2O even stored at 4^0 C. These 'aboriginal' microorganisms may survive at the beginning when the temperature is relatively low. With the increase of temperature, these microorganisms either perish or are outnumbered by the thermophilic bacterial species. These results, however, suggests the existence of anaerobic micro-site inside the waste particles even though aeration and aerobic conditions was employed during the composting process. (Yaowu et. al., 2000).

Emission of greenhouse gases is significantly decreased when composting is done by earthworms (More is discussed in Chapter 11).

CONCLUDING REMARKS

Everything from the kitchen wastes including the egg shells (but not the meat products) and the vacuum cleaner dust, the garden wastes (lawn clippings & dry leaves) and the farm wastes (cattle dung, straws & crop residues) can be composted into valuable fertilizer in a most convenient way & in a completely odorless environment. The best part is that it diverts massive organic wastes (a potential resource) from ending up in the landfills thus reducing the economic burden on the municipal councils. The compost can be used as an ideal soil conditioner and nutrient supplier for the farms, gardens and parklands.

In the eroded landscapes of world, which is prone to suffer from massive land degradation, composting is one of the keys strategies to maintain proper soil health in both rural and urban situations. Compost improves soil structure, improves biological activities and increases water holding capacity in soils which is much needed in the world today as the farmlands are becoming both physiologically (due to depleting soil moisture) and biologically (due to depleting biological organisms) dry in the wake of high-tech mechanical and chemical agriculture which heralded the green revolution. This is being referred to as the 'rape of the soil' to augment food productivity.

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SECTION-III

VERMICULTURE BIOTECHNOLOGY

Rediscovering the Environmental, Social and Economic Virtues of the Wonder Worms—Earthworms (Charles Darwin's Unheralded Soldier's of Mankind) Through Researches into Environmental Biotechnology

A revolution is unfolding in vermiculture studies (rearing of earthworms) for sustainable waste and land management, soil decontamination & fertility improvement, and for sustainable agriculture & organic farming. Earthworms body contains 65% protein (70-80 % high quality 'lysine rich protein' on a dry weight basis). They multiply very rapidly. Studies indicate that they double their number at least every 60 days. Given the optimal conditions of moisture, temperature and feeding materials earthworms can multiply by 2^8 i.e. 256 worms every 6 months from a single individual. Each of the 256 worms multiplies in the same proportion to produce a huge biomass of worms in a short time. The total life-cycle of the worms is about 220 days. They produce 300-400 young ones within this life period.

Charles Darwin (1881) called the earthworms as *'unheralded soldiers of mankind'*, *'natural ploughman'* and *'friend of farmer's'* working day and night for him in his farm soil. It is interesting to note that the incorporation of earthworms in soil was recommended by Surapala, the author of *Vrikshayurveda*, as early as in the 10th century AD for obtaining high quality pomegranate fruits (Verse 131). Presence of earthworms in soil is a 'biological indicator' of good soil fertility. One acre of fertile land may contain more than 50,000 earthworms of diverse species. Interest in vermiculture technology for sustainable agriculture grew in the recent times and several scientists have studied the impact of vermiculture on cereal, vegetable and fruit crops.

Earthworms have 600 million years of experience as waste and environmental managers managing bio-waste including human waste, soil and land on earth. Aristotle called them as *'intestine of earth'* meaning that they can digest a wide variety of waste materials from earth. About 4,400 different species of earthworms have been identified, and quite a few of them are versatile waste eaters and bio-degraders and feed on variety of organic wastes. Composting by earthworms is proving to be environmentally preferred process over the normal microbial composting and much more over the landfills as it is rapid and nearly odorless process, can reduce composting time significantly and above all, there is no emission of 'greenhouse gas methane' which plagues both these solid waste management options.

Earthworms body work as a 'biofilter' and they have been found to remove the BOD_5 by over 90%, COD by 80-90%, total dissolved solids (TDS) by 90-92% and the total suspended solids (TSS) by 90-95% from wastewater by the general mechanism of 'ingestion' and biodegradation. The vermi-filtered water is clean enough & also nutritive to be reused for farm irrigation and in parks and gardens. Many developing nations who cannot afford to construct and maintain costly Sewage Treatment Plants (STP's) can go for this low-cost option.

Earthworms in general, are blessed with high resistant power to several chemical contaminants in soil including the heavy metals and organic pollutants and have been reported to bio-accumulate them in specially created tissues by nature called 'chloragogen cells'. Vermiremediation biotechnology using earthworms is emerging as a low-cost and convenient technology for combating soil and land pollution. A 'wasteland' can be transformed into 'wonderland' by earthworms.

Vermiculture - vermi-composting, vermifiltration & vermiremediation, are all self-promoted, self-regulated, self-improved & self-enhanced, low or no-energy requiring zero-waste technology, easy to construct, operate and maintain. It excels all 'bio-conversion' technologies by the fact that it can utilize organics that otherwise cannot be utilized by others. It excels all 'bio-treatment' technologies because it achieves greater utilization than the rate of destruction achieved by other technologies. It involves about 100-1000 times higher 'value addition' than other biological technologies.

Vermiculture industry has grown considerably in recent years all over the world. For poor nations including in India, it is becoming a source of livelihood for the poor in rural areas, especially the women, and helping in poverty eradication programs.

10

VERMICULTURE FOR SUSTAINABLE AGRICULTURE & SAFE FOOD

Reviving the Traditional Plant Growth Promoting Roles of Earthworms in Farming Practices Through Researches into EBT

KEY WORDS

Earthworms Secrete Plant Growth Hormones; Earthworms Proliferate N_2 Fixing Bacteria; Earthworms Induce Biological Resistance in Plants & Reduce Pesticide Use; Earthworms Removes Soil Pollutants, Reduce Soil Salinity & Improves Fertility.

BACKGROUND INFORMATION

Presence of earthworms in soil is a 'biological indicator' of soil fertility. One acre of fertile land may contain more than 50, 000 earthworms of diverse species. Earthworms are natural biological agents that affect soil fragmentation, aeration, bring about soil turn and dispersion, digest organic matter, graze microflora and even reduce *Salmonella* bacteria to about 30 per cent. Earthworm activity is so prolific that, on average, 12 tonnes/ha/year soil or organic matter is ingested by this population, leading to upturning of 18 tons of soil/year, and world over at this rate it may mean a 2 inch humus layer over the globe (Bhawalkar & Bhawalkar, 1993).

Charles Darwin (1881) called the earthworms as 'unheralded soldiers of mankind', 'natural ploughman' and 'friend of farmer's' working day and night for him in his farm soil. They are continually ploughing and also manuring the soil in the farmland. The habit of burrowing and swallowing earth below the surface increases the fertility of the soil in many ways. The burrow permits the penetration of air and moisture into deeper layer of soil, make the soil porous, improve drainage and make easier the downward growth of the root. Earthworms also increase percolation of water into farm soil several times greater and simultaneously decrease surface run-off and reduce soil erosion.

It is interesting to note that the incorporation of earthworms in soil was recommended by Surapala, the author of *Vrikshayurveda,* as early as in the 10^{th} century AD for obtaining high quality pomegranate fruits (Verse 131) (Sadhale, 1996).

VERMICULTURE & REARING OF EARTHWORMS : THE BACKBONE OF SUSTAINABLE AGRICULTURE

Interest in vermiculture technology for sustainable agriculture grew in the recent times and several scientists have studied the impact of vermiculture on cereal, vegetable and fruit crops. Earthworms act as aerators, crushers, mixers, grinders, chemical degraders and biological stimulators. Earthworms harbour millions of nitrogen fixing bacteria in their gut. They help in renewing soil fertility and in soil regeneration, and also secrete valuable growth promoting substances for crop plants.

Palainsamy (1996) indicated that in the tropics earthworms improve the growth and yield of wheat grown with wormcasts. According to him, fertilizing soils with worms can increase crop yield by more than 40 per cent. Gunathilagraj (1996) noted that the association between crop plant and the earthworms induced significant variation among the crop plants. He reported that small doses of NPK fertilizers and earthworms + cowdung + mulch significantly increased the chlorophyll protein, potassium, iron, manganese and zinc contents in the field crops.

Bhawalkar and Bhawalkar (1993) experimented that an earthworm population of 0.2 - 1.0 million per hectare can be established within a short period of three months. This is the only key to a quick change over to sustainable agriculture without loss of crop yield.

LIVE EARTHWORMS IN SOIL : INDUCE BIOLOGICAL RESISTANCE IN PLANTS AND WORK AS RENEWABLE SOURCE OF NKP

Earthworms in soil has long been recognized as the farmer's friend and the bioindicator of soil fertility. They harbour millions of nitrogen fixing bacteria in their gut. They help in renewing soil fertility and in soil regeneration and also secrete valuable growth promoting substances for plants.

Earthworms swallow large amount of soil everyday, grind them in their gizzard and digest them in their intestine with aid of enzymes. Only 5-10 percent of the chemically digested and ingested material is absorbed into the body and the rest is excreted out in the form of fine mucus coated granular aggregates called 'vermicastings' which are rich in NKP (nitrates, phosphates and potash), micronutrients and beneficial soil microbes including the 'nitrogen fixers' and 'mycorrhizal fungus'. The organic matter in the soil undergo 'humification' in the worm intestine in which the large organic particles are converted into a complex amorphous colloid containing 'phenolic' materials. About one-fourth of the organic matter is converted into humus. The colloidal humus acts as 'slow release biofertilizer' in the soil.

In one of his works done at Indira Gandhi Center for Human Ecology at University of Rajasthan, Jaipur India, in 1998, the author found that those potted wheat plants which had rich population of 'live earthworms' in soil exhibited miraculous growth as compared to the control plants and also those grown on vermicompost or even on chemical fertilizer.

Presence of rich population of earthworms in farm soil have been found to reduce the incidence of pest attack in crops. This was also found by the author in his experiments in potted plants at University of Rajasthan, Jaipur, India (1988–1999). This gives reasons to believe that live earthworms population around plant roots induce 'biological resistance' in plants.

Earthworms Produce More Nutritive Vermi-compost Rich in Minerals, Plant Growth Regulators, Nitrogen Fixing Bacteria and Mycorrhizal Fungi

Earthworms contain millions of nitrogen fixing microbes in their guts and pass out microbes and contribute soil nutrients in the form of 'vermicasts'. They engineer the growth of beneficial decomposer bacteria, actinomycetes and 'mycorrhizal fungi', in the soil and can contribute between 20 to 40 kg nitrogen/ha/year in soil, in addition to other mineral nutrients and plant growth regulators, and increase soil fertility and plant growth by 30-200 % (Das & Patra, 1979). Excretion of droppings i.e. 'vermicastings', carry ammonia, nitrates, nitrogen, phosphorus, magnesium and other micronutrients and nitrogen fixing beneficial microbes. These have potential of generating NPK equal to 10 million tonnes annually in India as 1,000 tonnes of waste organic matter can be degraded to 300 tonnes of vermicompost (Bhawalkar & Bhawalkar, 1993).

Vermi-compost (end product of worm activities) is a nutritive plant food rich in NKP (1.16 % nitrogen, 1.34 % potassium and 1.22 % phosphorus) and micronutrients. They are plant-available nutrients. Earthworms also secrete polysaccharides, proteins and other nitrogenous compounds from their body. Most important is that they mineralize the nitrogen (N) in the organic waste to make it bio-available to plants as nutrients (nitrates). They ingest nitrogen from waste and excrete it in the mineral form as ammonium and muco-proteins. Patil (1993) found that earthworm recycle nitrogen in the soil in very short time. The quantity of nitrogen recycled is significant ranging from 20 to 200 kg N/ha/year.

The nitrogenous waste excreted by the nephridia of the worms is plant-available as it is mostly urea and ammonia. The ammonium in the soil is bio-transformed into nitrates. The mycorrhizal fungi encouraged by the earthworms transfer phosphorus by increasing solubilisation of mineral phosphate by the enzyme phosphatase.

Earthworms vermicasts contain enzymes like amylase, lipase, cellulase and chitinase, which continue to break down organic matter in the soil (to release the nutrients and make it available to the plant roots) even after they have been excreted. (Chaoui *et al.,* 2003).

TABLE 1

Nutrient value of vermicasts produced by earthworms in soil (in %)

Nutrients	(%)	Nutrients	(%)
1. N	7.37	6. Mg	0.38
2. P_2O_5	19.58	7. Zn	0.16
3. P	0.37	8. Cu	0.025
4. C	4.5	9. Fe	0.38
5. K	0.4		

Electrical Conductivity (EC) of Vermicast = 28.14%

Source : Radha Kale (1991).

TABLE 2

Comparative study of nutrient value of cattle dung compost and vermicompost

	Nutrient	*Cattle Dung Compost*	*Vermicompost*
1.	N	0.4-1.0	2.5-3.0
2.	P	0.4-0.8	1.8-2.9
3.	K	0.8-1.2	1.4-2.0

Source : Ph.D. Thesis, (Sunita Agrawal, 1999).

Earthworms Secrete Plant Growth Hormones

Neilson (1965) and Tomati *et al.* (1985) reported about biological substances like 'gibbrellins', 'cytokinins', and 'auxins' released by the earthworms. Kale and Bano (1986) also found that the worm cast significantly influence the vegetative growth of plants. It can promote massive flowering in ornamental plants. Earthworms and their vermicompost can even 'induce flowering' in those plants which fails to flower.

Earthworms Reduces Pesticide Use and Improves Nutritional Value

Presence of rich population of earthworms in farm soil have been found to reduce the incidence of pest attack in crops. Vermicompost produced by earthworms have been reported to contain some antibiotics and actinomycetes which help in increasing the power of biological resistance among the crop plants against pest and diseases. The actinomycetes fungus excreted by the earthworms in their vermicast produce chemicals that kill parasitic fungi such as *Pythium* and *Fusarium*. Studies have shown that use of vermicompost in crops inhibited the soil-born fungal diseases.

> Farmer in Sangli district of Maharashtra, India, grew grapes on eroded wastelands and applied vermicasting @ 5 tonnes/hectare. Cow dung and agricultural wastes were used as mulch. The grape harvest was normal with improvement in quality, taste and shelf life. Pesticide spray was reduced by 75 per cent. Soil analysis showed that within one year pH came down from 8.3 to 6.9 and the value of potash increased from 62.5 kg/ha to 800 kg/ha. There was also marked improvement in the nutritional quality of the grape fruits (Bhawalkar & Bhawalkar, 1993).

Earthworms Remove Soil Pollutants & Reduce Plant Parasites & Soil Nematodes

Due to the 'antibacterial' activity of 'coelomic fluid' the earthworms eradicates the soil pathogens and reduce plant parasites and soil nematodes by 30 %. As they have the capacity to store heavy metals and pesticides in their tissues, they can detoxify the polluted soils which is a growing problem these days with heavy use of agrochemicals (Ireland, 1986). Hence earthworms significantly contribute as soil conditioner to improve the physical, chemical as well as the biological properties of the soil and its nutritive value. (More has been discussed in Chapter 14).

Earthworms Reduce Soil Salinity

Earthworms not only help renew the natural soil fertility but also improves the soil pH and reduce 'soil salinity'.

> Farmers at Phaltan in Satara district of Maharashtra, India, applied vermiculture on his sugarcane crop grown on saline soils irrigated by saline ground water. The yield was 125 tonnes/hectare of sugarcane and there was marked improvement in soil chemistry. Within a year there was 37 % more nitrogen, 66 % more phosphates and 10 % more potash. The chloride content was less by 46 %.

Vermiwash (Residual Water from Earthworms Vermicomposting) Spray Can Prevent Pest and Disease in Crops

In a startling revelation in December 2006 the farmers near Pusa Agriculture University in Bihar, India, said that the 'vermiwash' (residual water collecting at the bottom during vermicomposting of farm waste) when sprayed over tomato and brinjal plants effectively controlled all incidences of pests and diseases and it completely eliminated the use of chemical pesticides. The plants were healthy and bore bigger fruits with unique shine over it and were sold in the market very quickly. These farmers were using vermicompost in all their crops since last 5 years completely giving up the use of chemical fertilizers. (Personal Communication of Author with Farmers, December 22, 2006, Bihar, India).

EXPERIMENTAL STUDIES OF VERMICULTURE ON POTTED WHEAT CROPS (PH.D STUDIES UNDER AUTHOR, UNIVERSITY OF RAJASTHAN, JAIPUR, INDIA, 1992-1999)

1. Experimental Study on Potted Wheat Crops

Bhatia (1998) studied the impact of 'live earthworms' on potted wheat crops at University of Rajasthan, Jaipur. Wheat seeds (*Triticum aestivum* Linn. No. Raj. 3077) and earthworms (*Elsenia foetida*) were obtained from Rajasthan Agricultural Research Institute, Jaipur. Three identical sets of pots with ten replicates of each were prepared from uniform soil of the same stock, which was assumed to be near neutral, i.e., without any manure. The first set of ten pots were kept as a control; to the second set of ten pots chemical fertilizers (104 gms urea, 0.2 gms potash and 0.75 gms single super phosphate) were added; to the third set of ten pots 50 earthworms were added to each pot and 250 gms of one week old cow-dung was added as feed material for the earthworms.

Vermicompost (20 gms) was also added to the soil of these pots of third set as a starting nutrient. After an interval of 15 days, 100 gms each of cow-dung and vegetable matter (kitchen wastes) were added to the third set of pots as additional feed for the earthworms. Ten seeds were sown in each pot (10 x 10 = 100 in ten pots) after mixing the soil with the respective fertilizers (Urea was added in two identical doses, one at the time of sowing and another 21 days).

The following parameters were studied :

- Percentage of seed germination
- Colour and texture of leaves
- Shoot and root length
- Dry weight of fruiting ear
- Internodal distance
- Health of seedlings
- Leaf length and chlorophyll content
- Length of fruiting ear
- Number of seed grains per ear
- Maturation and harvesting time

Findings and results

The earthworms + cow-dung fed wheat crops made good progress from the very beginning of seed germination up to maturation. Out of 100 seeds (in 10 pots), 90 germinated in the earthworms + cow-dung fed pots, 60 in the chemical fertilizer treated pots and 50 in the control.

> The earthworms + cow-dung fed seedlings and plants were the healthiest and greenest. The leaves were greener and broader, the shoots were thicker, node and internode were distinct and fruiting ears were much broader and longer. The number of seed grains counted per ear in the earthworms + cow-dung fed wheat plants were on average 31, in the case of chemical fertilizer fed plants 20, and in the control plants 12. In terms of seed numbers, root and shoot length, fruiting ear length and dry weight, the earthworms + cow-dung fed wheat plants made distinct progress over the other wheat plants. The growth of the wheat plants grown in soil containing live earthworms was several times better as compared to those grown in soil with chemical fertilizers and those without any input (Control).

TABLE 3

Comparative Study of the Impact of Chemical Fertilizers and Earthworms on Growth and Yield of Potted Wheat Crops (*Triticum aestivum* Linn.)

Parameters	Control	Soil with Chemical Fertilizers	Soil Containing Live Earthworms & Cow dung (as feed material)
(1)	(2)	(3)	(4)
(1) Percentage of seed germination	50	60	90
(2) Root length (cm)	7.13	9.32	16.46
(3) Shoot length (cm)	22.1	25.2	59.99
(4) Ear length (cm)	4.82	5.45	8.77
(5) Total height of plant (cm)	34.16	39.97	85.22

Contd. ...

Contd. ...

(1)	(2)	(3)	(4)
(6) Leaf length (cm)	12.73	14.19	26.37
(7) Dry weight of ears (gm)	0.135	0.171	0.466
(8) Number of seed grains per ear	11.8	19.9	31.1
(9) Chlorophyll content (mg/l)	0.783	1.947	3.486
(10) Number of tillers per plant	1	1-2	2-3

Source : Bhatia (1998).

2. Experimental Study on Potted Vegetable Crops

Sunita Agrawal (1999) studied the agronomic effects of vermiculture on brinjal *(Solanum melongena)* and okra *(Abelmoschus esculentus)* plants at University of Rajasthan, Jaipur. The study was made on potted vegetable crops with 5 replicas of each. In the first set of brinjal plants live earthworms were added; in the second set vermicompost was added; in the third set chemical fertilizers were added. The fourth set was kept as 'control' and no input was added. Tested seeds and seedlings were obtained from the horticulture department. 5 seeds of okra and 5 seedlings of brinjal were grown in each pot. Mixed species of earthworms consisting of *Elsinia foetida, Perionyx excavatus* and *Eudrilus euginae* were obtained from Rajasthan Agriculture Research Institute, Jaipur. 50 live earthworms were added in the first set of 5 pots with one week old cattle dung (250 gms) as feed material. Vermicompost was prepared indigenously by degrading kitchen wastes. 250 gms of vermicompost was used in each okra pots while 300 gms in brinjal pots. Chemical fertilizers were used in the form of urea (1.40 gm), S.S.P. (2.50 gm) and M.O.P. (1.04 gm) in each set.

Findings and Results

Findings were very exciting and the results were encouraging. Both vegetable crops performed exceedingly well in the presence of 'live earthworms' around their root zones. It made excellent impact on fruiting which is the main objective of any farmer. Vermicompost also sustained good growth and development and were still better over the chemical fertilizers. One more significant finding was the less incidence of pest and disease attack, better taste of fruits, flower colour and aroma of brinjal and okra crops grown on vermiculture.

TABLE 4

Agronomic Impact of Live Earthworms, Vermicompost and Chemical Fertilizer on Vegetative Growth and Fruit Development in Brinjal *(Solanum melongena)*

Treatment	Vegetative Growth in inches	Av. No. of Fruits/ Plant	Av. Wt. of Fruits/ Plant	Total No. of Fruits	Max. Wt. of One Fruit
(1)	(2)	(3)	(4)	(5)	(6)
1. Live Earthworms	28	20	675 gms	100	900 gms
2. Vermicompost	23	15	525 gms	75	700 gms

Contd. ...

Contd. ...

(1)	(2)	(3)	(4)	(5)	(6)
3. Chemical Fertilizer	18	14	500 gms	70	625 gms
4. Control	16	10	425 gms	50	550 gms

(N.B. Value of vegetative growth is taken which was achieved on the 90th day of the study, while the fruitings were estimated from the 45th day and ending with over 120 days).

Source : Ph.D Thesis (Sunita Agrawal, 1999).

TABLE 5

Agronomic impact of Live Earthworms, Vermicompost and Chemical Fertilizer on Vegetative Growth and Fruit Development in Okra *(Abelmoschus esculentus)*

Treatment	Vegetative Growth in inches	Av. No. of Fruits/ Plant	Av. Wt. of Fruits/ Plant	Total No. of Fruits	Max. Wt. of One Fruit
(1)	(2)	(3)	(4)	(5)	(6)
1. Live Earthworms	39.4	45	48 gms	225	70 gms
2. Vermicompost	29.6	36	42 gms	180	62 gms
3. Chemical Fertilizer	29.1	24	40 gms	125	48 gms
4. Control	25.6	22	32 gms	110	43 gms

(N.B. Value of vegetative growth is taken which was achieved on the 90th day of the study, while the fruitings were estimated after 45th day and ending with over 120 days.).

Source : Ph.D Thesis (Sunita Agrawal, 1999).

3. Experimental Study on Potted and Farm Wheat Crops

Reena Sharma (2001) studied the agronomic effects of vermiculture on the potted and field wheat crops (*Triticum aestivum*) at University of Rajasthan, Jaipur.The worms used were *Elsinia foetida, Perionyx excavatus* and *Eudrilus euginae.* The study was conducted in a farmland in Jaipur. The farm was divided into eight plots of 25 x 25 m^2 size. Three treatments were prepared -

(1) *Vermicompost* : In the pots 30 gms of vermicompost (VC) was used while in the field it was used @ 2.5 tonnes/ha.

(2) *NPK chemical fertilizer* : In the form of urea, single super phosphate, murete of potash, in three reducing doses for the pots and one for field; and

(3) *Live earthworms* : 50 live earthworms were used in each pot, while 1000 worms were released in the field plot of 25 x 25 sq.mt.

The fourth farm plot was kept as control. All the treatments were replicated twice. The wheat seed (Raj. 3077) was grown @ 100 kg/ha. Irrigation schedule was maintained as recommended for wheat. Chemical nitrogen (urea) was applied in two split doses (first half at the time of sowing and second half dose after 21 days of sowing) whereas the phosphate and potash fertilizers were applied as single dose at the time of sowing.

Findings and Results

The results were very encouraging. Growth of wheat crop performed well in all the treatments (vermicompost, live earthworms and chemical fertilizers (NPK)) over the control. In the pot experiments the best growth performance in respect of root length, shoot length, ear length and weight, weight of 1000 grains were observed in treatments where a combination of reduced dose (3/4) of chemical fertilizer (NPK-90:75:60) and vermicompost (VC 30 gms) were applied. However, the most significant finding was that both vermicompost and live earthworms positively influenced the total yield of the grains which is 19.2 and 19.1 grains/ear respectively .

TABLE 6

Agronomic Impact of Live Earthworms, Vermicompost and Chemical Fertilizers on Potted Wheat Crops

Treatments	Shoot Length	Ear Length	Root Length	Wt. of 1000 grains	Grains/Ear
1. Vermicompost	41.11	7.65	15.5	33.38	19.2
2. Live Earthworms (50 Nos.)	39.27	6.74	8.35	32.41	19.1
3. NPK (120:100:80) + VC	42.14	7.67	16.35	30.2	17.1
4. NPK (90:75:60) + VC	47.89	8.91	20.2	40.58	18.67
5. NPK (60:50:40) + VC	45.96	8.59	18.3	39.29	18.91
6. Control	37.01	5.83	7.18	27.28	15.4

Source : Ph.D Thesis (Reena Sharma, 2001). (VC= Vermicompost)

In the farm experiment the highest value was achieved where reduced doses (3/4) of chemical fertilizer (NPK-90 : 75 : 60) were applied with full dose of vermicompost (@ 2.5 tons/ha). But again, the total yield of the grain (grain/ear) obtained on exclusive use of vermicompost as well as the ear length and the weight of 1,000 grains in the field were very close and comparable to those obtained from the use of chemical fertilizers and vermicompost combined.

TABLE 7

Agronomic Impact of Live Earthworms, Vermicompost & Chemical Fertilizers on Farm Wheat Crops

Treatments	Shoot Length	Ear Length	Root Length	Wt. of 1000 grains	Grains/Ear
1. Vermicompost	83.7	13.14	23.5	39.28	32.5
2. Earthworms (1000 Nos.)	67.83	9.85	18.4	36.42	30.0
3. NPK (90:75:60) + VC	88.05	13.82	29.7	48.02	34.4
4. Control	59.79	8.91	12.11	34.16	27.7

Source : Ph.D Thesis (Reena Sharma, 2001).

Findings of the Complete Experimental Study on Agronomic Impact of Vermiculture

(1) In the pot experiments made by Bhatia (1998), Agarwal (1999) and Sharma (2001), vermiculture (especially the use of live earthworms in the pot soil) gave excellent results in both cereal as well as in the vegetable crops. It did not, however, perform well in the farm conditions which is actually needed for the farmers.

(2) In the farm, a judicious combination of chemical fertilizer + vermicompost, gave better results than the use of exclusive live earthworms and vermicompost.

(3) A 'transition period' is actually required in the farm as the 'nutrient hungry soil' has become 'addict' of chemical fertilizers and is ecologically degraded. Its 'natural fertility' is completely lost. Sustained application of live earthworms + vermicompost in the farm crops with increasingly reduced doses of chemical fertilizers (only as helping hand), over a period of time, would slowly improve the ecological status (biological health) of the soil and restore its 'natural fertility'.

(4) Vermicompost, besides providing NPK and micro-nutrients also work as a 'soil conditioner'. It also increases the organic carbon of the soil. It can help in reclamation of degraded croplands.

(5) Vermiculture can at least significantly reduce the use of 'economically and environmentally costly' chemical fertilizers in agriculture. It can be reduced even up to half the normal recommended dose for wheat farming. A correct combination of chemical fertilizer (as helping hand) and earthworms + vermicompost (as driving force) has to be identified through further experimentation in farm condition, which can bring greater sustainability in agriculture.

(6) In desert and drought prone areas vermicompost use can increase the moisture retention capacity of soils and assure food production ever under 20-30 per cent less rainfall.

(7) The farm produce after using vermiculture gives better taste, colour, aroma, appearance, nutrition and substantially improved shelf/storage life.

(8) Acceptability factor plays a crucial role in the introduction of any non-conventional farm input. The farmers have to be educated and supported with the development of infrastructure for vermiculture. This will be possible only when vermiculture is popularized by the agriculture department and become integral part of farm policy in India.

CONCLUDING REMARKS

Earthworms in soil has long been recognized as the farmer's friend and the bioindicator of soil fertility. They help in renewing soil fertility and in soil regeneration and also secrete valuable growth promoting substances for plants. With the heavy use of agro-chemicals these natural biofertilizers of earth soil has disappeared in several parts of world. Vermiculture is being promoted all over the world for mass production of live earthworms to be released into biologically depleted soils and restore their fertility.

Vermiculture biotechnology involves about 100-1000 times higher 'value addition' than other biological technologies can do in the natural ecosystems and the farm ecosystems. Obtaining earthworms from vermiculture farms would be one-time cost in vermiculture biotechnology as the earthworms multiply rapidly creating huge army of worms which carry out the job of soil enrichment and fertility improvement.

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11

VERMICOMPOSTING OF SOLID WASTES BY EARTHWORMS

A Rapid & Odourless Process While Reducing the Greenhouse Gas Emissions of Conventional Aerobic Composting by Microbes: A Significant Achievement of Researches into EBT

KEY WORDS

Rapid, Odor-Free Process; Reduction of Greenhouse Gas Emissions; Stimulation of Microbial Population for Enhanced Waste Degradation; Chemical & Pathogen Free Nutritive Bio-fertilizer; Vermi-composting of Coal Power Plants and Mining Wastes; Earthworm Biomass - Nutritive Feed for Fishery & Poultry Industry.

BACKGROUND INFORMATION

Millions of tons of municipal solid wastes (MSW) generated from the modern society are ending up in the landfills everyday, creating extraordinary economic and environmental problems for the local government to manage and monitor them (may be up to 30 years) for environmental safety (emission of greenhouse and toxic gases). Construction of engineered landfills incurs 20-25 million US dollars before the first load of waste is dumped.

A revolution is unfolding in vermiculture studies (rearing of earthworms) for efficient and sustainable waste and land management. It virtually creates 'wealth' from the 'waste' and converts the barren 'wasteland' into fertile 'wonderland'. (Martin, 1976; Satchell, 1983; Fraser-Quick, 2002).

Vermiculture and vermi-composting is a self-promoted, self-regulated, self-improved & self-enhanced, low or no-energy requiring zero-waste technology, easy to construct, operate and maintain. It excels all 'bio-conversion' technologies by the fact that it can utilize organics that otherwise cannot be utilized by others. It excels all 'bio-treatment' technologies because it achieves greater utilization than the rate of destruction achieved by other technologies. It involves about 100-1000 times higher 'value addition' than other biological technologies. (Appeholf, 1997).

The best part is that vermicomposting by worms decrease the proportion of 'anaerobic to aerobic decomposition', resulting in a significant decrease in the greenhouse gas methane (CH_4) which plagues the conventional composting methods by microbes.

EARTHWORMS : THE GREAT WASTE MANAGERS ON EARTH WITH 600 MILLION YEARS OF EXPERIENCE OF WASTE MANAGEMENT

Earthworms have 600 million years of experience as waste and environmental managers managing bio-waste including human waste, soil and land on earth. Aristotle called them as *'intestine of earth'* meaning that they can digest a wide variety of waste materials from earth. About 4,400 different species of earthworms have been identified, and quite a few of them are versatile waste eaters and bio-degraders and feed on variety of organic wastes. It promotes the growth of 'beneficial decomposer bacteria' in waste biomass and acts as an aerator, grinder, crusher, chemical degrader and a biological stimulator. (Dash, 1978; Sinha *et al.*, 2002).

All organic wastes by its very nature (chemical composition) is bound to disintegrate anaerobically in environment and generate greenhouse gas methane (CH_4). Only if they are allowed to degrade completely under aerobic conditions (which is readily facilitated by earthworms) that this can be prevented.

Earthworms feeds partly on the waste, but mostly on the microorganisms produced during decomposition. Study indicates that given the optimum conditions of temperature and moisture, about 5 kg of worms (numbering approximately 10,000) can vermiprocess 1 ton of waste into vermi-compost in just 30 days (ARRPET, 2005). Upon vermi-composting the volume of solid waste is significantly reduced from 1 cum (70-80 % moisture) to 0.5 cum of vermi-compost (about 30 % moisture).

Vermicomposting is saving over 13,000 cum of landfill space every year in Australia. (Komarowski, 2001). Some city councils in Australia are committed to 'no landfills' by 2008 and plan to achieve this target by vermi-composting the entire organic wastes of the residents.

EARTHWORMS : THE MIRACLE OF NATURE

Ecology

Earthworms are burrowing animals and form tunnels by literally eating their way through the soil. The distribution of earthworms in soil depends on factors like soil moisture, availability of organic matter and pH of the soil. They occur in diverse habitats specially those which are dark and moist. Organic materials like humus, cattle dung and kitchen wastes are highly attractive sites for some species. Earthworms are generally absent or rare in soil with a very coarse texture and high clay content or soil with pH < 4 (Gunathilagraj, 1996). Earthworms are very sensitive to touch, light and dryness. As worms breathe through their skin proper ventilation of air in soil medium is necessary. Water logging in the soil can cause them to come to the surface. Worms can tolerate a temperature range between 5· C to 29· C. A temperature of 20 · C to 25· C and a moisture of 60-75 % is optimum for earthworm function (Hand, 1988).

Biology

Earthworms are long, narrow, cylindrical, bilaterally symmetrical, segmented animals without bones. The body is dark brown, glistening and covered with delicate cuticle. They weigh over 1400-1500 mg after 8-10 weeks. On an average, 2000 worms weigh 1 kg and one million worms weigh approximately 1 ton. (ARRPET, 2005). Usually the life span of an earthworm is about 3 to 7 years depending upon the type of species and the ecological situation. Earthworms harbor millions of 'nitrogen-fixing' and 'decomposer microbes' in their gut. Their body contains 65 % protein (70-80 % high quality 'lysine rich protein' on a dry weight basis), 14 % fats, 14 % carbohydrates and 3 % ash.

Enormous Rate of Multiplication

Earthworms multiply very rapidly. They are bisexual animals and cross-fertilization occurs as a rule. After copulation the clitellum (a prominent band) of each worm eject lemon-shaped 'cocoon' where sperms enter to fertilize the eggs. Up to 3 cocoons per worm per week are produced. From each cocoon about 10-12 tiny worms emerge. Studies indicate that they double their number at least every 60 days. Given the optimal conditions of moisture, temperature and feeding materials earthworms can multiply by 2^8 i.e. 256 worms every 6 months from a single individual. Each of the 256 worms multiplies in the same proportion to produce a huge biomass of worms in a short time. The total life-cycle of the worms is about 220 days. They produce 300-400 young ones within this life period. (Hand, 1988).

A mature adult can attain reproductive capability within 8–12 weeks of hatching from the cocoon. Red worms takes only 4-6 weeks to become sexually mature. (ARREPT, 2005). Earthworms continue to grow throughout their life and the number of segments continuously proliferates from a growing zone just in front of the anus.

VERMICULTURE & VERMI-COMPOSTING : A GLOBAL MOVEMENT

Vermiculture movement was started in the middle of 20th century and the first serious experiments were established in Holland in 1970, and subsequently in England, and Canada. Later vermiculture were followed in USA, Italy, Philippines, Thailand, China, Korea, Japan, Brazil, France, Australia and Israel (Edward,1988; Riggle & Holmes, 1994). In UK large, 1000 mt vermi-composting plants have been erected in Wales (Frederickson, 2000). The American Earthworm Technology Company started a 'vermi-composting farm' in 1978-79 with 500 t /month of vermicompost production (Edward, 2000). Collier (1978) reported on the management of sewage sludge and effluents from intensively housed livestock by vermiculture in USA. Japan imported 3000 mt of earthworms from the USA during the period 1985-87 for cellulose waste degradation (Kale,1998).

The Aoka Sangyo Co. Ltd., has three 1000 t /month plants processing waste from paper pulp and the food industry (Kale,1998). This produces 400 ton of vermicompost and 10 ton of live earthworms per month. The Toyhira Seiden Kogyo Co. of Japan is using rice straw, municipal sludge, sawdust and paper waste for vermicomposting involving 20 plants which in total produce 2-3 thousands tons per month (Edward, 2000). In Italy vermiculture is used to biodegrade municipal and paper mill sludge. Aerobic and anaerobic sludge are mixed and aerated for more than 15 days and in 5000 cum of sludge 5 kg of earthworms are added. In about 8 months the sludge is converted into vermicompost (Ceccanti and Masciandaro, 1999). In France, 20 tons of mixed household wastes are being vermi-composted everyday using 1000 to 2000 million red worms (*Elsenia andrei*) in earthworm tanks. (ARRPET, 2005). Rideau Regional Hospital in Ontario, Canada, vermi-compost 375 kg of wet organics mainly food waste everyday. The worm feed is prepared by mixing shredded newspaper with the food waste. (ARRPET, 2005). In Wilson, North Carolina, U.S., more than 5 tons of pig manure (excreta) is being vermi-composted every week. (NCSU, 1997).

Vermiculture Movement in India : Providing Livelihood to the Poor and Helping in Eradication of Poverty

Bhawalker Earthworm Research Institute (BERI) in Pune, is most pioneer organization involved in vermiculture (vermicomposting of solid wastes and vermifiltration of sewage) in India since the 1970s.

> Almost all agricultural universities in India are now involved in vermiculture and a movement is going across the sub-continent especially involving the poor rural women with dual objectives of 'making wealth from waste' while cleaning the environment. Earthworms have enhanced the lives of poor. In several Indian villages NGO's are freely distributing cement tanks and 1000 worms and encouraging women to collect waste from villages, vermicompost and sell to the farmers. It has become good source of livelihood for many. (White, 1994; Hati, 2001).

In Tamil Nadu, at Periyar College of Technology for Women vermi-composting is being done on commercial scale in windrows. 350 surface level beds are made 3m (length) x 1m (width) x 0.5m (height) on a 5 acre land. Each bed has capacity to contain 1 ton of waste. The bed is prepared by filling alternate layers of waste and cow dung of 150-200 mm thickness. About 5 kg of earthworms (*E.fetida*) are introduced in each bed. 1 kg of worms has about 2000 adult worms in numbers. To ensure aerobic conditions periodical turning of the waste is done manually. First turning is given 4 to 7 days after filling and second after 5 to 10 more days. Water is sprinkled over the bed to ensure enough moisture. Complete decomposition occurs in about 30 days after which the degraded materials are excavated and sieved. About 30-40 % of the waste is recovered as vermi-compost while the earthworms grows in biomass to about 6 kg in these 30 days. The compost is sold to the farmers and part of the revenue earned is used in maintenance of the wormery. (ARRPET, 2005).

Vermiculture Movement in Australia : Reducing the Need of Landfills

Vermiculture is being practised and propagated on large scale in Australia as a part of the 'Urban Agriculture Development Program' which utilizes the urban wastes.

Australia's 'Worm Grower Association' is the largest in world with more than 1200 members.(RIRDC, 2003). The Sydney Waters in New South Wales has set up a vermiculture plant of 40 million worms to degrade up to 200 ton of urban wastes a week. Sydney's St. George Hospital is setting up plant to biodegrade its kitchen waste and fertilise its hospital gardens.

The Gayndah Shire Council in Queensland, Australia, is vermi-composting over 600 tons of organic waste into valuable organic fertilizer (vermi-compost) and selling to the local farmers. This fertilizer is completely non-toxic and odour free. They claim for 'NO LANDFILLS' by 2008. Other rural council in QLD Murgon Shire has also mounted a successful vermi-composting program. Redland Shire in QLD also did vermi-composting of biosolids with the aid of Sydney based company Vermitech Pty. Ltd. in 1997. The Hobart City Council in Tasmania, vermi-compost about 66 cum of biosolids every week, along with green mulch into 44 cum of vermicompost diverting them from landfills. (Datar *et al.*, 1997). Komarowski (2001), reports that Australia is saving over 13,000 cum of landfill space every year by vermicomposting.

Important works on vermiculture and its significance in environmental management is going on at Murdoch University, WA, University of Western Sydney, NSW, Southern Cross University, NSW, University of Queensland, Brisbane, and at the School of Environmental Engineering, Griffith University, Brisbane, QLD (under the supervision of author).

CATEGORIES OF ORGANIC WASTE SUITABLE FOR VERMICOMPOSTING BY EARTHWORMS

Earthworms can physically handle a wide variety of organic wastes from both municipal (domestic and commercial) and industrial (livestock, food processing and paper industries) streams. (Roe, 2002). Earthworms are highly adaptable to different types of organic wastes (even of industrial origin), provided, the physical structure, pH and the salt concentrations are not above the tolerance level. (Graff, 1981). Another matter of considerable significance is that the earthworms also partially 'detoxify' (by bio-accumulating any heavy metals and toxic chemicals) and 'disinfect' (by devouring on pathogens and killing them by anti-bacterial coelomic fluid) the waste biomass while degrading them into vermi-compost which is nearly sterile and odorless. (Pierre *et al.*, 1982; UNSW-ROU, 2002).

Municipal Organic Wastes

1. The food waste from homes (all raw and cooked kitchen waste- fruit and vegetable peels and cuts) and fast-food outlets and restaurants (preferably vegetable products & also egg shells);
2. The garden (yard) wastes (dry leaves and dry grass clippings) from homes and parks constitute an excellent feed stock for vermi-composting;
3. The sewage sludge from the municipal wastewater also provide a good feedstock for the worms. The worms digest the sludge and convert a good part of it into vermi-compost;

4. Paunch waste materials (gut contents of slaughtered ruminants) from abattoir also make good feedstock for earthworms.

Agriculture and Animal Husbandry Wastes

1. Farm wastes such as crop residues, dry leaves & grasses;
2. Livestock rearing waste such as cattle dung, pig and chicken excreta makes excellent feedstock for earthworms;

Some Industrial Organic Wastes Suitable for Vermi-composting

Solid waste including the 'wastewater sludge' from paper pulp and cardboard industry, brewery and distillery, sericulture industry, vegetable oil factory, potato and corn chips manufacturing industry, sugarcane industry, aromatic oil extraction industry, logging and carpentry industry offers excellent feed material for vermi-composting by earthworms. (Kale, *et al.*, 1993; Seenappa & Kale, 1993; Seenappa *et al.*,1995; Kale & Sunitha, 1995; Lakshmi & Vijayalakshmi, 2000; ARRPET, 2005.)

(a) Coal-Power Plant Waste : Saxena *et al*,. (1998) found that the 'fly-ash' from the coal power plants which is considered as a hazardous waste posing serious disposal problem due to heavy metal contents (of course it is also rich in nitrogen and microbial biomass) can be vermi-composted by earthworms. They found that 25 % of fly-ash mixed with sisal green pulp, parthenium and green grass cuttings formed excellent feed for *Elsenia fetida* and the vermi-compost was higher in NKP contents than other commercial manures. The earthworms ingest the heavy metals from the fly-ash while converting them into vermi-compost.

(b) Mining Waste : Kale and Sunitha (1995) found that the waste from the mining industry which contains sulfur residues and creates disposal problems can also be fed to the worms mixed with organic matter. Optimum mixing ratio of the sulfur waste residues to the organic matter was 4%.

Waste Materials Preferably to be Avoided for Vermi-composting

1. Heavily salted products unless soaked in water for 24 hours;
2. Excess citrus (orange & lemon peels and crushes) and onions wastes (might reduce pH and impair worm activity);
3. Feces of pets (may carry viral or bacterial toxins);
4. Fresh green grass (creates high temperature and can harm worms);
5. All non-biodegradable wastes;
6. Meat and dairy products and slaughterhouse waste are to be avoided in the initial stage till the number of earthworms become high enough in the composting bed (it may invite 'maggots' and also create bad odor for sometimes).

EARTHWORM SPECIES SUITABLE FOR VERMI-COMPOSTING

Long-term researches into vermiculture have indicated that the Tiger Worm *(Elsenia fetida)*, Red Tiger Worm (*E. andrei*), the Indian Blue Worm *(Perionyx excavatus)*,the African Night Crawler *(Eudrilus euginae)*,and the Red Worm *(Lumbricus rubellus)* are best suited for vermi-composting of variety of organic wastes under all climatic conditions. *E. fetida* and *E. andrei* are closely related. Our study has indicated that *E. fetida* is most versatile waste eater and degrader and an army of the above 5 species combined together works meticulously. These species are common to India and Australia, both nations being biogeographically very close. (Sinha, *et al.*, 2002).

MECHANISM OF WORM ACTION IN VERMI-COMPOSTING

Earthworms are versatile waste eaters and decomposers. It promotes the growth of 'beneficial decomposer bacteria' in waste and acts as an aerator, grinder, crusher, chemical degrader and a biological stimulator. (Sinha, et. al,. 2002). Most earthworms consume, at the best, half their body weight of organics in the waste in a day. *Elsenia fetida* is reported to consume organic matter at the rate equal to their body weight every day. (ARRPET, 2005). Vermicomposting involves following actions of worms :

(1) The food ingested is finely ground (with the aid of stones) into small particles to a size of 2-4 microns in their muscular gizzard and passed on to the intestine for enzymatic actions. The gizzard and the intestine work as a 'bioreactor';

(2) The worms secrete enzymes proteases, lipases, amylases, cellulases and chitinases in their gizzard and intestine which bring about rapid biochemical conversion of the cellulosic and the proteinaceous materials in the waste organics. Earthworms convert cellulose into its food value faster than proteins and other carbohydrates. They ingest the cellulose, pass it through its intestine, adjust the pH of the digested (degraded) materials, cull the unwanted microorganisms, and then deposit the processed cellulosic materials mixed with minerals and microbes as aggregates called 'vermicasts' in the soil. (Dash, 1978).

(3) Only 5-10 percent of the chemically digested and ingested material is absorbed into the body and the rest is excreted out in the form of fine mucus coated granular aggregates—the 'vermicasts' rich in nitrates, phosphates and potash.

(4) The final process in vermi-processing and degradation of organic matter is the 'humification' in which the large organic particles are converted into a complex amorphous colloid containing 'phenolic' materials. Only about one-fourth of the organic matter is converted into humus. The colloidal humus acts as 'slow release fertilizer'. (ARRPET, 2005).

Earthworms Reinforce Microbial Population and Act Synergistically Promoting Rapid Waste Degradation

Earthworms further stimulate and accelerate microbial degradation activities by increasing the population of aerobic decomposer microorganisms in the waste biomass and this they do by improving aeration by burrowing actions (Dash, 1978; Binet *et al.*, 1998). They hosts millions of decomposer (biodegrader) microbes in their gut (as they devour on them) and excrete them in soil along with nutrients nitrogen (N) and phosphorus (P) in their excreta (Singleton *et al.*, 2003). The nutrients N & P are further used by the microbes for multiplication and vigorous action.

Edward and Fletcher (1988) showed that the number of bacteria and 'actinomycetes' contained in the ingested material increased up to 1000 fold while passing through the gut. A population of worms numbering about 15,000 will in turn foster a microbial population of billions of millions. (Morgan & Burrows, 1982).

Singleton *et al.* (2003) studied the bacterial flora associated with the intestine and vermicasts of the earthworms and found species like *Pseudomonas, Mucor, Paenibacillus, Azoarcus, Burkholderia, Spiroplasm, Acaligenes,* and *Acidobacterium* which has potential to degrade several categories of organics. *Acaligenes* can even degrade PCBs and *Mucor* dieldrin.

Under favorable conditions, earthworms and microorganisms act 'symbiotically' to accelerate and enhance the decomposition of the organic matter in the waste. It is the microorganisms which breaks down the cellulose in the food waste, grass clippings and the leaves from garden wastes. (Morgan & Burrows, 1982; Xing *et al.* 2005)

VERMI-COMPOSTING STUDIES MADE AT GRIFFITH UNIVERSITY, BRISBANE

(a) Vermicomposting of Commingled Food Waste (Raw and Cooked) from Asian & Australian Homes & Restaurants

A comprehensive study on vermi-composting of mixed (commingled) food waste from kitchens of Australian and Asian homes, Indian restaurants & Fast Food Outlets' were carried out. It consisted of following waste components -

1. Rejects of raw and cooked potato and their peels;
2. Rejects of cabbage & lettuce leaves, celery, cucumber, spinach, beans, carrot cuts & peels, tomato & onion;
3. Leftovers of cooked / baked vegetables (okra, brinjal, beans, tomato, cauliflower & broccoli);
4. Rejects of fruits - apple, pear, kiwi fruits cuts & peel;
5. Leftovers of cooked chicken flesh with bones, egg shells;
6. Leftovers of rice, noodles, bread & buns and the Indian bread (chapatti).

Objectives

1. To understand the food preferences for earthworms from the kitchen waste;
2. To determine the sequence of degradation of the food waste components;
3. To understand the feeding behaviour of worms in raw vs. cooked food, and in oily and greasy foods;
4. To testify whether the worms can adapt to feeding on meat waste products;

The Vermicompost (VC) Bin

Vermi-composting was carried out in a specially fabricated plastic 'vermiculture bin'. It is about 1 m high and 50 x 50 cm in dimensions supported on 5 legs and strong enough to carry about 30-40 kg of organic waste and some soil at base. There is lid on the top with adequate provision for aeration from the side walls.

Preparing the Vermicomposting Bed

The bed was prepared with a thick layer of wet newspaper and maize leaves. About 6 inches of neutral soil obtained from nursery was spread over the paper bed and about 250 numbers of earthworms were released in this soil. About 100 gms of each of the food waste components were used. Mixed species of *Elsenia fetida, Eudrilus eugeniae* and *Perionyx excavatus* were used. The worms ranged between 3-6 cm in length and 0.1-0.2 cm in thickness. Moisture content was maintained at around 60 % by regular spray of water. Temperature within the bin was maintained at around 22-24· C.

Control Bin—Composting Without Earthworms

A control composting (CC) bin was organized with all above features except for the earthworms to compare and determine the role and efficiency of earthworms in biodegradation. Biodegradation was very slow as compared to earthworms and some had to be terminated after few weeks due to offensive odour.

Important Observations and Findings

1. There was initial odor problem in the VC bin which disappeared by second week. But this continued in the CC bin;
2. Fungus (blue, black and green mould) appeared on the waste biomass which disappeared after few weeks in the VC bin but continued in CC bin;
3. Maggots also appeared around the chicken waste (normal feature in meat products) which disappeared after sometimes in the VC bin but remained in the CC bin.
4. Bread & buns, rice & noodles were consumed very fast;
5. Cabbage & lettuce leaves, cucumber, spinach, carrot & fruit peels were also consumed faster;
6. Raw vegetable cuts were consumed still slower;
7. The baked and boiled vegetables were consumed faster than the cooked ones (may be due to use of oil and onion in cooking);
8. Surprisingly, raw potato was consumed very slowly & incompletely and their peels were NOT at all accepted till the end (over 14 weeks);
9. Similarly onions were also NOT accepted;
10. Cooked chicken flesh was consumed slowly but completely at the end (week 14), when no other food materials were available. Bones were left intact. Egg shells were consumed slowly.
11. The worms grew in size and number increased from 250 to about 500 adult worms along with baby worms and lots of cocoons. (Sinha *et al.*, 2005).

TABLE 1

Rate of Biodegradation of Food Waste Components (from Homes & Restaurants) With and Without Earthworms Under Identical Climatic Conditions (Food waste components about 100 gms each with 250 worms)

Food Waste Components	Weeks	% age Biodegradation (With Worms)	% age Biodegradation (Without Worms)
(1)	(2)	(3)	(4)
1. Bread, buns & naans	2-3	100%	Over 50%*
2. Rice, noodles & pasta	2-3	100%	Over 50%*

Contd. ...

Contd. ...

(1)	(2)	(3)	(4)
3. Raw cabbage, lettuce, spinach cucumber, tomato & carrot peels	2-4	100%	Over 20%**
4. Apple, pear, kiwi fruit cuts and peels	2-3	100%	Over 20%**
5. Baked & boiled potato, tomato, cauliflower, broccoli, brinjal & beans	5-6	100%	Over 30%**
6. Cooked potato, cauliflower, beans broccoli, brinjal & tomato (oily)	6-7	100%	Over 30%**
7. Raw rejects of cauliflower, okra, broccoli, celery, carrot & beans	7-8	100%	Over 20%**
8. Indian chapatti	8-9	100%	Over 30%**
9. Cooked chicken, lamb & beef (curry)	14	100%	(flesh, no bones)***
10. Crushed egg shells	14	30%	None
11. Raw potato cuts & peels	14	20%	(except peels) None
12. Raw onion cuts & peels	14	None	None

* 100 % degradation was achieved within the study period (Week 14) though with foul odor and heavy invasion of fungus;

** 100 % degradation could never be achieved, had odor problem and badly invaded by fungus (blue, black & green moulds) and had to be terminated;

*** Terminated in week 4 because of maggots, heavy fungus & offensive smell;

(b) Vermicomposting of Mixed Fried Food Waste from Fast Food Centres

They were obtained from restaurants in Griffith University Campus. It mostly consisted of deep fried items of fish, chicken, bacon, beef, potato fingers, and breads & buns. Similar nature of food wastes are generated at popular fast food centers like McDonald and Hungry Jacks. Such fried food wastes also contain very high amount of fat and oil. They are all ending up in landfills.

Vermi-composting was carried out in vermiculture (VC) bin described above and a control (CC) bin was also organized. The bedding was made of soaked newspaper with soil on top. About 9.5 kg of food waste from fast-food centers were used in both. They were placed on the bed for composting and 500 worms (same mixed 3 species) were used. Half of the food waste was crushed while the other half was left intact to see whether crushing made easier for worms to degrade faster. Moisture content was maintained at around 60 % by regular spray of water. Temperature within the kit was maintained at around 22-24· C

Important Observations & Findings

(1) Foul odor persisted in the first two weeks in the VC bin which declined towards the 3rd week and completely disappeared in 4th week. In the control (CC) bin foul odor continued and became more offensive and had to be terminated in week 6;

(2) Black, blue and yellow fungus (moulds) appeared on the disintegrating waste in both the bins but disappeared from the VC bin in week 4, while it increased in the CC bin;

(3) About 15 - 20 maggots appeared in both bins which was more pervasive in CC bin. While it disappeared in the VC bin in week 5, it grew in number in the CC bin.

(4) The earthworms grew in size (average 4-8 cm in length and 0.2-0.3 cm in thickness), looked very healthy, and their number increased between 600-650. (Patil, 2005).

Table 2

Rate of Biodegradation of Fried Food Wastes (from Fast Food Outlets) With and Without Earthworms Under Identical Climatic Conditions (Food waste used was about 9.5 kg with 500 worms)

Food Waste Components (Buns, bread, potato fries, bacon, beef, fish patty, chicken nuggets & calamari rings)	%age Biodegradation (With Worms) Crushed / Intact	%age Biodegradation (Without Worms) CONTROL Crushed / Intact	
Week 1-2	10% / ND *	ND / ND*	
Week 3-4	50% / 10% *	5% / ND**	
Week 5-6	80% / 50%	10% / 5%**	(Terminated)
Week 7-8	95% / 80%		
Week 9-10	100% / 90%		
Week 11-12	- / 95%		
Week 13-14	- / 100%		

ND = Not detectable * Mild foul odor ** Highly offensive foul odor

FACTORS AFFECTING OPTIMAL WORM ACTIVITY AND VERMI-COMPOSTING

1. Moderate Temperature

In general earthworms prefers and tolerates cold and moist conditions far better than the hot and dry ones. Most worms involved in vermi-composting require moderate temperature between 20 - 30 · C. Heat causes more problems in vermi-composting than the cold. Red worms are reported to become inactive above 29· C. They are at the highest levels of both waste degradation and reproduction activity as the weather cools in the fall and warms in the spring.

2. Adequate Moisture

Earthworms requires plenty of moisture for growth and survival ranging from 60 to 70 %. The bed should not be too wet as it may create anaerobic condition adversely affecting worm activity.

3. Adequate Aeration

Vermi-composting is an aerobic process and adequate flow of air in the waste biomass is essential for worm function. Worms breath through their skin and need plenty of oxygen in the surrounding areas. Although worms constantly aerate their habitat by burrowing actions, periodical turning of waste biomass can improve aeration and biodegradation.

4. Neutral pH (7.0)

Earthworms are sensitive to pH. The decomposition of organic matter produces 'organic acids' that lower the pH of the bedding soil and impair worm activity. Although the worms can survive in a pH range of 4.5 to 9 but functions best at neutral pH of 7.0. Although worms can lower pH of its medium by secreting calcium (Ca), it is suggestive to add ground limestone (calcium carbonate) powder to the bedding periodically. This would serve two purposes- maintain neutral pH and also supply the much needed calcium (Ca) to the worms for its metabolism.

5. Carbon / Nitrogen (C/N) Ratio of the Feed Material

High C/N ratio above 30 : 1 in waste biomass can impair worm activity and vermi-composting. Although earthworms help to lower the C/N ratio of fresh organic waste, it is advisable to add nitrogen supplements such as cattle dung or pig and goat manure or even kitchen waste (which are rich in nitrogen contents) when waste materials of higher C/N ratio exceeding 40 : 1 (grass clippings) are used for vermi-composting.

6. Adequate Supply of Calcium (Ca)

Calcium appears to be important mineral in worm biology (as calcarious tissues) and biodegradation activity. Although most organic waste contains calcium, it is important to add some additional sources of calcium for good vermi-composting. Egg shells are good source of natural calcium. Occasionally limestone powder should be added.

ADVANTAGES OF VERMI-COMPOSTING OVER CONVENTIONAL AERATED MICROBIAL COMPOSTING

Earthworms have real potential to both increase the rate of aerobic decomposition and composting of organic matter and also to stabilize the organic residues - removing the harmful pathogens and heavy metals from the compost.

> Vermicomposting by earthworms excels all other 'bio-degradation' and 'bio-conversion' technologies by the fact that - it can utilize waste organics that otherwise cannot be utilized by others; achieve greater utilization (rather than the destruction) of materials that cannot be achieved by others; and by the fact that it does all with 'enzymatic actions' and enzymes are biological catalysts giving pace and rapidity to all biochemical reactions even in minute amounts. It also keeps the system fully aerated with plenty of oxygen available to aerobic decomposer microbes. Aerobic processes are about 10 times faster than the anaerobic.

1. Vermi-composting is Nearly Odor-free Process

Earthworms create aerobic conditions in the waste materials by their burrowing actions, inhibiting the action of anaerobic micro-organisms which release foul-smelling hydrogen sulfide and mercaptans. They also release anti-bacterial coelomic fluids in the decaying waste biomass which arrest growth of anaerobic pathogens which promotes rotting.

2. Earthworms Destroy Any Pathogens Present in the End Product

The earthworms release coelomic fluids in the waste biomass which have anti-bacterial properties that destroy all pathogens. (Pierre *et al.*,1982). Earthworms also devour the protozoa, bacteria and fungus as food. Earthworms seems to realize instinctively that anaerobic bacteria and fungi are undesirable and so feed upon them preferentially, thus arresting their proliferation. In the intestine of earthworms some bacteria & fungus (*Pencillium* and *Aspergillus*) have also been found. They produce 'antibiotics' and kills the pathogenic organisms in the waste biomass making the medium virtually sterile.

3. Earthworms Remove Heavy Metals & Pesticides from End Products

Earthworms can bio-accumulate high concentrations of heavy metals & pesticides from the end products (vermicompost) rendering it free of any toxic chemicals. (More is discussed in Chapter 14).

4. Low Greenhouse Gas (Methane) Emission By Vermi-composting

> Composting by worms decrease the proportion of 'anaerobic to aerobic decomposition', resulting in a significant decrease in methane (CH_4) and volatile sulfur compounds which are readily emitted from the conventional (microbial) composting process.(Mitchell *et al.*, 1980). Vermi-composting of waste organics by using earthworms, therefore have distinct advantage over the normal composting as they do not allow greenhouse gas methane to be formed. Earthworms can play a good part in the strategy of greenhouse gas reduction and mitigation in the disposal of global organic wastes as landfills also emit methane resulting from the slow anaerobic decomposition of waste organics over several years.

However, recent researches done in Germany has found that earthworms produce a third of nitrous oxide (NOx) gases when used for vermicomposting. Molecule to molecule NOx are 296 times more powerful GHG than carbon dioxide (CO_2). This needs further studies and we are doing it at Griffith University.

5. Earthworms Produce More Nutritive Compost Rich in Minerals, Plant Growth Regulators, Nitrogen Fixing Bacteria and Mycorrhizal Fungi

End product (vermi-compost) is a nutritive plant food rich in NKP (1.16 % nitrogen, 1.34 % potassium and 1.22 % phosphorus), micronutrients, beneficial soil microbes like 'nitrogen-fixing bacteria' and 'mycorrhizal fungi', and also contain growth promoting hormone 'auxins' and flowering hormone 'gibberlins' (Tomati, et, al,.1985). The mycorrhizal fungi encouraged by the earthworms transfer phosphorus by increasing solubilisation of mineral phosphate by the enzyme phosphatase. Earthworms vermicompost contain enzymes like amylase, lipase, cellulase and chitinase, which continue to break down organic matter in the soil (to release the nutrients and make it available to the plant roots) even after they have been excreted. (Tiwary *et al.*, 1989; Chaoui *et al.*, 2003).

6. No or Low Energy Use in Vermi-composting Process

Conventional microbial composting requires energy for aeration (constant turning of waste biomass and even for mechanical airflow) and sometimes for mechanical crushing of waste to achieve uniform particle size. Vermi-composting do not involve such use of energy. Occasionally turning may be required for aeration which can be done manually.

ENVIRONMENTAL-ECONOMICS (EE) OF VERMICULTURE BIOTECHNOLOGY : GETTING GOLD FROM GARBAGE

Vermiculture technology involves about 100-1000 times higher 'value addition' than other biological technologies. Obtaining earthworms from vermiculture farms would be one-time cost in any vermiculture technology as the earthworms multiply rapidly creating huge army of worms which further promote and enhance the process. The resulting 'earthworm biomass' from the process also come as a very valuable byproduct as 'pro-biotic' nutritive food. Earthworms not only converts 'waste' into 'wealth' it itself becomes a valuable asset. Vermiculture is a flourishing industry all over the world. The cost of approx.1000 worms in Australian market is about $ 32.

Earthworms in dense population (high biomass) can physically handle most organic wastes and potentially at a fraction of the cost of conventional methods of solid waste management by landfill disposal. Moreover, landfills are not only an economic but also proving to be an environmental burden. Vermicomposting technology by earthworms is a self-promoted, self-regulated, self-improved, self-driven, self-powered & self-enhanced, low or no energy requiring zero-waste technology, easy to construct, operate and maintain. The greatest EE working in favour of vermicomposting of MSW is in the savings incurred in the reduction of landfills. The upfront cost of construction of an average secured landfill is estimated to be between US $ 15-25 millions before the first load of waste is dumped. This explains the dollar value of diverting every ton of MSW from the landfills by vermicomposting.

EE also work with respect to production and sale of the 'vermicompost' and the 'worm biomass' and the economic and environmental benefits that accrue from them. Both live worms and its excreta (vermicast) in soil tremendously improve its fertility and the productivity is simply doubled or even tripled.

Production of Earthworm Biomass : A Nutritive Earthworm Meal for Fishery & Poultry

Large scale vermiculture would result into tons of earthworms biomass every year. Studies indicate that under favorable conditions earthworms can 'double' their number at least every 60 - 70 days. A study found 5 kg of worms feeding on organic wastes increased to 6 kg within 30 days. (ARRPET, 2005). Potentially large quantities of worm biomass will be available as 'pro-biotic' food for the cattle and fish farming, after the first year of vermiculture. This can be a good source of nutritive 'worm meal' rich in essential amino acids 'lysine' and 'methionine'. It is superior to even 'fish meal'.

PROBLEMS ENCOUNTERED DURING VERMI-COMPOSTING & THEIR SOLUTIONS

1. Unpleasant Odor in Beginning

There may be initial odor problem in any vermi-composting process. This is mainly because the worms are overloaded with waste beyond its 'carrying capacity' at a given time and oxygen supply becomes insufficient leading to anaerobic conditions. Lesser number of worms per kg of waste biomass means less discharge of anti-bacterial coelomic fluid. But as the worms grow mature and multiplies in numbers it discharges more fluid, creates more aerobic conditions in the waste biomass by burrowing actions, and also devour the anaerobic microbes responsible for rotting and odor.

However, researches indicate that a natural insoluble mineral zeolite (aluminum silicate) when mixed with waste (3-5 %) reduces or eliminate the foul odor by absorbing the gases ammonia and hydrogen sulfide. Zeolite has additional advantages in vermi-composting. It has cage-like skeletal structure that allows to trap heavy metals which cannot then leach into environment to be extracted by plant roots (from vermicompost) or bio-accumulated by earthworms. Zeolite also helps to raise or lower pH of waste through cation exchange.

2. Fruit Flies, Maggots and Mites

Fresh food scraps attract fruit flies and mites that cause some nuisance. Sprinkling limestone powder or putting some vinegar in a cup in the bin drives them away. Maggots may appear initially if the organic waste contain meat products, but it disappear with time.

CONCLUDING REMARKS

Vermiculture industry has grown considerably in recent years all over the world. Composting by earthworms is proving to be environmentally preferred process over the normal microbial composting and much more over the landfills as it is rapid and nearly odorless process, can reduce composting time significantly and above all, there is no emission of 'greenhouse gas methane' which plagues both these solid waste management options. Vermicomposting of organic waste to divert massive domestic waste from the landfills are gaining importance and many municipal council in Australia are adopting this rapid and odorless vermicomposting technology using waste eater earthworms.

> Poor nations cannot even afford to construct and maintain a truly engineered landfill. Developing countries needs more options for safe waste management, and also low-cost, as they have several other social and developmental priorities with limited resources. Moreover, in the rural communities of both developed and developing world, centralized waste management system is never a good option. Individual households or a cluster of homes can treat their wastes better and also recover some resource (biofertilizer) more useful in the rural areas where farms are located.

Use of vermi-compost in soil eventually leads to increase in the number of earthworm population in the soil over a period of time, as the baby worms grow out from their cocoons contained in the vermicast. Studies have found that if 100 kg of organic waste with say, 2 kg of plant nutrients (NPK) are processed through the earthworms, there is a production of about 300 kg of 'fresh living soil' with 6 % of NPK and several trace elements that are equally essential for healthy plant growth. This magnification of plant nutrients is possible because earthworms produce extra nutrients from grinding rock particles with organics and by enhancing atmospheric nitrogen fixation. Earthworms activate this ground mix in a short time of just one hour. When 100 kg of the same organic wastes are composted conventionally unaided by earthworms, about 30 kg compost is derived with 3 % NPK. This usual compost thus has a total NPK of only about 1 kg. Rest 1 kg nutrient might have been leached or volatilized during the process of composting. (Bhawalker, 1995).

> Vermi-composting has the potential to be environmentally sustainable, cost-effective and economically rewarding for the abattoirs & meat processing industries who has to dispose their paunch wastes (high odor, high water & coarse forage) at high cost. Meat processors can reprocess the earthworm biomass generated into nutritive high protein 'worm meal' for sale and utilize their existing facilities to handle intractable water and reduce their greenhouse gas emissions.(Fraser-Quick, 2002).

The worm number and quantity (biomass) is a 'critical factor' for vermi-composting of organic wastes besides the optimal temperature and moisture which determines worm activity. A minimum of about 100-150 adult worms per kg of waste would be ideal to start with for rapid biodegradation and also odor-free process. Vermicomposting process driven by the earthworms tends to become more robust and efficient with time as the army of degrader worms grows and invade the waste biomass and further proliferating several battalions of aerobic decomposer microbial army.

Thus vermi-composting of organic waste is like 'hitting several targets in one shot'-

- Safe, fast and odorless disposal of much of the organic wastes;
- Making wealth (nutritive foods for the cattle and crops) out of waste;
- Reducing the need of costly landfills;
- Arresting the emissions of greenhouse gases that is inevitable in the disposal of organic waste.

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12

VERMIFILTRATION OF WASTEWATER BY EARTHWORMS

A Low-Cost Treatment Option for Wastewater and A Sustainable Alternative to the Costly Sewage Treatment Plants (STPs) for Developing Countries Through Researches into EBT

KEY WORDS

Vermifiltration; Vermifilter Bed; Earthworm Body as Biofilter; Stimulate Microbial Degradation; Odor-Free Process; Synchronous Sludge Treatment; Hydraulic Retention Time; Hydraulic Loading Rate; Biological Oxygen Demand; Chemical Oxygen Demand; Total Dissolved and Suspended Solids; Potential for Decentralization of Vermifiltration System

BACKGROUND INFORMATION

Vermifiltration of wastewater (municipal and some industrial) using waste eater earthworms is a newly conceived novel & cost-effective technology with several advantages over the conventional costly wastewater treatment systems. Earthworms body work as a 'biofilter' and they have been found to remove the 5 days BOD (BOD_5) by over 90%, COD by 80-90%, total dissolved solids (TDS) by 90-92% and the total suspended solids (TSS) by 90-95% from wastewater by the general mechanism of 'ingestion' and biodegradation of organic wastes, heavy metals and solids from wastewater and also by their 'absorption' through body walls. There is no sludge formation in the process which requires additional expenditure on landfill disposal. This is also an odor-free process and the resulting vermi-filtered water is clean enough & also highly nutritive, to be reused for farm irrigation and in parks and gardens. They create aerobic conditions in the waste materials by their burrowing actions, inhibiting the action of anaerobic micro-organisms which release foul-smelling hydrogen sulfide and mercaptans.

Many developing nations cannot afford to construct and maintain costly STP's. They need more options for sewage treatment at low-cost. In both developed and developing world, at least for new developments, centralized sewage treatment system may not fulfill sustainable wastewater management requirements in future due to ever-increasing demand. In cities of developed nations individual households or a cluster of homes can treat their domestic wastewater at source in a de-centralized manner so as to reduce the burden (BOD & COD loads) on the sewage treatment plants (STP's) down the sewer system.

CHARACTERISTICS OF MUNICIPAL WASTEWATER (SEWAGE)

Sewage is the cloudy fluid of human fecal matter and urine, rich in minerals and organic substances. Water is the major component (99%) and solid suspension amounts to only 1%. The biochemical oxygen demand (BOD) and oxygen consumption (OC) values are extremely high demanding more oxygen by aerobic microbes for biodegradation of organic matter. Dissolved oxygen (DO) is greatly depleted. Low oxygen in water leads to anaerobic decomposition of organic contents and emission of obnoxious gases like hydrogen sulfide, methane and carbon monoxide. The nitrogen (N) and phosphorus (P) contents are very high and there are heavy metals like cadmium (Cd) and significant amount of coliform bacteria. The total suspended solids (TSS) is also very high like the BOD and nutrients and this often leads to a high anaerobic microenvironment in the sediments.

BOD, COD and SS Values of Raw Sewage and the Acceptable Values of Treated Sewage

The average BOD (Biological Oxygen Demand) value of the raw sewage ranged between 200 - 400 mg/L, COD (Chemical Oxygen Demand) ranged between 116 -285 mg/L, the TSS (Total Suspended Solids) ranged between 300 - 350 mg/L and the pH ranged between 6.9 - 7.3. There is great fluctuation in these values depending upon catchment area, flow rate and season. Sewage from industrial areas can have high COD values, very low or high pH, due to accidental mixing of industrial wastewater. The normal acceptable values for BOD in treated wastewater is 1-15 mg/L, COD is 60-70 mg/L, TSS 20-30 mg/L and pH around 7.0.

MECHANISM OF WORM ACTION IN VERMIFILTRATION OF SEWAGE

Earthworms are versatile waste eaters and decomposers. It promotes the growth of 'beneficial decomposer bacteria' in wastewater and acts as an aerator, grinder, crusher, chemical degrader and a biological stimulator. (Sinha, *et al.*, 2002).

1. The two processes—microbial process and vermi-process simultaneously work in the vermifiltration system. Earthworms further stimulate and accelerate microbial activity by increasing the population of soil microorganisms and also through improving aeration (by burrowing actions) (Binet *et al.*, 1998). Earthworms hosts millions of decomposer (biodegrader) microbes in their gut. Edward and Fletcher (1988) showed that the number of bacteria and 'actinomycetes' contained in the ingested material increased up to 1000 fold while passing through the gut.

2. Vermifilters provide a high specific area—up to 800 sq m/g and voidage up

to 60%. Suspended solids are trapped on top of the vermifilter and processed by earthworms and fed to the soil microbes immobilized in the vermifilter.

3. Dissolved and suspended organic and inorganic solids are trapped by adsorption and stabilized through complex biodegradation processes that take place in the 'living soil' inhabited by earthworms and the aerobic microbes. Intensification of soil processes and aeration by the earthworms enable the soil stabilization and filtration system to become effective and smaller in size.

4. Earthworms intensify the organic loadings of wastewater in the vermifilter soil bed by the fact that it granulates the clay particles thus increasing the 'hydraulic conductivity' of the system. They also grind the silt and sand particles, thus giving high total specific surface area, which enhances the ability to 'adsorb' the organics and inorganic from the wastewater passing through it. The vermicast produced on the soil bed also offers excellent hydraulic conductivity of sand (being porous like sand) and also high adsorption power of clay. This is ideal for diluted wastewater like sewage. (Bhawalkar, 1995).

5. Earthworms also grazes on the surplus harmful and ineffective microbes in the wastewater selectively, prevent choking of the medium and maintain a culture of effective biodegrader microbes to function.

CRITICAL FACTORS AFFECTING VERMIFILTRATION OF SEWAGE

Hydraulic Retention Time (HRT) and Hydraulic Loading Rate (HLR) are two important factors which affect the treatment and quality of treated wastewater.

(a) Hydraulic Retention Time (HRT)

Hydraulic retention time is the time taken by the wastewater to flow through the soil profile (vermifilter bed) in which earthworms inhabits. It is very essential for the wastewater to remain in the vermifiltration (VF) system and be in contact with the worms for certain period of time. HRT depends on the flow rate of wastewater to the vermifiltration unit, volume of soil profile and quality of soil used. HRT is very critical, because this is the actual time spent by earthworms with wastewater to retrieve organic mater from it as food. During this earthworms carry out the physical and biochemical process to remove nutrients, ultimately reducing BOD, COD and the TDSS. The longer wastewater remains in the system in contact with earthworms, the greater will be the efficiency of vermi-processing and retention of nutrients. Hence the flow of wastewater in the system is an important consideration as it determines the retention of suspended organic matter and solids (along with the chemicals adsorbed to sediment particles). Maximum HRT can results from 'slower rate of wastewater discharge' on the soil profile (vermifilter bed) and hence slower percolation into the bed. Increasing the volume of soil profile can also increase the HRT. The number of live adult worms, functioning per unit area in the vermifilter (VF) bed can also influence HRT.

HRT of vermifiltration system can be calculated as—

$$\text{HRT} = (\rho \times V_s) / Q_{wastewater}$$

Where;

HRT = theoretical hydraulic retention time (hours)

V_s = volume of the soil profile (vermifilter bed), through which the wastewater flow and which have live earthworms (cum)

ρ = porosity of the entire medium (gravel, sand & soil) through which wastewater flows

$Q_{wastewater}$ = flow rate of wastewater through the vermifilter bed (cum / hr)

Thus the hydraulic retention time (HRT) is directly proportion to the volume of soil profile and inversely proportion to the rate of flow of wastewater in the vermifilter bed.

(b) Hydraulic Loading Rate (HLR)

The volume and amount of wastewater that a given vermifiltration (VF) system (measured in area and depth of the soil medium in the vermifilter bed in which the earthworms live) can reasonably treat in a given time is the hydraulic loading rate of the vermifilter (VF) system. HLR can thus be defined as the volume of wastewater applied, per unit area of soil profile (vermifilter bed) per unit time. It critically depends upon the number of live adult earthworms functioning per unit area in the vermifilter bed. The size and health of the worms is also critical for determining the HLR.

HLR of vermi-filtration system can be calculated as -

$$\mathbf{HLR = V_{wastewater} / (A \times t)}$$

Where :

HLR = Hydraulic Loading Rate (m / hr)

$V_{wastewater}$ = volumetric flow rate of wastewater (cum).

A = Area of soil profile exposed (sqm).

t = Time taken by the wastewater to flow through the soil profile (hr).

High hydraulic loading rate leads to reduced hydraulic retention time (HRT) in soil and could reduce the treatment efficiency. Hydraulic loading rates will vary from soil to soil. The infiltration rates depend upon the soil characteristics defining pore sizes and pore size distribution, soil morphological characteristics, including texture, structure, bulk density, and clay mineralogy.

SOME SUCCESS STORIES ON VERMI-FILTRATION OF SEWAGE

A pilot study on vermifiltration of sewage was made by Xing *et al,*. (2005) at Shanghai Quyang Wastewater Treatment Facility in China. The earthworm bed which was 1m (long) x 1m (wide) x 1.6m (high), was composed of granular materials and earthworms. The worm's number was kept at about 8000 worms/sqm. The average chemical oxygen demand (COD) value of raw sewage used was 408.8 mg/L that of 5 days biological oxygen demand (BOD_5) was 297 mg/L that of suspended solids (SS) was 186.5 mg/L. The hydraulic retention time varied from 6 to 9 hours and the hydraulic loading from 2.0 to 3.0 $m^3/(m^2.d)$ of sewage. The removal efficiency of COD ranged between 81-86%, the BOD_5 between 91-98%, and the SS between 97-98%.

Gardner *et al.*,(1997) studied on-site effluent treatment by earthworms and showed that it can reduce the BOD and COD loads significantly. Taylor *et al.*, (2003) studied the treatment of domestic wastewater using vermifilter beds and concluded that worms can reduce BOD and COD loads as well as the TDSS (total dissolved and suspended solids) significantly by more than 70-80%. Hartenstein & Bisesi (1989) studied the use of earthworm for the management of effluents from intensively housed livestock which contain very heavy loads of BOD, TDSS and nutrients nitrogen (N) and phosphorus (P). The worms produced clean effluents and also nutrient rich vermicompost.

VERMIFILTRATION STUDIES AT GRIFFITH UNIVERSITY, BRISBANE, AUSTRALIA

Extensive studies on vermiculture and vermifiltration of municipal and some industrial wastewater is being made at Griffith University, Brisbane, Australia under the supervision of author for over 5 years and has yielded very encouraging results which are discussed below in tables 1 to 4. The pilot study was carried out in a vermiculture kit containing about 30-40 kg of gravels with a layer of garden soil on top. This forms the vermifilter bed in which lies the earthworms. About 500 worms were used in the bed (13 cm x 13 cm x 6 cm). The soil and gravels in the vermifilter kit also work as the microbial - geological system of filtration. They also help in cleaning of wastewater by adsorbing the impurities. Gravels provide ideal sites for colonization by decomposer microbes which works to reduce BOD, COD and the TDSS from the wastewater. With more wastewater passing through the gravels there is more formation of 'biofilms' of decomposer microbes.

TABLE 1

Removal of 5 Day Biological Oxygen Demand (BOD_5) by Earthworms

(a) BOD_5 Removal Efficiency of Municipal Wastewater (Sewage) Treated by Earthworms (HRT : 1-2 hrs)

Expt. No.	Untreated Raw Sewage BOD_5 (mg/L)	Treated Sewage By Earthworms BOD_5 (mg/L)	% Reduction in BOD_5 by Earthworms
1	309	1.97	99.4
2	260	4.00	98.5
3	316	6.02	98.1
4	328	2.06	99.4
5	275	4.25	98.5

(See Graph in Appendix-1) Av. = 98.78%

(b) BOD_5 Removal Efficiency of Brewery Wastewater Treated by Earthworms (HRT : 3-4 hrs)

Expt. No.	Untreated Brewery Wastewater BOD_5 (mg/L)	Treated Brewery Wastewater By Earthworms BOD_5 (mg/L)	% Reduction in BOD_5 by Earthworms
1	6780	40.59	99.4
2	6600	45.57	99.3
3	5500	43.87	99.2
4	6240	29.26	99.5

(See Graph in Appendix-1) Av. = 99.35%

(c) BOD_5 Removal Efficiency of Dairy Wastewater Treated by Earthworms (HRT 6-10 hours)

Expt. No.	Untreated Dairy Wastewater BOD_5 (mg/L)	Treated Dairy Wastewater By Earthworms BOD_5 (mg/L)	% Reduction in BOD_5 by Earthworms
1	1,36,000	690	99.49
2	1,39,200	1180	99.15
3	1,03,200	1050	98.98

(See Graph in Appendix-1) Av = 99.2%

It can be seen from tables 1 (a, b & c) that the earthworms can remove BOD (BOD_5) loads of municipal wastewater by over 98% at hydraulic retention time of 1-2 hours while over 99% from the brewery wastewater at HRT 3-4 hours and the same from the dairy wastewater at higher HRT of 6-10 hours. Significantly, the earthworms can remove very high BOD loadings in thousands (brewery wastewater) and in millions (dairy wastewater). It requires higher hydraulic retention time (HRT) to remove high BOD loads. It is important to note that the earthworms degrade the high concentrations of wastewater organics (BOD loads) by 'enzymatic actions' (which work as biological catalysts bringing pace and rapidity in biochemical reactions) and that is the reason of high BOD removal which cannot be achieved by microbial degradation alone.

TABLE 2

Removal of Chemical Oxygen Demand (COD) by Earthworms

(a) COD Removal Efficiency of Municipal Wastewater (Sewage) Treated by Earthworms (HRT : 1-2 hrs)

Expt. No	Untreated Raw Sewage COD (mg/L)	Treated Sewage By Earthworms COD (mg/L)	% Reduction in COD by Earthworms
(1)	(2)	(3)	(4)
1	293	132	54.9
2	280	153	45.4

Contd. ...

Contd. ...

(1)	(2)	(3)	(4)
3	280	128	54.3
4	254	112	55.9
5	260	139	46.5

(See Graph in Appendix-1) Av. = 45.7%

(b) COD Removal Efficiency of Brewery Wastewater Treated by Earthworms (HRT 3 - 4 hrs)

Expt. No.	Untreated Brewery Wastewater COD (mg/L)	Treated Brewery Wastewater By Earthworms COD (mg/L)	% Reduction in COD by Earthworms
1	720	280	61.11
2	712	245	65.58
3	1042	143	86.24
4	1042	287	72.45

(See Graph in Appendix-1) Av = 71.3%

(c) COD Removal Efficiency of Milk Dairy Wastewater Treated by Earthworms (HRT 6 - 10 hours)

Expt. No.	Untreated Dairy Wastewater COD (mg/L)	Treated Dairy Wastewater By Earthworms COD (mg/L)
1	Above 15,000	561
2	Above 15,000	62
3	Above 15,000	319

Av. = 314

It can be seen from tables 2 (a, b & c) that the average COD removed from municipal wastewater by earthworms is over 45% at HRT 1-2 hours while that from brewery wastewater is over 71% at HRT 3-4 hours. The initial COD value of the milk dairy wastewater was higher than 15,000 mg/L and was beyond the range of estimation and hence percent reduction could not be accurately calculated. However, the earthworms significantly reduced its value from over thousands (5 digits) to tens & hundreds (2-3 digits). COD reduction was greatly affected by the hydraulic retention time. Higher HRT (above 9 hours) reduced the COD from above 15,000 mg/L to just 62 mg/L.

COD removal by earthworms is not as significant as the BOD, but at least much higher than the microbial system. Again, the enzymes in earthworms help in the degradation of several of those chemicals which otherwise cannot be decomposed by microbes.

TABLE 3

Removal of Total Suspended Solids (TSS) by Earthworms

TSS Removal Efficiency of Municipal Wastewater (Sewage) Treated by Earthworms (HRT 1 - 2 hrs)

Expt. No.	Untreated Sewage TSS (mg/L)	Treated Sewage By Earthworms TSS (mg/L)	% Reduction in TSS by Earthworms
1	390	28	92.82
2	374	24	93.58
3	438	22	94.97
4	379	27	92.87
5	407	25	93.85

(See Graph in Appendix-1) Av. = 92.97%

It can be seen from table 3 that the earthworms can significantly remove the suspended solids from municipal wastewater (sewage) by over 92%. Earthworms eat up the solids and also improve the 'adsorption' properties of the sands and soils by grinding them in their gizzard.

TABLE 4

Improvement in pH Value of Treated Sewage

Improvement in pH Value of Municipal Wastewater (Sewage) Treated by Earthworms

Expt. No.	Untreated Raw Sewage pH Value	Treated Sewage By Earthworms pH Value
1	6.58	7.05
2	5.76	7.35
3	6.65	7.00
4	6.67	7.25
5	6.05	7.06

Table 4 indicates that the pH value of raw sewage is almost neutralized by the earthworms.

A B C

Sewage Vermifiltration Studies Made at Griffith University (2005)

Bottle - A : The clear vermifiltered sewage treated by earthworms; **Bottle - B :** The hazy water from microbial-geological system without earthworms; **Bottle - C :** The turbid and cloudy untreated sewage

SIGNIFICANCE & ADVANTAGES OF VERMI-FILTRATION TECHNOLOGY OVER CONVENTIONAL SYSTEMS

Vermi-filtration system is low energy dependent and has distinct advantage over all the conventional biological wastewater treatment systems- the 'Activated Sludge Process', 'Trickling Filters' and 'Rotating Biological Contactors' which are highly energy intensive, costly to install and operate and do not generate any income. Since the conventional technologies are mostly the flow-processes and have finite hydraulic retention time (HRT) it always results into a 'residual stream' of complex organics and heavy metals (while only the simple organics are consumed) in the 'sludge' that needs further treatment (requiring more energy) before landfill disposal. This becomes unproductive. In the vermifilter process there is 100% capture of organic materials, the capital and operating costs are less, and there is high value added end product (vermicompost).

1. Vermi-filtration is Low Energy System

Some energy may be required in pumping the wastewater to the vermi-filtration unit if gravity flow is not adequate.

2. Synchronous Treatment of Wastewater and the Solids : End Products Become Useful Resource for Agriculture & Horticulture

Earthworms decompose the organics in the wastewater and also devour the solids synchronously and ingest the heavy metals from both mediums. It works as a 'sludge digester' where the sludge is softened by the grume excreted in the 'mouth' of the earthworms. In the esophagus it is neutralized by calcium (Ca) and then it comes to the intestine where it is absorbed and decomposed by the enzymes. This stabilized sludge is discharged in the vermifilter bed as 'excreta' (vermicompost) which is useful soil additive for agriculture and horticulture.

3. Vermifiltered Sewage is Free of Pathogens

Earthworms devour on all the pathogens (bacteria, fungus, protozoa & nematodes) found in both the wastewater and the sludge as they are their loved food. Some bacteria & fungi fostered by the worms also produce 'antibiotics' which kills the pathogenic organisms in the wastewater making the medium virtually sterile and odorless. The removal of pathogens, faecal coliforms (*E.coli*), *Salmonella* spp., enteric viruses and helminth ova from sewage and sludge appear to be much more rapid when they are processed by *E. fetida*. Of all *E.coli* and *Salmonella* are greatly reduced. (Bajsa *et al.*, 2003).

4. Vermifiltered Sewage is Free of Heavy Metals and Toxic Chemicals

Earthworms can bio-accumulate high concentrations of metals including heavy metals from the municipal wastewater. They readily bio-accumulate cadmium (Cd), mercury (Hg), lead (Pb) copper (Cu), manganese (Mn), calcium (Ca), iron (Fe) and zinc (Zn). Several studies have also found that earthworms can also either accumulate or degrade 'organochlorine pesticide' and 'polycyclic aromatic hydrocarbons' (PAHs) residues from the wastewater. (Ireland, 1983).

5. Earthworms Bioaccumulate Endocrine Disrupting Chemicals from Sewage Filter Beds

Markman *et al.* (2007) have reported significantly high concentrations of EDCs (dibutyl-phthalate, dioctylphthalate, bisphenol-A and 17β-estrdiol) in tissues of earthworms (*E. fetida*) living in sewage percolating filter beds and garden soil.

6. Vermifilter Treatment of Sewage is an Odorless System

There is no foul odor as the earthworms arrests rotting and decay of all putrescible matters in the wastewater and the sludge.

COMMISSIONING THE VERMIFILTRATION SYSTEM OF SEWAGE TREATMENT & DECENTRALIZATION

The real challenge is the commissioning of the vermifiltration technology for sewage treatment in homes. Many developing nations cannot construct and maintain costly STP's. They need more options for sewage treatment at low-cost, as they have several other social and developmental priorities with limited resources. Moreover, in the rural and urban communities of both developed and developing world, at least for new developments, centralized sewage treatment system may not fulfill sustainable wastewater management requirements in future due to ever-increasing demand. Individual households or a cluster of homes in cities can also treat their domestic wastewater better in a de-centralized manner so as to reduce the burden (BOD & COD loads) on the sewage treatment plants (STP's) down the sewer system.

All above results were obtained with approximately 500 worms in the vermifilter bed made in about 0.032 cubic meter (cum) of soil. This comes to approximately 16,000 worms per cum of soil. The initial number of worms must have increased substantially over the period of 7-8 weeks of experiment. If a vermifilter bed of 0.3 cum soil is prepared with approximately 5000 worms (over 2.5 kg) to start with, it can easily treat 950-1000 litres of domestic wastewater / sewage generated by (on an average) a family of 4 people with average BOD value ranging between 300-400 mg/L, COD 100-300 mg/L, TSS, 300-350 mg/L everyday with hydraulic retention time (HRT) of the wastewater in the vermifilter bed being approximately 1-2 hours.

Given that the worms multiply and double its number in at least every 60 days under ideal conditions of temperature and moisture, even starting with this number of earthworms a huge population (biomass) of worms with robust vermi-filtration system can be established quickly within few months which will be able to treat greater amount of wastewater generated in the family. An important consideration is the peak hour wastewater generation which is usually very high and may not comply with the required HRT (1 - 2 hrs) which is very critical for sewage treatment by vermi-filtration system. To allow 1 - 2 hrs HRT in the vermifilter bed an onsite domestic wastewater storage facility will be required from where the discharge of wastewater to the vermifilter tank can be slowly regulated through flow control.

ENVIRONMENTAL-ECONOMICS (EE) OF VERMIFILTRATION TECHNOLOGY : GETTING SILVER FROM SEWAGE

Earthworms can physically handle most organic wastewater and potentially at a fraction of the cost of conventional methods of wastewater treatment by sewage treatment plants (STP's). Moreover, STP's are not only an economic but also proving to be an environmental burden due to heavy 'sludge' formation which is a biohazard. Vermifiltration technology by earthworms is a self-promoted, self-regulated, self-improved, self-driven, self-powered & self-enhanced, very low energy & less chemical requiring zero-waste technology, easy to construct, operate and maintain. It has less operational cost since it requires energy only for pumping of wastewater and no skilled labour. Maintenance costs are also minimal as it does not involve any mechanical devices (except for pumps). EE of vermifiltration becomes highly positive also because of the synchronous treatment of sewage solids (sludge components) within the system, and the transformation of the sludge (a biohazard of negative economic value requiring huge cost in safe disposal either by landfill or incineration) into vermi-compost (a nutritive fertilizer of positive economic value) for use in agriculture & horticulture.

Obtaining earthworms for vermifiltration would be again one-time cost as the worms multiply rapidly creating huge population of worms which further promote and enhance the process. Furthermore, the technology creates two valuable byproducts. The vermifiltered water is almost crystal clear, nearly sterile and neutral in pH fit to be used for non-potable purposes (toilet flush, floor washing, cooling tower make water in industries etc.). As it also contains useful nutrients it is very suitable for irrigation (gardens, golf courses, fruits & vegetable farms).

CONCLUDING REMARKS

Vermifiltration is a logical extension of 'soil filtration' which has been used for 'sewage silviculture' (growing trees) since ancient days. Healthy soil is a bio-geological medium acting as an 'adsorbent' of organics, inorganic, pathogens and parasites. Vermifiltration technology can be a most cost-effective and odor-free process for sewage treatment with efficiency, economy and convenience and potential for decentralization. Any wastewater from the households and commercial organizations can be successfully treated by the earthworms and the technology can also be designed to suit a particular wastewater. It can treat dilute (less than 0.1% solids) as well as concentrated wastewater. It has an in-built pH buffering ability and hence can accept wastewater within a pH range of 4 to 9 without any pH adjustment.

Vermifiltration technology by earthworms excels all other 'bio-conversion', 'bio-treatment' and 'bio-degradation'- technologies by the fact that - it can utilize waste organics that otherwise cannot be utilized by others; achieve greater utilization (rather than the destruction) of waste materials that cannot be achieved by others; and by the fact that it does all with 'enzymatic actions' and enzymes are biological catalysts giving pace and rapidity to all biochemical reactions even in minute amounts. It also keeps the system fully aerated with plenty of oxygen available to aerobic decomposer microbes. Aerobic processes are about 10 times faster than the anaerobic.

> Vermifiltration process driven by the earthworms tends to become more robust and efficient with time as the army of degrader worms grows, proliferating further several battalions of microbial army (aerobic decomposers). It is also a compact biological wastewater treatment system as compared to other non-conventional system such as the 'constructed wetland system' which often suffer from limitations of oxygen for the decomposer aerobic microbes to act efficiently. Wetland based technologies involve mainly treatment and low utilization of waste materials and hence can be wasteful.

Vermi-filtration of wastewater must be started with higher number of earthworms, at least over 15,000-20,000 worms per cubic meter (cum) of soil in the vermifilter bed for good results. It is also important that they are mostly adult and healthy worms. In vermi-composting of solid waste, which is a continuous process (in days and weeks) the worms have to act 'gradually' in phases while their population (new army of bio-degraders) keeps on building up to intensify the biodegradation process. In vermifiltration of wastewater, the worms have to act 'instantly' as the wastewater flow past their body (degrading the organics, ingesting the solids and the heavy metals). That is why the wastewater has to be 'retained' (HRT) in the vermifilter bed for some appropriate period time (which has to be in hours and not in days) while the worms act on the wastewater.

> Although the worms have been found to remove very high BOD loads (10,000 - 1,00,000 mg/L often found in wastewater from food processing industries) within 4 to 10 hours of HRT (Sinha *et al*,. 2006) it can reduce the small BOD loadings of sewage (200-400 mg/L) within 30 - 40 minutes of HRT. But, in case of sewage treatment the objectives are not only to remove BOD and COD, but also to remove the heavy metals and pathogens from the wastewater. Hence greater hydraulic retention time (1-2 hours) is allowed so that the worms can ingest the heavy metals and also devour upon the pathogens completely while also killing them by discharging plenty of coelomic fluid (with antibacterial properties) in the wastewater. Greater interaction with wastewater components also provides better opportunity for the worms to eat all the solids and prevent any sludge formation.

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13

VERMISTABILIZATION OF SEWAGE SLUDGE BY EARTHWORMS

Converting a Potential Biohazard into Pathogen Free, Nutritive Organic Fertilizer : Significant Achievement of Researches into EBT

KEY WORDS

Earthworms as Sludge Digester; Earthworms Stimulate Microbial Degradation; Sewage Sludge - A Biohazard & a Potential Health Hazard; Bioaccumulation of Heavy Metals by Earthworms; Pathogen Destruction by Earthworms; Mineralization of Nutrients from Sludge; Reduction of Coliforms; Reduction in Volatile Solids; Decrease in Total Organic Carbon (TOC); Grade A Stabilized Sludge by Earthworms

BACKGROUND INFORMATION

Sludge is an inevitable hazardous and odorous byproduct from the conventional water and wastewater treatment plants which eventually require safe disposal either in landfills or by incineration incurring heavy cost. When the sludge is dewatered and dried they are termed as 'biosolids'. Management of the biosolids remains problematic due to the high cost of installing sewage sludge stabilization reactors and dehydration systems.

The chemical and biological composition of sewage sludge is unpredictable as they may contain toxic chemicals and pathogens. They are potential health hazard as they have been found to contain high numbers of cysts of protozoa, parasitic ova, fecal pathogens like *Salmonella, Shigella* and *E. coli* and also heavy metals such as zinc (Zn), cadmium (Cd), mercury (Hg) and copper (Cu). However, they also contains organics and essential plant nutrients like nitrogen (N), phosphorus (P), potassium (K) and various trace elements. Sludge is stabilized to reduce or eliminate pathogens and heavy metals, eliminate offensive odor, and reduce or eliminate the potential for putrefaction. Stabilized sewage sludge can become good source of organic fertilizer and soil additive.

Earthworms feed readily upon the sludge components, rapidly convert them into vermicompost, reduce the pathogens to safe levels and ingest the heavy metals. Volume is significantly reduced from 1 cum of wet sludge (80 % moisture) to 0.5 cum of vermicompost (30 % moisture). (Eastman, 1999).

Vermicomposting has been successfully used for treating & stabilizing municipal as well as industrial (paper mill, dairy and textile industry) sludge, diverting them from ending up in the landfills (Elvira *et al.* 1998; Ndegwa & Thompson 2001; Fraser-Quick, 2002; Contreras-Ramos *et al.*, 2005). Collier (1978) and Hartenstein and Bisesi (1989) have reported on the management of sewage sludge and effluents from intensively housed livestock by vermiculture in USA. In Italy vermiculture is used to biodegrade municipal and paper mill sludge. Aerobic and anaerobic sludge are mixed and aerated for more than 15 days and in 5000 cum of sludge 5 kg of earthworms are added. In about 8 months the sludge is converted into vermicompost (Ceccanti and Masciandaro, 1999).

VERMI-STABILIZATION OF SEWAGE SLUDGE : A SUCCESS STORY FROM AUSTRALIA

Vermiprocessing of sludge (biosolids) from the sewage and water treatment plants is being increasingly practiced in Australia and as a result it is saving over 13,000 cum of landfill space every year in Australia. (Komarowski, 2001). Redland Shire in QLD did vermi-composting of sludge (biosolids) from sewage and water treatment plants with the aid of Sydney based company Vermitech Pty. Ltd. in 1997. The facility received 400-500 tons of sludge every week with 17 % average solid contents and over 200 tons of vermicast is produced every week by vermicomposting. (Vermitech, 1998).

The Hobart City Council in Tasmania, Australia, vermicompost and stabilize about 66 cum of municipal biosolids (from sewage sludge) every week, along with green mulch diverting them from landfills. Zeolite mixed with the biosolids helps balance the pH and also in absorbing ammonia and odor. About two-third of this volume (44 cum) becomes 'vermi-compost' which is sold to public. The City Council is saving AU $ 56,000 per year just from avoiding landfill disposal (transport & tipping fees etc.). They are earning an equal amount as revenue from the sale of vermi-compost. (Datar *et al.*, 1997).

EARTHWORM SPECIES SUITABLE FOR VERMI-COMPOSTING OF SEWAGE SLUDGE

Long-term researches into vermiculture have indicated that the Tiger Worm *(Elsenia fetida)*, Red Tiger Worm (*E. andrei*), the Indian Blue Worm *(Perionyx excavatus)*,the African Night Crawler *(Eudrilus euginae)*,and the Red Worm *(Lumbricus rubellus)* are best suited for vermi-composting of sewage sludge under all climatic conditions. *E. fetida* and *E. andrei* are closely related. The army of the above 5 species combined together works meticulously. *E. fetida* appears to be most suitable as it is highly resistance to toxic substances with high rate of multiplication.

MECHANISM OF WORM ACTION IN STABILIZATION (DIGESTION) OF SEWAGE SLUDGE

Vermistabilization of sewage sludge is not a true process of composting as it does not involve heat. It is a very complex mechanical, chemical and biological transformation and the resultant product has higher stabilization and soil supplement value than traditional composting that relies on mechanical incorporation of sludge with green waste in large compost heaps. The earthworms acts as an aerator, grinder, crusher, chemical degrader and a biological stimulator. (Sinha, et. al,. 2002). It decompose the organics in the sewage sludge, mineralize the nutrients, ingest the heavy metals and devour the pathogens (bacteria, fungus, nematodes and protozoa) found in sludge making them chemicals and pathogen free ready to be reused as soil additive and organic fertilizer.

Essentially, earthworms works as a 'sludge digester' which is accomplished in the following steps—

1. The sludge is softened by the grume excreted in the 'mouth' of the earthworms and from there it goes to the 'esophagus'.
2. In the esophagus the softened sludge components is neutralized by calcium (Ca) (excreted by the inner walls of esophagus) and passed on to the gizzard and the intestine for further action.
3. In the muscular gizzard it is finely ground (with the aid of stones) into small particles to a size of 2-4 microns and passed on to the intestine for enzymatic actions. The gizzard and the intestine work as a 'bioreactor';
4. In the intestine the ground and pulped sludge components are decomposed by the enzymes proteases, lipases, amylases, cellulases and chitinases secreted here and then absorbed.
5. The final process in vermi-processing and degradation of sludge is the 'humification' in which the large organic particles are converted into a complex amorphous colloids containing 'phenolic' materials. Finally this stabilized sludge is discharged as 'excreta' (vermicast / vermi-compost).

Earthworms Reinforce Microbial Population in Sewage Sludge and Act Synergistically in Sludge Stabilization

Earthworms further stimulate and accelerate microbial activity by increasing the population of decomposer microorganisms and through improving aeration by burrowing actions (Dash, 1978; Binet *et al.*, 1998). They hosts millions of decomposer (biodegrader) microbes in their gut as they devour upon on them as their food.

Under favorable conditions, earthworms and microorganisms act 'symbiotically' to accelerate and enhance the decomposition of the organic matter in the sludge. (Morgan & Burrows, 1982; Xing *et al.*, 2005).

FACTORS AFFECTING OPTIMAL WORM ACTIVITY AND SLUDGE VERMI-COMPOSTING

The role of earthworms in stabilization of municipal sewage sludge is greatly linked to the sludge age and nutrient content, aerobic conditions, the moisture content, temperature, ash content and the loading rate. (Dominguez *et al.*, 2000; Masciandaro *et al.*, 2000).

1. Sludge Age and Nutrient Content

Nutritional value of the sludge play important role in the stabilization of the sludge and growth of worms in it. The major concern being the nitrogen (N) and phosphorus (P) content in the sludge. As the sludge ages, its nutritive value to the earthworms declines rapidly after about 12 weeks of removal from the digester. Other possibilities to make the sludge more palatable to the worms is to aerate the sludge in rotor drums to encourage the growth of aerobic microbes before feeding the earthworms. However, the ash content of the sludge increases with time which is an indication of sludge stabilization (Loehr *et al.*, 1998).

2. Adequate Aeration

Vermi-stabilization is an aerobic process and adequate flow of air in the waste biomass is essential for worm function. Worms breath through their skin and need plenty of oxygen in the surrounding areas. Although worms constantly aerate their habitat by burrowing actions, periodical turning of the sludge can improve aeration and biodegradation. Aeration also makes the sludge more palatable to worms.

3. Adequate Moisture

Both excessive and insufficient moisture can adversely affect earthworm performance in stabilization process. About 75 % moisture is recommended for sludge digestion. Neuhauser et al,.(1988) recommends 80-90 %. If it is too wet, it may create anaerobic condition adversely affecting worm activity.

4. Moderate Temperature

In general earthworms prefers and tolerates cold and moist conditions far better than the hot and dry ones. Most worms involved in vermi-composting require moderate temperature between 10 - 30 · C. The ideal temperature for vermicomposting is between 15 and 25· C (Neuhauser *et al.*, 1988). Heat causes more problems in vermi-composting than the cold. They are at the highest levels of both waste degradation and reproduction activity as the weather cools in the fall and warms in the spring.

5. Neutral pH (7.0)

Earthworms are sensitive to pH. The decomposition of organic matter produces 'organic acids' that lower the pH of the bedding soil and impair worm activity. Although the worms can survive in a pH range of 4.5 to 9 but functions best at neutral pH of 7.0. Although worms can lower pH of its medium by secreting calcium (Ca), it is suggestive to add ground limestone (calcium carbonate) powder to the bedding periodically. This would serve two purposes- maintain neutral pH and also supply the much needed calcium (Ca) to the worms for its metabolism.

6. Appropriate Carbon / Nitrogen (C/N) Ratio of the Feed Material

C/N ratio of the feed material affects the decomposition process by earthworms. A C/N ratio of 25 is considered good for vermicomposting of sewage sludge and reduction of volatile solids. Sewage sludge may have low C/N ratio due to higher nitrogen content and can affect vermicomposting adversely. C/N ratio can be improved by mixing of carbon rich 'bulking materials' such as straw and decaying leaves. This also prevents the loss of nitrogen (N) content by volatilization.

7. Adequate Supply of Calcium (Ca)

Calcium appears to be important mineral in worm biology (as calcarious tissues) and biodegradation activity. Although most organic waste including sewage sludge contains calcium, it is important to add some additional sources of calcium for good vermi-composting. Egg shells are good source of natural calcium. Occasionally limestone powder should be added.

ADVANTAGES OF VERMI-STABILIZATION OF SEWAGE SLUDGE BY EARTHWORMS

1. Nearly Odor-free Process

Earthworms create aerobic conditions in the sludge by their burrowing actions, inhibiting the action of anaerobic micro-organisms which release foul-smelling hydrogen sulfide and mercaptans. They also release anti-bacterial coelomic fluids in the decaying waste biomass which arrest growth of anaerobic pathogens which promotes rotting.

2. Destroy Pathogens

The earthworms release coelomic fluids in the waste biomass which have anti-bacterial properties that destroy all pathogens. (Pierre *et al.*,1982). Earthworms also devour the protozoa, bacteria and fungus as food. They seems to realize instinctively that anaerobic bacteria and fungi are undesirable and so feed upon them preferentially, thus arresting their proliferation. In the intestine of earthworms some bacteria & fungus (*Pencillium* and *Aspergillus*) have also been found. They produce 'antibiotics' and kills the pathogenic organisms in the sewage sludge making it virtually sterile. The removal of pathogens, faecal coliforms (*E.coli*), *Salmonella* spp., enteric viruses and helminth ova from sewage and sludge appear to be much more rapid when they are processed by *E. fetida*. Of all *E.coli* and *Salmonella* are greatly reduced. (Bajsa *et al.*, 2003).

Lotzof (2002) also revealed that the pathogens like enteric viruses, parasitic eggs and *E.coli* were reduced to safe levels in sludge vermicast. Cardoso and Remirez (2002) reported a 90 % removal of fecal coliforms and 100 % removal of heliminths from sewage sludge and water hyacinth after vermicomposting. Contreras-Ramos et, al., (2005) also confirmed that the earthworms reduced the population of *Salmonella* spp. to less than 3 CFU/gm of vermicomposted sludge. There were no fecal coliforms and *Shigella* spp. and no eggs of helminths in the treated sludge. (Eastman *et al.*,2001; Kumar & Sekaran, 2004). Our studies, Brahmbhatt (2006) has also confirmed complete removal of coliforms by earthworms.

> In conventional composting system pathogens are also removed, but due to increase in temperature. This is called 'thermophilic composting'. Vermiculture falls in the category of 'mesophilic composting' where composting do not increase temperature beyond 50· C.

3. Remove Heavy Metals and Toxic Chemicals

Earthworms can bio-accumulate high concentrations of metals without affecting their physiology and this particularly when the metals are mostly non-bioavailable.

Studies indicate that they can take up and accumulate in their tissues heavy metals such as cadmium (Cd), mercury (Hg), lead (Pb) copper (Cu), manganese (Mn), calcium (Ca), iron (Fe) and zinc (Zn). They can ingest and accumulate in their tissues extremely high amounts of zinc (Zn) and cadmium (Cd). Contreras-Ramos et, al.,(2005) also confirmed that the earthworms reduced the concentrations of chromium (Cr), copper (Cu), zinc (Zn) and lead (Pb) in the vermicomposted sludge (biosolids) below the limits set by the USEPA in 60 days.

Our studies at Griffith University, Brahmbhatt (2006) has also confirmed significant removal of lead (Pb) and cadmium (Cd) from vermicomposted sewage sludge. Some metals are bound by a protein called 'metallothioneins' found in earthworms which has very high capacity to bind metals. The chloragogen cells in earthworms appears to mainly accumulate heavy metals absorbed by the gut and their immobilization in the small spheroidal chloragosomes and debris vesicles that the cells contain. (Nelson *et al.*, 1982; Ireland, 1983).

Several studies have found definite relationship between 'organochlorine pesticide' residues in the soil and their amount in earthworms, with an average concentration factor (in earthworm tissues) of about 9 for all compounds and doses tested. (Ireland, 1983).

4. Low Greenhouse Gas (Methane) Emission

Composting of sewage sludge by worms decrease the proportion of 'anaerobic to aerobic decomposition', resulting in a significant decrease in methane (CH_4) and volatile sulfur compounds. (Mitchell *et al.*, 1980).

5. Mineralization of Nutrients from the Sludge

Earthworms accelerates the decomposition of the sludge and mineralization of the organic compounds in it. Most important is that earthworms mineralize the nitrogen (N) and phosphorus (P) in the sludge to make it bio-available to plants as nutrients. They ingest nitrogen from the sludge and excrete it in the mineral form as ammonium and muco-proteins. The ammonium in the soil is bio-transformed into nitrates. Phosphorus (P) contents increased in the vermicomposted sludge treated with earthworms but decreased in the samples without earthworms, while the nitrogen (N) content did not show much difference (Parvaresh, *et al.*, 2004). Elvira *et al.*, (1998) reported increase in the potassium (K) content of the sludge vermicompost.

6. Decrease in Total Organic Carbon (TOC) & Lowering of C/N Ratio of Sludge

This has significance when the composted sludge is added to soil as fertilizer. Plants cannot absorb and assimilate mineral nitrogen unless the carbon to nitrogen (C/N) ratio is about 20:1 or lower. Mineralization of organic matter in the sewage sludge by earthworms lead to significant decrease in total organic carbon (TOC) content thus lowering the C/N ratio. This they do by consuming and breaking carbon compounds during respiration. Elvira *et al.*, (1996) found that vermicomposting of paper-pulp-mill sewage sludge for 40 days decreased carbon (C) content by 1.7 fold. Contreras-Ramos *et al.*, (2005) found that carbon content decreased by 1.1 to 1.4 fold in two months. Our work (Brahambhatt, 2006) also indicated that earthworms reduce TOC from composted sludge.

7. Reduction in Volatile Solids (VS)

Maximum reduction of the volatile solids (VS) is the goal of any sludge stabilization system and reduced VS is an indicator of stabilized sludge. Study found that *Elsenia fetida* increases the rate of volatile solid sludge (VSS) destruction when present in aerobic sludge and this reduces the probability of putrefaction occurring in the sludge due to anaerobic conditions. (Loehr, et al,1998). Earthworms creates and maintains good aerobic conditions in the sludge due to its burrowing actions and this enhances the process of VSS destruction. Hartenstein and Hartenstein (1981) reported a 9 % reduction in volatile solids over 4 weeks of sludge vermicomposting by earthworms, which was higher than that of control by almost one-third. Fredrickson *et al.*, (1997) found a VS reduction of 30 % in compost after 4 months of conventional composting, whereas, the reduction was 37 % after only 2 months of vermicomposting. Higher decrease means more stable product and earthworms plays important role.

8. No or Low Energy Use in Vermi-stabilization Process

Conventional microbial composting requires energy for aeration (constant turning of sludge and even for mechanical airflow). Vermi-stabilization do not involve such use of energy. Occasionally turning may be required for aeration which can be done manually.

SOME STUDY ON SEWAGE SLUDGE STABILIZATION BY VERMICOMPOSTING

Contreras-Ramos *et al.* (2005) studied the vermicomposting of biosolids (dried sewage sludge) from various industries but mainly from textile industries and some households (municipal) mixed with cow manure and oat straw. 1,800, 1,400 and 1000 gms of aerobically digested biosolids were mixed with 800, 500 and 200 gms of cow manure and 200, 100 & 0 (zero) gms of oat straw in triplicate set up. A control was also kept with only biosolids. Cow manure was added to provide additional nutrients and the oat straw to provide bulk. 50 earthworms (weighing 40 gm live weight) were added in each sample and the species used was *Elsenia fetida*. They were vermicomposted at three different moisture contents - 60 %, 70 % and 80 % for two months (60 days). The best results were obtained with 1,800 g biosolids mixed with 800 g of cow manure and no (0) straw at 70 % moisture content. Volatile solids of the vermicompost decreased by 5 times, heavy metals concentrations and pathogens (with no coliforms) were below the limits set by USEPA (1995) for an excellent biosolid, carbon content decreased significantly due to mineralization of organic matter, and the number of earthworms increased by 1.2 fold.

EXPERIMENTAL STUDY OF SEWAGE SLUDGE (BIOSOLIDS) STABILIZATION BY EARTHWORMS AT GRIFFITH UNIVERSITY, BRISBANE, AUSTRALIA

Sewage sludge (biosolids) was obtained from local sewage treatment plant in Brisbane and earthworms were obtained from Bunnings hardware. It contained mixed species of *Elsenia fetida, Perionyx excavatus* and *Eudrillus eugeniae*. Cow dung was obtained from cattle farm in Ipswich. Both sludge and the cow manure was partially air dried for 5 days to prevent any methane & hydrogen sulfide generation that might harm the worms. Vermicompost was obtained from our vermiculture lab in the School of Environmental Engineering. Five sets of experimental bins (40 litre HDPE containers) with biosolids were prepared with one as CONTROL and were studied for morphological (color & texture), biological (pathogens) and chemical (heavy metals) changes over the 12 weeks period. The bins were organized as under and covered with moist newspaper and placed under shaded condition to prevent moisture loss. Hole was made in the bin bottom to drain any excess of water.

Experimental Set Up

Bin 1 : Biosolids (10 kg as CONTROL)

Bin 2 : Biosolids (10 kg) + Earthworms (250 numbers)

Bin 3 : Biosolids (10 kg) + Earthworms (250) + Cow Dung (5 kg)

Bin 4 : Biosolids (10 kg) + Cow Dung (5 kg)

Bin 5 : Biosolids (10 kg) + Organic Garden Soil (5 kg)

1. Bin 1 (control with only biosolids) was organized to know the fate of sludge upon 'natural ageing' with time and the natural microbial activity occurring in them even if they are not treated.
2. Bins 2 & 3, in which 250 worms were added was organized to know the fate of sludge upon vermi-composting and worm action on sludge if it has to feed only on sludge components (Bin 2) and when it is provided with additional feed materials (cow dung in Bin 3) which they love to feed upon.
3. Bins 4 & 5 were organized to know the fate of sludge upon conventional composting by 'enhanced microbial degradation' due to addition of cow dung and organic rich garden soil respectively. Both cow dung and organic soil are rich in decomposer microbes. Cow dung is also rich in nutrients for the microbes.

Objectives of the Study

1. To assess the rapidity of sludge stabilization and maturity under different composting methods;
2. To assess the status of heavy metals cadmium (Cd) and lead (Pb) in the untreated biosolids (upon natural ageing), in the vermicomposted and conventionally composted biosolids;
3. To assess the status of Coliforms and *E.coli* in the untreated biosolids (upon natural ageing), in the vermicomposted and conventionally composted biosolids;
4. To asses the % age of total organic carbon (TOC) in the untreated biosolids (upon natural ageing), in the vermicomposted and conventionally composted biosolids;

Important Observations

1. There was no significant changes in the Control Bin 1 up to several weeks. There was rapid moisture loss (and water had to be sprinkled frequently) and

foul odor continued up to week 12. This was just about 20 % stabilized by week 12.

2. Most significant and rapid changes were observed in Bins 2 and 3 which contained earthworms. Foul odor disappeared by week 2 and the color and texture of the sludge started changing like soil which is an indication of stabilization process that started in week 2. By week 12, the black and brittle biosolid became a homogenous and porous mass of brown vermicast with light texture. The worms were active and agile, they grew in size but not much in numbers. There was enough moisture and the biosolid appeared to be stabilized by over 80 % by week 12.
3. Worm activity was more vigorous in Bin 3 where cow dung was provided as additional feed for the worms. They worms were much healthier and more in numbers with several cocoons and baby worms indicating rapid rate of reproduction. By week 10, the black biosolid became a homogenous and porous mass of brown vermicast with lighter texture. There was greater retention of moisture. It was over 90 % stabilized by week 12.
4. The changes occurring in Bin 4 (biosolid mixed with cow dung) was slow, with odor continuing up to week 12. It was about 50 % stabilized by week 12.
5. The changes occurring in Bin 5 (biosolid mixed with organic soil) was also slow, with odor continuing up to week 12. This too, was about 40 % stabilized by week 12.

Findings

1. Status of Coliform Pathogens in Untreated (But Aged) Biosolids, Vermicomposted & Conventionally Composted (By Microbial Degradation) Biosolids: Test for pathogens in untreated (but aged) and vermicomposted biosolids were made by 'Colilert Reagent' which is a low-cost method with fast results indicating the presence (positive) and absence (negative) of coliforms in a medium. White granules of Colilert powder is added to the samples and incubated for 24 hrs at 35· C. The samples are tested under Ultraviolet (UV) Lamp for color changes after 24 but before 28 hours. Yellow colour indicates the presence of total coliforms and the yellow colour with fluorescent indicates the presence of *E.coli*. If there is no color change, it is free of pathogens.

(1) One gram of sample from Bin 1 (12 weeks aged biosolid) was taken in a polycarbonate sterile container and diluted with 100 ml of sterile water in a laminar flow machine and tested under UV lamp. The colour was yellow with fluorescence indicating the presence of total coliforms in the untreated biosolids even after ageing for 12 weeks;

(2) One gram sample from Bins 2 and 3 (vermicomposted biosolids) were taken and subjected to same operation. There was NO COLOUR change indicating complete absence of coliforms and *E.coli*.

(3) One gram sample from Bins 4 and 5 (composted biosolids) were taken and subjected to same operation. There was change in color (yellow) indicating the presence of coliforms and *E.coli.*

TABLE 1

Status of Coliforms in the Untreated (Control), Vermicomposted and Conventionally Composted (By Microbial Degradation) Biosolids After 12 Weeks

Nature of Sample Tested	Time and Percent of Colilert Test	Stabilization Under UV Lamp
1. Biosolids (CONTROL)	(12 Wks) 20%	Positive
2. VC Biosolids by Earthworms	(12 Wks) 80%	Negative
3. VC Biosolids by Earthworms + Cow Dung	(12 Wks) 90%	Negative
4. *Composted Biosolids by Cow Dung	(12 Wks) 50%	Positive
5. *Composted Biosolids by Organic Soil	(12 Wks) 40%	Positive

VC= Vermicomposting; * Microbial Composting

Interpretation of the Results and Discussion

Results clearly shows that the earthworms significantly reduce or almost eliminates the pathogens from the digested (composted) sludge (biosolids). Biosolids treated with earthworms (with or without feed materials) only showed 'negative results' by the Colilert test under the UV lamp. And this was achieved in just 12 weeks.

As discussed above, the earthworms release coelomic fluids in the waste biomass which have anti-bacterial properties that destroy all pathogens. (Pierre *et al.*,1982). They also devour the protozoa, bacteria and fungus as food. Bacterium *Pencillium* and fungus *Aspergillus* present in the gut of earthworms produce 'antibiotics' that kills the pathogens.

It also infers that under the conventional composting systems by enhanced microbial degradation the pathogens will remain in the biosolids for longer period of time until it is completely dry with all food and moisture exhausted making them difficult to survive. That is why they are classed as 'biohazard' and landfilled or incinerated.

2. Status of Heavy Metals Cd & Pb in Untreated (But Aged) Biosolids, Vermicomposted & Conventionally Composted (By Microbial Degradation) Biosolids: Test for presence of heavy metals cadmium (Cd) and lead (Pb) were done by using AA Spectrophotometer. Samples were heated in oven at 60· C for 48 hours, cooled in dessicators and grinded by hand to 1 mm particle size. 1 gm of air dry sample was digested with 5 ml of concentrated nitric acid (HNO_3) in 75 ml digestion tube for 3 hours at 145 ·C. The solution was allowed to cool down for 48 hours after which it was filtered through glass fiber in a funnel into 25 ml volumetric flask. The solution is then transferred into 50 ml Falcon Tubes which is centrifuged at 3000 rpm and the supernatant is tested for heavy metals in the AAS.

TABLE 2

Status of Heavy Metals Cadmium (Cd) & Lead (Pb) in the Untreated (Control), Vermicomposted & Conventionally Composted Biosolids (mg/kg of soil) in 12 Weeks

Nature of Sample Tested	Cd	(% Reduction)	Lead	(% Reduction)
1. Untreated (Aged) Biosolids (12 Weeks)	2.6	(-)	83.2	(-)
2. Vermicomposted Biosolids by by Earthworms (12 Wks)	2.1	(19.23 %)	67.2	(19.2 %)
3. Vermicomposted Biosolids by Earthworms With Cow Dung (12 Wks)	1.1	(57.70 %)	37.1	(55.4 %)
4. *Composted Biosolids by Mixing With Cow Dung (12 Wks)	2.6	(NC)	83.2	(NC)
5. *Composted Biosolids by Mixing With Organic Soil (12 Wks)	2.6	(NC)	83.2	(NC)

* Conventional Composting by Microbial Degradation; NC = No Change in Value

Interpretation of the Results and Discussion

Results clearly show that the earthworms reduce the heavy metals cadmium (Cd) and lead (Pb) from the digested sludge. There was no change in the values of heavy metals between the untreated sludge (Bin 1) and those treated by adding cow dung and by organic soil (Bins 4 and 5) to enhance microbial composting. Although biosolids can be slowly stabilized by microbial degradation over a period of time, the heavy metals will remain in the system for quite sometimes after which it may leach into soil or get bound with soil organics by chemical reactions occurring in the soil.

Though there was not very significant removal of heavy metals by earthworms in the 12 weeks of experiment yet their role in heavy metal removal cannot be undermined. Providing additional feed materials (Bin 3) enhanced worm activity and also their number (stimulating reproduction) and led to greater removal of heavy metals. It infers that over a period of time and with enhanced worm activity the heavy metals can be completely removed from the biosolids.

Of all the metals Cd and Pb appears to bio-accumulate in most species of earthworms at greater level. They can particularly ingest and bio-accumulate extremely high amounts of cadmium (Cd) which is very mobile and may be readily incorporated into soft and non-calcareous tissues of earthworms. Cadmium levels up to 100 mg per kg dry weight have been found in tissues. Ireland (1983) found that cadmium (Cd) and lead (Pb) are particularly concentrated in chloragogen cells in *L. terrestris* and *D. rubidus*, where it is bound in the form of Cd-metallothioneins & Pb-metallothioneins respectively (i.e. bio-transformed) with small amounts deposited in waste nodules.

3. Status of Total Organic Carbon (TOC) in Untreated (But Aged) Biosolids, Vermicomposted & Conventionally Composted (By Microbial Action) Biosolids : Total organic carbon (TOC) was measured by heating 3 gm of samples in crucibles (whose dead weight was recorded) in oven at 500· C for one hour so that all the carbon containing organic matters got volatilized from the samples. They were then cooled on dessicators for 30 minutes. The weight of the samples were recorded again to determine the amount of carbon contents that volatilized. The difference in initial and final weights gave the value total organic carbon in the samples.

TABLE 3

Total Organic Carbon (TOC) in the Untreated (Control), Vermicomposted and Conventionally Composted (By Microbial Action) Biosolids in 12 Weeks

Nature of Sample Tested	TOC (%)
1. Untreated (Aged) Biosolids (12 Weeks)	64.15
2. Vermicomposted Biosolids by Earthworms (12 Wks)	52.36
3. Vermicomposted Biosolids by Earthworms With Cow Dung (12 Wks)	41.36
4. *Composted Biosolids by Mixing With Cow Dung (12 Wks)	58.16
5. *Composted Biosolids by Mixing With Organic Soil (12 Wks)	58.18

* Microbial Composting

Interpretation of the Results and Discussion

Results clearly shows that vermicomposting of biosolids by earthworms can reduce the total organic carbon of sludge (which has very high carbon content) in much lesser time. This has significance in lowering the C/N ratio so that nitrogen can be made bio-available to the plants.

ADVANTAGES OF VERMI-STABILIZATION OF SLUDGE BY EARTHWORMS

More Homogenous End Products Rich in Nutrients

The advantages for employing vermi-composting to process and stabilize sewage sludge over conventional composting by microbial action are that the end product are more homogenous, rich in plant nutrients and the levels of contaminants are significantly reduced. Lotzof, 1999). McCarthy (2002) asserts that vermicomposting of sewage sludge has converted them into an end product that is safe for agricultural use. Vermicompost has very 'high porosity', 'aeration', 'drainage' and 'water holding capacity' and also contains 'plant-available nutrients'. The resulting product appears to retain more nutrients for longer period of time and also greatly increases the water holding capacity of the farm soil (Hartenstein & Hartenstein, 1981; Appelhof, 1997).

ENVIRONMENTAL-ECONOMICS (EE) OF VERMISTABILIZATION OF SLUDGE : CONVERTING BIOHAZARDOUS WASTE INTO WEALTH

Earthworms in dense population (high biomass) can physically handle most organic wastes municipal and industrial, and potentially at a fraction of the cost of conventional methods of solid waste management by landfill disposal. Moreover, landfills are not only an economic but also proving to be an environmental burden (emitting greenhouse and toxic gases & threat of groundwater contamination by leachate discharge).

Vermicomposting technology by earthworms is a self-promoted, self-regulated, self-improved, self-driven, self-powered & self-enhanced, low or no energy requiring zero-waste technology, easy to construct, operate and maintain. Any vermiculture technology involves about 100-1000 times higher 'value addition' than other biological technologies. Obtaining earthworms from vermiculture farms would be one-time cost in any vermiculture technology as the earthworms multiply rapidly creating huge army of worms which further promote and enhance the process.

The greatest environmental-economics working in case of vermi-stabilization of sewage & water treatment sludge is in the savings incurred in the reduction of landfills as the sludge which is a 'biohazard' has to be necessarily disposed in landfills for environmental safety. Some nations incinerate sewage sludge and the cost of incineration is both economic (in terms of high use of energy) and environmental (in terms of pollution). Komarowski (2001), reports that Australia is saving over 13,000 cum of landfill space every year by vermicomposting. The upfront cost of construction of an average secured landfill is estimated to be between US $ 15-25 millions before the first load of waste is dumped. This explains the dollar value of diverting every cubic meter of sludge from the landfills by vermicomposting.

Environmental-economics also work with respect to production and sale of the 'vermicompost' and the 'worm biomass' and the economic and environmental benefits that accrue from them. This has been discussed above. Both live worms and its excreta (vermicast) in soil tremendously improve its fertility and the productivity is simply doubled or even tripled. The Hobart City Council in Tasmania, Australia, stabilize about 66 cum of municipal sewage sludge by earthworms every week and about 44 cum becomes 'vermi-compost'. The Council is saving AU $ 56,000 per year just from avoiding landfill disposal and earning an equal amount (about $ 55,000) from the sale of vermi-compost to public. (Datar *et al.*, 1997). Redland Shire in Queensland, Australia, vermi-composted 400-500 tons of sludge every week and produced over 200 tons of vermicast per week. (Lotzof, 2000). It was reported to be saving over $ 50,000 every year and earning an equal amount from the sale of vermicompost. Earthworms not only converts 'waste' into 'wealth' it itself becomes a valuable asset.

CONCLUDING REMARKS

Earthworms have real potential to both increase the rate of aerobic decomposition and composting of organic matter in the sewage sludge and also to stabilize the organic residues - removing the harmful pathogens and heavy metals from them. It may not be able to remove the toxic substances completely, but at least change the 'chemical make-up' of the sludge to make it harmless to the soil. This method have been found to comply with grade A standards for sludge stabilization (Basja, *et al.*, 2003).

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14

VERMIREMEDIATION OF POLLUTED LANDS BY EARTHWORMS

A Low-Cost Nature's Biotechnology for Combating Soil Pollution for Land Reclamation With Fertility Improvement : Converting Wastelands into Wonderlands Through Researches into EBT

KEY WORDS

Earthworms - Detoxifying Agents in Nature; Earthworms Bio-accumulate Toxic Chemicals; Chloragogen Cells - Special Cells for Storage of Heavy Metals; Earthworms Biodegrade Toxic Chemicals into Simpler Compounds : Earthworms Bio-transform Toxic Chemicals into Harmless Compounds; Earthworms Immobilize Soil Contaminants; Earthworms Convert Wastelands into Wonderland;

BACKGROUND INFORMATION

Large tract of arable land is being chemically contaminated due to mining activities, heavy use of agro-chemicals in farmlands, landfill disposal of toxic wastes and other developmental activities like oil and gas drilling. No farmland of world especially in the developing nations are free of toxic pesticides, mainly aldrin, chlordane, dieldrin, endrin, heptachlor, mirex and toxaphene. According to National Environment Protection Council there are over 80,000 contaminated sites in Australia-30,000 in NSW, another 30,000 in Queensland, 10,000 in Victoria, 4000 in South Australia, another 4000 in Western Australia, 1000 in Northern Territory and 500 each in Tasmania and the Australian Capital Territory (ACT). There are 40,000 contaminated sites in US; 55,000 in just six European countries and 7,800 in New Zealand. There are about 3 million contaminated sites in the Asia-Pacific. These also include the abandoned mine sites along with the closed landfills. The contaminated sites mostly contain heavy metals cadmium (Cd), lead (Pb), mercury (Hg), zinc (Zn) etc. and chlorinated compounds like the PCBs and DDT. Cleaning them up mechanically by excavating the huge mass of contaminated soils and disposing them in secured landfills will require billions of dollars. There is also great risk of their leaching underground (aggravated by heavy rains) and contaminating the groundwater.

> Vermiremediation biotechnology using earthworms is a low-cost and convenient technology for combating soil and land pollution. Earthworms in general (specially *E. fetida*) are highly resistant to many chemical contaminants including heavy metals and organic pollutants in soil and have been reported to bio-accumulate them in their tissues. Certain species of earthworms such as *Elsenia fetida, Aporrectodea tuberculata, Lumbricus terrestris, L. rubellus, Dendrobaena rubida, D. veneta, Eiseniella tetraedra, Allobophora chlorotica* have been found to remove heavy metals, pesticides and lipophilic organic micropollutants like the polycyclic aromatic hydrocarbons (PAH) from the soil (Contreras-Ramos et. al, 2006). *E. fetida* has been used as the test organisms for different soil contaminants and several reports indicate that *E. fetida* tolerates 1.5% crude oil while *L. terrestris* did not survive in 0.5% of it (OECD, 2000; Safwat *et al.*, 2002). Earthworms also inhibit the soil borne pathogens and work as a detoxifying agent for polluted soil. It reduces *Salmonella* population in soil to about 30%.

EARTHWORMS IMPROVES QUALITY OF SOIL AND LAND IN TOTALITY

Significantly, vermiremediation leads to total improvement in the quality of soil and land where they inhabit. During the vermi-remediation process, the population of earthworms increases significantly benefiting the soil in several ways. A 'wasteland' is transformed into 'wonderland'.

Earthworms Bio-accumulate Heavy Metals from Soil

Earthworms can bio-accumulate high concentrations of metals including heavy metals in their tissues without affecting their physiology and this particularly when the metals are mostly non-bioavailable. Studies indicate that earthworms can take up and bio-accumulate heavy metals like cadmium (Cd), mercury (Hg), lead (Pb) copper (Cu), manganese (Mn), calcium (Ca), iron (Fe) and zinc (Zn). They can particularly ingest and accumulate extremely high amounts of zinc (Zn) and cadmium (Cd).(Hartenstein *et al.*, 1980). Cadmium is very mobile and may be readily incorporated into soft and non-calcareous tissues of earthworms. Cadmium levels up to 100 mg per kg dry weight have been found in tissues. Of all the metals Cd and Pb appears to accumulate in most species of earthworms at greater level.

Earthworms collected from the roadsides and mining sites show higher amounts of heavy metals than those from the other sites. Thus earthworms can also work as a 'bioindicator' of heavy metal contamination in soil.

Earthworms Bio-accumulate Organochlorine Pesticides & Polycyclic Aromatic Hydrocarbons from Soil

A study found that after only one application of the pesticide aldrin to soil, more than 34% was found to be present in the soil 5 years later. Most of the remaining 66% must therefore have remained in soil, either as unchanged aldrin or in the form of other closely related chemicals formed by the decomposition of aldrin. Several studies have found that earthworms can also either accumulate or degrade 'organochlorine pesticide' and 'polycyclic aromatic hydrocarbons' (PAHs) residues in the medium in which it feeds. (Ireland, 1983).Several studies have found definite relationship between 'organochlorine pesticide' residues in the soil and their amount in earthworms, with an average concentration factor (in earthworm tissues) of about 9 for all compounds and doses tested. (Davis, 1971).

MECHANISM OF WORM ACTION IN VERMIREMEDIATION : THE UPTAKE OF CHEMICALS FROM SOIL AND IMMOBILIZATION BY EARTHWORMS & REINFORCEMENT OF MICROBIAL ACTION

Earthworms uptake chemicals from the soil through passive 'absorption' of the dissolved fraction through the moist 'body wall' in the interstitial water and also by mouth and 'intestinal uptake' while the soil passes through the gut. Earthworms eat large volume of soil with microbes and organic matter during the course of their life in soil. Earthworms apparently possess a number of mechanisms for uptake, immobilization and excretion of heavy metals and other chemicals. They either 'bio-transform' or 'biodegrade' the chemical contaminants rendering them harmless in their bodies.

1. Biotransformation of Chemical Contaminants in Soil

Some metals are bound by a protein called 'metallothioneins' found in earthworms which has very high capacity to bind metals. Ireland (1979) found that cadmium (Cd) and lead (Pb) are particularly concentrated in chloragogen cells in *L. terrestris* and *D. rubidus*, where it is bound in the form of Cd-metallothioneins & Pb-metallothioneins respectively (i.e. bio-transformed) with small amounts deposited in waste nodules. The chloragogen cells in earthworms appears to mainly accumulate heavy metals absorbed by the gut and their immobilization in the small spheroidal chloragosomes and debris vesicles that the cells contain. After removal of the gut contents, the tissues in *L. terrestris* contained 90-180 mg lead /gm dry weight. In *L.rubellus* and *D. rubida* it was 2600 mg /gm and 7600 mg / gm dry weight. Zn, Mn, and Fe were shown to be excreted through the calciferous glands. (Ireland, 1983).

2. Biodegradation of Chemical Contaminants in Soil : Earthworms Promote Microbial Activity for Biodegradation

Ma *et al.*, (1995) found that earthworms biodegrade organic contaminants like phthalate, phenanthrene and fluoranthene. It may be noted that several soil microorganisms especially bacteria and fungi also biodegrade several categories of chemicals including hydrocarbons in soil. However, when earthworms are added to the soil they further stimulate and accelerate microbial activity by increasing the population of soil microorganisms and also through improving aeration (by burrowing actions) in the soil, and in totality enhance the rate of biodegradation.

Many soils contain abundance of pores with diameters of 20 nm or less. Such pores are too small to allow the smallest bacterium (1 um), protozoa (10 um) or root hairs (7 um) to penetrate and attack the chemicals. A chemical contaminant residing in such fine pores in soil is thus completely protected from attack by a microbe in the soil for biodegradation action. In other words such chemical contaminants are not 'bio-available' for any biological action. Earthworms play a very important and critical role here by enlarging the pores through continuous 'burrowing actions' in the soil, thus allowing the microbes to enter into the pores and act on the contaminants. It also stimulate the population of decomposer microbes to several folds for enhanced biodegradation action. The 'gizzard' in the earthworms helps to grind the food very thoroughly with the help of tiny stones swallowed by the worms into smaller particles 2-4 µm in size. This grinding action may serve to make the chemical contaminants sequestered in the soil 'bio-available' to decomposer microbes for degradation.

EXPERIMENTAL STUDY OF VERMIREMEDIATION OF SOIL CONTAMINATED BY PAHs AT GRIFFITH UNIVERSITY, BRISBANE

This study was carried out by bachelor students of environmental engineering for their 30 CP projects under the supervision of author. PAHs contaminated soil was obtained from a former gas works site in Brisbane where gas was being produced from coal. The initial concentration of total PAHs compounds in the soil at site was greater than 11,820 mg/kg of soil. (Ryan, 2006). The legislative requirements for soil PAHs concentration is only 100 mg/kg for industrial sites and 20 mg/kg for residential sites.

As described in earlier chapters, PAHs are group of over 100 chemicals of which anthracene, benzo (a) pyrene, naphthalene, pyrene are common. US EPA has identified 16 PAHs as 'priority pollutants' with potential for high 'bio-magnification', and therefore, toxic to living organisms. Earthworms have been found to accelerate the process of PAH biodegradation by stimulating and reinforcing microbial activity in soil while also removing them by passive absorption and bioaccumulation. (Eijsackers *et al.*, 2001; Tang *et al.*, 2002).

Earlier Studies

Contreras-Ramos et.al, (2006) studied the uptake of three PAHs viz. phenanthrene, anthracene and benzo(a)pyrene at different concentrations by *E. fetida* and measured the PAHs concentrations in the soil and in the tissues of earthworms exposed to the PAHs for 11 weeks. 10 earthworms per 50 gms of soil (equivalent to 200 worms per kg of soil) was added and sufficient moisture was maintained. The concentration of anthracene decreased by 2-fold after addition of earthworms and the average removal was 51% which was only 23% by microbes alone when the earthworms were not added to the soil. On an average the concentration of benzo(a)pyrene decreased by 1.4- fold and the average removal was 47% which was only 13% by microbes when earthworms were not present. Phenanthrene was completely removed (100%) by earthworms when the amount of the chemical was < 100 mg/kg of soil, while only 77% was removed by microbes in absence of earthworms. However, no earthworms survived when the chemical was added at the rate of 150 mg/kg of soil.

Earthworms Bio-accumulate PAH Compounds

Earthworms have been found to bio-accumulate the PAH compounds in the fatty deposits in their body (Contreaus-Ramos *et al.*, 2006). Parish *et al.*, (2005) studied the bio-accumulation of PAHs in earthworms from contaminated soils obtained from disused manufacturing gas plant and found that earthworms readily accumulated 3 to 4 ring PAH compounds but not above that.

Hydrophobic organic contaminants are taken up by the earthworms in two ways—

(i) By passive diffusion from the soil solution through the worms outer membrane;

(ii) By intestinal re-sorption of the compounds from the soil while it passes through the gut (by digestion) and then their degradation by enzymatic activity called 'Cytochrome P 450' system. This enzymatic activity have been found to operate particularly in *Elsenia fetida* which survive the benzo(a)pyrene concentration of 1,008 mg/kg of soil (Achazi et al,1998).

The passive diffusion is driven by the difference between the pore water in soil and within the earthworm's tissues (Jager et al, 2003). The accumulation increases when the concentration of PAHs in their surrounding soil water or in their food increases (Belfroid et, al. 1995). Johnsen *et al.*, (2005) also reported that the earthworms may take PAHs up through absorption by the body surface and also by feeding and ingestion, since PAHs sorb to the soil organic detritus, which the worms feed on.

Earthworms Promote PAHs Biodegradation by Microbes

Biodegradation of PAHs occur when microorganisms break the aromatic rings of benzene and produce aliphatic compounds that readily enters the tricarboxylic acid cycle (metabolic activity) operating in living cells. *Cunniughamela elegans* and *Candida tropicalis* have been reported to degrade PAHs. (Kanaly & Harayama, 2000). Degradation products of PAHs are, however, not necessarily less toxic than the parent compounds.

Singleton *et al.* (2003) studied the bacterial flora associated with the intestine and vermicasts of the earthworms and found species like *Pseudomonas, Paenibacillus, Azoarcus, Burkholderia, Spiroplasm, Acaligenes* and *Acidobacterium*. Some of them, such as *Pseudomonas, Acaligenes* and *Acidobacterium* are known to degrade hydrocarbons. Some fungi such as *Pencillium, Mucor* and *Aspergillus* have also been found in the intestine of earthworms and they degrade hydrocarbons. *Acaligenes* can even degrade PCBs and *Mucor* dieldrin. (Johnsen *et al.*, 2005).

TABLE 1

Removal of Soil PAH by Earthworms (*Elsenia fetida*) and by Microbial Degradation (Contamination < 100 mg/kg of Soil With 10 Worms / 50 gm of Soil for 11 Weeks)

PAH	Soil With Earthworms		Soil Without Earthworms (Microbial Action)
	Amount Decrease	% Removal	% Removal
1. Anthracene	2 fold	51%	23%
2. Benzo(a) pyrene	1.4 fold	47%	13%
3. Phenanthrene	Complete	100%	77%

Source : Ma *et al.* (1995)

Ma *et al.* (1995) also found that the removal of phenanthrene and fluoranthene (also a PAH) in soil was accelerated by earthworms. After 56 days (8 weeks), 86% of the phenanthrene was removed.

Experimental Set Up

10 Kg of contaminated soil was taken in each of the four 40 litre black HDPE containers and made into three sets 1,2, 3 and 4. The first remained as control and no treatment was done. In the second container approximately 500 earthworms (mixed species of *E.fetida, Perionyx excavatus* and *Eudrillus euginae*) of varying ages and sizes were added to the soil. (Worms were contained in about 2 kg of primary feed materials bought from Bunning Hardware). To this was added 5 kg of semi-dried cow dung as secondary feed material. In the third container 5 kg of kitchen waste organics were added as secondary feed material to the 500 worms. In the fourth container only 5 kg of organic compost was added to the contaminated soil and no worms. This was set up to assess the effect of only microbial action on the contaminated soil as any organic compost is known to contain enormous amount of decomposer microbes.

In all the four containers enough water was added time to time, to maintain the moisture content between 70-80% and were allowed to stand for 12 weeks. They were kept under shade thoroughly covered with thick and moist newspapers to prevent any volatilization or photolysis of the PAH compounds in the soil.

Thus, containers 2 and 3 had total of 17 kg materials (10 kg contaminated soil + 2 kg of primary feed materials with worms + 5 kg of secondary feed material added additionally). Container 4 had 15 kg (10 kg soil + 5 kg compost). Due to addition of feed materials (cow dung and kitchen waste) and compost in the contaminated soil significant dilution of PAH compounds are expected to be made and has to be taken into consideration while determining the impact of earthworms and the microbes in the removal of PAH compounds.

Extraction of PAHs from the Contaminated Soil and the Instruments & Methods Used in Analysis and Quantification of PAH Compounds

The extraction of PAHs from soil samples was carried out by the Soxhlet extraction method. Five (5) grams of soil from each container - the first untreated and used as 'control', the second treated by earthworms with cow dung as feed material and the third treated by earthworms with kitchen waste as feed material was added to 5 grams of anhydrous sodium sulphate and taken in a glass extraction thimble. This was placed into an extraction chamber which then underwent 3 hours of extraction using acetonitrile. After extraction, the samples are cleaned up (purified) to remove any unwanted contaminants.

The gas chromatography-mass spectrometer (GS-MS) and high performance liquid chromatograph (HPLC) which utilize UV - visible or fluorescent light detection, were the two main types of instruments were used to quantify the extracted PAHs (Ryan, 2006). The chromatograph was calibrated by running 100 ppb standard solution (for 35 mins) through the machine which contained known PAH compounds like benzo (a) pyrene, benzo (a) anthracene, chrysene, benzo (b) flouranthene, benzo (k) flouranthene, anthracene, pyrene, phenthracene, flouranthene, dibenzo (1,2.3,c,d) pyrene and benzo (g,h,i) pyrene. Different PAHs had different retention times of light (wavelength 295 nm) with the heavy molecular weight PAH taking longer time to pass through the Hypersil column. The different retention times were used to determine the type of PAH compound in the soil solution after comparing with known standard ones. Only seven PAH compounds indicated in table -2 were quantified. Dibenzo (a,h) pyrene and benzo (g,h,i) pyrene had identical retention time and hence the values have been entered together.

Observations

1. There was no noticeable change in the contaminated soil kept as control in container 1 over the 12 weeks period. There was continuous emission of hydrocarbon odour till the end of the experiment;
2. Earthworms were very active in container 2 and the hydrocarbon odour disappeared after 2-3 weeks. The soil color slowly changed to brown and became more porous and lighter due to formation of worm vermicast;
3. The soil in container 3 containing kitchen waste as feed material was invaded by moulds (fungus) and maggots (as it contained some meat products) which disappeared after few weeks. Worms were active and the hydrocarbon odour disappeared after some times. Here too, the soil color slowly changed into brown and became more porous and lighter due to formation of worm vermicast.
4. The soil in container 4 appeared little brown from the very beginning due to addition of compost but there was no apparent change. Hydrocarbon odor disappeared slowly.

Results and Discussion

Results of the experiment is tabulated in tables 2 and 3.

TABLE 2

Removal of Some PAH Compounds from Contaminated Soil by Earthworms Provided With Different Feed Materials

(10 Kg Contaminated Soil + 500 Worms* in 2 kg Feed Materials With Additional Feed Materials Cow Dung (5kg) & Kitchen Waste (5kg) for 12 Wks) and compost (5Kg).

Extracted PAH Compounds	Set-1 Only Soil (CONTROL) Initial Value (mg/kg)	Set-2 Soil+Worms +Cow Dung After 12 weeks (mg/kg)	Set-3 Soil+Worms +Kitchen Waste After 12 weeks (mg/kg)	Set-4 Soil+Compost (NO WORMS) After 12 weeks (mg/kg)
1. Benzo (a) anthracene	3945	929	1128	2476
2. Chrysene	2694	897	456	1589
3. Benzo (b) flouranthene	3067	318	101	1087
4. Benzo (k) flouranthene	315	31	64	189
5. Benzo (a) pyrene	726	83	162	367
6. Dibenzo (a,h) pyrene & Benzo (g,h,i) pyrene	1072	179	492	497
Total Measure	**11,819**	**2,437**	**2,403**	**6,205**

* Mixed species of worms (*E.fetida, Perionyx excavatus & Eudrillus eugeniae*) were used

TABLE 3

Percent Removal of Some PAH Compounds from Contaminated Soil by Earthworms Provided With Different Feed Materials

(10 Kg Contaminated Soil + 500 Worms* in 2 kg Feed Materials With Additional Feed Materials Cow Dung (5kg) & Kitchen Waste (5kg) for 12 Wks)

Extracted PAH Compounds	Set - 2 Soil + Worms + Cow Dung After 12 weeks		Set - 3 Soil + Worms + Kitchen Waste After 12 weeks		Set - 4 Soil + Compost (NO WORMS) After 12 weeks	
1. Benzo (a) anthracene	76%	(58%)	71%	(56%)	37%	(6%)
2. Chrysene	67%	(49%)	83%	(68%)	41%	(12%)
3. Benzo (b) flouranthene	90%	(72%)	97%	(82%)	65%	(47%)
4. Benzo (k) flouranthene	90%	(72%)	80%	(65%)	40%	(10%)
5. Benzo (a) pyrene	89%	(71%)	78%	(63%)	49%	(24%)
6. Dibenzo (a,h) pyrene & Benzo (g,h,i) pyrene	83%	(65%)	54%	(39%)	54%	(30%)
Av. =	**79%**	**(61%)**	**80%**	**(65%)**	**47.5%**	**(21%)**

(%) Values within bracket are those after taking the dilution factor (due to mixing of feed materials) into account. This is just in 12 weeks period.

Results confirm the decisive role of earthworms in PAH removal which can be by both activities - bioaccumulation, and by promoting microbial activities. When microbial activity in the soil is enhanced alone (without aided by earthworms) by adding microbe rich organic compost to the soil (Set 4) the removal rate of PAHs are not very significant. This indicates that earthworms acts in a different manner and contributes decomposer microbes for hydrocarbons which otherwise is normally not available in soil or in ordinary compost. Singleton *et al.* (2003) has reported some 'uncultured' bacterial flora tightly associated with the intestine of the earthworms. Some of them, such as *Pseudomonas, Acaligenes* and *Acidobacterium* are known to degrade hydrocarbons.

Providing the earthworms with additional feed materials (in the form of cow dung and kitchen waste - both organics) must have played important role in raising worm activity and in its reproductive behavior, and also in stimulating the soil microbial activity. There was not much significant difference in the impact of two types of feeds. Ma *et al.* (1995) showed an increase in PAH loss from polluted soil when the worms were denied of any additional source of food. They concluded that earthworms increase oral intake of soil particles when driven by 'hunger stress' and consequently ingested more PAHs polluted soil. While this may be a temporary phenomenon in short time (9 weeks of study) it can never be a long-term strategy because any bioremediation treatment of polluted soil is time-taking - in months and years. Worms would starve and die by then. And, adding organic feed materials to the polluted soil has several other advantages. It promotes microbial activities in soil and when the worms ingest them and excrete - the excreted products are nutrient rich organic fertilizers.

ENVIRONMENTAL-ECONOMICS OF VERMIREMEDIATION TECHNOLOGY: CONVERTING 'WASTELAND' INTO 'WONDERLAND'

Vermiremediation technology by earthworms is a self-promoted, self-regulated, self-improved, self-driven, self-powered & self-enhanced, low or no energy requiring zero-waste technology, easy to construct, operate and maintain. Any vermiculture technology involves about 100-1000 times higher 'value addition' than other biological technologies. Obtaining earthworms from vermiculture farms would be one-time cost in any vermiremediation technology as the earthworms multiply rapidly creating huge army of worms which further promote and enhance the process.

Vermiremediation of chemically polluted / contaminated soils / lands would cost about $ 500 - 1000 per hectare as compared to $ 10,00C - 15,000 per hectare by mechanical excavation of contaminated soil & its landfill disposal. Of considerable economic and environmental significance is that the worm feed used in vermiremediation process is necessarily an 'organic waste' product. This means that it would also lead to reuse and recycling of vast amount of organic wastes which otherwise end up in landfills for disposal at high cost. And what is of still greater economic and environmental significance is that the polluted land is not only 'cleaned-up' but also 'improved in quality'. Earthworms improves the fertility of soil by adding its excreta - the vermicast (as vermicompost) which contains rich nutrients (NKP and micronutrients), enzymes and growth hormones and beneficial nitrogen-fixing and decomposer microbes. The soil becomes lighter and porous rich in biological activities and the productivity is increased to several times. Earthworms not only convert the 'wasteland' into 'wonderland' it itself becomes a valuable asset. Vermiculture is a flourishing industry all over the world today.

CONCLUDING REMARKS

Earthworms have great potential in removing hydrocarbons from contaminated soil, even the PAHs like benzo(a)pyrene which is very resistant to degradation. They are extremely resistant to toxic PAHs and tolerate concentrations normally not encountered in the soil. It is important to mention here that nearly 80% removal (60-65% if the dilution factor is taken into account) of seven important PAH compounds was achieved in just 12 weeks period and that too with only about 500 worms (of both mature and juvenile population) in 10 kg of soil (50 worms/kg of soil). And this was during the winter season in Brisbane (March - May 2006) when the biological activities of worms are the lowest ebb. Increasing the number of worms per kg of contaminated soil to about 100 mature adult worm /kg of soil, and the time of remediation up to 16 weeks could have completely (100%) removed the PAH compounds. Contreras-Ramos et. al, (2006) studied with 10 worms / 50 gms of contaminated soil (which is equivalent to 200 worms/kg of soil) in about 11 weeks and got 50-100% removal of some PAHs.

Vermi-remediation may prove very cost-effective and environmentally sustainable way to treat polluted soils and sites contaminated with hydrocarbons in just few weeks to months. With the passage of time, the remedial action is greatly intensified. As the worms multiply at an enormous rate it can quickly achieve a huge arsenal for enhanced degradation of PAHs in much shorter time. Comparing the cost incurred in mechanical treatment by excavation of contaminated soils and their safe dumping in secured landfills (as hazardous wastes), this technology is most economic. More study will be needed on the PAH removal activities of earthworms with and without additional feed materials and upon different categories and doses of organic feed.

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SECTION-IV

RENEWABLE BIOLOGICAL RESOURCES

Biological Products For Environmental Safety, Human Health & Sustainability : Achievements of Researches into Environmental Biotechnology

Biological resources are 'heavenly assets' and a great gift of nature to mankind. Biological resources has ceased to be a challenge only for the mere preservation of threatened species. It is a multi-sectorial issue and now looked upon more as the scientific challenge for bioprospecting of the 'biological resources' (specially the wild genetic resources) of world for the social and economic gains and progress of mankind and for the eradication of poverty, disease and hunger. Wild species and their genetic variations contribute worth millions of dollars to agriculture, medicine, and other industries as biological raw materials every year.

We use biological raw materials from the wild species every time we apply a shampoo or a sunscreen, every time we paint a wall or varnish a table, every time we use a golf ball or jet engine, or oil drilling equipment, every time we employ goods containing tin-plate or glycerine, or every time we get to the doctor to take vaccine or to the dentist to make a mould of teeth. Each time we take a medicine, there is one chance in two, that our purchase owes its origin, in some way or other, to start point materials from the wild plants from forest, wetland, or the ocean. The product may be an analgesic, an antibiotic, a diuretic, a laxative, a tranquilliser, or a cough postile. The importance of 'morphine' and 'codeine' in human life is already known to civilization. A host of microbial, anti-viral, cardioactive and neuro-physiological substances have been derived from the marine plants and animals. Great majority of modern Western medicines owe their existence to biotechnological researches on the natural chemical compounds that plants, animals and microbes produce. Relatively few of the 250,000 kinds of plants in the world have been fully examined for compounds with possible biological efficacy. So it stands to reason that the remaining species contain many unknown compounds of probable therapeutic value.

Biomass is a form of renewable solar energy stored in all plant materials everyday in the process of photosynthesis. The world forest and vegetation produce 220 billion tones (dry weight) of plant biomass every year by photosynthesis with energy content of 1500 to 2400 quadrillion Btu. This represents some 10 times the world's current energy usage. Biomass energy is in fact a living, renewable and cleaner alternative to the non-living, non-renewable and polluting dirty fossil fuels and also much cheaper. Biomass is the product of current plant photosynthesis, while the fossil fuels are of those ancient plants, which photosynthesized in the remote past (Carboniferous age of the Paleozoic era). One ton of wet biomass has the energy equivalent to one barrel of oil. Another advantage is that like fossil fuels bio-fuels obtained from the biomass can be stored, and made available when required.

Advances in environmental biotechnology has raised the economic value of global biodiversity (biological resources of earth) because any species can now become 'raw material' for genetic engineering. By speeding the chemical screening process that undergrids the development of new drugs from plants, biotechnology has made screening natural biological products a low-cost, but potentially lucrative activity. When a promising chemical is found from a plant source, large quantities of that chemical can be reproduced simply by transferring those 'plant genes' responsible for the production of such chemicals into bacterial host without harvesting those plants from nature or having to cultivate and propagate those plants to extract chemicals from them. The host bacteria becomes the 'drug/chemical factory' instead. Human insulin is being produced in bacterial host in the similar way.

Unfortunately, the gene rich developing countries are poor in biotechnology and cannot make best use of their own genetic resources and have to depend upon the developed nations who take great advantage of the situation.

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Biological Resources : Potential for Big Business

Developing Environmentally Safer & Renewable Alternative to the Geochemical Resources for Socio-economic Development : Significant Contribution of Researches into EBT to the Global Economy

KEY WORDS

Biological Resources - A Heavenly Asset; Biochemicals for Pharmaceutical Industries & Modern Medicine; Phytochemicals for Cosmetic Industries; Herbal Dyes for Dyeing Industries; Plant Hydrocarbons (from Petro-crops) for Petroleum Industries; Biodiesel - Environmentally Safer Alternative to Mineral Diesel; Bio-plastics - Environmentally Biodegradable Plastics; Biofertilizers & Biopesticides - Environmentally Safer Agro-Chemicals.

BACKGROUND INFORMATION

Biological resources are 'heavenly assets' and a great gift of nature to mankind. Biological resources has ceased to be a challenge only for the mere preservation of threatened species. It is a multi-sectorial issue and now looked upon more as the scientific challenge for bioprospecting of the 'biological resources' (specially the wild genetic resources) of world for the social and economic gains and progress of mankind and for the eradication of poverty, disease and hunger. Wild species and their genetic variations contribute worth millions of dollars to agriculture, medicine, and other industries as biological raw materials every year.

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BIOCHEMICAL COMPOUNDS FOR MODERN MEDICINE & THEIR ECONOMIC VALUE

Worldwide medicines from the wild products worth some 40 billion U.S. dollars a year is being utilized by mankind. WHO has listed over 21,000 plant species which have medicinal use around the world. Vast profits are being made by the multinational corporations (dealing with food and drug development) of the rich North nations from the use of 'genetic materials' obtained from and conserved by the poor South nations. The rich developed countries of North particularly the U.S. have earned billions of dollars by using the genetic resources obtained both legally and 'illegally' from the South developing nations in the development of new sources of foods, medicines and industrial raw materials.

According to UNEP (1992), about 5% of the gross domestic product (GDP) in the U.S. which is equivalent to some US $ 87 billion per year is attributable to the harvest of the wild species in the form of plants with food and medicinal values. Interestingly, most of the wild genetic resources came from the gene rich developing countries of the South. According to United Nation Development Program (UNDP) the developed countries of North owe at least US $ 5.3 billion a year in royalties to the developing countries for the 'crop genetic resources' and the associated 'indigenous knowledge' given to the global seed industries by the farmers of the South.

The Madgascar periwinkle (*Vinca rosea* Syn. *Catharanthus roseus*) yielding the drugs 'vincristine' & 'vinblastine' for the treatment of leukaemia forms the basis of a million dollar drug industry in the U.S. today yielding about US $ 160 million worth of drug sales each year. The Pacific yew *(Taxus wallichiana)* and its Himalayan relative *Taxus buccata* from the Indo-Nepal region has yielded a drug 'taxol' which makes great promise to treat ovarian and breast cancer in women and its value is worth several million dollars now. The American company W.R. Grace has patented the product. Another medicinal plant is the Cameroon twiner *Ancistrocladus korupensis* whose product 'Michellamine-

B' was patented by the National Cancer Institute of New York. The drug inhibit the multiplication of AIDS virus. Once it has passed the clinical trial the value of the drug will be worth billion of dollars. (UNEP Reports, 1992-2006).

A 1997 World Bank report says that revenue earned by India from the export of crude herbal drugs in 1994-95 was US $ 53.2 million. Worldwide, medicines from wild species originating mostly in the developing countries of South are worth some US $ 40 billion a year of which over 80% goes to the US drug industries. U.S. is earning yearly royalty of US $ 260 million from the drug 'reserpine' obtained from *Rawolfia serpentina*. It was the first drug to be used for the treatment of schizophrenia and other forms of mental disorders and for lowering blood pressure in India.

The American Company Fox Chase Cancer Research Centre is earning millions of dollars from another Indian medicinal plant *Phyllanthus niruri* whose product is very effective against viral hepatitis. German company Hoechsts is earning millions of dollars by developing drugs to treat cardiac diseases from the Indian medicinal plant *Coleus forskohlii*. It holds at least 6 patent claims for *C. forskohlii* which is proving very potent for the treatment of cardiac problems. Another German company Boehringer is earning millions of dollars for developing anti-cancerous drugs from *Nothatodytes foetida*.

Salix purpurea provided the information for the synthesis of 'acetyl salicylic acid' (aspirin), which is a prized drug today (doing multi-million dollar business every year) all over the world for rheumatism and dissolving blood clots in arteries. *Papaver somniferum* and *P. bracteatum* which gave 'morphine' and 'codeine' respectively provided the chemical structure of the modern pain killers which dominated the 20th century medical prescription all over the world yielding billions of dollars to the drug industry. The anti-spasmodic drug 'atropine' from *Atropa belladonna* and *A. acuminata* is also yielding huge revenues to the industries. 'Diosgenin' from the Mexican yam (*Dioscorea deltoidea*) the source of sex hormones are valued up to US $ 1 billion per annum (Ayensu, 1986). The cost of margosa (*Azadirachta indica*) biopesticide 'azadirchtin' developed by Max Planc Institute of Germany could be about US $ 50 million per annum.

In 1973 natural products from the wild organisms or cultivated plants accounted for 41.2% of the total prescriptions dispensed in community pharmacies in the U.S. and were retailed at US 1.6 billion. Figures from 1980 put the value of herbal drugs at US $ 4 billion and those drugs dispensed from U.S. government agencies, hospitals and other legitimate channels at US $ 8.1 billion. Most of those plants originated in the developing countries of South and the value of illegally procured herbal drugs from the developing countries is, of course, incalculable. (Oldfield, 1984).

In 1995 the New York Botanical Garden (NYBG) and Pfizer Drug Company signed a million dollar contract to scan the NYBG collections. The 'wealth' of medicinal herbs were in fact brought from different tropical countries by Americans on several goodwill missions. National Biodiversity Institute (INBio) in Costa Rica, established in 1989, is a private non-profit institution whose goal is to manage and sustainably use the biological wealth of Costa Rica. It signed US $ 1 million agreement with Merck & Company of U.S. under which the INBio is providing Merck with plants and animal extracts for screening for medicinal use. INBio in turn will get a share of the royalties from any drug that is developed from the extract. The company's best selling cardio-vascular drug is '*mevacor*' obtained from a plant found in Costa Rica.

BIOFERTILIZERS : ENVIRONMENTALLY SAFER & ECONOMICALLY CHEAPER ALTERNATIVE TO THE CHEMICAL FERTILIZERS & POTENTIAL TO SAVE BILLIONS OF DOLLARS EVERY YEAR

Biofertilizers are nitrogen rich metabolic products of plants, animals and friendly microbes in our environment. Production and delivery of all types of nitrogenous biofertilizers as an effective alternative to the chemical urea is one of the greatest achievements of researches into environmental biotechnology. The Microbiological Resource Centers (MIRCENS) in Kenya, Senegal, Egypt and Brazil are exploring biofertilizers based on nitrogen-fixing bacteria, which it is estimated, could cut the world's chemical fertilizer bill by US $ 15 billion a year and arrest millions of tons of greenhouse gases entering into the environment every year. (UNEP Reports, 1996-2006). (More about biofertilizers are in Chapter 19).

THE BIOPESTICIDES : ENVIRONMENTALLY SAFER AND ECONOMICALLY CHEAPER ALTERNATIVE TO THE CHEMICAL PESTICIDES

Production and delivery of all types of biological pesticides as an effective and environmentally safer alternative to the chemical pesticides is one of the greatest achievements of researches into environmental biotechnology. Several plants and microbes have come to light that have potent 'insecticidal' and 'pesticidal' properties. The one which has revolutionized the agricultural industries is 'azadirachtin' from the Indian margosa (neem) tree (*Azadirachta indica*) isolated by the Max Planck Institute of Germany.

The viral pesticides are particularly proving very useful 'bio-weapon' in agriculture, horticulture and forestry. Hundreds of viruses have been found pathogenic to insects pests and a few of them have been commercially developed as 'viral pesticide' in USA. Among the bacterial pesticides, the *Bacillus thuringiensis* is especially a goldmine acting on wide range of pests. There are more than 500 fungal species which infects insect pests.

The Microbiological Resource Centers (MIRCENS) at Cairo, Egypt is examining the use of microbes in 'pest & vector control'. (UNEP Reports, 1996-2006). The significant thing is that like the chemical pesticides the herbal pesticides do not kill the 'natural enemies' of pests and also do not induce 'resistance' in organisms. (More about biopesticides are in Chapters 20).

THE HERBAL DYES : ENVIRONMENTALLY SAFER ALTERNATIVE TO THE CHEMICAL DYES

Ever since chemical dyes were shown to be an environmental hazard and toxic to human health search is on to revive the traditional herbal dyes and discover new ones from the wild plant species through researches in environmental biotechnology. Some of the more promising plant sources are—

1. *Arnebia hispidissima* : Leaves & stems yield coloured dyes.
2. *Bixa orellana* : Yield red dye from the fruit pulp.
3. *Butea .monosperma* : Flowers yield red and pink colour dye.
4. *Catharanthus tinctorius* : Yield orange and red dye from the flowers.
5. *Curcuma longa* : Rhizomes (roots) yield yellow color dye.
6. *Dactylopius coccus* : Yield red cochineal dye from the insect reared on *Opuntia.*
7. *Haematoxylon campechianum* : Purplish red dye obtained from the heart-wood.
8. *Indigofera* spp. : Brilliant blue dye obtained from the leaves of various species. (It is in use by civilization for over 4000 years now).
9. *Isatis tinctoria* : Blue dye obtained from the leaves.
10. *Juglans regia* : Leaves yield ash color dye.
11. *Lawsonia inermis* : Orange dye obtained from the leaves. (It is being used since centuries called as 'Henna')_
12. *Mehuwia sikkimensis*: Bark of the tree yield yellow dye.
13. *Rubia cordifolia* : Root and leaves yield purple color dye.
14. *Rumex neplensis* : Root and stems yield brown color dye.

THE BIODEGRADABLE PLASTICS AND POLYESTERS : ENVIRONMENTALLY SAFER ALTERNATIVE TO THE HAZARDOUS PLASTICS

Plastics have permeated the human society in almost every sphere of life. They have wide applications from homes to industries and have replaced several water and energy intensive, waste and pollution generating metals (aluminum, iron and copper) and materials (timber and paper). Plastics are everywhere, as the furniture and fixtures, bottles, bags and containers in our homes and institutions, as stationary items and office equipments, as the component parts of our household appliances specially the electronic equipments, as the component parts in our vehicles and automobiles, as irrigation pipes and equipments in our agriculture farms, as sports goods, on the sole of our shoes and slippers, and sometimes even in our bodies as soluble stitches and artificial heart valves.

However, safe disposal of plastic wastes is one of the biggest techno-economic problems faced by the civilization. Being non-biodegradable, all plastics can remain in the environment for decades without being degraded. All plastics cannot be recycled and recycling of plastic wastes is a hazardous process which gives out toxic fumes during melting. Efforts are being made to create environmentally friendly biodegradable plastics.

Bioplastics from Carbohydrates

Biodegradable plastics are now being produced from starch (obtained from corn, wheat and potato) which is a 'natural polymer'. As it is soluble in water its use in polymer production has some limitations. This problem has been overcome by microbial transformation of starch into 'lactic acid', which is a monomer. The lactic acid monomer is chemically treated to link their molecules into long chains - the polymers. The plastic made from bonding of this polymer is called 'polylactide' (PLA). PLA is in commercial use in Australia since 1990 in medical implants, sutures and drug delivery systems because of their properties to dissolve over time.

Environmental Biotechnology has developed a new 'biodegradable polyester' called 'politrimethylene terephthlate'. It has been made by fermentation of carbohydrates from corn, beet and potato and agricultural wastes.

A single microbe has been genetically tailored which possess all the enzymes for conversion of sugar into 'glycerol' and to 'polyester'. The fibers are heat-settable and stable to moisture. There is no use of heavy metals, petroleum products or any toxic chemicals in the process and no generation of hazardous waste. *Bacteria can produce biodegradable plastics through fermentation but the process is five times more expensive than the production of conventional non-biodegradable plastics made from petroleum.* Plants are much more efficient in converting carbon into plastic as they fix carbon directly from the atmosphere in the process of photosynthesis. (UNEP Reports, 1996-2006),

Bioplastics by Bacterial Fermentation

Genetically tailored bacteria has been produced which can make granules of polymers called 'polyhydroxyalkanoate (PHA) inside their cells. Bacteria are grown in cultures and the biopalstic granules are harvested. Biotechnology has provide the tool to isolate and stitch the genes from these bacterial cells into corn plants, which then become capable of synthesizing these polymer granules into their cells. Like PLA, PHA is still more expensive to produce.

Bacteria can produce biodegradable plastics through fermentation and transformation but the process is five times more expensive than the production of conventional non-biodegradable plastics made from petroleum. Plants are much more efficient in converting carbon into plastic as they fix carbon directly from the atmosphere in the process of photosynthesis.

1. The Central Tuber Crop Research Institute (CTCRI) in Kerala, India is has produced a biodegradable plastic by mixing low density polyethylene with starch. Bacteria would eat up the polyethylene with starch.

2. Indian scientists at the National Chemical Laboratory (NCL) in Pune, have discovered a cheaper way to make plastics biodegradable by exploiting the sweet eating properties of bacteria. If small amount of sugar is added to the plastics during manufacture they degrade within days, instead of lasting for decades in landfills.

3. Australia has already commercialized the production and use of biodegradable plastic grocery and shopping bags. Australian scientists at CSIRO in collaboration with Swinburn University and University of Queensland, Brisbane, have developed a cost-effective biodegradable packaging material made from wheat and sugarcane starch.

Wheat and sugarcane starch are mixed with polymers to make it biodegradable. Scientists at the Bureau of Sugar Experiment Station in Queensland have isolated genes that bacteria use to produce plastic precursors from sugarcane starch. The opaque material which looks like a normal plastic sheet biodegrade within 4 to 6 weeks. The material can be used for manufacture of grocery shopping bags, to pack vegetables, as baking trays and as mulch sheeting for farming and gardening to conserve water. The polymer structure is broken down by normal microorganisms in the environment.

> 4. The giant agriculture company Monsanto of U.S. has produced a biodegradable 'agro-plastic' PHBV (poly 3-hydroxybuyrate-co-3-hydroxyvalerate) from cress and oil seed rape plants through genetic manipulation of four bacterial genes and the metabolic pathways of amino acids and fatty acids synthesis. Commercialization will take time but the door has been opened.

BIOLOGICAL RAW MATERIALS FOR THE INDUSTRIES

Industrial raw materials derived from the wild include fats, oils, waxes, latexes, pectins, gums, resins and other exudates, vegetable dyes and tannins, lignin, cellulose, starch, fibres, hydrocarbons and host of biochemical compounds now needed in chemical industries to replace the mineral-based and synthetic toxic chemicals.

1. Guayule (*Parthenium argentatum*) is a wild perennial shrub from the deserts of Mexico. It is a rich source of hydrocarbons potential for rubber production. Purified guayule rubber is chemically similar to para rubber and the yield is about 12% by dry weight.
2. Another wild desert plant from Mexico - *Euphorbia antisyphilitica* yield a wax called 'candelilla wax'. It has been found suitable for manufacture of explosives.
3. The gumweed (*Grindelia camporum*) hold a big promise as 'wood resin' for the manufacture of adhesives, paper sizings etc.
4. Crambe (*Crambe abyssinica*) from the Mediterranean sea holds a big promise as a source of erucic acid - a long chain fatty acid used in the production of lubricants and plasticizers.
5. Kenaf (*Hibiscus cannabinus*) from East Central Africa can yield 25-40 metric tons (dry weight) of fibre from stems per hectare per year. The pulp is better than the wood pulp and about half the cost.

1. Plant Oils

Plant oils are finding new applications in several economic development programs. Palm oil is used in hundred products from lipstic to tinplate, ice-cream to jet engines. Some 28,000 species of palm exist all over the world.

> Jojoba oil from Jojoba (*Simmondsia chinensis*) a wild shrub from the Mexican desert is of great economic value today. The seeds contain 50% by weight of 'liquid wax', which is equivalent to the precious 'sperm whale oil' in property. It is widely used in the manufacture of cosmetics and as a high performance lubricants required to stand extreme pressure and even as transmission fluid. With the ban imposed on sperm whale oil under CITES (Convention on International Trade in Endangered Species) the economic and industrial importance of jojoba oil has increased significantly.

The essential oil extracted from the leaves and inflorescence of *Cymbopogon martini* var *motia* has been found to have great perfumery properties. The oil is in great demand in the perfumery industry, especially for its high 'geraniol' content.

The root of vetiver (*Vetiveria zizanoides*) produces an essential oil called 'vetiver oil' which is used in perfumery industry. The south Indian genotype is specially useful in oil production. The Department of Natural Resources in Australia is producing world class perfume 'Guerlain'. Vetiver oil is also an 'insect repellant'.

2. Plant Hydrocarbons for Petroleum Industry : Environmentally Safer and Renewable Alternative to the Fossil Fuels

Some wild desert plants from of India, U.S., and elsewhere in world have been found to yield latex, which are rich source of hydrocarbons (C-15 compounds) and have potential to be processed into petroleum products replacing the mineral-based oils. *Euphorbia caducifolia, Jatropha curcus* and *Calotropis procera* are important petro-plant species. *Euphorbia* burns fiercely upon combustion and their latex is now being processed in the U.S. to obtain petroleum products. The oil obtained from the latex of *Jatropha* is very close to the diesel oil in chemistry and can become an renewable source of bio-diesel.

3. Phyto-chemicals for the Cosmetic Industry : Replacing the Neurochemicals

AVEDA Corporation was founded in 1978 with a vision to replace hundreds of neurotoxic synthetic chemicals used in cosmetic industry by natural plant-based phyto-chemicals.

Aveda produces plant based hair, skin, body, aroma, make-up and lifestyle products. Since 1993, Aveda developed business partnerships with traditional and indigenous communities - the custodians of biodiversity all over the world. They grow and harvest ingredients for use in the cosmetic products of Aveda and gets equitable share in profit. One such group is the Yawanawa tribe in Brazil who provide a natural plant pigment called 'uruku' used in lipistics and in botanical line of color-enhancing shampoos and conditioners. In Peru, Aveda work with Conservation International and the Ese'eja community to obtain Brazil nuts that are processed into proteins and then used in shampoos, conditioners and treatments. In another million dollar project Aveda is working with Quebradeiras de Coco women who collects 'babassu nuts' whose oil is turned into a gentle cleansing agent. This replaces many of the existing toxic chemical cleansing agents used in shampoos and facial and body cleansers.

An important environmental benefit of this project has been the reduced dependence on the coconut materials which is grown to fulfill the high demand by coconut producing countries by converting their rainforest into coconut plantations.

> At Aveda they have proved that beauty products could come from Mother Nature by their 'conservation' and not by 'exploitation'. They have also demonstrated that economic profitability and environmental sustainability are synergistic goals and can be achieved together. In 1997, Aveda discovered that the sandalwood oil in its product '*Love Pure-Fume*' was not 100% traceable and the majority of Indian sandalwood was being unsustainably harvested- in some cases poached. They immediately suspended production and started searching for sustainable sources. In 2002, they re-launched the product with fully traceable oil, sustainably harvested from trees in Western Australia. They are however, searching fully traceable source of Indian sandalwood oil.

In 2002 only, Aveda joined forces with UNESCO, UNEP, the UN Foundation, and an innovative organization RARE Center for Tropical Conservation, to help conserve some of the world's most important protected areas (sites) and their indigenous people. These are Sian Ka'an and El Vizcaino Biosphere Reserves in Mexico, Tikkal National Park in Guatemala, Rio Platano Biosphere Reserve in Hondura, and Komodo and Ujung Kulon National Park in Indonesia.

Aveda also worked with its suppliers of plastic bottles to increase the post-consumer recycled content of the bottles to nearly 80%. This translates into saving of nearly 150 tones of virgin high density polyethylene (HDPE) in more than 16 million plastic bottles they use annually. This also means significant reduction of waste and pollution in the plastic bottle manufacturing industries.

VALUABLE PLANT PRODUCTS WITH INCREASED BIOMASS FROM TISSUE CULTURE TECHNIQUE : A SIGNIFICANT ACHIEVEMENT OF RESEARCHES INTO ENVIRONMENTAL BIOTECHNOLOGY

Tissue culture (TC) is a powerful and rapid mean to propagate plants and also increase their biomass in a short time from their cells and tissues with the aid of specific plant hormones and appropriate nutrition.

> Tissue culture has become very useful tool provided by the environmental biotechnology for regenerating those economically important plant species which has become 'endangered in nature' and is not propagating by itself or are very slowly reproducing. Many such plants of great medicinal value are now endangered. Prime example is *Rawolfia serpentina*. In order to meet the growing requirement of 'biomass' (for bio-fuel, biochemical, fodder & timber) all over the world tissue culture is the only alternative.
>
> Fourteen (14) trees have been identified in India for large-scale micro-propagation by TC. They are required in re-forestry plan of denuded lands. Through TC some commercially important but slow growing trees, like the teak tree (*Tectona grandis*) and the sandalwood tree (*Santalum album*) have been made to grow faster.

One of the earliest success in environmental biotechnology, in India, was in the area of tissue culture in the 1980's. It included the test-tube culture of cells, protoplast and pollen grains, leading to their regeneration into full plants. These techniques allowed multiplication of 'selected elites' - the economically & environmentally important and useful plants such as the papaya, coconuts, turmeric, ginger, sugarcane, tamarind, tobacco, cardamom, sandalwood, bamboo, rose and citrus fruits in large numbers, in a much shorter time than the conventional propagation techniques.

One of the most remarkable achievements in tissue culture in India have been the 'induction of flowering' in bamboo (*Bambusa* spp.) in the test-tube. In nature bamboos flowers only once anytime between 15 to 120 years and then dies. This was for the first time in world. This has led to conservation and propagation of bamboo species and its diversity. Some species of bamboo grows very fast - as much as 4 cm in an hour. Bamboo occupies great significance as a valuable 'biological resource & raw material' for diverse industries. It is a structural material for rural houses in several developing countries, used for manufacture of modern furniture and baskets. Tender bamboo shoots are used as a delicacy in food items in some cultures. The biggest commercial use is in the manufacture of paper.

Major works on tissue culture of endangered species to save them from 'extinction' in future is being carried out at M.S. Swaminathan Research Institute in Chennai, India. The National Chemical Laboratory (NCL), Pune and the Tata Energy Research Institute in New Delhi are involved in bulk multiplication of elite forest tree species by TC for reforestation. They have potential to produce millions of 'plantlets' every year.

CONCLUDING REMARKS

Biodiversity based biological raw materials are finding more and more uses in modern economic development activities. Being renewable resources, they are also environmentally safe and sustainable.

The economic stakes in obtaining biochemical compounds from plants used in traditional medicine for biotechnological development and commercialization in modern medicine are quite substantial. Plant derived compounds are big business in world medicine and health development programs. Plants drug often serve as a chemical blueprints and starting materials for the commercial production of related synthetic substitutes for which now many pharmaceutical companies all over the world, especially in the U.S. are eagerly looking for. Study made by United Nations in 1980 estimated world trade of medicinal plants at US $ 550 million. Hong Kong is the largest herbal drug market in world. In 1979 the value of herbal drug imported into Hong Kong was US $ 189 million of which *Panax ginseng* accounted for US $ 53 million.

Knowledge in environmental biotechnology has developed more and more biological products into useful raw materials replacing the environmentally destructive mineral based materials. Extraction of mineral resources from earth crust often results into deforestation and biodiversity erosion, land subsidence, soil erosion, disruption of underground water circulation (when virgin ores are extracted in mining industries); water pollution, acid drainage and tailings generation (when ores are washed and concentrated); air pollution and solid waste generation (when ores are processed in metallurgical industries).

If environmental biotechnology can commercialize the production of 'biodegradable plastics' it would help in reducing over half the use of toxic materials and generation of hazardous wastes on Earth.

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16

HERBAL PRODUCTS FOR HUMAN HEALTH & SUSTAINABILITY

Developing Herbal Medicines & Health Promoting Products from the Wonder Herbs : A Great Achievement of Researches into EBT

KEY WORDS

Healing Herbs; Herbal Medicines; Plants Combating Cancers; Plants Combating AIDS; Plants Combating Degenerative Diseases; Plants Combating Leukemia; Plants Promoting Sleep - Aromatherapy; World Health Organization Promoting Pant Based Herbal Medicines;

BACKGROUND INFORMATION

Wild plant parts (roots, leaves, stems, fruits and seeds) have provided many of our medicines in the traditional medicine since centuries, and now in modern medicine too after science discovered that their biochemical compounds can have beneficial biological effects on human body. With the chemical and industrial revolution of the 19^{th} century several medicinal plants of antiquity used by the traditional medicinemen have found wide acceptance and a place of pride in modern medicine. Our knowledge in environmental biotechnology has given us the tool to use those plants of medicinal value for human health more sustainably and without harming the environment and its biodiversity.

THE HERBAL MEDICINES : POTENTIAL FOR DEVELOPMENT OF MODERN MEDICINES

Each time we take a medicine, there is one chance in two, that our purchase owes its origin, in some way or other, to start point materials from the wild plants from forest, wetland, or the ocean. The product may be an analgesic, an antibiotic, a diuretic, a laxative, a tranquilliser, or a cough postile. The importance of 'morphine' and 'codeine' in human life is already known to civilization. A host of microbial, anti-viral, cardioactive and neuro-physiological substances have been derived from the poisonous marine wildlife.

LIFE SAVING MEDICINES FROM PLANTS : THE FORTUNE FOR THE FUTURE

The human civilization knows the value of the drug 'quinine' obtained from the bark of cinchona tree (*Cinchona officinalis*) which saved millions of lives of malaria victims in the 1940s and 50s; the value of antibiotic 'pencillin' discovered by Alexander Fleming from the fungus blue-mould (*Pencillium notatum*) which saved millions of lives after the World War II. Unfortunately the bugs which they killed in the 1940s and 50s have now become resistant to them.

> The chemical compound 'digitoxin' from foxglove (*Digitalis purpurea*) gave a valuable drug to the modern medicine, which has saved and prolonged the life of millions of people suffering from 'congested heart failures'. It stimulate heart function and regulate its tone and rhythm, improves blood supply to heart and even kidney thus removing any renal obstructions.

Potential Plant Drugs for Combating Leukaemia

1. In 1960, a child suffering from leukaemia had only one chance in five (5) to survive. Now the child has four (4) chances in five, due to the treatment with drugs 'vinblastine' and 'vincristine' obtained from the Madagascar periwinkle (*Vinca rosea* Syn. *Catharanthus roseus*).

2. Lymphoid leukaemia once killed 90 per cent of its child victim within six months. Now the disease is being effectively treated with the chemical 'tylocrebin' derived from the Australian native vine *Tylophora erecta*.

Potential Plant Drugs for Combating Cancers

The National Cancer Institute (NCI) in Maryland, U.S. is randomly screening plants from all over the world but especially from the tropical nations for anti-cancer properties.

1. In 1991, The Pacific Yew (*Taxus walichiana*) was found to contain a chemical compound named as 'taxol' that shows great promise against breast cancer, ovarian and uterine cancer in women. Its molecule is structurally unique and there is no way it could have been visualised and synthesised by the biochemist of world if it were not to be discovered in nature. The Himalayan Yew (*Taxus buccata*) also yield the drug taxol and has come as substitute after the decline of Pacific Yew due to its over-exploitation.

> The Department of Biotechnology (DBT) in India is screening other species of *Taxus* e.g. *T. bractus* which grows abundantly in the north-eastern region of Arunachal Pradesh for their 'taxol' content. This plant is being propagated through tissue culture techniques.

2. 'Colchicine' derived from *Colchicum luteum* is getting new attention in cancer treatment in recent times. The drug has shown to arrest cell division of the cells in cancerous tissues and also make them more susceptible to X-ray treatments.

3. *Withania somnifera* better known as the 'Indian Ginseng' yield two chemical compounds 'withanolide-D' and 'withaferin-A'. They exhibit significant anti-tumour activity in-vivo against Sarcoma-180 cells in mice. They also inhibit RNA biosynthesis in them.

4. The mayapple tree (*Podophyllum peltatum*) used by North American Indians has yielded a new drug called *VePesid.* It is a semi-synthetic derivative of 'podophyllotoxin' – a natural extract of mayapple. It is proving to be a boon for treatment of testicular cancer with 47% recovery rate.

National Cancer Institute (NCI) of New York has also found *Achyoronychia baueri, Brucea antidisenterica* and *Helitropium indicum* as very promising anti-cancer agents. The boadest anti-cancer spectrum is possessed by the chemical 'acronycine' from *A. baueri.*

Other plants like bloodroot (*Sanguinaria canadensis*) used by North American has been confirmed by modern science to be active against malignant tumours while the bark of *Croton lechleri* used by the tribal people of South America is proving useful in treatment of stomach cancer. 'Isohexenylnaphthazarins' isolated from the roots of *Arnebia hispidissima* a desert plant are new class of chemicals and some of them possess 'anti-cancer activity'. (WWF Report, 1984 (Huxley); UNEP Reports, 1992-2006).

Potential Plant Drugs for Combating AIDS

National Cancer Institute of U.S. is also screening large number of plants from the tropical countries to identify chemicals, which can inhibit the HIV in human body.

1. A novel chemical compound named as 'Michellamine-B' from the African vine *Ancistrocladus korupensis* from the forest of Cameroon have been found to exhibit remarkable range of anti-HIV activity. *A. tectorius* discovered from the Andaman & Nicobar Islands in India have also been found to possess this property.

2. The chemical from the Australian Moreton Bay Chestnut (*Castanospermum australe*) has assumed great reputation in the treatment of AIDS.

3. The plant *Homolanthus nutans* from the Samoa Island in South Pacific yield the chemical 'prostatin' which shows great promise to combat HIV AIDS.

WORLD HEALTH ORGANIZATION (WHO) RECOMMENDING FOR PLANT BASED MEDICINES

The World Health Organization (WHO) has advised the nations of world to take major initiative in launching biotechnological surveys for locating those plants which will be earnestly required by the humanity in future for combating various health problems. WHO has asked to locate plants which can –

1. Accelerate brain function, memory and intelligence in children and the aged;
2. Reduce serum cholesterol, triglycerides and sugars in blood;
3. Influence the nerves and the central nervous system (CNS) and combat neurological disorders;
4. Stimulate heart function, improve cardiac and cerebral blood flow and dissolve blood clots in arteries;

5. Protect liver and promote liver function;
6. Restore and strengthen the human immune system;
7. Selectively arrest cell division in abnormally growing cells and tissues;
8. Inhibit the multiplication of HIV causing AIDS;
9. Increase stamina and endurance, improve performance, reflexes and concentration;
10. Accelerate growth in children and retard the process of aging in the old;
11. Adapt human body to overcome physical and mental stress and body fatigue;
12. Produce anti-fertility affects in both males and females;
13. Have anti-bacterial, anti-viral and anti-protozoal properties without inducing resistance in organisms;
14. Combat degenerative diseases, arthritis and rheumatism ;
15. Act as 'anti-oxidants' to protect healthy cells from damage by 'free-radicals';
16. Act against asthmatic and bronchial problems;
17. Protect skin from the UBV rays damage and regenerate dead skin cells;
18. Act as 'anti-implantation' agent;
19. Delay cataract formation in eyes;
20. Help in de-addiction and against build-up of narcotic substances in body.

REVIEW OF PLANTS FROM INDIA & THE TROPICAL COUNTRIES OF SOUTH WITH POTENTIAL FOR USE IN MODERN PHARMACOPOEIA

Several plants from Indian and also found different parts of world especially in the tropical countries of the South have been found to possess chemical compounds, which exhibit above biological properties. They were used in the traditional medicine practiced by the indigenous communities of those countries. Many of them finds mention in the ancient Indian literature of medicine the *'Ayurveda'* and also in *'Charak Samhita'* and were used by the ancient Indian sages and the indigenous people. The Lucknow based Central Drug Research Institute (CDRI) of India has screened over 4000 plants for broad biological activities and have confirmed 450 plants which have potential to be used by modern medicine. CDRI has also developed drugs for commercial use from some of them. Some of the very important plants are mentioned in this text.

1. The snakeroot plant (*Rawolfia serpentina*) has been widely accepted in modern medicine for the preparation of standard drug 'reserpine' for the treatment of high blood pressure and for certain neurotic disorders due to its strong sedative and hypotensive properties. The drug has been shown to be 'phenotropic' influencing the functions of mind and behaviour.

2. Ginseng (*Panax ginseng*) from Korea and China referred in the pharmacopeia as the wonder drug and 'cure for all disease'. It is now being actively investigated all over the world for its effects on tumours, corneal opacity and for increasing body resistance against infection.

Researches reveal that ginseng increases the endocrine function and metabolic activity, stimulates circulatory and digestive systems, counter the aging process by restoring the tolerance level of anaerobic metabolism.

Japanese scientist has found that the drug protect liver from damage caused by intake of excessive alcohol and exerted anti-cancer effects on 'Ehrlich abdominal ascites' cancer cells.

3. *Trichopus zeylanicus* also called as the 'Indian Ginseng'. It has been shown to exhibit 'anti-fatigue' effects on human body and also strengthen the human immune system. It is said to have 'cure for all disease'.

4. *Withania somnifera* from India is another 'wonder drug'. It has significant anti-tumour activity and the root powder has 'anabolic' properties accelerating growth in children and retarding the process of aging in older people.

5. *Ginkgo biloba* - an archaic tree on the verge of extinction, gained prominence in modern medical therapy as a cure for several ailments. The drug 'flavone' has vascular dilating properties and increase cerebral blood supply and thus have potential to fight 'senile dementia', Alzheimer's disease and other age related mental problems of the old people.

Recent researches indicate *Ginkgo's* possible indirect role in improving memory, maintaining the health of capillaries and improving blood circulation in extreme organs like hands and feet. Another research show that the 'flavoneglycosides' found in *Gingko biloba* may inhibit the PAF (platelet activation factor) and thereby reduce the frequency and severity of asthma attacks.

It also act as a strong anti-oxidant scavenging the free radicals especially in the circulatory system and in the eyes, ears and brain thereby preventing damage to the protective myelin sheath on nerves and other vital cells in the brain.

6. The margosa tree (*Azadirchta indica*) has found a place of pride and honour in modern medicine. Scientific investigations reveal it has immuno-modulatory, contraceptive, antiseptic and antimicrobial activities. Max Planc Institute of Germany isolated the drug 'azadirachtin' which is active against more than 200 pests and insects and several microorganisms.

7. *Centella asiatica* yielding the drug 'asiaticoside' has anti-leprotic properties inducing fast growth of hairs, nails, and skin in humans. It also find mention in Ayurveda as 'Brahmi'.

More significant is that *Centella asiatica* improves memory power and the general ability and behavioural pattern of mentally retarded children. CDRI, Lucknow has developed a drug called 'Memory Plus' for commercial use. It is a memory-enhancing tonic based on the extracts of the plant.

8. The Kangaroo apple (*Solanum aviculare* and *S. laciniatum)* found in Australia contains a remarkable chemical 'solasodine' which is use to prepare a steroid that helps

the body adapt to stress and balance the body fluids. It also helps control inflammations and promotes tissue regeneration.

9. The fungus *Tolypocladium inflatum* found in Norway, has been found to produce. 'cyclosporin' a substance vital in organ transplants as it prevents rejection of tissues. Sponges from the Caribbean, have also been found to yield drug that combat rejection of organ transplants.

10. *Eleutherococcus senticosus* from Russia is a 'divine gift' of nature working to increase the stamina, performance, endurance, reflexes and concentration. It is highly useful where high degree of mental and physical alertness is required such as for pilots and astronauts, long distance truck drivers and research scientists. Russians use it for athletes, divers, climbers, rescuers, explorers, factory workers and truck drivers.

The plant product has been found to act on the 'hypothalamus' of brain ultimately leading to the production of stress steroids which acts against extensive stress factor.

11. The French Martime Pine (*Pinus pinaster*) has assumed great medicinal value today. The bark extract has been found to be useful as immune booster, as anti-aging factor, in improving blood circulation and protecting heart. It has also been shown to protect skin cells against damage by UBV rays.

Clinical studies indicate that *Pinus pinaster* can reduce clumping and aggregation of blood platelets in arteries, which normally occur in heavy smokers and in diabetic patients or under severe stress thus reducing the risk of arterial blockage and cardiac arrest. The drug has gained great reputation in the U.S. and sold as 'Pycnogenol'.

12. 'Artemesin' from *Artimesia annua* (a Chinese plant) has been found to be the only drug effective against all strains of *Plasmodium falciparum* causing malaria. Ironically it has become resistant to quinine and others malarial drugs.

13. Gymnemic acid from the plant *Gymnema sylvestre* have been found to biologically rejuvenate the 'beta cells' of the pancreas to produce insulin. It will be a boon for the diabetes patients working more as a preventive than a curative medicine.

14. *Glycyrrhiza glabra* yielding 'glycyrrhizin' is very effective as anti-inflammatory, anti-allergic and ant-ulcer agent. Japanese scientist has shown that the compound is quite promising as 'anti-viral' herbal agent inducing interferon like activity.

15. *Mucuna pruriens* seed extract and powder combines several health benefits. It significantly promote peristalitic movement of the intestine, lowers blood pressure and slows down heart beat. It has also been found to have 'hypoglycaemic' and 'anti-parkinsonian' activities.

16. The senna leaves (*Cassia angustifolia*) yielding 'sinnosides A & B' is a wonder drug for the stomach, has strong laxative action and increase the peristalitic movement of the intestine.

17. *Picrorhiza kurroa* has yielded a drug called 'picroliv' developed at Cental Drug Research Institute, Lucknow. It is a hepato-protective drug protecting the liver from damage caused by carbon tetrachloride, paracetamol, galactosamine and the virus causing hepatitis-B. It also stimulates gastric secretion and bile production by liver.

Recent researches indicate that picroliv possess significant immuno-stimulant activity.

18. *Terminalia arjuna* has been found to be an anti-stress adaptogenic tonic and a mental and physical restorative. It reduces serum cholesterol, increases cardiac output without affecting the heart rate or rhythm.

19. *Commiphora wightii* (also *C.mukul*) has yielded a hypolipidemic drug called 'gugulipid'. It is a resinous gum exuded by the plant and also finds mention in the *Ayurveda* as 'guggal'. It has bee shown to significantly reduce serum cholesterol and phospholipid levels in blood. The active principle is effective against wide range of disorders like rheumatoid arthritis, obesity and also work as an internal antiseptic and anti-inflammatory agent.

CDRI, Lucknow has developed a blood cholesterol-reducing drug branded as 'Gugulipid' for commercial use and has been found to be as effective as the conventional drugs with better tolerance and 'no side-effects'.

20. *Coleus forskohlii* has yielded a drug 'coleonol' developed at CDRI, Lucknow. Recent investigations show that 'coleonol' has potent hypotensive (blood pressure reducing) agent and also appears effective for treatment of 'glaucoma' of eye for which there is no other cure.

It aroused great interest for modern medicine as the drug provides a new mechanism to potentiate hormonal response in human body.

21. *Bacopa monniera* have yielded drug, which is a wonderful tonic for nerves and nervous disorders and as a mental tonic to help increase 'memory power' and relieve from mental fatigue and stress. It also finds mention in '*Ayurveda*'.

Researches have revealed that *Bacopa* facilitates the performance of rats in various learning experiments, indirectly giving scientific proof of its possible role in aiding memory.

22. *Phyllanthus nuriri* came into prominence when the Fox Chase Cancer Centre of Philadelphia, U.S. claimed that the drug from the plant can cure even Hepatitis-B for which there is no other cure. Traditionally it was used for cure of liver disorders.

23. *Aloe vera* came into prominence when its extract was shown to have remarkable healing effects on dead epithelial cells of skin damaged by solar radiation and stimulate the growth of new cells. It quickly heals even the 3rd degree burns of skins.

Aloe vera has assumed great significance today when the depletion of ozone in the stratosphere has increased the threat of UV rays reaching the Earth's surface.

24. *Valeriana officinalis* has gained reputation as a sedative for modern medicine. The drug acts as a depressant on the CNS.

25. *Evolvulus alsinoides* yield drug that has 'psychedelic' and 'psychelytic' effects on human beings relieving from anxiety neurosis and mental stress. It is also considered as a brain tonic improving memory in children.

26. *Ephedra foliata* yield the drug 'ephedrine' which is now being used in modern medicine for curing bronchial asthma and also to stimulate heart function.

27. *Convolvulus microphyllus* yield drugs that can reduce serum triglycerides and serum LDL-cholesterol in experimental animals. It can thus protect human beings too from cholesterol-induced artheroisclerosis and prevent plaque formations.

28. *Ocimum sanctum*, the plant used as 'home remedy' for treating cold and cough has gained prominence in the treatment of 'ischemic heart disease'. Recent researches indicate that it can also enhance resistance against stressful conditions.

29. *Boswellia serrata* has given the drug for combating degenerative diseases like joint pains, arthritis and backaches. Sunova Dabur India Ltd. is preparing the drug as 'Napane Capsules'.

30. *Thevetia nerifolia* yield a chemical 'peruvoside' which has been introduced into modern medicine and marketed in Germany under the name of 'Encordin'. It is highly effective in insufficiency of heart and weak heart.

31. *Papavar somniferum* yielded the alkaloid 'morphine' discovered by the German biochemist Serturner in 1806.

> *Papavar somniferum* provided the chemical blueprint and starting material for the synthesis of all those class of 'analgesic', which the modern medicine have today.

32. *Vitex negundo* has gained great importance as a drug for good eyesight and improving intelligence and increasing memory power in children.

THE ALPINE HERBS & THEIR BENEFITS TO MENTAL HEALTH OF MANKIND - REDUCING STRESS, INDUCING CALMNESS & SOUND SLEEP : POTENTIAL FOR COMMERCIALIZATION

The Alps in Europe is like the Himalayas in Asia in terms of biodiversity & biological resources. It is home of great variety of herbs and studies have revealed that many of them are of great value to human health. Recently some seventeen (17) plants have been identified which when used in combination have been found to exert following mental & psychological health benefits to human beings—

1. Induce soothing effects and 'calmness';
2. Promote 'relaxation' of body and mind;
3. Reduce mental and physical 'stress'
4. Alleviate body pains;
5. Promote 'optimal breathing'; and
6. Induce 'sleep'.

Above health benefits are most needed these days in modern materialistic age, full of stress and strain in day to day living. Investigations reveal that if such herbs (in dried forms) are kept around in homes especially in bedrooms, their aroma and flavour upon inhalation can provide several of the above health benefits and even induce 'sound sleep' in night. This has been termed as 'aromatherapy' by Prof. Hademar

Bankhoffer of Germany. Biotechnological and biomedical researches indicated that when these dried herbs were used in the bed mattresses as an 'underlay' to get their aroma while in bed, it induced sound sleep overnight, made people to achieve 'deep sleep' more quickly, and they felt more rejuvenated next morning.

The herbs studied are—

1. Mother of Thyme (*Thymus serpyllum*)
2. Hops (*Humulus lupus*)
3. Lavender (*Lavendula officinalis*)
4. Lemon balm Leaf (*Melissa officinalis*)
5. Rose (*Rosa damascena*)
6. Eucalyptus (*Eucalyptus globules*) (Leaves are used)
7. Hay Flower (*Flow graminis*)
8. Hyssop (*Hyssopus officinalis*)
9. White Willow (*Salix alba*)
10. Fern (*Aspidium filix*)
11. Horestail (*Equisetum arvense*)
12. Elder flower (*Sambucus nigra*)
13. Shepherd's Purse (*Capsella bursa pastoris*)
14. Arnica Flower (*Arnica Montana*)
15. Nettle Wort (*Urtica dioca*)
16. Milfoil (*Achillea millefolium*)
17. Chamomile (*Matricaria chamomilla*)

Study reveal that aroma of the mixture of these seventeen plants together bring the above health benefits. Some of them exert calming of the autonomic nervous system (ANS), some help in neuralgia & muscle pains, while others have anti-inflammatory and anti-rheumatic effects on human body. Several of these plants are of global occurrence.

> The scientific explanation given about the effects of seventeen herbal mixture is that due to the body heat emitted nigh after night during sleep, essential oils are released from the underlay herbs in the mattress which penetrate the body through the skin pores and bring about the beneficial effects on tissues, muscles, bones and joints. Vapours (aroma) from the herbal oils when breathed in, reach the olfactory cells with the nose which is connected to human brain. The sensory cells take up the stimulation and conduct the aroma via nerve fibres to the autonomic nervous system (ANS) and further on to certain brain cells, and eventually to the 'limbic system' which induce sound sleep. (Wenatex, 2007).

The Alpine herbs are now commercialised for manufacturing mattresses with 'herbal underlay' - a new concept in the Western world for inducing sound sleep bio-medically and with other related health benefits to reduce the stress in the stressful modern living and lifestyle. The Wenatex SilverMed Company manufacturing mattresses in Austria has come of with one such products (info@wenatex.com.au or www.wenatex.com.au)

CONCLUDING REMARKS

Great majority of modern Western medicines owe their existence to researches on the natural chemical compounds that plants, animals and microbes produce. Relatively few of the 250,000 kinds of plants in the world have been fully examined for compounds with possible biological efficacy. So it stands to reason that the remaining species contain many unknown compounds of probable therapeutic value.

> Once the natural lead compounds have been discovered in wild plants then the chemists can proceed with synthetic modifications to improve upon the natural lead drug. Some 119 pure chemical substances extracted from about 90 species of plants are used in modern drugs. More than 40 of the 90 species listed as plants useful in modern medicine have origin in Asia, Africa, and Latin America. (Myers, 1986).

World Health Organization (WHO) has listed over 21,000 plant species, which have medicinal value and have potential to be used in the modern medicine too. The Chinese use more than 5,000 species of their plants for medicinal purposes and the Indians use 7,500 species. Of this about 500 species are used in the Indian market yielding handsome revenues. (UNEP Report, 1992-2006). Sustainable use of biodiversity through knowledge in environmental biotechnology has made it possible and much more will be added to the list in future. This is an appropriate example where 'development, environment, human health and sustainability' goes hand-in-hand.

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Useful Website

www.wenatex.com.au (About the New Mattress Manufactured in Austria with Herbal Underlay to Induce Sound Sleep)

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17

DEVELOPING BIO-FUELS FROM BIOMASS ENERGY

The Cheaper & Cleaner Renewable Alternative to Fossil Fuels : A Great Achievement Towards Sustainable Energy Development by Researches into EBT

KEY WORDS

Bioenergy Plantations; Biogas (Methane) from Waste Biomass; Biogas from Waste Landfills; Electricity from Biogas; Bio-alcohol (Ethanol) from Waste Biomass; Bio-diesel from Slaughterhouse Waste; Biodiesel from Petrocrops; Biodiesel from Waste Vegetable Oils;

BACKGROUND INFORMATION

Traditionally the biomass, specially fuel wood plants has been used as an important source of energy since pre-history days. Biomass is an organic substance living or dead that has potential biochemical energy stored in its carbon -hydrogen - oxygen (C-H-O) bonds. This is a form of renewable solar energy stored in all plant materials everyday in the process of photosynthesis. The world forest and vegetation produce 220 billion tones (dry weight) of plant biomass every year by photosynthesis with an energy content of 1500 to 2400 quadrillion Btu. This represents some 10 times the world's current energy usage. Types of biomass used as energy sources are wood and wood wastes; purpose grown energy crops; agricultural wastes such as cotton ginning trash, bagasse, nut shells, oil seeds, rice and wheat straws and husks, corn stover, prunings and weeds; animal dung, human excreta and sewage sludge; food processing wastes; waste paper and cardboards; major fraction of municipal solid wastes (MSW).

Biomass energy is in fact a living, renewable and cleaner alternative to the non-living, non-renewable and polluting dirty fossil fuels and also much cheaper. Biomass is the product of current plant photosynthesis, while the fossil fuels are of those ancient plants, which photosynthesized in the remote past (Carboniferous age of the Paleozoic era). One ton of wet biomass has the energy equivalent to one barrel of oil. Another advantage is that like fossil fuels bio-fuels obtained from the biomass can be stored, and made available when required.

Biomass provides about 14-15% of the world's energy, and is the main source of energy for half the world's population in the developing countries, mostly as fuel-wood. More than two billion people, or the majority in the developing world, still rely on fuel wood. Modern biotechnology has tremendous potential for providing cheap, clean and green energy (biofuel) to the civilization in the form of bio-ethanol, bio-diesel, biogas etc. Bio-ethanol and bio-diesel made at both small and industrial scale - passed 3.3 billion litres in 2004 (UNEP, 2006).

FUELWOOD PLANTATIONS HIGH IN BIOMASS ENERGY : TRADITIONAL ENERGY SOURCE STAGING A COMEBACK IN MODERN SOCIETY

The traditional fuel wood is staging a coming back as a convenient source of energy into our modern society. All industrialized nations of world, specially in the colder regions of Europe, America and Scandinavia, and Tasmania in Australia still use fuel-wood for heating their homes. For the poor people in the developing countries, fuel-wood is the most economical and easily available means of energy for cooking and heating.

Through botanical studies several plant species have come to light that have very high calorific value (heat of combustion) and high growth potential. These are-

1. *Casuarina equisetifolia,*
2. *Trema orientalis,*
3. *Lucaena leucocephala,*
4. *Ailanthus excelsa* and
5. *Azadirchta indica.*

They have very high energy content ranging between 3900 Kcal / gm to 4500 Kcal/gm. *Trema orientalis* is particularly very promising fast growing fuel wood species. It produces 54 tones of dry biomass per hectare only after 3 years of plantation. *Ailanthus excelsa* attain large biomass very quickly. These fuel-wood species can be grown even on the wastelands.

CONVERSION OF BIOMASS ENERGY INTO CLEANER BIO-FUELS BY EBT

Biomass energy can be converted into cleaner bio-alcohol (ethanol) through appropriate fermentation technologies or to combustible biogas (methane) by gasification technology or to bio-diesel through biochemical conversion - all involving the knowledge of modern environmental biotechnology.

Production of biogas, bio-alcohol and bio-diesel from biomass enhance the utilization efficiency of biomass energy making it economically and environmentally more sustainable.

What is more exciting is that these bio-fuels can even be extracted from the waste biomass ,even from the wastes in the landfills by bioreactor technology. If a significant percentage of flammable products currently derived from petroleum can be produced from living or waste biomass, the major industrial economies will improve their energy security by reducing their dependence on Middle Eastern oil and all countries, developed and developing, will spend far less on oil imports, dramatically reduce greenhouse gas emission, and build a new bio-based industry worth hundreds of billion dollars worldwide every year.

1. Biogas (Methane) from Biomass : The Clean Burning Gas

Biogas technology is an integrated environmental biotechnology, mainly based on effective utilization of cattle dung, farm and kitchen wastes, all vegetable wastes and even human excreta and sewage sludge. It was conceived in India and China in the 1950s for rural development program and with multiple objectives to provide the rural people with two important necessities of life-'fuel' and 'fertilizers' and to prevent rural deforestation (extraction of fuel-wood from the community forest). Biogas which is essentially methane (CH_4) is a cheap, clean and convenient source of energy generated from local vegetable resources for multipurpose uses like heating, cooking, lighting and also electricity generation.

Biogas can be produced from a variety of plant materials (vegetable biomass) and even from the waste biomass of municipal solid waste (MSW) with high carbon/nitrogen (C/N) ratio. Production of bio-gas from biomass enhances the utilization efficiency of biomass energy. Each ton of organic waste biomass by dry weight yields about 36 cum of biogas and 350 kg of biomanure. The gas production is high in case of materials which are rich in cellulose and hemi-cellulose, and also contain sufficient proteinaceous substances. Complex polysachharides are more favourable for biogas production. The vegetable and fruits wastes from the green groceries, food processing and dairy waste, cattle dung and the animal matters from the slaughter houses which contains about 24% solid contents can be readily utilized to generate biogas. Even the sewage sludge (biosolids) and. the human excreta rich in organic matter and high C/N ratio can be efficiently used to yield biogas. The monstrously growing noxious aquatic weed water hyacinth *(Eichhornia cressipes)* has assumed great significance in biogas production with rich methane content. Each kg of the weed by dry weight yields about 370 litres of biogas with average methane content between 69-91%. When mixed with cattle dung and algal weeds, the yield of gas is much greater. The calorific value of the gas is about 22,000 KJ/cum.

The biogas contains approximately 63% methane (CH_4), 27% carbon dioxide (CO_2), 10% hydrogen (H_2), traces of hydrogen sulfide (H_2S) and nitrogen (N_2). Methane is colourless, tasteless and non-poisonous gas and on combustion yields 550 Btu of heat per cubic feet of its volume. Methane value of the biogas ranges from 55 to 90% depending upon the nature of the feed-stock. The greater the methane content in biogas, lower the value of the CO_2, better is the thermal efficiency of the gas and higher is its fuel value.

Countries Using Biogas (Methane) Energy

Bio-gas plants are now used in over 46 developing countries and the technology is being improved and popularized in several parts of world specially in the tropical countries to prevent rural deforestation. A biogas program started in Nepal in 1992 has resulted in over 110,000 household biogas plants, with 20,000 additional plants being installed each year by private companies. (GEO, 2006).

Biogas for Electricity Generation

Developed countries including U.S. and Australia have also realized the importance of this simple rural biotechnology for effective utilization of their piling municipal waste, generation of clean source of energy to displace coal for electricity generation and reduce the emission of greenhouse gas.

> The Whittier Company of California, USA is reported to be generating 2.8 MW of electricity by the biogas obtained from 10,000 tones of 'city trash' using 'dual-fuel' IC engines, with diesel and biogas in 50:50 ratio. Engines using 100% of biogas for electricity generation is being developed. Removal of carbon dioxide (CO_2) from the biogas and increasing the methane content, improves the efficiency of the engines and electricity generation.

The Townsville City Council in Queensland, Australia has installed two 115 kW biogas-fuelled power plants at a cost of $ 54,000. Biogas is being produced from the sewage sludge obtained from the Citiwater Sewage Treatment Plants at Cleveland Bay and Mount St John. The electricity produced is used back by the wastewater treatment plants. This is displacing about 300 tones of coal and eliminating the emission of 700 tones of greenhouse gas carbon dioxide (CO_2) every year since 2000. Brisbane City Council in Australia has been using methane generated from the Luggage Point waste treatment plant since 1979 to power 3.2 MW power station

Retrieving Biogas from Landfills for Power Generation : Reducing the Economic and Environmental Burden of Landfills

Several U.S. companies are using landfill methane for power generation. The Capstone Turbine Corporation has installed 50 microturbines at the Lopez Canyon landfill in north Los Angeles. The first commercial landfill gas energy recovery project was developed at Palos Verdes Landfill in Rolling Hills, California in 1975. The average size of an LFG energy recovery project in the US is about 3 MW with typically over 95% availability. The number of commercial LFG power plants have increased from 4 in 1981 to about 130 in 1996.

> The ReOrganic Energy Swanbank in Queensland, Australia is generating electricity from the landfill methane. By end of April 2002, over 2,300 MWh of electricity had been generated (equivalent to providing electricity to about 6,000 homes from coal power plants). It is projected that in the period from 2002 to 2016, some 500,000 MWh of electricity will be generated. Another 3 MW power generating facility was launched at Rochedale Landfill site in Brisbane. This is expected to supply electricity to 5000 homes.

Biogas in India and China

Biogas technology is working wonder in India and China. China has about 8 million biogas plants dotting its entire rural countryside. India has over 10 million biogas plants mostly installed in the rural areas. The Khadi and Village Industries Commission of India provides subsidy to farmers for installing biogas plants. It has also created jobs (for installation and maintenance) for about 8 million man days per year for both skilled and unskilled workers. The Central Mechanical Engineering Research Institute, Durgapur, and the Central Institute of Fisheries Technology, Cochin, have developed improved technologies of biogas production from algal biomass and water hyacinths. In the NE state of Assam two 1 MW power plant is being established which would run on biogas generated from bamboos. Nearly 80 million tons of this giant grass in grown in India each year.

The New Delhi Municipal Corporation in India, is generating biogas from the sewage sludge at Okhla Sewage Treatment Plant. The gas is being supplied to 6000 homes in the locality at a nominal cost. The UNEP supported Sulabh International in India is generating biogas from 'human excreta' in several cities and producing electricity for street lighting.

2. Bio-alcohol from Biomass : The Cleaner Liquid Fuel

The blatant air pollution unleashed by the combustion of oil, and the 'oil shock' of 1970s made the world to rethink about the virtues of alcohol as a cleaner fuel. Ethanol is assuming great value all over the world as an environmentally cleaner fuel and a good substitute of petroleum products as it is a highly inflammable liquid. Ethanol derived from plant sources have clear potential to be sustainable, low cost and high performance, are compatible with present and future automobiles and transportation systems, and provide near-zero net greenhouse gas emissions.

All biomass, even the waste biomass rich in starch and cellulosic materials are good raw materials for ethanol production by enzymatic fermentation carried out by microorganisms 'yeast' and some bacteria. Bagasse is most appropriate raw material. 6000 kg of bagasse upon fermentation yields 1000 litres of ethanol of 95% strength. The paper waste is comprised of 61% cellulose; 16% hemicellulose; 21% lignin; and 2% protein, ash and so on. With this composition the waste paper is ideally suited as a feedstock for ethanol production. The 'molasses and bagasse' from the sugarcane industries, the waste from the paper and pulp industries, saw dust and waste from the saw mills, rice and wheat straws, and even the aquatic weeds can be converted into ethanol. It has been found that 112 kg of total fermentable solids from the sugar can industries could yield about 54 litres of ethanol.

Biotechnology has engineered yeasts, enzymes and bacteria - capable of breaking down plant products (biomass) into complex sugars from which a wide variety of bio-based products including bio-alcohol ethanol.

New bioconversion technologies based on the knowledge of environmental biotechnology could open the gate to the cost-effective use of a wide variety of feed-stocks including agricultural waste products like corn stalks, rice and wheat straws and perennial grasses to produce bio-fuel ethanol and other products, such as chemicals and plastics that are currently derived from fossil fuels. More significantly, the plastics would be 'agro-plastic' and 100% biodegradable. These technologies would allow farmers to harvest double dividends - selling wheat and corn grains, and converting the leftover crop wastes to bio-alcohol ethanol for use in transport and energy industries. The net emissions of greenhouse gas carbon dioxide (CO_2) from the use of bio-fuel ethanol (from plant and agricultural wastes) in automobiles would be near zero. The CO_2 emitted by the ethanol driven automobiles would be ultimately absorbed by the agricultural crop plants as they grow in the farms.

The Brazilean Experiment and Success Story

Brazil is already using 'ethanol' as an auto-fuel (gasohol) since 1975. It grows sugarcane to convert it into ethyl alcohol (ethanol) by fermentation. Its 'National Alcohol Program' is being scrutinized all over the world but mainly in the western nations. Starting in 1975, it aimed at making 10.7 billion litres of 'gasohol' a year by 1985 and substituting this for perhaps 40% of the petroleum consumption. Ethanol provided 44% of all non-diesel motor vehicle fuel consumed in Brazil in 2004. Eventually, Brazil intends that Cassava should replace sugarcane as the main gasohol feedstock.

Mixed with petrol, it increases the energy efficiency. Standard car engines run on a blend of up to 15% gasohol with little or no tuning, and can be modified to use up to 40% gasohol. New cars have been designed in Brazil that will run on gasohol alone. Brazil has gasohol fuel stations named as 'ALCOOL' which offers motorists a choice of fuel grades.

The main byproduct of gasohol production is a very rich organic soup, which could be useful in manufacture of cattle feed, fertilizer or biogas fuel. Unfortunately it is dumped in rivers causing massive pollution.

In the U.S., Australia and several European nations ethanol is now being blended with petrol or diesel to reduce both the amounts of these fossil fuels used and also to reduce the emission products (pollutants). In 2004, ethanol was being blended with 30% of all gasoline sold in the U.S. Currently ethanol accounts for less than 2% of U.S. petrol consumption. The new bioenergy (bio-alcohol) technology could dramatically increase that figure, producing as much as 150 billion litres of ethanol- the equivalent of one quarter of our current petrol use (UNEP Report, 2004).

3. Bio-diesel from Biomass : Cleaner Alternative to Mineral Based Diesel

An alternative cleaner fuel for diesel engines brewed from organic feed-stocks, such as animals waste fats (tallows), lard and waste cooking oils are being produced in Australia and other countries on commercial scales. In Europe about 2 billion litres of biodiesel is being consumed annually. Germany and France are the biggest producers, followed by Italy and recently the the Czech Republic. Australia has a total combined capacity of 50,000 litres a day which is expected to go up to 250,000 litres a day. A Canadian company is projected to produce 35,000 kilolitres of bio-diesel a year. Biodiesel is also being produced from offal at a turkey-processing plant in the U.S. It can now be produced from household waste and used tyres which is mounting all over the world.

Rudolf Diesel, the man who invented diesel oil wrote as early as in 1911- '*The diesel engine can be fed with vegetable oils and would help considerably in development of agriculture of the countries which will use it*'.

Bio-diesel grew by an average of 25% a year in the last decade. It is completely non-toxic and biodegradable, almost free of sulfur and aromatics, and have lower CO_2 emissions than the mineral oil derived diesel. It can be used in existing fuel engines without any modifications. The emissions from bio-diesel are 100% lower in sulfur, 96% lower in total hydrocarbons (HC), 80% lower in polycyclic hydrocarbon (PAH), 45% lower in carbon monoxide (CO) and 28% lower in suspended particulate matters (SPM). It is much less combustible with a flash point greater than 150 °C (73 °C higher than diesel). The ozone potential of speciated HC is 50% lower (AEN, 2002).

Any vegetable oil can form the feedstock to produce bio-diesel. It can produced by recycling the waste oil from fast-food restaurants and the deep friers of French fries which generate huge amount of waste vegetable oils. Bio-diesel is the methyl (or ethyl) ester of fatty acids and can be used in pure form or blended with regular diesel. At present, 3:1 mixture of sunflower oil and diesel is similar in efficiency to pure diesel.

Bio-diesel is produced by combing the natural oils or fats with alcohols. The production process involves heating the feedstock to 60 °C and adding ethanol or methanol and potassium hydroxide (KOH) working as chemical reagents. This changes the chemical composition of the feedstock and separates them into biodiesel and glycerine. In low, and ultra-low sulfur diesel fuel, the addition of biodiesel significantly improves the fuels's lubricity.

Biodiesel from Petro-Crops

Another novel idea is to grow 'petro-crops'. They contain rich hydrocarbons (C-15 compounds) in their latex. *Jatropha curcus, Euphorbia* spp., *Calotropis procera, C. gigantea, Jojoba simmondsia, Pongamia* spp. etc. are identified petro-crops and can be grown even in the deserts and wastelands. Their hydrocarbon containing milky latex can be processed to get biodiesel. The oil obtained from the latex of *Jatropha curcus* is close to diesel oil in chemistry. *Euphorbia* burns fiercely upon combustion and their latex is being processed in the U.S. to obtain biodiesel.

Biodiesel oil obtained from the petro-crops or from the vegetable oil plant can be considered as renewable and cleaner alternative to fossil fuels and their use will result into 'net zero emission' of carbon dioxide. They have already absorbed greater amount of carbon dioxide from the atmosphere (while growing) what they have emitted upon combustion of their oil. Estimates suggest that each ton of biodiesel produced and consumed from petro-crops leads to a reduction of greenhouse gases by 3 times i.e. the petro-crops would absorb three times of carbon dioxide that is what emitted upon using their biodiesel.

CONCLUDING REMARKS

Biomass energy can be used in a variety of ways to supply heat, steam, green electricity, combustible gases (hydrogen and methane), and even transport fuel (as ethanol). Biomass is a local energy source, and a biomass based power plant in rural area can create several jobs opportunities for local people. Capital requirements for establishment of biomass power plant will be much less. Small biomass power plants can be more decentralized and transmissional losses as compared to centralized coal-fired plants will be greatly reduced. Environmental-economics of waste biomass generated energy will still be more positive as it has several environmental and economic benefits. It is a situation where waste becomes a resource. It is like getting 'gold from garbage'.

One of the major cause of rural deforestation in the developing countries is the extraction of fuel wood from the native forest and vegetation by the rural poor.

Deforestation leads to a chain reaction ending up with soil erosion and desertification. The social impact of deforestation and desertification is poverty and disease which has become order of the rural society in several Asian and African countries.

Although fuel-wood combustion emits greenhouse gas carbon dioxide (CO_2), it do not add to the net increase in CO_2 value of the atmosphere. Regrowth of the biomass takes back an equivalent amount of carbon dioxide from the atmosphere in the process of photosynthesis.

> It is estimated that if a coal powered plant of 1000 MW is fuelled by biomass it would displace an equivalent amount of coal and the net carbon dioxide emission would be reduced by 7.4 million tones a year. But this ECBA will work positive only for those biomass fuelled power plants in which the biomass is obtained through 'bio-energy plantations' (agroforestry) with fast growing fuel wood species or the biomass is obtained from the waste (MSW). It will be disastrously negative if the biomass is obtained by cutting trees from the existing forest and vegetation. This would mean removing the 'carbon sink' (trees) on one hand and increasing the 'carbon source' (combustion of wood) on the other.

Unlike fossil fuels, biomass contain no or negligible amount of sulfur and hence no threat of sulfur dioxide (SO_2) emission and acid rains. The ash left after combustion contains negligible amount of toxic metals and hence can be used as soil conditioner. However, fuel wood combustion emits toxic and carcinogenic suspended particulate matters (SPM). Indoor air pollutants from biomass smoke have been found to contain formaldehyde and polycyclic aromatic hydrocarbons (PAH). They have been linked with the heart and lung diseases, cancer of lungs and nasopharynx, acute respiratory infections in children and low birth weights if pregnant mothers were exposed to wood smoke for long hours. However, the biogas, bio-alcohol and bio-diesel are most sustainable form of biomass energy.

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SECTION-V

ENVIRONMENTAL BIOTECHNOLOGY : THE BACKBONE OF SUSTAINABLE AGRICULTURE

Producing Safe & Protective Foods for Mankind Without Recourse to Hazardous Agro-chemicals

In the coming decades and by the year 2020, there will be an extra 1.5 billion people to be fed. This is another challenge, and as great as it was faced 50-55 years ago which necessitated the first green revolution in the 1950's and 60's. If the increasing threat of soil degradation, food contamination, crop genetic erosion and food shortages in world has to be avoided, there has to be a pragmatic changes in the farm technology for ushering in an era of 'sustainable agriculture' based on the principles of environmental biotechnology.

And, it is just not 'enough' to produce sufficient food to feed the people but to produce a high quality of food which should be 'safe' (chemical free) and also 'protective' (good combination of macro and micro nutrients and vitamins) and to do it in a sustainable manner so as to ensure 'food security' for all, but most importantly in the poor Third World nations in the long term. In the developed nations 'food security' is not a major issues as it is in the developing nations.

'Food safety' is a major issue everywhere in the world. According to United Nation Environment Program (UNEP) and the World Health Organization (WHO) nearly 3 million people suffer from 'acute pesticide poisoning' and some 10 to 20 thousands people die every year from it in the developing countries. *US scientists predict that up to 20,000 Americans may die of cancer, each year, due to the low levels of 'residual pesticides' in the chemically grown food.* (UNEP Reports, 1992-2006). The extreme evolution in the quality of safe and protective food could be the development of 'medicafood' containing oral vaccines. Since the early 1990's scientists have been working on developing 'oral vaccines' based on engineered food crops.

In the wake of 'Green Revolution' of the 1960's indiscriminate use of chemical pesticides all over the world has destroyed the 'natural predators' and 'pollinators' and other 'non-target' species, and increased biological resistance in target pests. To date more than 400 species of insect pests are believed to have become resistant to pesticides. This has necessitated the development of alternative method of pest and disease control. Researches into environmental biotechnology has provided some environmentally safer methods where pests can be killed even without recourse to pesticides. It has also led to development of environmentally safer alternatives to chemical pesticides—the 'biopesticides' made from plants and microbes. The Microbiological Resource Centers (MIRCENS) at Cairo, Egypt is also examining the use of microbes in 'pest & vector control'.

Production and delivery of all types of 'nitrogenous biofertilizers' as an effective alternative to the chemical urea is one of the greatest achievements of researches into environmental biotechnology. The Microbiological Resource Centers (MIRCENS) in Kenya, Senegal, Egypt and Brazil are exploring biofertilizers based on nitrogen-fixing bacteria, which it is estimated, could cut the world's chemical fertilizer bill by US $ 15 billion a year and arrest millions of tons of greenhouse gases entering into the environment every year. Biological nitrogen fixation through microbial labour force holds huge potential for future food production by 'organic farming' (by use of environmentally safe 'organic nitrogen' instead of chemical nitrogen). Each year about 139 billion tones of organic nitrogen are added to the earth soil through 'biochemical fixation of atmospheric nitrogen' by certain class of microorganisms. The concept of biofertilizer technology is to domesticate some of these organisms in the agricultural production system and enhance their capacity for atmospheric nitrogen fixation (where 78 % of the air is nitrogen) through gene manipulations by biotechnology.

Environmental biotechnology has increased the value of crop biodiversity in agriculture. The distant wild relatives of modern crops once considered useless for breeding purposes contain unique traits that can now be bred into commercial elite varieties within a matter of years. Agri-biotechnology and genetic engineering has opened up new avenues of unlimited potentiality for increasing food production without the use of agro-chemicals.

Planning for sustainable agriculture requires a judicious combination of some new technological inputs especially those resulting from the researches in 'agricultural biotechnology', with the 'traditional wisdoms' of farming practices of ancient times based on biodiversity. It will amount to embarking on a 'Second Green Revolution' with 'biotechnological inputs' as the driving force. We will need further genetic improvement in our conventional crop plants-not only for increasing quantity but also nutritional quality of the food, resistant not only to 'pest and diseases' but also to 'drought and salinity' which is growing all over the world. Salinity resistant crop plants will have further advantage of being cultivated by saline seawater.

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Agri-biotechnology : The Revolution in Food Production

Production of Safe and Protective Foods With In-built Mechanism for Protection from Pest and Diseases : A Significant Achievement in the Realm of EBT

KEY WORDS

Food Security; Transgenic Crops; Medica Food; Super Rice; Super Potato; Super Tomato; Introduction of NIF (Nitrogen Fixing) Genes in Crops; Production of Drought, Freeze and Salinity Resistant Crops by Gene Manipulation; Gene Silencing Technology

BACKGROUND INFORMATION

In the coming decades and by the year 2020, there will be an extra 1.5 billion people to be fed. This is another challenge, and as great as it was faced 50-55 years ago which necessitated the first green revolution in the 1950's and 60's. If the increasing threat of soil degradation, food contamination, crop genetic erosion and food shortages in world has to be avoided, there has to be a pragmatic changes in the farm technology for ushering in an era of 'sustainable agriculture' based on the principles of environmental biotechnology.

And, it is just not 'enough' to produce sufficient food to feed the people but to produce a high quality of food which should be 'safe' (chemical free) and also 'protective' (good combination of macro and micro nutrients and vitamins) and to do it in a sustainable manner so as to ensure 'food security' for all, but most importantly in the poor Third World nations in the long term. In the developed nations 'food security' is not a major issues as it is in the developing nations. In general, over the last 15 years the output of food and fibre in OECD countries has risen by 20%, on 2% less land and with 8% fewer workers. Despite short-term fluctuations, the price of food has declined over the long term. There has been on average 5% decline in nutrients surpluses from agriculture while pesticide uses has fallen in all but three OECD countries over the last decades. (UNEP-DTIE, 1999). Moreover, in the affluent societies people have a variety of foods to choose from, but the poor depends just on handful of few staple foods like rice, wheat and corn that they can easily afford.

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THE DAWN OF ENVIRONMENTAL AGRI-BIOTECHNOLOGY FOR SUSTAINABLE AGRICULTURE

Agri-biotechnology and genetic engineering has opened up new avenues of unlimited potentiality for increasing food production without the use of agro-chemicals. Genetic engineering took off in the US in the early 1970s with companies like Biogen, Cetus and Genentech doing pioneering works. Genes believed to determine specific traits, such as height, tolerance to frost or drought, protein or fatty acid composition, are spliced into plants from unrelated organisms, such as animals, other plants, fungi or bacteria, in the belief that the genetically engineered plant will exhibit the desired trait.

Genetic material from chickens and silk moths have been spliced into potatoes to confer resistance against bacterial diseases.

With the development of 'genetic engineering' it became possible to 'synthesize and transfer' the desired 'useful genes' directly to the crop plants and get the results quickly. Genetic engineering offer a faster route than conventional plant breeding, to increase yield levels, decrease the use of pesticides and raise the nutrient value of basic food crops. It can also provide the farmers on less favourable lands, with plant species that have greater tolerance to drought, salinity, flooding, freezing and lack of soil nutrients. It can also produce plants which are resistant to pests and diseases, herbicides use, salt and toxic heavy metals in soil (Conway, 2000).

Hundreds of field tests of genetically engineered crops have been taking place since 1987, mainly in the United States and Britain. In plant field release trials carried out between 1993-1994 in the 14 OECD countries, herbicide tolerance was the most common genetically engineered trait being tested (36%); insect resistance was second at 32%, while tests for virus resistance and quality traits (altered fruit ripening, for example, or increased solid content in fruits and tubers) accounted for 14% each, leaving 4% to 'others' (including disease and male sterility in plants).

PRODUCTION OF GENETICALLY ENGINEERED FOOD : THE TRANSGENIC CROPS

Genetically engineered/modified variants (GMs) of most of the world's major food, fibre and fruit crops have now been produced including corn, wheat, rice, potato, soybean, sunflower, oilseed rape, cotton and tomato (Steinbrecher, 1996). In 1996, Zeneca successfully introduced its GM tomato puree in the UK, and Mosanto the GM soya.

Several genetically engineered crops called 'transgenic crops' have been produced. In the past decades, the genetically engineered food crops have begun to leave the laboratory and enter the agro-ecosystems of world. These are 'transgenic corn', 'transgenic cotton', 'transgenic soybean', 'transgenic potato' and 'transgenic rice' etc. Worldwide over 40 million hectares were planted with genetically engineered crops in 1999, mostly in the US. China is growing transgenic crops in over 1,00,000 hectares. It is growing 'transgenic tobacco' on commercial scale since 1992.

The claims made for the 'gene revolution' of the 1990s echo those of the 'green revolution' of the 1960s—increased food production from new, higher-yielding seed varieties with world hunger becoming a thing of the past, the only difference being that this time the seed varieties are newly-created rather than newly-bred.

1. The Super Tomato

The first transgenic plant variety to be marketed in world was the 'flavor-savor' (Flr-svr) tomato in 1994. Its main achievement was long shelf life, allowing the harvest of ripened fruits instead of green ones and thus developing a better savour. These tomatoes also have a higher dry matter content, which is appreciated by the food processor for tomato ketchup industries.

2. The Super Rice

A new rice variety called 'golden rice' has been developed by Swiss Federal Institute of Technology in Zurich and the University of Freiburg with research support from Rockefeller Foundation who succeeded in inserting 7 new genes in rice. This has led to increase in yield by 15 to 25 per cent higher. But the most significant achievement is that genes which produce 'beta-carotene' (precursor of vitamin A) has been introduced in the rice grain. Another gene from the French bean has been added which increases the iron content over three fold. This was achieved by the International Rice Research Institute (IRRI), Philippines. These new genes encode enzymes and proteins that gives rice the ability to make and store beta carotene in the kernels and allow the kernels to accumulate extra absorbed iron.

This is truly a manmade 'miracle rice' a protective food, which will be highly nutritional and save millions of women and children in the rice eating countries who suffers from vitamin A and iron deficiencies.

Another new variety of rice called 'Nerica' have been developed by a NGO of Africa with research support from UNDP. The new variety yield 50 per cent larger crops without fertilizers, have higher amount of protein, are more resistant to diseases and tolerant to acid soils. Their broad blades that grow more quickly, cut out light and thus help stop growth of weeds. They also resist some of the most damaging insect pests. Similarly, the German scientists have produced vitamin rich rice 'T-30 D' by transferring genes from 'daffodil' and a bacterium into 'Japonica' paddy.

3. The Super Potato

Biotechnological researches done at Jawaharlal Nehru University, New Delhi, under the leadership of Prof. Ashish Dutta has isolated a gene from the traditional crop 'ramdana' (*Amaranthus hypochondriacus*) which codes for a novel protein that contains all ten essential amino acids. The gene named as 'AMA-1' gene (amaranthus seed albumin) has been patented in India and has been successfully implanted into potato. The new 'super potato' is being grown by Potato Research Institute, Simla. It is rich in protein (like *Amaranthus*), gives higher yield and is resistant to several diseases.

If the AMA-1 gene is successfully implanted in other cereal and pulse crops it would be a significant achievement. The National Centre for Plant Genomic Research (NCPGR) at JNU, New Delhi has taken up studies on the expression of AMA-1 gene and another gene called OXDC (oxalate decarboxylase) for production of transgenic cereals with high nutritional value. (Basu & Khan, 2001; Sharma, 2002).

4. The New Soybean : A Protective Food With Better Taste & Easy Digestability

Domesticated by Chinese 5000 years ago, the food value of soybean has increased tremendously in the past years and it stands 4^{th} after rice, wheat and corn in global consumption. The 1999 world soy bean harvest was over 154 million tons.

> There are several health benefits associated with soybean, which is mainly due to the rich 'isoflavones' that has medical value in cardiovascular diseases, cancer, osteoporosis, etc. Soya is also major source of 'dietary saponins'. Studies show that saponins have the ability to lower cholesterol in blood vessels, inhibit growth of cancer cells, eliminate digestive toxins and strengthen the immune system.

Having all these benefits many people do not enjoy its taste and digestibility is another issue. A new soybean with 90% less stachyose and raffinose (sugars difficult to digest) has been developed. High oleic acid content has also been developed in soybean. Not only this soybean provide better taste, but it also has a healthier fatty acid profile (high in mono-unsaturated fatty acids) and hence soybean oil is a healthy vegetable oil than other.

ECONOMIC & ENVIRONMENTAL BENEFITS OF TRANSGENIC CROPS

There have been several economic and environmental benefits of the transgenic crops developed by genetic engineering. It has helped in the preservation of the quality of air, water and the soil in the farm ecosystem everywhere in the world where it has been introduced.

1. Significant Reduction in Use of Chemical Pesticides

There has been significant reduction in the use of chemical pesticides where transgenic crops are grown. These crops have 'in-built *Bt* genes' (transferred from *Bacillus thuringiensis*) to produce 'toxins' which work as 'natural pesticide and fungicide' against pests and fungus attack. They mostly kill pest caterpillars, leaving beneficial insects unharmed. They are also resistant to 'herbicide' and therefore, profitable in weed control. 'Endotoxin' gene from *B. thuringiensis* and 'trypsin inhibitor gene' from cowpea have been transferred to mustard, chickpea and rice for conferring resistance against insect pest (Basu & Khan, 2001). It was also transferred to melon which became resistant to 'cucumber mosaic virus'. An Australian survey shows that the use of 'transgenic cotton' led to a 16-86% decrease in insecticide use in various products in 1996 / 97 and 1997/ 98. (UNEP-DTIE, 1999).

2. Preventing Soil Erosion

The revolutionary 'zero' or 'minimum tilling' technology is most sustainable way to maintain the soil health. However, in zero or minimum tilling system major limiting factor is 'weeds'. The development of 'herbicide tolerant' varieties is one of best solution to overcome the problem. It has been reported that the use of transgenic soya beans in no-till production system decreased soil erosion by 90% (UNEP-DTIE, 1999).

3. Preservation of Air and Water Quality in the Farm Ecosystem

Herbicide tolerant cultivars (HTC) facilitate 'no-till' or 'zero till' farming (discussed later), improving both air and water quality. Water quality is improved by reducing the amount of sediments reaching the surface water and the concentration of nitrogen leaching through soil to groundwater. HTC also allow the use of environmentally-friendly products such as 'glyfosate' and 'glyfosinolate' which are quickly broken down in the soil and do not move to groundwater. The major air pollutants from agriculture production are 'pesticide shifts', dust and the greenhouse gas CO_2. The cultivation of pest and disease tolerant cultivars (PDTC) would reduce both pesticides and pesticide shifts, while the cultivation of HTC and continued 'no-till' farming can reduce atmospheric dust loading from croplands to near zero and also the emission of CO_2 (discussed above). Indeed no-till soils can work as a 'carbon sink'.

4. Overcoming the Problems of Soil Toxicity by Aluminium

The Rockefeller Foundation has funded Mexican researchers who added genes to rice and corn to increase tolerance to aluminium - a soil toxicity problem that blights vast areas of the tropics. (UNEP-DTIE, 1999).

5. Overcoming Problems of Submergence and Dryness

In India scientists have added two genes to rice that appears to help plants that are submerged for long periods, a common problem is Asia. Similarly attempts are being made to introduce drought resistant/tolerant genes in crops. In the events of erratic rainfall, drought and deluge due to climate change and global warming this may become a regular problem and only such ecologically adapted crops will survive. (UNEP-DTIE, 1999).

5. Apprehensions about 'Transgenic Crops'

However, there are serious apprehensions that genetically engineered plants may increase the use of herbicides and pesticides and accelerate the evolution of 'super-weeds' and 'super-bugs'. Crucially, major environmental risks associated with genetically engineered plants are the unintended transfer to plant relatives of the 'trans-genes' and the unpredictable effects. By using genetic engineering, corporate scientist are attempting to transform nature to their own blueprint. There is a serious apprehension that in the attempt, the technology may backfire on the environment and the agricultural ecosystems in a myriad of ways. The terminator gene technology which produced 'terminator seeds' could play havoc with farmers and their crops. If this genetic package seeped to other crops, it could cause mass sterility in them. Fortunately, this monstrous technology has now been banned all over the world.

Potential health and environment risks associated with such transgenic 'food crops' are—

1. The crops can themselves become pest in farms or in the wild because of their improved ability to survive;
2. These crops act as a conduit for new 'foreign' genes to move into wild plants;
3. Crops engineered to produce toxic substances such as drugs or pesticides may present dangers to other organisms like birds feeding on the crops as well as to the humans;
4. Crops engineered to tolerate harmful pesticides may increase the use of these chemicals, further polluting water and poisoning wild plant species;

Testing of transgenic crops are being done in over 45 countries, among which Argentina, Canada, Australia, Mexico, South Africa, Spain, France, U.S. and China are leading.

Safety of 'Transgenic Foods' and the Risk Assessment

Risk assessment about the safety of 'transgenic foods' is technically different from the 'transgenic crops'. While the crops constitute the part of the agricultural ecosystem and may have suspected impacts on the natural ecosystem, their products (foods) are actually consumed and may impact the human physiology and biochemistry. In assessing safety of food produced from transgenic crops, there is no place for complacency. In general, two main concerns with TFs are -

1. Potential allergenicity (allergic reactions in human body); and
2. Use of antibiotic resistance markers genes for resistance against ampicillin and kemanycin.

It must be realized that the first TF as a 'super tomato' appeared in the market in 1994 followed by rice, corn, potato, soybean and variety of other foods and by now they have been eaten by hundreds of millions of people in Europe, America, Australia and Asia without any known problem, indicating that the assessment of allergencity is working satisfactorily.

> As regards antibiotic resistance markers, it must be noted that a plant engineered to contain a gene for antibiotic resistance do not cause antibiotic production in that plant, and also does not make the person eating the food from that plant resistant to the antibiotic. Thus no antibiotics are present in the foods produced from plants containing these genes, and thus there is no risk of affecting the beneficial microbes in the intestine. There was also apprehension that the antibiotic resistant genes from the transgenic plants could be transferred in nature to bacteria harmful to humans. To date, stable gene transfer from plant genomic DNA to microbes has never been shown to occur under natural conditions. Scientific Committees at European levels have completed the safety assessment of a transgenic corn plant containing an 'amikacin marker' and cleared it.

Joint FAO/WHO Codex Alimentarius Commission, which is in charge of defining food standards, at its 23^{rd} session in July 1999, decided to establish a 'Task Force on Food Derived from Agri-biotechnology'. That Task Force should help define and harmonise requirements for assessing the safety of transgenic foods (TFs), thus allowing consumers to reap the benefits of biotechnology and genetic engineering researches in agriculture worldwide. Transgenic crop plants will very likely contribute to achieving sustainability in food safety for the world, and food security for the developing country with improvement in quality as a nutritive and protective food.

GENE SILENCING BIOTECHNOLOGY : PRODUCING RESISTANCE AGAINST BACTERIAL DISEASES IN CROPS

Prof. Abhay Dandekar of University of California, Davis, USA, have developed a technique called 'gene silencing' to produce resistance to bacterial diseases in plants especially the 'crown gall disease' caused by the common soil bacterium *Agrobacterium tumefaciens*. Galls can damage the plants by blocking transport of nutrients and water up and down the plant stem or tree trunk. Gene silencing works by interrupting or suppressing the activity of a targeted gene in the host plant and preventing it from coordinating with the infecting bacterial gene in the production of specific proteins which lead to over production of plant growth hormones, resulting in uncontrolled cell growth and gall (tumour) formation. The work was done on tomato plant.

> The 'gene silencing' technique holds promise for creating genetically engineered trees and vines that can stave off 'crown gall', a costly disease that afflicts many perennial fruit and nut crops, including walnuts, apples and grapes. The plants could still be infected by *A. tumefaciens* but would not produce the hormones that lead to gall formation.

OTHER PROMISES OF ENVIRONMENTAL AGRI-BIOTECHNOLOGY

1. Enhancing Biological Nitrogen Fixation : Improving Upon the Nature's Biotechnology

Biological nitrogen fixation through microbial labour force holds huge potential for future food production. Each year about 139 billion tones of 'biological nitrogen' (which is 'organic' and environmentally benign as compared to the 'chemical nitrogen) are added to the earth soil through 'biochemical fixation' of 'atmospheric nitrogen' only by certain class of microorganisms. They are free-living terrestrial and aquatic blue-green algae; the 'symbiotic bacteria' *Rhizobium* (*aerobic*) living in the root nodules of legumes; the free-living bacteria *Azatobacter* (*aerobic*) and *Azospirillum* living in close association with the roots of several crop plants; free living bacteria *Closteridium* (*anaerobic*), and all those microbes living in the gut of earthworms. They have 'nitrogen fixing' (NIF) genes and produce an array of enzymes to assimilate the 'molecular nitrogen' from the atmosphere and convert it into 'organic nitrogen'. The key enzyme is a molybdenum (Mo) and iron (Fe) containing protein called 'nitrogenase'. The biochemical process is essentially a reduction of nitrogen to ammonia (NH_3) and subsequent biosynthesis of amino acids, proteins and other nitrogenous biomolecules.

Inoculation of crop plants with nitrogen fixing microbes has become an accepted biotechnology in US, Germany, Brazil, Israel, Egypt, China and India. In Egypt, scientists have discovered that a species of *Rhizobium* can also colonise rice crops, making them more productive. They provide about 0.5 to 0.8 kg of nitrogen/day/hectare. BGA biotechnology producing 'blue-green algae biofertilizer' has emerged as a cost-effective technology for rice cultivation. It can provide up to 30 to 40 kg of organic nitrogen / hectare / season.

Efforts to Transfer NIF Genes in Crop Plants : A Great Feat of Environmental Agri-biotechnology

Mukherjee (1986) through his painstaking works in India has established the technological superiority of the natural biological nitrogen fixation system over the man-made synthetic (abiological and chemical) industrial nitrogen fixation system. (Dadarwal & Yadav, 1989).

Taking lessons from the nature, biotechnologists all over the world are trying to synthesize and transfer the 'nitrogen fixing (NIF) genes' directly into the major food crops – rice, wheat and maize to provide them the direct power of 'nitrogen assimilation' from the atmosphere. Nature deprived all other green plants on Earth of the 'wonder NIF genes' and the scientists are trying to do what nature could not do. The day this biotechnological feat is achieved it would revolutionize cleaner food production in world. It would not only significantly reduce the cost of food production by eliminating the need of costly chemical resources but also save the environment from the ill effects of the chemical production by use of nitrogenous fertilizers.

2. Introducing Cold Regulation Genes in Crops to Extend Growing Seasons

Another significant achievement is the introduction of 'cold regulation gene' switch which would control the plant cell defence responses to low temperatures. *Increasing plant tolerance to the cold could extend growing seasons and expand the regions supporting agriculture for some crops.*

3. Introducing Drought, Freeze and Salinity Resistant Genes in Crops

Search is also on for genes resistant to drought, freezing and salinity. When plants are subjected to such harsh environmental conditions, they naturally produce some 'defensive proteins' to combat and overcome them. These proteins have been identified and named as 'LEA Protein', 'RAB Protein', 'Dehydrin', 'H.S. Protein', 'Osmotene' and

'Anaxin' etc. The genes producing these proteins have also been isolated and transferred to rice, mustard, tomato and potato. *The team of Prof. V.L. Chopra at Pusa Institute, New Delhi and at Delhi University have successfully implanted 'drought resistant genes' into mustard and rice to produce their drought resistant varieties.* The new variety of mustard called 'Jai Kisan' has become very popular and are grown in the desert districts of Rajasthan. (Basu & Khan, 2001).

At M.S. Swaminathan Research Foundation, Chennai, a gene has been isolated from the Mangrove plants growing on the sea coast which tolerate saline water. *This 'salinity resistant gene' has been successfully transferred into tobacco, and now in rice too. Such transgenic crops grown in the coastal areas can withstand salinity and saline sea water can be used for irrigation of such crops with salinity resistant genes* (Sharma, 2002). Such salinity resistant crops will also be boon for the saline soils of Thar desert in Rajasthan and Gujarat.

4. Introducing Genes for Improving Storage Life After Harvest

Genes are also being searched for fruits and vegetable crops which would enable them to be stored for longer time after harvesting. They can be kept green for longer time and ripening can be prolonged. Pusa Institute in New Delhi has produced a tomato which is much tastier and also stable for longer time.

5. Genes to Enhance the Rate of Photosynthesis in Crop Plants

Food production can also be significantly increased by improving the efficiency of 'photosynthesis' in conventional crop plants by synthesis and transfer of genes which can double the rate of carbon dioxide (CO_2) fixation and also eliminate the process of 'photo-oxidation' which destroy the synthesized carbohydrates under high light intensity and oxygen pressure. Nature has provided some grasses which operate more efficient mechanism for CO_2 fixation called C_4 pathway. Sugarcane also possess those genes. Their genes could be bred into our major food crops rice and wheat which also belong to the grass family.

NEW SOURCES OF NUTRITIVE AND PROTECTIVE FOODS THROUGH BIOTECHNOLOGICAL STUDIES

Future of human food security is also dependent on domestication of new sources of food from the wild. Just three (3) crop species - wheat, rice and maize provides half the world food; another five potato, barley, sweet potato, cassava and now soybean bring the total to more than three quarters. Such overwhelming dependence on few food crops may prove dangerous for the civilization. Disease can wipe out this handful of crops as it happened in the case of Irish Potato famine in the 1840s, causing a fifth of the country's people to die of starvation.

Some 500 plant species have been used as food by the indigenous people across the world. They need to be studied and domesticated. *Amaranthus polygamous, Fagopyrum esculentum* and the 'buckwhets' are pseudo-cereals rich in good protein particularly amino acid 'lysine' which is lacking in modern wheat varieties. Seeds of *Cicer songaricum* a wild plant from Ladhak Himalayas has high protein and phospholipids and about 1% lecithin. They can all enter into our modern food culture.

New sources of plant food (with high nutritional but low caloric value) from the wild have come to light. These are—

1. Buffalo gourd from North America rich protein content, 65.3% of lineolic acid and 23% oleic acid.
2. Winged beans from New Guinea has 34% palatable protein (like soybean) and 18% oil content. The seeds, pods, leaves and tuberous roots- all are edible. The leaves taste like spinach, the sautéed flowers like mushrooms and the seeds like peas. It is now being grown in more than 70 countries;
3. Yeheb nut from Somalia and Kenya has nutritious seeds with pleasant sweet flavour. More exciting is that it is drought-resistant and hence can be grown in regions with low rainfall and can cope in future in the event of global warming;
4. Pummelo from South-east Asia is a large sweet-flavoured citrus fruit. It has been found that some varieties of pummelo are salt tolerant, opening up the possibilities of cultivating citrus fruits on saline soils and on irrigation by seawater;
5. Amaranth is the mystical crop of Aztecs in Mexico used in the 15^{th} century. Grain is rich in proteins with high 'lysine' (valuable amino acid) content. It has potential to comeback in modern food system;
6. Job's Tears is very nutritious cereal which could possibly replace wheat and rice in disease prone areas;
7. The blue-green algae *Spirulina* is emerging as a new source of nutritive food with short-harvest cycle of mass production in open ponds or closed polythene tubes. It out-performs all of the major crops with yield ten times higher than wheat. It has very high protein value - 70% by dry weight and vitamins A, B, C, D & E, β carotene and rich minerals. One gram of *Spirulina* is equivalent to 1 kg of assorted food. Global production of *Spirulina* is over 1,500 tons a year. It is also rich in gamma linolenic acid (GLA) which dissolves low density (LDL) cholesterol in arteries;
8. The 'tree tomato' from South America and 'cope goosberry' from South America- all are potential food plants for the future;
9. A plant from Paraguayan produces 'calorie free' substance 300 times sweeter than sugar;
10. A coffee entirely free from 'caffeine' has been discovered on the island of Comoros near Madagascar. (WWF Reports, 1984).

CONCLUDING REMARKS

Environmental biotechnology has increased the value of crop biodiversity in agriculture. The distant wild relatives of modern crops once considered useless for breeding purposes contain unique traits that can now be bred into commercial elite varieties within a matter of years.

Planning for sustainable agriculture requires a judicious combination of some new technological inputs especially those resulting from the researches in 'agricultural biotechnology', with the 'traditional wisdoms' of farming practices of ancient times based on biodiversity. It will amount to embarking on a 'Second Green Revolution' with 'biotechnological inputs' as the driving force. We will need further genetic improvement in our conventional crop plants- not only for increasing quantity but also nutritional quality of the food, resistant not only to 'pest and diseases' but also to 'drought and salinity' which is growing all over the world. Salinity resistant crop plants will have further advantage of being cultivated by saline seawater.

Further, we not only need to genetically improve our conventional crop plants but also to 'conserve their genetic diversity' and search for 'new sources of alternative food crops' from the wild with 'high nutritional value' and 'shorter harvest cycle' and bring it into our modern food culture.

Implantation of genes for atmospheric nitrogen fixation, controlling the ripening of fruits and seeds, extending the growing season, can also increase human food supply and also reduce the consumption of nitrogenous fertilizer.

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19

BIOFERTILIZERS : FOR FOOD SAFETY AND HEALTH SECURITY

An Ecologically Safer & Economically Cheaper Alternative to the Hazardous & Costly Chemical Fertilizers : A Significant Achievement of Researches into EBT for Sustainable Agriculture

KEY WORDS

Food Safety & Security; Biofertilizers - Source of Slow Release Nutrients; Biofertilizers - Source of Environmentally Safe Organic Nitrogen; Biofertilizers - Source of Plant Growth Hormones; Biofertilizers - Source of Natural Pesticides; Blue-green Algae Biofertilizer; Rhizobium Biofertilizer; Azatobacter Biofertilizer; Azospirillium Biofertilizer; Mycorrhizal Biofertilizer; Azolla Biofertilizer;

BACKGROUND INFORMATION

The industrial fixation of atmospheric nitrogen (N_2) to produce chemical urea (NH_2CONH_2) is proving highly destructive to the environment, both during their production in factory and their use in farms. Worldwide consumption of chemical fertilizers peaked in 1998 at 137 million tons in the urge to raise food productivity. On an average production of every 1 kg of nitrogenous chemical urea emits 2,500 grams of carbon dioxide (CO_2), 10 grams of nitrous oxide (N_2O) and 1 gram of methane (CH_4) - all greenhouse gases. (Dwivedi, 2006). Molecule to molecule methane absorbs 20 times more radiation than CO_2. Seldom more than 50% of the N applied is taken up by the crops and the rest is either stored in the soil or lost into the environment. With annual environmental loadings significant amount of this N eventually migrates to the groundwater. The nitrifying bacteria (*Urobacteria, Nitrosomonas & Nitrobacter*) in the soil convert the chemical nitrogen into 'nitrate' which is a potential human health hazard in groundwater. Being highly soluble nitrate readily leaches through soil and move into groundwater. The denitrifying bacteria (*Bacillus, Chromobacter, Pseudomonas, Spirillium* etc.) in soil convert back the 'nitrates' into gaseous nitrogen (N_2) and this phenomenon again release greenhouse gases 'nitrous oxide' (N_2O) and carbon dioxide (CO_2). N_2O is also destructive to stratospheric ozone.

> 'Food safety and security' is a major issue everywhere in the world and we have to find a more safer and sustainable alternative to the 'chemical agriculture' whose products have threatened the health of millions of people in both developed and the developing countries. The challenge is also to maintain the productivity (to feed the growing millions, especially in the developing countries where 'food security' is also a big issue) but, without compromising with their health security. All kinds of biofertilizers (and also the biopesticides discussed in next Chapter 20) which are products of plants, animals and microbes, and which have been obtained through painstaking researches into environmental biotechnology appears to make a big promise towards achieving that goal.

Biofertilizers are nitrogen rich metabolic products of plants, animals and friendly microbes in our environment. Biological nitrogen fixation through microbial labour force holds huge potential for future food production by 'organic farming' (by use of environmentally safe 'organic nitrogen' instead of chemical nitrogen). Each year about 139 billion tones of organic nitrogen are added to the earth soil through 'biochemical fixation of atmospheric nitrogen' by certain class of microorganisms. (UNEP Reports, 1992-2006). The concept of biofertilizer technology is to domesticate some of these organisms in the agricultural production system and enhance their capacity for atmospheric nitrogen fixation (where 78% of the air is nitrogen) through gene manipulations by biotechnology.

> Production and delivery of all types of nitrogenous biofertilizers as an effective alternative to the chemical urea is one of the greatest achievements of researches into environmental biotechnology. The Microbiological Resource Centers (MIRCENS) in Kenya, Senegal, Egypt and Brazil are exploring biofertilizers based on nitrogen-fixing bacteria, which it is estimated, could cut the world's chemical fertilizer bill by US $ 15 billion a year and arrest millions of tons of greenhouse gases entering into the environment every year. (UNEP Reports, 1996-2006).

MICROBIAL BIOFERTILIZERS : MICROBES ASSIMILATING ATMOSPHERIC NITROGEN (N_2) & MOBILIZING PHOSPHORUS (P)

The microbes endowed with the property of atmospheric nitrogen (N_2) fixation and assimilation are of broadly of two types-

1. The Blue-green algae (Cyanobacteria)

1. *Anabaena spp.*
2. *Aphanotheca spp.*
3. *Aulosira spp.*
4. *Calothrix spp.*
5. *Gleocapsa spp.*
6. *Gleotrichia spp.*

7. *Lyngbya spp.*
8. *Nostoc spp.*
9. *Plectonema spp.*
10. *Tolypothrix spp.*
11. *Wollea spp.*

2. The Bacteria

1. *Azatobacter spp.*
2. *Azospirillum lipoferum*
3. *Beijerinckia spp.*
4. *Chroococcus spp.*
5. *Closteridium spp.*
6. *Cylindrospermum spp.*
7. *Digitaria clecumbens*
8. *Klebsiella spp.*
9. *Microsystis spp.*
10. *Rhizobium spp.*
11. *Rhodospirillum spp.*

These microbes provides organic nitrogen to plants (as biofertilizer) in several ways. They are as—

1. The free-living terrestrial and aquatic blue-green algae;
2. The symbiotic bacteria *Rhizobium* (*aerobic*) living in the root nodules of legumes;
3. The free-living bacteria *Azatobacter* (*aerobic*) and *Azospirillum* living in close association with the roots of several crop plants;
4. The free living bacteria *Closteridium* (*anaerobic*) in soil, and
5. Microbes living in the gut of earthworms.

They all have 'nitrogen fixing' (NIF) genes and produce an array of enzymes to assimilate the molecular nitrogen from the atmosphere and convert it into organic nitrogen. The key enzyme is a molybdenum (Mo) and iron (Fe) containing protein called 'nitrogenase'. The biochemical process is essentially a reduction of nitrogen to ammonia (NH_3) and subsequent biosynthesis of amino acids, proteins and other nitrogenous biomolecules.

Environmental biotechnologists are working to transfer this NIF genes into crop plants. The day this biotechnological feat is achieved, it will herald a new revolution in human food production and sustainable development with safety of food (for the consumers) and security for the farm and the farmers (the producers). A Nobel Prize awaits for this scientific achievement.

THE MECHANISM OF NITROGEN FIXATION & BIOFERTILIZATION BY MICROBES

1. The Symbiotic Nitrogen Fixing Rhizobium Biofertilizer : The root nodules of leguminous plants are considered to be miniature factories operated by the plant and engineered by the bacterium *Rhizobium* spp. Based on host plant specificity, different *Rhizobium* species have been placed in seven (7) cross inoculation groups namely:

1. Alfa alfa (*R. meliloti*);
2. Clover (*R. trifolii*);
3. Pea (*R. leguminosarum*);
4. Bean (*R. phaseoli*);
5. Lupine (*R. lupini*);
6. Soybean (*R. japonicum*); &
7. Cowpea (*Rhizobium* spp.).

Rhizobium bacteria have the capacity to fix free atmospheric nitrogen of the soil in nodule. It enters the roots througl the root hairs or at the point of origin of the secondary roots and colonizes the cortex of the root and becomes polymorphic forming bacteroids. The host synthesizes a pink substance 'leghaemoglobin', which surrounds the bacteroids enabling them to metabolize molecular nitrogen. These bacteria utilize the free atmospheric nitrogen and synthesize it into new nitrogenous compounds, which are utilized by plants for their growth; the bacteria in turn get their food from these plants.

Rhizobium biofertilizer can fix 50-200 kg of nitrogen per hectare per year. It increases yield by 25-30 per cent and 40-80 kg nitrogen in the soil if left over in the field useful for subsequent crops (Sarkar, 1987).

> In India, since 1970 many private agencies and government organizations have started producing *Rhizobium* biofertilizers. For large scale crop inoculation, carrier based inoculums are prepared. The pure cultures of different *Rhizobia* are first multiplied in shake cultures or fermentors and then mixed with sterilized carrier material like peat, charcoal and lignite. A good quality inoculums has the titre value of about $10^{8\text{-}9}$ cells per gram of the carrier material and a shelf life of over 4 months. Coating the seeds with the inoculums, popularly known as 'seed pelleting' is the easiest way to inoculate the crop. For acid, saline and saline-alkali soils, an additional coating of the seeds with gypsum or lime is recommended to protect the bacterial cells. *Rhizobium* possess nitrogen fixing genes (NIF-genes). In Egypt, scientists have discovered that a species of *Rhizobium* bacteria which fixes nitrogen in legumes can also colonise rice plants, making them more productive (Vishnoi, 1998).

The legumes with *Rhizobium* also works as a green biofertilizer which can supply nitrogen to companion plants, if grown together and to the succeeding generations of crop plants if ploughed back into the fields. Most legumes fix between 100 to 350 kg N/ha/year. In the earlier days when there was no conventional fertilizers, farmers use to plough back into their fields the green leguminous crops (after harvesting their seeds and weeds). The legumes possessed rich flora of nitrogen fixing bacteria in their root nodules. The decaying leguminous plants would release plenty of minerals and organic matter in the soil. Usually thick growing leguminous crops like cowpea, sunn-hemp, berseem, varieties of pulses and lobia are good source of organic matter and nitrogen. These crops are grown and ploughed in the field at a time when their stem is still green and have attained sufficient height and reasonable green leafy matter. This supplies

8 tonnes of organic matter, 40 kg of nitrogen per hectare. Approximately 2/3rd of nitrogen is fixed by bacteria living in the root zone of these green manuring crops. The most commonly used green manures in India are sunn hemp (*Crotolaria juncea),* dhaincha (*Sesbania aculeate),* cluster bean (*Cyamopsis tetragonoloba*), cow pea (*Vigna catjang*), horse gram (*Dolichos biflorus*), berseem (*Trifolium alexandrinum*) and lentil (*Lens esculenta*).

2. The Free Living Nitrogen Fixing *Azotobacter* Biofertilizer : For non-leguminous crops like cotton, sorghum, pearl millet, maize and other cereals suitable nitrogen fixing microorganisms has been in search for many years. In the former USSR, *Azotobacter* have been used as biofertilizer. It is a dependable source and has proved to be effective in several laboratories for pot culture and field experiment. Tamil Nadu Agricultural University has been advocating for the use of this bacterium for rice crop and other cereals. However, *Azotobacter* population in soil or near the root zone of crop is very low as compared to other soil bacteria. It hardly exceeds a few hundred to a few thousand per gram of soil or root. Substantial amount of energy is required for *Azotobacter* to fix nitrogen and the possible source of energy is the soil organic matter. In Indian soils the level of organic matter is very low (0.1 to 0.2%) and hence *Azotobacter* soon runs short of energy source and ceases to fix nitrogen. The most efficient strains of *Azotobacter* needs about 1000 kg organic matter for fixing 30 kg of N/ha. For these reasons scientists have become skeptical in the use of *Azotobacter* and have been looking for better organism (Sarkar, 1987).

3. **The Symbiotic *Azospirillum* Biofertilizer :** Recently, a soil bacterium, *Azospirillum* possessing many advantages over *Azotobacter* has been recognized. It lives in close association with the root system of plants. Almost all crop plants harbour this bacterium in their roots. Its association in cereals e.g. sorghum, maize, barley, oat, wheat, cumbu and minor millets and also in fodder grasses is interesting. Those bacteria colonising the roots not only remain on the surface but also a sizeable proportion penetrate into the root tissue and live in harmony with the plants. *Azospirillum* has wonderful capacity of nitrogen fixation often between 0.5 to 0.8 kg of nitrogen/day/hectare. *Azotobacter and Azospirillum secrete antibiotics which act as pesticides.* Hence biofertilizers can also act as 'bio-pesticides'. These biofertilizers also supply growth regulators such as IAA, IBA, NAA, GA_1, to GA_3 (Sarkar, 1987).

Barbara and Thomas Hurek of the Max Planck Institute for Terrestrial Microbiology in Germany have come across a new group of nitrogen-fixing bacteria inside the roots of Kallar grass *(Leptochloa fusca)* grown in the Indian sub-continent. This grass grows wild in salty soil, and does not need fertilizer. Scientists have named this micro-organism *'Azoarcus'*. It produces a nitrogen-fixing enzyme inside the roots of rice plants which seem to grow 10 to 20 per cent more than plants without the bacteria (Vishnoi, 1998).

BIOFERTILIZERS MADE FROM PLANT AND ANIMALS PRODUCTS (BIOMANURE)

Farmers of ancient days had developed through their traditional experience various methods by which the fertility of the soil could be restored, enhanced and maintained. They used all kinds of plants and animals waste products (dung & urine), bone marrows, blood of animals, fish and fish washings, residues of crops etc. as manure. Since these manures are products of biological origin, they are categorised as biofertilizer. Four types of biomanures are generally recognized :

(1) Farm Yard Manure (FYM)

(2) Concentrated Organic Manures

(3) Compost & Vermicompost

(4) Green Manures from the Legumes

Their production and use is being revived through the modern knowledge in environmental biotechnology.

1. Farm Yard Manure (FYM)

Produced form the farm waste, the FYM are rich in soil nutrients and also provide food to millions of soil microogranisms, which in themselves are good and ready source of soil nutrients after their death and decay. They contribute significantly to soil fertility and also regenerate physio-chemical properties of the soil. FYM are generally decomposed before being applied to the fields. They contain dung, urine, straw, farm waste, household waste and various other organic and inorganic materials.

Ten tonnes of FYM/acre supplies 45 kg nitrogen, 19 kg phosphate and 45 kg potassium. FYM contains 0.5-1.5 per cent N, 0.4-0.8 per cent P_2O_5 and 0.5-0.9 per cent K_2O (Table-2). FYM also contains various micro-nutrients essential for maintenance of soil fertility. It releases soil nutrients for plant growth in a slow but continuous process. Thus the nutrients for plant growth are available for full period of growing season, and some nutrients are retained for next crop also. India's large cattle population contributes large quantity of dung and urine. Annual production of FYM is nearly 7300 kg per animal (Singh, 1992).

2. Concentrated Organic Manures (COM)

Concentrated organic manures are those materials that are organic in nature and made from raw materials of animal and plant origin. These includes non-edible oil cake, edible oil cake and other materials like blood meal, fish meal, bones, horn and hoof meal. These are the waste products of mills, factories and butcher houses. They have high nutrient content. Cotton seed cake has 6.4 per cent N, 2.9 per cent P_2O_5 and 2.2 per cent K_2O (Singh, 1992).

3. Composts

Composts are aerobically decomposed products of organic wastes such as animal dung, decaying and rotting vegetable matters. They supply balanced nutrients to plant roots and stimulate growth; increase organic matter content of the soil and thus improve their physical and chemical properties; provide food for soil micro-organisms and thus increase their capacity of renewing fertility.

One ton of compost may contain 10 lbs of nitrogen (N), 5 lbs of phosphorus (P_2O_5) and 10 lbs of potash (K_2O). They are not all available to plant roots in the first year because nitrogen and phosphorus in organic matter are resistant to decay. Nitrogen is about one

half effective as compared to chemical fertilizer, but phosphorus & potassium are as effective as chemical fertilizers. With continued application of compost the organic nitrogen tends to be released at constant rate from the accumulated 'humus' and the net overall efficiency of nitrogen over a period of years is considerably greater than 50 per cent of that of chemical fertilizers. Availability of phosphorus is sometimes much greater (Reaganold *et al.*, 1990; Bombatkar, 1996).

Cattle urine contains more percentage of nitrogen (N) and potash (K_2O) than the dung, and the urine of goats, sheep and horses have the greatest amount of N and K_2O, pig dung are rich in P_2O_5. Compost made from poultry droppings contain highest nutrient level among all compost. On dry weight basis it contains 1.46 per cent N, 1.17 per cent P_2O_5 and 0.62 per cent K_2O (Singh, 1992).

Vermicompost are nutritionally more rich and efficient compost produced by the action of waste eater earthworms. This has attained great significance these days as a viable alternative to chemical fertilizers. Bhawalkar & Bhawalkar (1993) have found that if 100 kg of organic waste with say, 2 kg of plant nutrients (NPK) are processed through the earthworms, there is a production of about 300 kg of fresh living soil with 6 per cent of NPK and several trace elements that are equally essential for healthy plant growth. This magnification of plant nutrients is possible because earthworms produce extra nutrients from grinding rock particles and by enhancing atmospheric nitrogen fixation. When 100 kg of the same organic wastes are composted unaided by earthworms, about 30 kg compost is derived with 3 per cent NPK. This normal compost thus has a total NPK of only about 1 kg. Rest one kg nutrient might have been leached or volatilised during the process of composting.

4. Green Manures (GM)

The whole legume plant with *Rhizobium* in their root nodules, works as a 'green manure' which can supply nitrogen to companion plants, if grown together and to the succeeding generations of crop plants if ploughed back into the fields. Most legumes fix between 100 to 350 kg N/ha/year. In the earlier days when there was no conventional fertilizers, farmers use to plough back into their fields the green leguminous crops (after harvesting their seeds and weeds).

The legumes possessed rich flora of nitrogen fixing bacteria in their root nodules. The decaying leguminous plants would release plenty of minerals and organic matter in the soil. Usually thick growing leguminous crops like cowpea, sunn-hemp, berseem, varieties of pulses and lobia are good source of organic matter and nitrogen. These crops are grown and ploughed in the field at a time when their stem is still green and have attained sufficient height and reasonable green leafy matter. This supplies 8 tonnes of organic matter, 40 kg of nitrogen per hectare. Approximately 2/3rd of nitrogen is fixed by bacteria living in the root zone of these green manuring crops (Singh, 1992).

The most commonly used green manures are—

1. Sunn hemp (*Crotolaria juncea*),
2. Sesbania (*Sesbania aculeate*),

3. Cluster bean (*Cyamopsis tetragonoloba*),
4. Cow pea (*Vigna catjang*),
5. Horse gram (*Dolichos biflorus*),
6. Berseem (*Trifolium alexandrinum*) &
7. Lentils (*Lens esculenta*)

THE BLUE-GREEN ALGAE BIOFERTILIZER : POTENTIAL FOR COMMERCIALISATION AS SUSTAINABLE ALTERNATIVE TO THE CHEMICAL FERTILIZERS

Blue-green algae (BGA) are 'nitrogen-fixing' and 'phosphate solubilizing' biofertilizers. They can be good substitute to synthetic nitrogenous fertilizers especially for the wet and semi-dry crops. BGA are unicellular or filamentous, microscopic plants inhabiting freshwater or moist soils. Terrestrial species of BGA withstand dry conditions through the formation of resting spores. They multiply by vegetative reproduction and can rapidly produce a large nitrogen-rich biomass in soils.

Major researches on this valuable biotechnological product is being carried out at the Indian Agriculture Research Institute (IARI) in New Delhi and at Kamraj Madurai University in Tamil Nadu. Author and his Ph.D students also did some work on BGA biofertilizer on wheat crops at Indira Gandhi Centre for Human Ecology, University of Rajasthan, Jaipur between 1992-1999. It was a pioneer attempt to study the agronomic impact of BGA on semi-dryland crop like wheat.

The nitrogen-fixing property of BGA and their utility as biofertilizers was first shown in India by De (1939) and then by Singh (1961). BGA can fix as much as 40 kg of organic nitrogen per hectare per season, equivalent to more than 100 kg of chemical urea. BGA cells contain blue and green pigments and nitrogenase enzyme systems, which assimilate free nitrogen. There are more than a hundred nitrogen-fixing genera of BGA. The important ones found in India include *Nostoc, Oscillatoria, Spirulina, Rivularia, Tolypothrix, Scytonema, Calothrix, Aulosira* and *Anabaena*. One species of *Anbaena* (*Anbaena azollea*) grows in symbiotic association with the water fern *Azolla*. *Azolla* can fix 60-80 kg of organic nitrogen per hectare.

TABLE 1

Nitrogen Fixation Potential of BGA Under Cultural Conditions

Species	Nitrogen fixed (mg 100 / ml)
Aulosira fertilissima	8.7
Anabaena ambigua	5.6
A. azollae	3.5
A. cycadae	3.1
A. oryzae	4.4

Contd. ...

Contd. ...

Species	Nitrogen fixed (mg 100 / ml)
A. variabilis	5.7
A. novelis	3.6
Cylindrospermum gorakhporens	5.0
C. sperica	5.2
Tolypothrix tenuis	5.2
T. campilonemoids	6.5
Calothrix brevissima	3.4
Anabaenopsis circularis	2.1
Nostoc spp.	1.1

Source : Kaushik (1997) '*Cyanobacterial Nitrogen Fixation*'.

(i) BGA Improves Phosphate Uptake by Crops : BGA are also capable of dissolving insoluble phosphates in soil, giving double benefit to the crops. Algalization also increases the available phosphorus in soil, possibly because of excretion of organic acids by the BGA cells. Organic acids solubilizes the insoluble calcium phosphate. *Anabaena, Nostoc, Aulosira, Toypothrix* have been found to solubilize 'extra-cellular insoluble phosphate' up to 2.27 mg P_2O_2/50 ml/20 days. This attains significance in view of the fact that most of the phosphatic fertilizer, when applied to the soil is immediately converted into 'insoluble calcium phosphate' and becomes unavailable to plant roots.

(ii) BGA Secretes Plant Growth Hormones : BGA release a variety of biological substances such as growth regulators auxins (IAA, IBA, NAA) and gibberlins (GA, to GA_3) and vitamins, which promote crop growth (Venkatraman and Neelkanthan, 1967). The growth pattern of rice seedlings treated with algal filtrate from *Aulosira fertilissima* resembled seedlings treated with gibberellic acid (Singh and Trehan, 1973). Extracts of *Cylindrospermum muscicola* produced positive effects on root growth of rice crops similar to that produced by the vitamin B_{12} (Venkatraman & Neelkanthan, 1967).

(iii) BGA Scavenge Salt from Soil and Combat Soil Salinity : BGA also have the ability to scavenge sodium from salt-affected soils. The organic acids excreted by BGA solubilizes the $CaCo_3$ nodules in salt affected soils. The released Ca replaces Na on the soil complex. Algalization of saline and alkaline soils lead to remarkable decrease in soil pH, electrical conductivity (EC), and exchangeable sodium (Subhashini and Kaushik, 1981). This property of BGA can be utilized for the reclamation and productivity of saline wastelands.

(iv) BGA Improves Efficiency of Mineral Utilization : BGA improve the mineral utilizing efficiency of the soil. They concentrate nutrients such as nitrogen, phosphorus, fixed carbon (by photosynthesis) and trace elements around the root zones of plants for their better absorption (Goyal, 1987). BGA reduce the oxidizable matters of the soil, remove soil compaction, narrow the C/N ration and facilitate aeration in the rhizosphere.

(v) BGA Improves Soil Binding and Moisture Holding Capacity : BGA have good soil-binding and moisture-holding capacities, which improve the physical nature of the soil (Aiyer *et al.*, 1971). Algalization with BGA in neutral to alkaline soils has resulted in an overall improvement in physico-chemical properties of soil. It improves soil aggregation due to the liberation of complex polysachharides—glucose, galactose, xylose, arabinose, rhamnose etc. The improvement of soil aggregation under field conditions may vary from 50 to 98%. An increase of organic matter by 68.7%, water holding capacity by 34.7%, exchangeable Ca by 58.3%. Rate of infiltration were also reported to be increased due to algalization of saline and alkaline soils (Singh, 1961; Kaushik *et. al*, 1981).

(vi) BGA Reduces Incidence of Pests and Diseases : Egyptian scientists have recently observed a reduction in the incidence and severity of rice leaf and neck infection by 'blast fungus' (*Pyricularia oryzae*) when BGA inoculums were used in rice fields. In our studies made on wheat crops in Jaipur, we observed that the crops grown on BGA biofertilizers were less susceptible to pests and disease as compared to the one grown on chemical fertilizers.

BGA biofertilizer maintain a constant supply of organic nitrogen to the soil besides enriching the plant roots with other valuable nutrients. BGA also appear to reinforce the nitrogen-fixing abilities of the soil microorganisms *Azatobacter* and *Closteridium* and help renew the natural fertility of soil. There is also no need for repeated application of BGA biofertilizers in farm soil because the algae 'regenerate' and keeps on multiplying with little soil moisture.

BGA can also thrive on dry soils in the form of perennating spores which germinate and initiate vegetative growth as soon as moisture returns. *Nostoc*, *Scytonema* and *Tolypothrix* are specially adapted to thrive in dry conditions. The only limiting factor to the natural growth of BGA in the field crop is the availability of phosphates and calcium. A number of farmers in Tamil Nadu and Andhra Pradesh in South India are producing and applying BGA in their paddy fields.

Farm Production of BGA

BGA biofertilizers can be produced on a commercial scale at low cost, even by poor and marginal farmers in developing countries on their farm wastelands and in pits and depressions. They can also be produced in plastic or metallic troughs in the backyards. The process is quick and convenient and requires no special technique or great investment. In a 40 m^2 area, hundreds of kilograms of blue-green algae can be produced every month. The land is bounded and flooded with water and flooding is repeated to keep the water standing. Superphosphate is added to the soil before flooding at the rate of 12 kg/40 m^2 together with a small amount of insecticide to prevent mosquito breeding. The BGA inoculum is added to the standing water at the rate of 2 kg/40 m^2 pit. In clay soils growth of algae occurs in about two to three weeks on clear sunny days and in loam it takes three to four weeks. Maximum growth occurs in the summer months between April and June, when the average yield of BGA per harvest period ranges from 15-30 kg/40 m^2. Once the algae have grown to form a floating mat, they are sun dried and the algal flakes are collected to be used as biofertilizers and also inoculum for further production of BGA. They have a long storage life and repeated harvests from the same pit are achieved by reflooding the pit and applying superphosphates. Further addition of algal inoculums is not required (Kaushik, 1993).

THE AZOLLA BIOFERTILIZER TECHNOLOGY : SYMBIONT OF BGA

Another category of biofertilizer which is increasingly attracting attention is the water-fern, *Azolla* which harbours a blue-green algae *Anabaena azollea* in symbiotic association. *Azolla* has been used in the far East for centuries indeed long before its capacity to fix nitrogen was realized. *Azolla* grows very fast and can readily double its biomass in 3-5 days and fix 60-80 kg of nitrogen per hectare. It can produce up to 1 ton of green biomass per hectare per day containing up to 3 kg of fixed nitrogen. *Azolla pinnata* contains 2.33-3.83 per cent nitrogen (N), 0.196 per cent phosphorus (P_2O_5) and 0.153 per cent potash (K_2O). The use of *Azolla* can provide yield as high as those obtained from chemical fertilizers.

CASE STUDIES OF USE OF BGA AS A SUBSTITUTE TO CHEMICAL FERTILIZER FOR RICE FARMING

Positive responses of algal biofertilizer on rice crops have been reported from Japan, Egypt, Philippines, USSR, Sweden, Nepal, Sri Lanka, China and Burma. Experiments done in The Philippines gave very encouraging results on the responses of different rice varieties grown on *Nostoc commune* as biofertilizer (Venkatraman and Shanmugusundram, 1992).

BGA has proved to be an excellent source of renewable organic nitrogenous fertilizer for commercial production of rice crops replacing the chemical fertilizers up to 70 per cent. Use of BGA generally results in a saving of up to $1/3^{rd}$ of the inorganic fertilizer nitrogen without affecting the yield. They also help in preventing the loss of inorganic nitrogen through percolation, run-off and denitrification (Goyal, 1993). Under favourable conditions of soil moisture and availability of calcium and phosphorus it has the potential to supply 0.7 to 0.8 million tons of 'utilizable organic nitrogen' equivalent to 1.5 to 1.7 mt of inorganic nitrogen. Superimposed application of the algae for 4-5 consecutive seasons, lead to a significant build-up of algal population in the soil to such an extent that the algal effect is sustained over a considerable period of time.

TABLE 2

Rice Yield With and Without Algal Application at Different Nitrogen Levels

N	Treatment		Grain Yield Kg/ha	
	P (Kg/ha)	*K (Kg/ha)*	*Adt. 31*	*1R-20*
(1)	(2)	(3)	(4)	(5)
0	50	50	4175	4106
0	50	50 + BGA	4650	4715
25	50	50	4675	4814
25	50	50 + BGA	5025	5518

Contd. ...

Contd. ...

(1)	(2)	(3)	(4)	(5)
50	50	50	4900	5611
50	50	50 + BGA	5200	6638
75	50	50	5150	6311
75	50	50 + BGA	5550	7091
100	50	50	5575	6948
100	50	50 + BGA	6000	7819

Source : Srinivasan and Ponnaya (1978).

TABLE 3

Responses of Different Rice Varieties to the Application of *Nostoc commune* (BGA) in the Philippines

Grain Yield (Kg/ha)

	Control	NPK	NPK + BGA	BGA
First crop (C-168)	5,376	7,888	7,027	6,272
Second crop (IR-30)	4,704	4,352	5,248	4,832
Third crop (Ir-28)	3,984	5,328	4,960	5,168
(IR-30)	3,536	6,144	5,200	6,256
(IR-30)	4,816	5,232	5,120	4,992
Polled data% increase	22,416	28,944	27,600	27,520
Over control	–	29.1%	23.1%	22.7%

Source : Venkataraman & Shanmugasundaram-'*Algal Biofertilizer Technology for Rice*', 1992.

EXPERIMENTAL STUDIES OF USE OF BGA BIOFERTILIZERS ON WHEAT CROPS (PH.D STUDIES UNDER AUTHOR AT UNIVERSITY OF RAJASTHAN, JAIPUR, INDIA, 1992-1999)

1. Experiment on Potted Wheat Crops

Five identical sets of pots with ten (10) replicas of each were prepared from uniform soils of the same stock which were assumed to be near neutral, i.e. without any manure. First set was kept as control. To the second set, powdered BGA flakes (50 gms) were added. To the third set, chemical fertilizers, i.e. diamonium phosphate (DAP) (10 gms), potash (5 gms) and urea (5 gms), were added. A fourth set was prepared in which BGA was added as 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 gms respectively in the ten pots. In the fifth set BGA was mixed with chemical fertilizers (CF). But the doses of each component of the CF i.e. DAP, urea and pottash were reduced to half (50%). Twenty seeds were sown in each pot after mixing the soil with the respective fertilizers. Wheat seeds (*Triticum aestivum*) were obtained from the Durgapura Agricultural Research Station at Jaipur and dried blue-green algae flakes (*Nostoc* spp.) were obtained from National Centre for Conservation and Utilization of Blue-Green Algae, IARI, New Delhi.

In the fertilizing schedule only two applications of BGA (50 gms each) were made during the entire period of growth from January to April, while the chemical fertilizers were applied four times within that period. The irrigation schedule was kept uniform, i.e. 500 cc of tap water every morning, which was reduced to 200 cc in the BGA applied wheat crops as the demand declined.

Following aspects of plant growth were studied :

- Percentage of seed germination;
- Health of seedlings;
- Colour and texture of leaves;
- Shoot & root length and dry wt.
- Dry weights of the fruiting ears;
- Length of the fruiting ear;
- No. of seed grains/ear;
- Water requirement and soil condition
- Maturation and harvesting time.

Findings and Results

The BGA + CF applied wheat crops made good progress from the very beginning of seed germination up to maturation. The BGA + CF fed seedlings and plants were the healthiest and greenest—the leaves were thicker and greener and the fruiting ears were much broader and longer. In terms of seed numbers, root and shoot lengths, root and shoot dry weights, fruiting ear length and dry weights, the BGA + CF fed wheat plants made distinct progress over the other two. One crucial observation was that, although seed germination started late in BGA fed soils, the plants grew faster and matured earlier. The fruiting ears started appearing after five weeks, and were ripe with seeds by the end of the third month (Sinha & Yadav, 1994; Vishnoi, 1998).

TABLE 4

Agronomic Impact of BGA and Chemical Fertilizers (CF) on Potted Wheat Crops

Parameters	Control	BGA Fed Soil (50 gms)	CF Fed Soil	BGA* fed Soil (10-100 gms)	BGA + CF (in half dose)
1. Percentage of seed germination	62.50	76.00	74.00	81.50	91.00
2. Root length (cms)	7.10	8.70	9.09	8.50	9.60
3. Dry weight of root (gms)	0.06	0.07	0.07	0.07	0.08
4. Shoot length (cms)	32.90	38.60	40.30	38.60	42.30
5. Dry weight of shoot (gms)	0.14	0.19	0.20	0.19	0.21
6. Ear length (cms)	5.40	7.20	7.80	7.10	8.60
7. Dry weight of ears (gms)	0.14	0.16	0.16	0.16	0.18
8. Number of seeds/ear	20	26	27	26	30

* Average rate of growth of wheat crops in 10-100 gms of BGA was determined.

Source : Vishnoi (1998).

2. Experiment on Farm Wheat Crops

The study on farm wheat crops was carried out at Rajasthan Agriculture University Research Station, Jaipur. Five farm plots of the size 5 x 5 m were prepared for the study. The fertilizer schedule was maintained as follows :

Plot 1 : 37.5 gms of BGA (@ 15 kg/ha (which is recommended for wet crop rice) and 468 gms of super phosphate (SSP).

Plot 2 : 75 gms of BGA (@ 30 kg/ha (we recommended for semi-dry crop like wheat) and 468 gms of SSP.

Plot 3 : 75 gms of BGA, 162.5 gms of Urea (in two identical doses, one at the time of sowing and other at the time of first irrigation), 235 gms of SSP and 62.5 gms of MOP (Murete of Pottash). (N.B. The chemical fertilizers were applied at half of the normal dose (50%) recommended by the agriculture department for wheat crops).

Plot 4 : 325 gms of Urea, 468 gms of SSP and 125 gms of MOP. (N.B. This was full dose of chemical fertilizer recommended by the agriculture department. No BGA biofertilizer was added).

Plot 5 : No fertilizer was added in this plot and kept as 'control'.

The whole experiment was completed in 4 months and 10 days. Seeds of wheat (*Triticum aestivum* var Raj 3077) were obtained from State Seed Corporation and BGA flakes were obtained from IARI, New Delhi. Chemical fertilizers were obtained from Rajasthan Agricultural University Research Station

Findings and Results

The best growth of wheat crop was achieved under the combined doses of BGA biofertilizer with reduced dose of chemical fertilizers by 50% (Plot 3) in terms of percentage of seed germination, height and structure of plant, foliage development, root length, shoot length, chlorophyll contents of leaves, texture of leaves, yield (number of grains/ear) and the biomass of each grain. The total yield of wheat grains after the harvest was @ 48 quintal / ha in this plot which is good and significant because even with full dose of chemical fertilizer the standard average yield is 50 quintal/ha anywhere in India under irrigated conditions. In our experiment the yield of wheat crops with exclusive chemical fertilizers (Plot 4) was 45.6 quintal/ha. Even the crop yield in plots 1 and 2 with exclusive BGA which was between 40-42 quintal / ha is highly significant because although some superphosphate was added to them no chemical nitrogen was added. (Vishnoi, 1998).

> The most exciting and interesting part of the study was the behaviour and growth performance of the wheat crops grown in the corner of Plot 1 (BGA 15 kg/ha + SSP), which was closer to the source of water point for irrigation and therefore maintained overall better moisture regime throughout the period of study. Wheat crops of about 2 x 2 m plot area near the edge were remarkably distinct from their other companions in the same plot. They showed growth performance comparable to or even better than those wheat crops grown on full doses of chemical fertilizers. This was an accidental discovery.

The only one factor for this remarkable growth was obviously the maintenance of higher moisture regime around these plants. This gave excellent opportunity to the blue-green algae inoculums to multiply faster into nitrogen rich biomass, fix more organic nitrogen and also secrete growth promoting substances. Since the 'remarkable wheat plants' were few in numbers, their yield were not quantified.

One very interesting finding of considerable significance was the taste and flavour of the 'chapattis' (baked Indian bread) made from the flour of wheat grains grown on BGA fertilizer. A random test was done on few individual guests about the bread that they were served in the meal. The guests were caught unaware when they questioned about the source of wheat flour as the breads they were eating tasted 'distinctly different'.

TABLE 5

Comparative Agronomic Impacts of BGA Biofertilizer & Chemical Fertilizers on Growth & Yield of Farm Wheat Crops

Parameters	Plot 1 BGA (15 kg/ha) + SSP	Plot 1* BGA (15 kg/ha) + SSP*	Plot 2 BGA (30 kg/ha) + SSP	Plot 3 BGA + CF	Plot 4 CF	Plot 5 Control Plot
Percentage of Seed Germination	92.000	95.000	92.000	96.000	95.000	90.000
Height of Plant (cm)	87.000	109.000	97.000	110.000	101.000	64.700
Root length (cm)	13.500	14.600	13.500	15.000	14.300	9.900
Dry weight of root (gm)	0.080	0.082	0.079	0.083	0.081	0.068
Shoot length (cm)	65.200	81.700	74.000	82.000	74.800	46.900
Dry weight of shoot (gm)	0.177	0.193	0.182	0.195	0.184	0.161
Leaf length (cm)	20.300	27.300	20.200	28.600	24.100	16.900
Ear length (cm)	8.800	12.700	10.500	12.900	11.500	7.800
Dry weight of ear (gm)	0.221	0.226	0.223	0.227	0.224	0.210
No. of Grains/Ear	32.000	40.000	35.000	43.000	37.000	24.000
Weight of 100 grains (gm)	4.292	4.457	4.301	5.150	4.419	3.214
Crop yield (q/ha)	40	–	42	48	45.600	30
Chlorophyll content (mg/litre)	3.341	4.523	3.492	4.988	4.405	2.027

* Wheat crops grown on BGA (near the source of water) with high moisture regime.

Source : Experiment made at Durgapura Agricultural Research Station, Jaipur, India (1997-98) : Vishnoi & Sinha (1998).

Soil Profile of BGA Applied Farm Plots

Soils from those plots in which BGA was added, showed improvement in conductivity, presence of organic carbon and phosphate. Presence of organic carbon also gives indirect evidence of presence of nitrogen in the soil. This can be understood because BGA is endowed with both the properties of carbon fixation (by photosynthesis) and nitrogen fixation. Increase in the amounts of the phosphates in the soil in only one case, i.e., in plot 3 where combined dose of BGA and CF was added is of some significance. The original value of phosphate in the soil was at the rate of 60 kg / ha which increased to 65 kg / ha. It is again consistent with the properties of BGA which has a unique property of dissolving the insoluble phosphates present in the soil and making it available to the plant roots. (Vishnoi, 1998).

TABLE 6

Soil Profile of BGA Applied Farm Plots

	PH	Conductivity (mm/cm)	Organic Carbon (Per cent)	Phosphate (Kg/ha)	Potash (Kg/ha)
1. Before the Experiment (November, 98)	7.8	0.29	0.10	60	230
2. In between the Experiment (February, 98)					
(A) Plot A (BGA 15 Kg/ha + SSP)	7.8	1.00	0.14	20	200
(B) Plot B (BGA 30 Kg/ha + SSP)	7.3	0.40	0.16	22	220
(C) Plot C (BGA + CF)	7.2	0.29	0.16	65	190
(D) Plot D (CF)	7.2	0.30	0.14	44	220
(E) Plot E (Control Group)	7.3	0.30	0.14	40	230
3. After the Experiment (April, 98)					
(A) Plot A (BGA 15 Kg/ha + SSP)	8.1	0.28	0.14	32	140
(B) Plot B (BGA 30 Kg/ha + SSP)	8.0	0.24	0.24	25	200
(C) Plot C (BGA + CF)	8.0	0.23	0.14	28	210
(D) Plot D (CF)	8.0	0.30	0.16	26	180
(E) Plot E (Control Group)	8.0	0.27	0.14	24	190

Source : Experiment made at Durgapura Agricultural Research Station, Jaipur, India (1997-98) : Vishnoi & Sinha (1998).

THE MYCORRHIZAL BIOFERTLIZER TECHNOLOGY : POTENTIAL FOR COMMERCIALIZATION

Mycorrhizae are the fungal associations with the roots of higher plants forming a major interphase between the soil and the roots. Fungi mainly belonging to Basidiomycetes and Zygomycetes form a mantle around the roots. The mycelium may be exogenous (ectomycorrhizae) and endogenous (endomycorrhizae). In the latter, some of the hyphae enter the roots and form bladder like structures (vesicles) and finely divided profusely branched systems (arbuscules). These endomycorrhizae are known as vesicular-arbuscular mycorrhizae (VAM). The mycobiont in VAM are generally the members of Zygomycetes belonging to genera *Acaulospora, Entrophospora, Gigaspora, Glomus, Sclerocystis* and *Scutellospora.*

The VAM fungi are prevalent on a wide range of vascular plants. They penetrate living cells of plant root without harming them, and their hyphae at the same time range far into the bulk soil, establishing intimate contacts with microbiota of soil aggregates. Incorporation of VAM fungus is specifically important for cash crops, horticultural and plantation crops of commercial value, tree species important in agro-forestry, and for wasteland regeneration and reclamation. They provide several benefits to the host plant (Sarkar, 1987). The VAM fungi produce chemicals which prevents aggregation of the soil particles making them more porous. They provide following benefits to the crops and the farm soil—

1. Increase the host-nutrient acquisition as they extend beyond the nutrient depletion zone of the rhizosphere. The hypae of mycorrhizae penetrate deep into the soil and nearby surroundings for increased absorption of phosphorus, micronutrients and water for the host plant.
2. Improve soil structure, especially the ectomycorrhizae which bind the soil particles together.
3. The symbiosis enhances the activity of the associated nitrogen fixers and increase the plant-water interaction.
4. Secrete the enzyme 'phosphatases' which help in the solubulization and utilization of organic phosphates.
5. Scavenge the 'metal pollutants' from the immediate surroundings of the plant roots and accumulate them in their hyphae. They provide resistance against drought, salinity and pollution stresses, which is very important for crop plants these days.
6. Reduce the incidence of root diseases. There are also instances where the fungus directly interact with the pathogen and render them ineffective.
7. Produce 'plant growth hormones' in the mycorrhized roots and promote general growth in plants.
8. Mycorrhizal root systems increase the absorptive capacity and the absorbing area of roots from 10 to 1000 times thereby greatly improving the ability of the plants to utilize the soil resources.

The Centre for Mycorrhizal Research, Cadila Pharma Limited, Ahmedabad had developed a dual culture of mycorrhizal root segments containing the fungus *Glomus intraradices* with carrot roots genetically transformed by *Agrobacterium rhizogenes* (Wild-type strain A4). The selected VAM fungus *G. intraradices* has a wide host range capable of infecting a wide range of crops and has a high degree tolerance to adverse soil and environmental conditions. After culture, the lag phase of 4 weeks is followed by a rapid increase in spore production and further infection. The number of spores formed after 6 months of culture averages 10,000. The dual culture supports extensive internal root colonization with many arbuscules and vesicles being formed in the host root. They can be readily incorporated into the root systems of several important crops of food and ornamental value to improve their efficiency of nutritional uptake from the soil and other growth benefits (Cadila, 2002).

ENVIRONMENTAL & ECONOMIC ADVANTAGES OF BIOFERTILIZER OVER CHEMICAL FERTILIZER

It is often questioned whether the biofertilizers can become an effective substitute for the quick acting and convenient chemical fertilizers. However, the question is becoming insignificant and irrelevant as the damage done to the soil and the human health due to chemical fertilizers is becoming more evident. The very objective of boosting crop productivity by chemical fertilizer is becoming self-defeating and unsustainable. Biofertilizers may be slow acting but sustainable over a longer period and also much cheaper farm input as compared to the chemical fertilizer. The environmental cost of production and use of chemical fertilizers is even greater. It is also possible to make mass production of biofertilizers in quantities comparable with chemical fertilizers with longer storage life. Being of biological origin, several of them are capable of rapid multiplication and self-renewing in soil.

Whereas chemical fertilizers provide only few minerals, the combination of biofertilizers and organic fertilizers can provide all essential macro and micronutrients with vitamins and growth promoting substances in balanced forms. Organic fertilizers also release nutrients more slowly than the inorganic (chemical) fertilizers. This can be an advantage, as nutrients are less likely to be leached from the soil.

1. Biofertilizers also Provides Phosphorus to Plants

Phosphorus is a very important crop nutrient because it is a constituent of many vital enzymes involved in various metabolic activities. Although, most of the soils contain enough phosphorus, but it is generally present in unavailable form. All the blue-green algae and organisms like *Bacillus megaterium* var. *phosphaticum*, *B. polymyxa*, *Pseudomonas striata*, *P. rathonis*, *Aspergillus niger*, *Aspergillus awamori*, *Penicillium digitatum* and *Trichoderma curvularia* have been found to solubilize the insoluble inorganic and organic phosphates in soils. Trials using these organisms with wheat, rice, gram, soybean, cowpea and potato have shown 10-50 per cent increase in the crop yield. (Sarkar, 1987).

2. Biofertilizers Provide Growth Hormones to Plants

Some biofertilizers (e.g. BGA, VAM, *Azotobacter* and *Azospirillum*) supply growth regulators such as IAA, IBA, NAA & GA_1 to GA_3 and vitamins.

3. Biofertilizers Secrete Antibiotics Working as Natural Pesticides

The *Azotobacter* and *Azospirillum* secrete antibiotics which act as natural pesticides.

TABLE 7

Comparison of Chemical Nitrogen Fertilizer and Biofertilizer

Parameters	Chemical Nitrogen Fertilizer	Biofertilizer
1. **Production Scale and centralized**	Industrial; large scale decentralized & on farm	Biological; small scale,
2. **Production Process**	Haber-Bosch Process	Biological nitrogen fixation
3. **Raw Materials Used in Production**	Fossil fuels	Atmospheric nitrogen
4. **Energy Use in Production**	Energy intensive	Energy conservative

Contd...

Contd...

Parameters	Chemical Nitrogen Fertilizer	Biofertilizer
5. Global Production Potential	About 40 million tonnes	About 139 million tonnes
6. Efficiency of Use by Crops	Subject to loss due to volatilisation, leaching, denitrification	Slow release, since it is a biological process
7. Mode of Fertilization	Linear fertilization	Cyclic fertilization
8. Ecological Benefits to Farms	Nil	Builds soil, C, N, P and other trace elements; enhances soil aggregation
9. Environmental Impacts	Polluting	Non-polluting
10. Production Cost	High (for hectare at 30 kg N will cost about Rs. 150)	Low (for hectare to provide 30 kg N, biofertilizer will cost Rs. 10-30)
11. Targeted Crops	All	Several
12. Application to Plants	Good	Good
13. Shelf Life	Long	Short for bacteria; long for algae
14. Accessibility	Rich farmers	All, even small and marginal farmers
15. Rural Employment Potential	None	Good (can be produced in villages)

Source : Venkatarman and Shanmugasundaram-'*Algal Biofertilizer Technology for Rice*', Madurai Kamraj University, 1992.

CONCLUDING REMARKS

Biofertilizer increase soil's physical properties such as structure and texture; chemical properties such as water holding capacity, cation exchange capacity and its buffering capacity. The mycorrhizal biofertilizer (VAM) have the unique property of scavenging metal pollutants from the soil and provide resistance to crops against salinity, drought and pollution stresses. This assumes great agronomic and ecological significance these days in wake of massive environmental degradation which is occurring in all agro-ecosystems everywhere in the world. *Azolla* biofertilizer not only supplies nitrogen but also increases organic matter in the form of biomass and thus enhance soil fertility. They proliferate useful soil micro-organism and are also cost-effective. They are highly efficient and renewable farm input, ecologically friendly, technologically feasible and socially acceptable by the farmers, especially the poor and the marginalised.

Inoculation of crop plants with nitrogen fixing microbes has become an accepted technology in US, Germany, Brazil, Israel, Egypt, China and India. Biofertilizers may be slow acting but sustainable over a longer period and also much cheaper farm input as compared to the chemical fertilizer. The environmental cost of production and use of chemical fertilizers is even greater. It is also possible to make mass production of biofertilizers in quantities comparable with chemical fertilizers with longer storage life. Being of biological origin, several of them are capable of rapid multiplication and self-renewing in soil.

But the disadvantages of biofertilizers is that they can vary greatly in their nutrients levels, composition and water content. One of the biggest variables in the content of organic fertilizers / biofertilizers is the amount of nitrogen (N) they contain. They are not able to meet the nitrogen (N) requirement in the same quantity as it is met by the chemical fertilizers. Researches into environmental biotechnology and genetic engineering promises to solve that problem very soon.

However, the natural 'organic nitrogen' provided by the biofertilizers to the agro-ecosystem do not pose any health risk to humans which is being seriously apprehended by the use of chemical and synthetic 'inorganic nitrogen'. They are accumulating into the agro-ecosystem and the human ecosystem like a 'time bomb'.

The scientific community has provided an alternative to the destructive chemical fertilizers. However, much depends upon the decisions makers who has to adopt it into their farm policy and implement it. Some Indian scientist working at the Centre for Blue Green Algae Facility, IARI, New Delhi, were in tears while narrating how the government overlooked their hard laboured research product. The industrial lobby producing chemical fertilizers always prevails on government. (Personal Communication of Rajiv Sinha with Dr. B.D. Kaushik at IARI, New Delhi, 1996)

To achieve agronomic, social, ecological and economic sustainability in agriculture, the production of all categories of biofertilizers must be commercialised. The various combinations of biofertilizers should become the main 'driving force' for agriculture and the chemical fertilizers can still remain as the 'helping hand'.

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20

Biotechnological Control of Crop Pest & Diseases

Killing Pests Without Pesticides & Development of Environmentally Friendly Alternatives to the Hazardous Chemical Pesticides—The Biopesticides : Significant Achievement of Researches into EBT for Sustainable Agriculture

KEY WORDS

Natural Predators; Predator Birds; Parasitoids; Benevolent Insects; Vermiwash; Herbal Pesticides; Viral Pesticides; Bacterial Pesticides; Fungal Pesticides; Actinomycetes - The Natural Plant Antibiotics;

BACKGROUND INFORMATION

In the wake of 'Green Revolution' of the 1960's indiscriminate use of chemical pesticides all over the world has destroyed the 'natural predators' and 'pollinators' and other 'non-target' species, and increased biological resistance in target pests. To date more than 400 species of insect pests are believed to have become resistant to pesticides. This has necessitated the development of alternative method of pest and disease control. Researches into environmental biotechnology has provided some environmentally safer methods where pests can be killed even without recourse to pesticides. It has also led to development of environmentally safer alternatives to chemical pesticides - the 'biopesticides' made from plants and microbes.

'Food safety' is a major issue everywhere in the world. According to United Nation Environment Program (UNEP) and the World Health Organization (WHO) nearly 3 million people suffer from 'acute pesticide poisoning' and some 10 to 20 thousands people die every year from it in the developing countries. *US scientists predict that up to 20,000 Americans may die of cancer, each year, due to the low levels of 'residual pesticides' in the chemically grown food.* (UNEP Reports, 1992-2006).

Production and delivery of all types of biotechnological pest control means and methods as an effective alternative to the chemical control is one of the greatest achievements of researches into environmental biotechnology. The Microbiological Resource Centers (MIRCENS) at Cairo, Egypt is examining the use of microbes in 'pest & vector control' which it is estimated, could cut the world's chemical pesticides bill by several million US dollars every year, prevent pesticide pollution of soil and water, save thousands of farmers (especially in the poor developing nations) from pesticide poisoning and arrest millions of tons of greenhouse gases generated during their production. (UNEP Reports, 1996-2006).

INTRODUCTION OF BT GENES (FROM *BACILLUS THURINGIENSIS*) IN CROPS TO PRODUCE IN-BUILT RESISTANCE & NATURAL PESTICIDE & FUNGICIDE AGAINST PESTS & DISEASES

There has been significant reduction in the use of chemical pesticides where transgenic crops are grown. These crops have 'in-built *Bt* genes' (transferred from *Bacillus thuringiensis*) to produce 'toxins' which work as 'natural pesticide and fungicide' against pests and fungus attack. An Australian survey shows that the use of 'transgenic cotton' led to a 16-86% decrease in insecticide use in various products in 1996/97 and 1997/98. (UNEP-DTIE, 1999). (Details were discussed in Chapter 18 of this Section).

BIOCONTROL OF INSECT PESTS : THE NEWLY REVITALIZED TRADITIONAL BIOTECHNOLOGY FOR KILLING PESTS WITHOUT USE OF PESTICIDES

It is newly revitalized traditional biotechnology that marshalls 'benevolent insects', 'soil microbes' and 'predator birds' to combat the enemies of crop plants. There are about 10 lakh species of insects in world of which 10 thousands are pests creating problems to the human society. Among them, over 100 are harmful crop pests. '*Heliothis*' and '*Helicoverpa*' are most serious pests. There are some 'parasitoids' and other 'predatory' insects which devour on the crop pests.

1. Parasitoids

Significant achievements have been made on the use of egg parasitoids particularly *Trichogramma chilonis* and *T. brasilinensis* for the control of *Helicoverpa armigera* infecting the chickpea. These tiny benevolent insects can be multiplied cheaply in large numbers and released in the chickpea crop fields to control *Helicoverpa*. This was also reported to control the infection of *Helicoverpa* on pigeonpea. Parisitism levels in larvae of *H. armigera* collected from pigeonpea were generally greater than those collected from chickpea. While the 'dipteran' parasitoids were predominant on pigeonpea, the 'hymenopterans' were abundant on the chickpea early in the season (Sharma, 1998). Many nematode species are endoparasites of agricultural pests. Besides killing the host, this parasitic association may result in sterility, reduced fecundity, delayed development and aberrant behaviour of hosts. Three major families namely Steinernematidae, Heterorhabditidae and Mermithidae of the phylum Nematoda are known to have insecticidal properties (Sharma, 1998).

2. Predators

Nineteen (19) species of predatory insects and several species of spiders and birds preying on the eggs and larvae of *Helicoverpa* have been reported. Adults of the pod-fly (*Melanagromyza obtusa*) is trapped in spider webs. Mud wasps such as Delta spp. carry off many large *Helicoverpa* larvae to feed their young. The 'Ladybird beetles' vociferously feed on the sucking pests (*Prodenia* and *Heliothis* larvae). The local versatile herb *Calotropis procera* are grown around the crop lands to provide host for the Ladybird beetles. Their leaves are then spread in the crop fields for the predators to attack the preys.

THE HERBAL PESTICIDES FROM PLANTS

Several plants have come to light that have potent 'insecticidal' and 'pesticidal' properties. The one which has revolutionized the agricultural industries is 'azadirachtin' from the Indian margosa (neem) tree (*Azadirachta indica*) isolated by the Max Planck Institute of Germany. Neem tree leaves and kernels were used in India since centuries to eradicate crop pests and diseases.

> Neem has 'cure for all' problems in agriculture. Neem extracts are undergoing bio-efficacy tests all over the world. *Studies in U.S. have shown that the neem extracts are highly effective against 128 species of crop pests including 'gypsy moth', 'aphids', 'tobacco budworm', 'Japanese beetle' and 'boll weevil'.*
>
> International Centre of Physiology and Ecology, Philippines have found that the neem extract is a 'contact deterrent' and a potential anti-feedant for rice pests like 'brown and green hopper' and *Sogastella* . However, neem does not kill the natural enemies of plant hoppers and leaf hoppers. In fact, crop fields treated with neem have shown a higher population of natural enemies than untreated fields (Singh & Singh, 1993).

Indian Agricultural Research Institute, New Delhi have developed granular formulations of neem kernels effective against corn and sorghum 'stem borers'. 'Azadirachtin' isolated from the Indian neem have been found to inhibit egg development and molting in pest. Other biological weapon from neem for action against pests are 'meldinin' and 'nimbin'. Three forms of neem extracts having 'biocidal activities' have already come in emulsifiable concentrate, granular formulations and a wettable powder. Neem extract have been used to arrest the mycelial growth of the sorghum anthracnose pathogen *Colletotrichum graminicola* in Nigeria (Akpa *et.al.*, 1991). When paddy is sprayed with neem oil, the number of 'brown plant hoppers' are reduced and the pests fails to transmit the 'grassy and rugged stunt viral' diseases. It also prevents the transmission of the 'rice trungro virus' by green leaf hoppers. Spraying with neem oil deforms the body appendages of the 'rice ear-cutting caterpillar'. The 'rice leaf folder' larvae develop abnormalities within 24 hours of treatment with neem.

Other potent herbal pesticides are—

1. 'Pyrethrins' isolated from *Chrysanthemum cineriifolium;'*
2. 'Napthaquinones' from *Plumbago capensis, '*
3. 'Amorphin' from *Amorpha fruticosa.*

4. 'Rotenone' (a potent insecticide next to pyrethrins) from *Derris elliptica, Parthenium argentatum, Tephrosia villosa, Sophora spp. & Lonchocarpus utilis*
5. 'Anabasin' from *Anabasis aphylla*
6. 'Filicin' from *Dryopteris filise*

Other biological insect repellents are 'vetiver oil' from *Vetiveria zizanoides*, 'citronella oil' from *Cymbopogon nardus, Trigonella* spp. and *Tagets erecta* etc. An East African medicinal plant *Ajuga* spp. yields a chemical called 'phytoecdysones' which kills caterpillars.

> 'Pyrethrin', extracted from *Chrysanthemum cineriifolium*, is a poison more deadly to insects than DDT, yet hardly toxic to mammals or plants. It is also non-persistent and hence environmentally safe. It can be safely used as an insecticide in the house where food is processed and stored. The plants are now grown widely in uplands in Rwanda, Kenya, Tanzania, Ecuador, Japan and Australia.

Derris, a genus of tropical woody climber, kills beetles and weevils as well and is also harmless to mammals. Certain species of marigold (*Tagetes*), release into the soil a sulfur compound that can destroy nematode worms (eelworms) within a radius of a meter. Early South American farmers kept eelworms at bay from their potatoes by interplanting marigolds.

Several plants of Solanacea family have been found to produce a new class of chemicals called 'withanolides' (secondary metabolites). They act as repellents, feeding deterrents and toxins to a wide variety of pests. A wild potato species produce a biochemical '2-tridecenone' that kills tobacco hornworms. Some plants control pests by suppressing their appetites.

THE MICROBIAL PESTICIDES FROM VIRUSES, BACTERIA, FUNGI AND THE ACTINOMYCETES

Several microbes - the viruses, bacteria, fungi and the actinomycetes have come to light that devour on the crop insects and pests but are not pathogenic to human beings or the farm animals. They have potential to be developed into 'microbial pesticides' for crop pest control. The Microbiological Resource Centers (MIRCENS) at Cairo, Egypt is examining the use of microbes in 'pest & vector control'.

1. The Viral Pesticides

Predator viruses and bacteria working like 'viral and bacterial pesticides' devour the 'devil microbes' when they are mixed into the soil or injected into plants. The viral pesticides are particularly proving very useful 'bio-weapon' in agriculture, horticulture and forestry.

> Hundreds of viruses have been found pathogenic to insects pests and a few of them have been commercially developed as 'viral pesticide' in USA. The viruses kill specific target pest species and have no adverse effect on useful insects, pollinators, parasites and predators and warm blooded animals including human beings. They infect and kill the host (pest) by gaining entry through mouth and in the digestive tract.

Several types of viruses e.g. 'baculoviruses', 'cytoplasmic polyhedrosis viruses', 'granulosis inclusion viruses', 'entomogenous viruses' and the 'nuclear polyhedrosis viruses' have been developed for commercial use. The nuclear polyhedrosis virus is very effective in controlling the popular pest *Clostera fulgurita* which practically defoliates the poplar tree (Bhagat & Bala, 1991).

2. The Bacterial Pesticides

Among the bacterial pesticides, the *Bacillus thuringiensis* is especially a goldmine acting on wide range of pests. The bacterium is gram negative and found naturally in soil. It exists in several strains. A gene from this bacterium called '*CRY gene*' produce a 'toxin' and each strain produce a specific toxin active against wide range of pests. Different strain of bacteriam attack on different pests thus sparing the friendly insects. Some kill 'weevils' and other the 'beetles'. Some kill the insects of Lepidoptera family while others eradicated the 'American ballworms', the 'nematodes' and 'flatworms'. There are over 100 crops pests which are most harmful. Among them '*Heliothis*' and '*Helicoverpa*' are most serious. *Bacillus popilliae*, cause milky disease in the larvae of 'chafer grubs' and kill. The toxin from *B. thuringiensis*' even kill them. In USA *Bacillus thuringiensis* is used to control 'cabbage looper disease' caused by *Trichoplusia* spp., and *Entomophtora ignobillis* is used to control the 'green peach aphid' (*Myzus persica*) of potatoes.

> So far more than 80 CRY genes (Bt genes) producing different toxins have been isolated and about 30 of them have been successfully transferred into commercial crops including cotton, mustard, chickpea, corn, rice and potato to confer resistance in them against pest attack. Attempts are being made to transplant these Bt genes into tomato, brinjal, cauliflower, cabbage and tobacco to confer resistance in them against wide range of pests (Sharma, 2002). The toxin crystalls dissolve in the midgut of insects and kill (Canby, 1993).

Central Drug Research Institute (CDRI), Lucknow, has come out with a very effective 'biocide' to control insect pests. It is produced from *Bacillus sphaericus*. *Unlike Bacillus thuringiensis that requires repeated applications like conventional pesticides, B. sphaericus persists for longer period in the environment* (Basu & Khan, 2001). The natural soil bacteria *Azotobacter* and *Azospirillum* also secrete 'antibiotics' which acts as bio-pesticide.

3. The Fungal Pesticides

There are more than 500 fungal species which infects insect pests. Some of them or their products are being used as 'fungal pesticides'. In UK *Verticillum lecanii* is produced commercially for use against 'aphids' and other pests of glasshouse crops. Russians use 'boverin' obtained from the conidia of fungus *Beauveria bassiana* to control the 'Colorado Potato Beetle' (*Leptinotarsa decemlineata*).

Among the entomogenous fungi the *Beauveria tenella* is a parasite causing heavy mortality in larvae of *Eutectona machaeralis* pest which caused severe epidemic in trees of Melghat Wildlife Sanctuary in 1978. The *Beauveria bassiana* species of the fungal pathogen kills larvae of *Hypsipyla robusta, Eligma narcissus, Philosamia cynthia, Atteva febriciella* and Scarabaeid beetles in nature causing large scale mortality. These biological pesticides can be dusted on roots, on seeds, mixed with potting soil or spread on fields by ordinary farm tool.

> Researches have indicated that an outstanding strain of soil borne fungus *Aspergillus niger* (AN 27) can eradicate and control several devastating soil-borne diseases like 'wilt', 'root rot', 'damping off', 'blight', 'charcoal rot' and 'black scurf' diseases caused by diverse groups of pathogenic fungi like *Fusarium oxyporum, F. solani, Macrophomina phaseolina, Pythium aphanidermatum, Rhizoctonia solani,* and *Sclerotinia selerotiorum* in diverse groups of crop plants like maize, paddy, millet, pulses, oilseeds, cotton, castor, banana, sugarcane, several fruits and vegetables, tuber crops; ornamental, fodder and fibre crops. It also eats up the dead pathogenic hyphae of funguses and induces resistance in plants by accelerating its defence enzymes.

Aspergillus niger (AN 27) have been commercially produced and called as 'kalisena'. It comes in a coarse granular formulation, is non-pathogenic to human beings and has extraordinary long shelf life of 2 years. It is applied as seed dressing just before sowing @ 8gm/kg seed. It can survive in all types of soil with temperature range of 0 - 45° C with no specific pH and moisture requirement. Another significant property of *A. niger* (AN 27) is that it produces two growth promoting substances and also help in phosphate uptake by solubilising the phosphorus present in the soil.

> A new bio-pesticide for sugarcane called 'Bio-Cane' has been developed in Australia to replace the chemical pesticide. It is a fungal product cultured on broken rice grains. The biocane granules are particularly effective against 'greyback cane grub' disease in sugarcanes.

4. Actinomycetes : The Natural Antibiotics in Crop Plants

Actinomycetes are benevolent microbes that colonise the wheat crops naturally as it grow and enter the plant through seed. They work as *'antibiotic of sorts'* for wheat plants which suffers from 'low early vigour' due to the build up of some of the early diseases and funguses.

> Researchers at Flinder's University in Australia have found that if the wheat seeds are coated with actinomycetes and grown, the microbe grow along with the wheat plants and protect it from infection by the fungus from the very early stages. They have isolated about 12 strains of actinomycetes that live with wheat plants naturally (Anonymous, 2001). This is a natural biotechnology to control plant diseases and reduces the use of chemical pesticides in wheat crop.

Wheat seeds coated with actinomycetes bacteria have been sown in the field in the South Australian west coast.

THE VERMI CONTROL OF PESTS BY EARTHWORMS AND THEIR VERMIWASH (RESIDUAL WATER) FROM VERMICOMPOSTING

Presence of rich population of earthworms in farm soil have been found to reduce the incidence of pest attack in crops. This was also found by the author in his experiments in potted plants at University of Rajasthan, Jaipur, India (1988 - 1999). Earthworms 'coelomic fluid' exhibit 'antibacterial' activity that hep eradicate the soil pathogens and reduce plant parasites and soil nematodes. The 'vermiwash' from earthworms vermicomposting also exhibited strong pesticidal properties.

CONCLUDING REMARKS

Some 200 insects and half a dozen weeds have since been brought under control by predators and parasites introduced for purpose. We need to identify and promote the multiplication of all those benevolent predator birds and insects in nature that are natural enemies of destructive pests which destroy our crop plants.

The use of microbial pesticides developed for purpose can significantly reduce (if not totally eliminate) the production and use of chemical pesticides of which about 137 different formulations already exist and several million tones have been produced and stocked piled on earth since the onset of green revolution in the 1950s-60s. The microbial pesticides acts on the target pests (not on benevolent organisms in farms) and there is no 'residual pesticides' (like the persistent chemical pesticides) remaining on the raw food products (grains, fruits & leafy vegetables). Even if they fall on the soil, they do not cause soil or water pollution and do not pass into human food chain like the chemical pesticides.

Herbal & microbial pesticides are gaining importance all over the world. The significant thing is that like the chemical pesticides the they do not kill the 'natural enemies' of pests (benevolent predator organisms) and also do not induce 'resistance' in organisms.

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21

HYDROPONICS : THE SOIL-LESS SUSTAINABLE AGRICULTURE

Producing Food Crops Without Soil, Minimum Use of Water While also Using Saline Sea Water : A Significant Achievement of Researches into EBT for Water Conservation in Agricultural Production

KEY WORDS

Soil-less Agriculture & Food Production; Inorganic Hydroponics; Organic Hydroponics; Salinity Resistant Crops; Aquaponics; Aquaculture & Aquatic Foods;

BACKGROUND INFORMATION

Hydroponics is the biotechnology of growing plants without soil. It has also been referred as '*soil-less agriculture*' in which plants are grown without the medium of soil and gets all its nutrient requirements through the medium of water. The nutrients are provided in the form of 'inorganic nutrients' dissolved in water. The US army used 'sand-culture hydroponics' in the Pacific Islands and Japan during the WW II to supply fresh vegetables to the troops.

> It only needs one-tenth of the water used by the soil crops and can be very successfully practised in the cities, in every house on the roof-tops, thereby significantly reducing, not only the cost of transportation of fruits and vegetables from the distant farms, but also reducing the water requirement which is becoming a dearer commodity everywhere but much so in the cities (Douglas, 1985).

THE TECHNOLOGY OF HYDROPONICS

With the revolutionary advancement in plastic technology, hydroponics has become a very convenient method of food production. Circular or semi-circular plastic pipes of 3-4 inches diameter, with slots to hold plant roots or plastic troughs with floating plastic platforms having holes to hold plant roots are some convenient and cheaper methods to practice hydroponics. The pipes can be assembled in any open area in the backyard or on the roof top or in transparent glass-houses. Often artificial lighting, sterilized water and carbon dioxide is used to promote growth in the closed environments. Also used are disease suppressing pro-biotic micro-organisms and ozone.

DEVELOPING ORGANIC HYDROPONICS

Attempts are being made to develop 'organic hydroponics' based on 'organic municipal wastes' by capturing soluble plant nutrients from the waste materials. Scientist in Singapore used 'municipal wastewater' to successfully grow lettuce through hydroponics. The experiment also showed that the plant was able to remove 95 per cent of waste materials from the water in just 16 days.

Another exciting branch is 'aquaponics' in which 'aquaculture' (fish and shrimp farming) is combined with hydroponics so that the 'excreta' and the food wastes from fish and crustaceans in water becomes food (nutrients) for the vegetable crops. The fish waste is converted into plant-useable 'organic nutrient' by bacteria which exist within the system. The pro-biotics are also used for disease control. This dual use of water reduce water use by more than 50 per cent per kg of plant and animal food produced (El-Shinawy *et al.*, 1999).

In recent years three organic manures from chicken, pigeon and buffalo were tested and compared with a standard inorganic salt formulae for the growth of lettuce plant. The electrical conductivity was maintained between 1.8 and 2.0 $dScm^{-1}$, and the pH ranged from 5.5 to 6.5. The manure solutions were prepared by taking 1 kg of manure, which was shaken for 15 minutes in 4 litres of distilled water and then incubated for two days. The suspension material was filtered and the tea was used as the stock nutrient solution. Inorganic fertilizer solution was used in the control treatments.

TABLE 1

Mineral composition of the organic nutrient solution made from manure (in ppm)

Source	N	P	K	Ca	Mg	Fe	Mn	Zn	Cu
1. Chicken Manure	94.0	80.0	1598	106	81.6	0.99	0.76	0.220	0.180
2. Pigeon Manure	109.0	51.6	1024	148	212.0	3.36	0.39	0.390	0.192
3. Buffalo Manure	45.4	12.8	1424	118	156.0	0.92	0.17	0.127	0.120
4. Control (Inorganic)	151.0	17.0	150	115	17.0	1.60	0.60	0.040	0.030

Source : El-Shinawy *et al.*, (1999).

TABLE 2

Agronomic impact of organic nutrient on lettuce plant

Manure Solution Used	Average Weight Per Head (in grams)	
	Season One	Season Two
1. Chicken Manure	303	409
2. Pigeon Manure	267	276
3. Buffalo Manure	146	108
4. Control (Inorganic)	573	445

Source : El-Shinawy *et al.*, (1999).

The results showed that in both seasons the yield of lettuce in grams per plant was highest in the inorganic salt nutrients, followed by the chicken manure nutrient.

In the inorganic fertiliser salt nutrient nitrogen is in the form of nitrate (NO3) while in the manure solutions (which has not been processed) nitrogen is mostly available in the form of ammonium (NH_4) which cannot be taken directly by the plant roots and need to be first converted into nitrates by bacterial action. This is the main cause of loss of weight. The ammonical nitrogen can be converted into nitrate by composting or by vermiculture technology. One of the major drawbacks in organic solutions for hydroponics is that it needs to be fully decomposed using bacteria or other organisms like earthworms before the minerals are converted into plant useable forms. Another problem with some types of organic nutrients is that when used in hydroponics there is a rapid growth of bacteria and fungi, which smothers the plant root system and remove all oxygen from the solution.

But the good thing is that the 'nitrate content' in the leaf tissues of all the lettuce plants grown on organic solution was 'low' as compared to those grown on inorganic fertiliser solution. This is the major advantage of the organically grown foods from the human health point of view. High nitrate levels in human foods grown conventionally in farms by heavy use of nitrogenous chemical fertilisers is a potential health risk. Food production through hydroponics would also reduce the use of dangerous pesticides and herbicides.

COUNTRIES PRACTICING HYDROPONICS

Hydroponics is popular in US, Japan and Israel but the most productive 'hydroponics farms' are located in Auckland in New Zealand where they annually earn NZ $ 400,000 from the fruits and vegetables produced. Since 1990, hydroponics cultivation in Korea has expanded to about 550 hectares supporting 1,656 farms. They export roses and vegetables to Japan. Solvenia, (formerly part of Yugoslavia) has 30,000 square meters of hydroponics farms producing tomatoes. It plans to extend it to 80,000 square meters. Inorganic hydroponics using industrial fertilizers is also big business in Australia, with about 5000 companies providing fruits and vegetables to the supermarkets. The 'Big Banana' at Cox Harbour in NSW, Australia, is growing 'huge lettuce' (weighing over 2 kg), tomatoes, peas, spinach and egg-plants on commercial scale by hydroponics.

The most successful story of hydroponics comes from the desert nation Israel which imported food till few years back and has now become the net exporter of food and flowers. It grows 95 per cent of its roses for export earning by hydroponics. Israel has also pioneered in using 'saline water' for hydroponics culture. It has about 12,000 hectares in soil-less culture using hydroponics. This has led to the 'blooming of their deserts' for producing food for export. It hosted the *'World Congress for Soil-less Culture'* in May 2000 where more than 150 scientists working on hydroponics gathered to signify the importance of hydroponics for sustainable food production in the future. Hydroponics is now being practised in Latin America too.

SEARCH FOR SALT TOLERANT CROPS BY EBT FOR SALINE AGRICULTURE USING SEAWATER

Apprehending shortage of fresh water for agriculture in future an alternative technology called 'Saline Agriculture' is being developed to grow salt tolerant and resistant species of crops with seawater. The National Research Council of US in its report *'Saline Agriculture : Salt Tolerant Plants for Developing Countries'* have identified over 100 salt tolerant plant species which grow well on saline soils irrigated by saline/ sea water. In Bhavnagar, Gujarat pearl millet (*Pennisetum typhoides*) has been successfully cultivated in sand nurtured with seawater. The yield is promising with up to 1 ton of harvest of pearl millet per hectare.

> Abraham and Thomas (1997) reported a salt tolerant paddy variety 'pockali' from Kerala. It is widely cultivated by farmers. Crops like *Aspargus* and some varieties of wheat, cotton, barley and rice are being genetically bred or selected for salt tolerance. A number of halophyte food plants were harvested by ancient cultures but never gained acceptance in modern food culture. Researches into environmental biotechnology (EBT) is trying to bring those plants into modern food culture.

Saline agriculture is meeting with good success in Israel and Pakistan and other Middle East countries. Israeli farmers are growing tomatoes and cotton in commercial scale irrigated by saline water. Mexican farmers are also cultivating crops irrigated by saline water.

CONCLUDING REMARKS

Organic and inorganic hydroponics is now being commercially used as an environmentally sustainable alternative to the 'conventional method of chemical farming on land' specially for the production of fruits and vegetables like tomato, lettuce, spinach, strawberries and egg-plants and ornamental flowers like roses, tulip and gladiolas etc.

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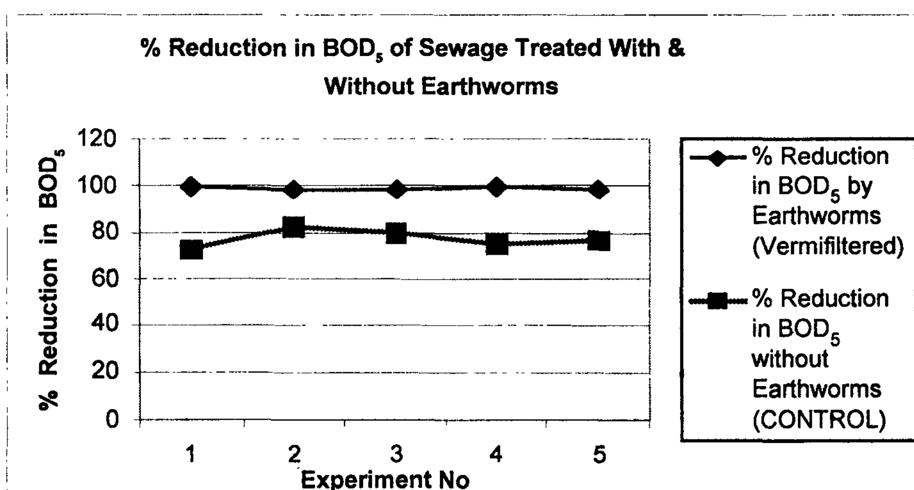
APPENDIX

VERMIFILTRATION OF WASTEWATER BY EARTHWORMS (CHAPTER-12)

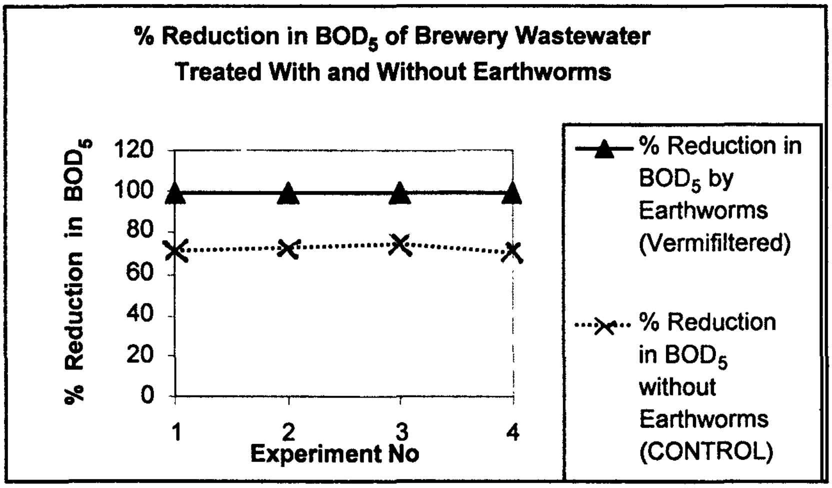
(1) Removal of 5 Day Biological Oxygen Demand (BOD_5) by Earthworms

(a) BOD_5 Removal Efficiency of Municipal Wastewater (Sewage) Treated by Earthworms (HRT: 1- 2 hrs)



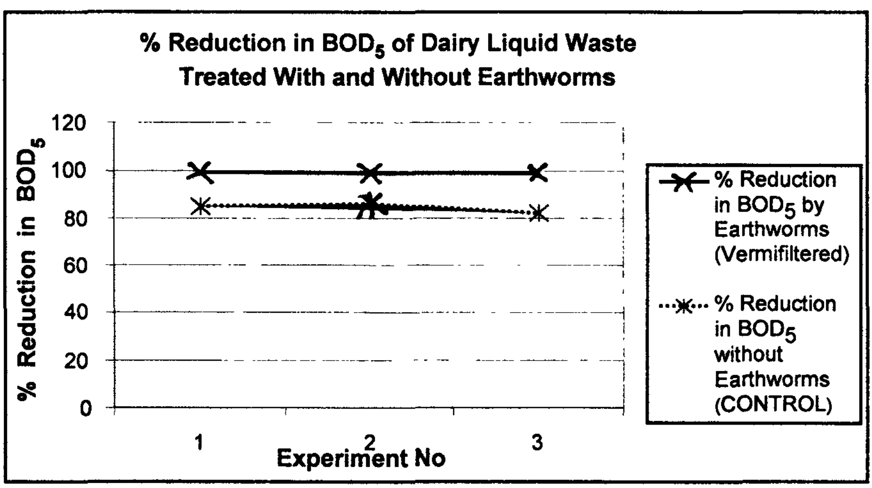
(Graphical representation of Table-1 (a) on page 187. It also shows the value of BOD_5 reduction in control when earthworms are not present in the system. Presence of earthworms does make a difference in BOD reduction in municipal wastewater).

(b) BOD_5 Removal Efficiency of Brewery Wastewater Treated by Earthworms (HRT : 3-4 hrs)



(Graphical representation of Table-1 (b) on page 188. It also shows the value of BOD_5 reduction in control when earthworms are not present in the system. Presence of earthworms does make a difference in BOD reduction of brewery wastewater).

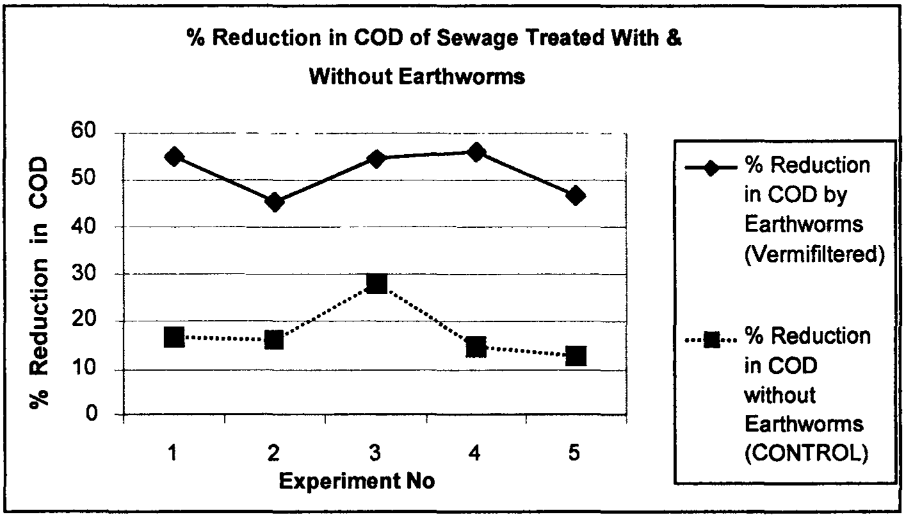
(c) BOD_5 Removal Efficiency of Dairy Wastewater Treated by Earthworms (HRT 6-10 hours)



(Graphical representation of Table-1 (c) on page 188. It also shows the value of BOD_5 reduction in control when earthworms are not present in the system. Presence of earthworms does makes a difference in BOD reduction of milk dairy wastewater).

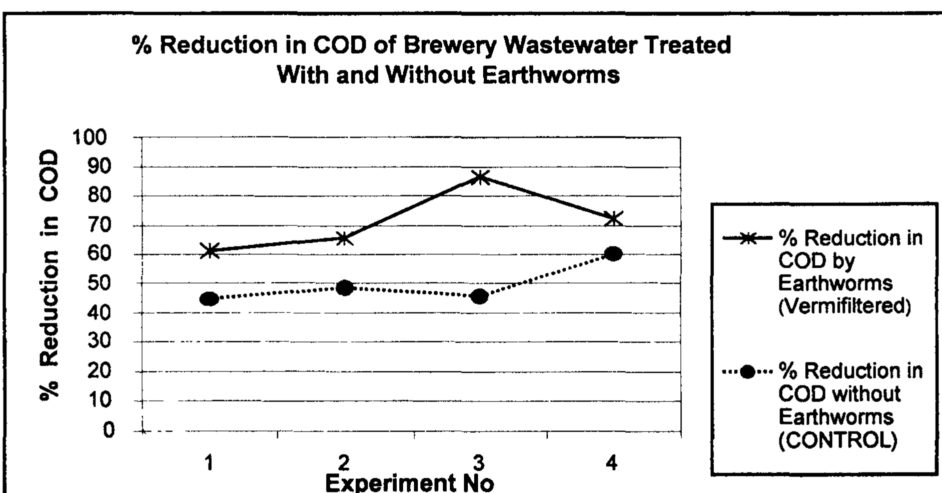
(2) Removal of Chemical Oxygen Demand (COD) by Earthworms

(a) COD Removal Efficiency of Municipal Wastewater (Sewage) Treated by Earthworms (HRT : 1-2 hrs)



(Graphical representation of Table–2 (a) on page 188. It also shows the value of COD reduction in control when earthworms are not present in the system. Presence of earthworms makes a significant difference in reduction of COD of wastewater).

(b) COD Removal Efficiency of Brewery Wastewater Treated by Earthworms (HRT 3-4 hrs)

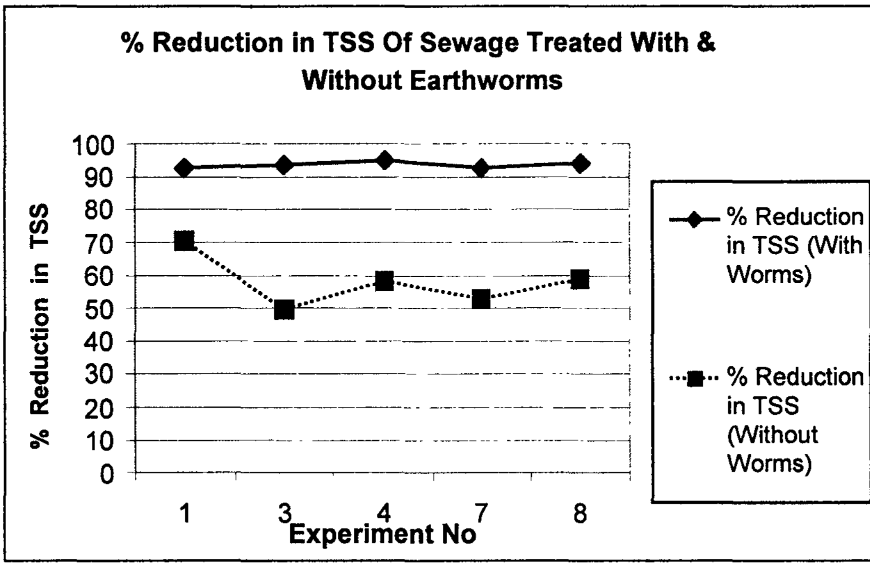


(Graphical representation of Table–2 (b) on page 189. It also shows the value of COD reduction in control when earthworms are not present in the system. Again, the presence of earthworms makes a significant difference in reduction of COD of wastewater)

(c) Graphs could not be plotted for COD removal of milk dairy wastewater as the initial value of COD of dairy wastewater was higher than 15,000 mg/L and beyond the range of our estimation and calculation for percent reduction in COD value)

(3) Removal of Total Suspended Solids (TSS) by Earthworms

TSS Removal Efficiency of Municipal Wastewater (Sewage) Treated by Earthworms (HRT 1 - 2 hrs)



(Graphical representation of Table - 3 on page 190. It also shows the value of TSS reduction in control when earthworms are not present in the system. Again, the presence of earthworms makes a significant difference in reduction of TSS of wastewater)

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