

Aquatic Pollutants and Bioremediations [Review]

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Abstract: Around 1500 substances have been listed as pollutants in freshwater ecosystems, and each of them occurs in the following types of freshwater pollutants: Acids & alkalis; Anions; Domestic sewage and farm manures; Detergents; Gases (e.g. chlorine, ammonia); Oil and oil dispersants; Organic toxic wastes (e.g. formaldehyde, phenols); Heat; heavy metals; Food processing wastes; Nutrients (especially phosphates, nitrates); Pesticides; Polychlorinated biphenyls; Pathogens; Radionuclides; etc. The different pollutants put forth different problems to different freshwater ecosystem (Mostly, expressed in the amount of oxygen that is available for fish and other species). This sometimes results in habitat destruction and extinction of local populations. Fish react to stress in different ways, depending on the severity and length of exposure to the stressor. Fish may die almost immediately from shock if the stressor is sufficiently severe or, at the other extreme, they may adapt to a mild or slow developing stressor and suffer no long-term effects. Fish may also respond to a stressor by altering their physiology to the point that natural resistance and immunity to disease is affected with varying degrees. Many transgenic plants with increased resistance and uptake of heavy metals were developed for the purpose of phytoremediation. Once the rate-limiting steps for uptake, translocation, and detoxification of metals in hyperaccumulating plants are identified, more informed construction of transgenic plants would result in improved applicability of the phytoremediation technology. Immobilization processes may enable metals to be transformed *in situ* and are particularly applicable to removing metals from aqueous solution. This contribution will outline selected microbiological processes which are of significance in determining metal mobility and which have actual and potential application in bioremediation of metal pollution. These include autotrophic and heterotrophic leaching mechanisms, reductive precipitation, sulfate reduction and metal sulfide precipitation. The present review will discuss the methods used as bioremediation for heavy metals, pesticides and organic xenobiotics.

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1.Introduction

Heavy metals are the main group of inorganic contaminants and a considerable large area of land is contaminated with them due to use of sludge or municipal compost, pesticides, fertilizers, and emissions from municipal waste incinerators, car exhausts, residues from metalliferous mines, and smelting industries [Garbisu & Alkorta, 2003; Halim *et al.*, 2003]. Although metals are present naturally in the Earth's crust at various levels and many metals are essential for cells (e.g. copper (Cu), iron (Fe), manganese (Mn), nickel (Ni), zinc (Zn)), all metals are toxic at higher concentrations. Specifically, any metal (or metalloid) species may be considered a "contaminant" if it occurs where it is unwanted, or in a form or concentration that causes a detrimental human or environmental effect [McIntyre, 2003]. The threat of heavy metals to human and animal health is aggravated by their long-term persistence in the environment [Gisbert *et al.*, 2003]. For instance, Pb, one of the more persistent metals, was estimated to have a soil retention time of 150–5000 years and was reported to maintain high concentration for as long as

150 years after sludge application to soil [Nanda Kumar *et al.*, 1995]. Also, the average biological half life of Cd has been estimated to be about 18 years [Forstner, 1995] and 10 years once in the human body [Salt *et al.*, 1995]. Another reason for toxic heavy metals causing concern is that the metals may be transferred and accumulated in the bodies of animals or human beings through food chain, which will probably cause DNA damage and carcinogenic effects by their mutagenic ability [Knasmuller *et al.*, 1998], e.g. some species of Cd, Cr, and Cu have been associated with health effects ranging from dermatitis to various types of cancer [Das *et al.*, 1997; McLaughlin *et al.*, 1999].

Immobilization processes may enable metals to be transformed *in situ* into insoluble and chemically inert forms and are also applicable to removing metals from aqueous solution. It should also be noted that metal removal/transformation processes are intrinsic although less appreciated components of traditional means of water/sewage treatment as well as reed bed, lagoon and wetlands technologies [Webb *et al.*, 1998; Stephen and Macnaughton, 1999].

Molecular and genetic analysis is now furthering understanding of microbial metal metabolism, including those aspects which are of environmental and biotechnological relevance [Chen *et al.*, 1999; Nies, 1999].

Bioremediation of heavy metals

The mechanisms by which microorganisms effect changes in metal speciation and mobility are fundamental components of biogeochemical cycles for metals as well as all other elements, including carbon, nitrogen, sulfur and phosphorus, with additional implications for plant productivity and human health [Gadd, 1999, 2002]. The ability of microorganisms to affect metal speciation stems from their ability to effect and/or mediate mobilization or immobilization processes that influence the balance of metal species between soluble and insoluble phases. Mobilization of metals can be achieved by, e.g., protonation, chelation, and chemical transformation while immobilization can occur by precipitation or crystallization of insoluble organic or inorganic compounds or by sorption, uptake and intracellular sequestration. Redox reactions can mobilize or immobilize metals depending on the metal species involved. As well as being an integral component of biogeochemical cycles for metals, these processes may be exploited in environmental biotechnology [Gadd, 1999, 2000a,b, 2002; Lloyd and Macaskie, 2000; Schiewer and Volesky, 2000; Lloyd and Lovley, 2001; McLean *et al.*, 2002]. Almost all metal-microbe interactions have been examined as a means for removal, recovery or detoxification of inorganic and organic metal or radionuclide pollutants [Lovley and Coates, 1997; Francis, 1998; Stephen and Macnaughton, 1999; Eccles, 1999]. In such a context, solubilization may enable removal from solid matrices, such as soils, sediments, dumps and industrial wastes.

Plants have constitutive and adaptive mechanisms for accumulating or tolerating high contaminant concentrations in their rhizospheres. The use of such plants to cleanup soils and water contaminated with pollutants, a technique known as phytoremediation, is emerging as a new tool for in situ remediation. Phytoremediation takes advantage of the fact that a living plant acts as a solar-driven pump, which can extract and concentrate certain heavy metals from the environment [Raskin *et al.*, 1997]. This remediation method maintains the biological properties and physical structure of the soil. The technique is environmentally friendly, potentially cheap, visually unobtrusive, and offers the possibility of bio-recovery of the heavy metals [Yang *et al.*, 2002]. Phytoremediation strategies can offer suitable approaches for decontaminating polluted soil, water, and air by trace metals as well as organic substances. As early as the 19th century, Baumann [Baumann *et al.*, 1985] identified plants capable of accumulating

uncommonly high Zn levels. Minguzzi and Vergnano, 1948 identified plants capable of hyperaccumulating up to 1% Ni in shoots. Following the identification of these and other hyperaccumulating species, a great deal of research has been conducted to elucidate the physiology and biochemistry of metal hyperaccumulation in plants [Yang *et al.*, 2002]. Plants ideal for phytoremediation should be: (a) fast-growing, (b) have high biomass, (c) extensive root system, (d) be easy to harvest, and (e) tolerate and accumulate a range of heavy metals in their harvestable parts. While no such plant has been described so far, high biomass non-accumulators that are fast-growing can be engineered to achieve some of the properties of the hyperaccumulators [Clemens *et al.*, 2002]. Determining the molecular mechanism of metal accumulation will be key point to achieving this goal. The hyperaccumulating plants show extraordinary ability to absorb metals from the soil and accumulate them in the shoots under low and high metal levels [Ma LQ *et al.*, 2001; Yang *et al.*, 2002]. Most of heavy metals have low mobility in soils, and are not easily absorbed by plant roots. For instance, there was no significant correlation between Zn accumulation and total Zn in soil for *Thlaspi caerulescens* [Knight *et al.*, 1994], but a close relationship was noted between shoot Zn accumulation and soil extractable Zn level [Romheld, 1991]. The bioavailability and plant uptake of heavy metals in the soils are affected by metal content, pH, Eh, water content, organic substances, and other elements in the rhizosphere. Plant roots and soil microbes and their interaction can improve metal bioavailability in rhizosphere through secretion of proton, organic acids, phytochelatins (PCs), amino acids, and enzymes.

Secretion of protons by roots could acidify the rhizosphere and increase the metal dissolution. Bernal *et al.*, 1994, found that pH affected proton release and plant growth of the Ni hyperaccumulator (*Alyssum murale*) under solution culture condition. However, the difference seemed not large enough for totally explaining the acidification of rhizosphere and improved metal dissolution. It was observed that the pH in the rhizosphere soil of the Cu accumulating plant species (*Elsholtzia splendens*) was significantly lower than in the bulk soil when plants were grown in Cu and other metal contaminated soil under field experiment conditions [Peng *et al.*, 2005]. The proton extrusion of the roots is operated by plasma membrane H⁺-ATPase (E.C. 3.6.3.6) and H⁺-pumps. The molecular bases and effects on these membrane proteins by other factors have been researched.

In Lupin, P deficiency induces citrate exudation by enhancing the activity of plasma membrane H⁺-ATPase and H⁺ export [Ligaba *et al.*, 2004]. AtHMA4 is an *Arabidopsis thaliana* P-1B-ATPase which

transports Zn and Cd. Verret *et al.*, 2004 demonstrated that AtHMA4 is localized at the plasma membrane and expressed in tissues surrounding the root vascular vessels. The ectopic overexpression of AtHMA4 improved the root growth in the presence of toxic concentrations of Zn, Cd, and Co. A null mutant exhibited a lower translocation of Zn and Cd from the roots to shoot. In contrast, the AtHMA4 overexpressing lines displayed an increase in Zn and Cd accumulation by shoots. The secretion of organic acids can mobilize heavy metals and enhance root absorption. Krishnamurti *et al.*, 1997, reported that Cd-organic complexed Cd took about 40% of the total in the soil and was positively related to phytoavailability of Cd. Many low molecular organic acids could influence Cd release from absorbed Cd in the soil and increase Cd solubility through forming of Cd- LMWOA complexes. Cieslinski *et al.*, 1998, found many low molecular weight organic acids, such as acetic acid and succinate in the rhizosphere of the Cd-accumulating genotype of wheat (Kyle), but not the non-accumulating genotype (Arcola). Al-induced exudation of maleate, as a basis for the mechanism of Al tolerance in wheat, was found to be accompanied by changes in PM surface potential and the activation of H⁺-ATPase [Ahn *et al.*, 2004]. However, contrasting results were reported on the role of root exudation for metal hyperaccumulation in *Thlaspi caerulescens*. Some researchers concluded that there was no relationship between root exudation and metal hyperaccumulation in *T. caerulescens* [McGrath *et al.*, 1997, McGrath *et al.*, 2001, Zhao *et al.*, 2001], whereas other groups reported that root-microbe interaction changed soil conditions in the rhizosphere and increased the solubility of the retained Zn in the rhizosphere soil of the Zn hyperaccumulator *T. caerulescens*. We found that root exudates of the Zn/Cd hyperaccumulating plant species *Sedum alfredii* Hance could extract more Zn and Pb from the contaminated soil [Li TQ *et al.*, 2005]. The transport and hyperaccumulation of Ni were enhanced by amino acid histidine in *Allysum* [Kramer *et al.*, 1997]; the biochemical characterization of root exudates and molecular bases of root exudation in increasing heavy metal mobilization in the rhizosphere need to be further studied.

Genetic engineering for phytoremediation

Metal hyperaccumulators are notorious for small size and slow growth. These properties have an adverse impact on the potential for metal phytoextraction and severely restrict the employment of effective agronomic practices, such as mechanical harvest [Tong *et al.*, 2004].

To overcome these disadvantages, conventional breeding approaches have been proposed to improve plants for metal extraction [Li TQ, *et al.*, 2004].

Unfortunately, the success of this approach may be precluded due to sexual incompatibility caused by anatomical differences between parents.

Biotechnology has the potential to overcome this limitation by allowing direct gene transfer [Kramer, Chardonnes, 2001 99]. Research data indicate that manipulation of relevant plant features, including metal tolerance, is a realistic possibility.

Cellular mechanisms for metal tolerance can be classified into two basic strategies. One strategy is to keep the concentration of toxic metal ions in the cytoplasm low by preventing the metal from being transported across the plasma membrane, either by increased binding of metal ions to the cell wall or by reduced uptake through modified ion channels, or by pumping the metal out of the cell with active efflux pumps, a mechanism that is widespread in metal-tolerant bacteria [Tong *et al.*, 2004]. The other strategy is to detoxify heavy metal ions entering the cytoplasm through inactivation via chelation or conversion of a toxic ion into a less toxic or easier to handle form and/or compartmentalization. Modification or overexpression of the enzymes that are involved in the synthesis of GSH and PCs might be a good approach to enhance heavy metal tolerance and accumulation in plants. Zhu *et al.*, 1999, overexpressed the *Escherichia coli* counterparts of g-ECS and GSH synthetase in Indian mustard plants that accumulate more Cd than wild-type plants. Rugh *et al.*, 1998, modified yellow poplar trees with two bacterial genes, merA and merB, to detoxify methyl-Hg from contaminated soil. In transformed plants, merB catalyzes the release of Hg²⁺ from methyl-Hg, which is then converted to Hg⁰ by merA. Elemental Hg is less toxic and more volatile than the mercuric ion, and is released into the atmosphere. Pilon-Smits *et al.*, 1999, overexpressed the ATP-sulfurylase (APS) gene in Indian mustard. The transgenic plants had four-fold higher APS activity and accumulated three times more Se than wild-type plants. Recently, Dhankher *et al.*, 2002, reported a genetics-based strategy to remediate As from contaminated soils. They overexpressed two bacterial genes in Arabidopsis. One was the *E. coli* AsrC gene encoding arsenate reductase that reduces arsenate to arsenite coupled to a light-induced soybean rubisco promoter. The second gene was the *E. coli* g-ECS coupled to a strong constitutive actin promoter. The AsrC protein, expressed strongly in stem and leaves, catalyzes the reduction of arsenate to arsenite, whereas g-ECS, which is the first enzyme in the PC-biosynthetic pathway, increases the pool of PCs in the plant. The transgenic plants expressing both AsrC and g-ECS proteins showed substantially higher As tolerance; when grown on As, these plants accumulated a 4–17-fold greater fresh shoot weight and accumulated 2–3-fold more As than wild-type plants.

Bioremediation of Pesticides

Today, intensification of agriculture has increased the risk of losses due to improper crop health making agriculture sector heavily dependent upon the use of pesticides to prevent losses from pests. Pesticides are usually applied as a spray over the crop in aqueous or some non-polar solvent medium of which only 5 per cent is estimated to be utilized for the intended purpose and the rest remains in the environment as residues. These residues may get washed off and either seep into the ground water or reach water bodies along with the runoff. Once reaching the water bodies, the process of biomagnification begins. **Vaccari et al. (2006)** estimated that pesticide Dichlorodiphenyldichloroethane (DDD) may get accumulated 85,000 times more in a predatory fish than at concentration it enters in water (**Viklar et al., 2013**).

Some pesticides may get decomposed sooner after they are dissolved in a solvent but the most commonly used organochlorides have a very long (half-life making them threatening to the ecosystem and human beings. Pesticides may get accumulated in the human adipose tissue which enter the system orally, through inhalation and some are even absorbed dermally. In humans, pesticides may cause irritation, affect mental health, affect digestion and even cause carcinosis (**Green and Hoffnagle, 2004**). Organic pesticides have a very long half-life and are recalcitrant. UNEP's (United Nation Environment Programme) list of persistent organic pollutants, including aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, hexachlorobenzenes, mirex and toxaphene.

Sometimes the pesticide used may be less toxic than the degraded product that is produced from it. Hence, an effective bioremediation technique would be one that acts fast so as to prevent the degradation process and the end product that results from bioremediation is either non-toxic or less toxic. Bioremediation of metal contaminants or hydrocarbon contaminants is easier as the organisms that can survive in excess of metals and hydrocarbons can be naturally found but this is not the case with pesticide as these are artificial chemicals intended to kill. Hence, identification of organisms that may help in bioremediation process is crucial. Usually four remediation technologies are followed at the pesticide contaminated regions-Low temperature desorption, Incineration, Bioremediation and Phytoremediation. All these techniques have their own advantages and disadvantages. While incineration and low temperature desorption are faster technologies they are usually very expensive. Bioremediation and phytoremediation on the other hand are very efficient and cheaper technologies but the time taken for remediation is very long and hence a major drawback. Since our concern is aquatic habitat, bioremediation and phytoremediation

are by far the best technologies that can remove pesticide load from water and the water-soil interface.

White-rot fungi, particularly those of the family Phanerochaete, are becoming recognized for their ability to efficiently biodegrade toxic contaminants. Most studies focus on the ability of *Phanerochaete chrysosporium* to degrade persistent compounds, but *Phanerochaete sordida*, *Pleurotus ostreatus*, *Phellinus weirii*, and *Polyporus versicolor* have also been successful in laboratory studies [**Safferman et al., 1995**]. **Watanabe et al. (2007)** reported anaerobic microbial strains that have the ability to degrade various types of POPs, such as HCB, dieldrin, endrin, aldrin, and heptachlor. Amongst the aerobic organic pesticide degrading bacteria, *Pseudomonas sp.*, *Bacillus sp.*, *Trichoderma viride* [**Matsumura and Boush, 1967**], *Aerobacter aerogenes* [**Wedemeyer 1968**], *Mucor alternans* [**Anderson et al., 1970**], and *Trichoderma koningi* [**Bixby et al., 1971**] were isolated as dieldrin-degrading and *Pseudomonas sp.*, *Micrococcus sp.*, and several other unidentified bacteria and yeast [**Matsumura et al., 1971**] were found to be endrin degrading microorganisms.

Principal compound among the organic solvent-soluble metabolites was 6,7-trans-dihydroxydihydroaldrin produced by *Pseudomonas sp.*, *Bacillus sp.* [**Matsumura and Boush, 1967**], *A. aerogenes* [**Wedemeyer, 1968**], and *T. viride* [**Matsumura et al., 1968**]. Microbial gene (*atz*, *trz*, *psb*, *tri*, *tfd*, *piouh*, and *ndo*) encoding different groups of enzymes like dehalogenase, dehydrogenase, dehydro-chlorinase, hydrolase, haloperoxidase, urease, cytochrome P450, deaminase, dioxygenase, isomerases, reductases, and glutathione S transferases were found to have been involved in herbicide degradation and are also involved in pesticide degradation [**Hussain et al., 2009**]. **Sutherland et al. (2002)** reported gene *Esd* in *Mycobacterium spp.* capable of mineralising a-endosulfan. *Ese* gene from *Arthrobacter spp.* isolated by **Weir et al. (2006)** is capable of mineralizing both a and a form of endosulfan and endosulfate. A group of *lin* genes (*lin*, *linA*, *linB*, *linC*, *linD*, *linE*, *linX*), which encode several enzymes like dehalogenase, dehydrogenase, dehydrochlorinase, and hydrolase, have been reported in numerous Gram-negative Hexachlorohexane degrading soil bacteria [**Boltner et al., 2005**; **Ceremonie et al., 2006**].

Nocardia spp. was identified to have *trzN* gene responsible for initial dechlorination of atrazine into hydroxyatrazine which is further dealkylated in two step reaction [**Smith et al., 2005**]. Gene *atzC* is required for ring cleavage and was found in *Agrobacterium tumefaciens*, *Caulobacter crescentus*, *Pseudomonas putida*, *Sphingomonas yanikuyae*, *Nocardia sp.*, *Rhizobium sp.*, *Flavobacterium*

oryzihabitans, and *Variovorax paradoxus*. Similarly many, other genes have been discovered that characterize the utility of microbes for bioremediation of organic pesticides. Such genes have been summarized by **Hussain et al. (2009)**.

As mentioned earlier, there are different methods to carry out remediation of pesticide contamination and which method to adopt is crucial. Bioremediation techniques are effective and can act even on very small amount of contamination but when the contamination is severe and affects the immediate survival of organisms in the affected area then other methods like incineration and desorption may have to be used so as to provide immediate relief and the residues from such treatments may be left for bioremediation. Therefore, different techniques may be used so as to effectively remove the pesticide contaminants.

Bioremediation of Organic Xenobiotics

Organic xenobiotics in aquatic habitats have a varied origin - industrial effluents, incomplete combustion of fuels, forest and grass fires, biosynthesis of hydrocarbons by aquatic or terrestrial organisms, post-depositional transformation of biogenic precursors, diffusing from the mantle, petroleum source rocks or reservoirs [**Perelo, 2010**]. Persistent organic pollutants (POP) are a major source of concern amongst these organic xenobiotics due to very long half-life that makes them almost indestructible for years and years under natural conditions. **Perelo, (2010)** classified organic xenobiotics in aquatic sediments under four headings:-(a) Polycyclic aromatic hydrocarbons (PAH), (b) Polychlorinated biphenyls (PCBs), (c) Polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs), and (d) others.

(a) Polycyclic aromatic hydrocarbons (PAH)

Over hundred PAH have been identified which have their origin from incomplete combustion of organic substances and rarely are of industrial use, except for a few PAHs used in medicines and the production of dyes, plastics and pesticides [US-EPA, 2008]. They are highly hydrophobic making them insoluble in water, hence, they tend to get adsorbed on the aquatic sediments where they usually do not get decomposed and get accumulated later on in aquatic flora and fauna. PAH are carcinogenic and highly mutagenic [**Perelo, 2010**]. Chronic toxic effects from high PAH concentrations in sediments on benthic and aquatic organisms have been reported.

(b) Polychlorinated biphenyls (PCBs)

These are used widely in industry and get into the aquatic system through industrial discharge and spillage. Perhaps, they are most toxic and highly persistent nature and are classified as the most dangerous of all the POPs. They are toxic and carcinogenic, have wide distribution and degrade at very slow rate. As per a very old NRC report there are

hundred thousand tonnes of commercial PCB persistent in aquatic sediments and the quantity might have magnified since then [**NRC, 1979**].

(C) Polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs):

Though they were deposited decades ago under the sediments but are still found buried due to their high recalcitrant nature and non-bioavailability. Chlorine substitutions at 2, 3, 7 and 8 position makes them highly toxic and carcinogenic to humans [**Kaiser, 2000**].

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