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Review Article

Arsenic detoxification by phytoremediation

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ABSTRACT

Heavy metals pollution is amongst the commonest form of environmental pollution. These metals have accumulated over time from the smelting and mining activities of man, from poor waste disposal practices and from modernization. Recently the impact of heavy metal pollution of the environment is stirring up serious concerns since the discovery that some edible plants accumulate these metals to a level, toxic to both themselves and to the animals that consumes them. Common features of heavily polluted soil include barrenness, desertification, erosion, and this usually result in developmental stagnation in areas with such pollution. More researches have recently been stepped up in the field of remediating soils polluted with heavy metals. Traditional method includes excavation of the top soil, capping of the soil, stabilization of the polluting heavy metals, soil washing. In recent time, emphases have been drawn to the use of plants that has high metal accumulating and tolerating capacity to remediate metal-contaminated soil. This mini-review highlights the different conventional and recent practices in the control of heavy metal pollution.

Keywords: Environmental pollution, Heavy metals, Arsenic

INTRODUCTION

Arsenic, a silver-gray brittle crystalline solid represented by symbol As, has an atomic weight of 74.9216, atomic number of 33, specific gravity of 5.73, melting point of 817°C (28 atm) and sublimes at 613°C. It is 20th abundant element in the earth's crust, notorious poisonous metalloid it exists in different allotropic forms that are yellow,

black and gray. In the natural environment, arsenic is rarely encountered as a free element. It usually occurs as a component of sulfiolic ores as metal arsenide. Arsenic exists in variable oxidation states in the environment i.e., -3, 0, +3 and +5. Under aerobic conditions arsenic exists as As (+5) whereas reducing environment is congenial for the existence of elemental Arsenic (0), Arsenite (+3) and Arsine (-3). Arsenicals, both trivalent and pentavalent, are

soluble over a wide pH range and are found routinely in the surface as well as groundwater. Arsenic and many of its compounds are especially potent poisons. Arsenic disrupts adenosine triphosphate (ATP) production through several mechanisms. At the level of the citric acid cycle, arsenic inhibits pyruvate dehydrogenase and by competing with phosphate it uncouples oxidative phosphorylation, thus inhibiting energy-linked reduction of NAD+, mitochondrial respiration, and ATP synthesis. Hydrogen peroxide production is also increased, which might form reactive oxygen species (ROS) and oxidative stress. These metabolic interferences lead to death from multi-system organ failure probably from necrotic cell death, not apoptosis. A postmortem reveals brick red colored mucosa, due to severe hemorrhage. Although arsenic causes toxicity, it can also play a protective role.1

Organic arsenic is 500 times less harmful than inorganic arsenic and is a minor problem compared to the groundwater situation which affects many millions of people. Seafood is a common source of the less toxic organic arsenic in the form of arsenobetaine. The arsenic reported in 2012 in fruit juice and rice by consumer reports was primarily inorganic arsenic. Persistent contact to Arsenic is linked with a broad variety of neurological disorder, cardiovascular disease, dermatologic and carcinogenic effects, Peripheral neuropathy, diabetes, ischemic heart disease melanosis and keratosis and impairment of liver function.²

In humans, inorganic arsenic is reduced nonenzymatically from pentoxide to trioxide, using glutathione (GSH), or it is mediated by enzymes. Reduction of arsenic pentoxide to arsenic trioxide increases its toxicity and bioavailability, methylation occurs through methyltransferase enzymes. S-adenosylmethionine (SAM) may serve as methyl donor. Various pathways are used, the principal route being dependent on the current environment of the cell. Resulting metabolites are monomethylarsonous acid (MMA[III]) and dimethylarsinous acid (DMA[III]). Methylation had been regarded as a detoxification process. While in fact reduction from +5 As to +3 As may be considered as a bioactivation instead. Another suggestion is that methylation might be a detoxification if "As(III) intermediates are not permitted to accumulate" because the pentavalent organoarsenics have a lower affinity to thiol groups than inorganic pentavalent arsenics. Gebel (2002) stated that methylation is a detoxification through accelerated excretion. With regard to carcinogenicity, it has been suggested that methylation should be regarded as a toxification.3

MECHANISM

Arsenite inhibits not only the formation of Acetyl-CoA but also the enzyme succinic dehydrogenase. Arsenate can replace phosphate in many reactions. It is able to form Glc-6-Arsenate *in vitro*; therefore it has been argued that hexokinase

could be inhibited (eventually this may be a mechanism leading to muscle weakness in chronic arsenic poisoning). In the glyceraldehyde-3-P-dehydrogenase reaction arsenate attacks the enzyme bound thioester. The formed 1-arseno-3phosphoglycerate is unstable and hydrolyzes spontaneously. Thus, ATP formation in glycolysis is inhibited while bypassing the phosphoglycerate kinase reaction (moreover, the formation of 2,3-bisphosphoglycerate in erythrocytes might be affected, followed by a higher oxygen affinity of hemoglobin and subsequently enhanced cyanosis). As shown by Gresser, submitochondrial particles synthesize adenosine-5'-diphosphate (ADP) arsenate in the presence of succinate. Thus, by a variety of mechanisms arsenate leads to an impairment of cell respiration and subsequently diminished ATP formation. This is consistent with observed ATP depletion of exposed cells and histopathological findings of mitochondrial and cell swelling, glycogen depletion in liver cells and fatty change in liver, heart and kidney. Experiments demonstrated enhanced arterial thrombosis in a rat animal model, elevations of serotonin levels, thromboxane A and adhesion proteins in platelets while human platelets showed similar responses. The effect on vascular endothelium may eventually be mediated by the arsenic-induced formation of nitric oxide (NO). It was demonstrated that +3 As concentrations substantially lower than concentrations required for inhibition of the lysosomal protease cathepsin L in B-cell line TA3 were sufficient to trigger apoptosis in the same B-cell line, while the latter could be a mechanism mediating immunosuppressive effects.4

CARCINOGENICITY

It is still a matter of debate whether DNA repair inhibition or alterations in the status of DNA methylation are responsible for the carcinogenic potential of As. As vicinal sulfhydryl groups are frequently found in DNA-binding proteins, transcription factors, and DNA-repair proteins, interaction of arsenic with these molecules appears to be likely. However, in vitro, most purified DNA repair enzymes are rather insensitive to arsenic, but in cell culture, As produces a dose-dependent decrease of DNA ligase activity. This might indicate that inhibition of DNA repair is an indirect effect due to changes in cellular redox levels or altered signal transduction and consequent gene expression. In spite of its carcinogenicity, the potential of arsenic to induce point mutations is weak. If administered with point mutagens it enhances the frequency of mutations in a synergistic way. Its comutagenic effects may be explained by interference with base and nucleotide excision repair, eventually through interaction with zinc finger structures. DMA showed to effectuate DNA single stand breaks resulting from inhibition of repair enzymes at levels of 5-100 mM in human epithelial Type II cells. +3 DMA is able to form ROS by reaction with molecular oxygen. Resulting metabolites are the dimethylarsenic radical and the dimethylarsenic peroxyl radical. Both +5

DMA and +3 DMA were shown to release iron from horse spleen as well as from human liver ferritin if ascorbic acid was administered simultaneously. Thus, formation of ROS can be promoted. Moreover, arsenic could cause oxidative stress by depleting the cell's antioxidants, especially the ones containing thiol groups. The accumulation of ROS like the cited above and hydroxyl radicals, superoxide radicals and hydrogen peroxides causes aberrant gene expression at low concentrations and lesions of lipids, proteins and DNA in higher concentrations which eventually lead to cellular death. In a rat animal model, urine levels of 8-hydroxy-2'-desoxyguanosine (as a biomarker of ROS DNA damage) were measured after treatment with DMA. In comparison to control levels, they turned out to be significantly increased. This theory is further supported by a cross-sectional study which found elevated mean serum lipid peroxides in the As exposed individuals which correlated with blood levels of inorganic arsenic and methylated metabolites and inversely correlated with non-protein sulfhydryl levels in whole blood. Another study found an association of As levels in whole blood with the level of reactive oxidants in plasma and an inverse relationship with plasma antioxidants. A finding of the latter study indicates that methylation might in fact be a detoxification pathway with regard to oxidative stress: the results showed that the lower the As methylation capacity was, the lower the level of plasma antioxidant capacity. As reviewed by Kitchin, the oxidative stress theory provides an explanation for the preferred tumor sites connected with arsenic exposure. Considering that a high partial pressure of oxygen is present in lungs, and +3 DMA is excreted in gaseous state via the lungs this seems to be a plausible mechanism for special vulnerability. The fact that DMA is produced by methylation in the liver excreted via the kidneys and later on stored in the bladder accounts for the other tumor localizations.

Regarding DNA methylation, some studies suggest interaction of As with methyltransferases which leads to an inactivation of tumor suppressor genes through hypermethylation, others state that hypomethylation might occur due to a lack of SAM resulting in aberrant gene activation. An experiment by Zhong et al. with arseniteexposed human lung A549, kidney UOK123, UOK109 and UOK121 cells isolated eight different DNA fragments by methylation-sensitive arbitrarily primed polymerase chain reaction (PCR). It turned out that six of the fragments were hyper and two of them were hypomethylated. Higher levels of DNA methltransferase mRNA and enzyme activity were found.⁵ Kitchin proposed a model of altered growth factors which lead to cell proliferation and thus to carcinogenesis. From observations, it is known that chronic low-dose arsenic poisoning can lead to increased tolerance to its acute toxicity. Multidrug resistance-associated proteins (MRP1)-overexpressing lung tumor GLC4/Sb30 cells poorly accumulate arsenite and arsenate. This is mediated through MRP1 dependent efflux. The efflux requires

GSH, but no As-GSH complex formation. Although many mechanisms have been proposed, no definite model can be given for the mechanisms of chronic arsenic poisoning. The prevailing events of toxicity and carcinogenicity might be quite tissue-specific. Current consensus on the mode of carcinogenesis is that it acts primarily as a tumor promoter. Its co-carcinogenicity has been demonstrated in several models. However, the finding of several studies that chronically arsenic-exposed Andean populations (as most extremely exposed to ultra violet light) do not develop skin cancer with chronic arsenic exposure is puzzling. Another aspect is the similarity of arsenic effects to the heat shock response. Short-term arsenic exposure has effects on signal transduction inducing heat shock proteins with masses of 27, 60, 70, 72, 90, 110 kDa as well as metallotionein, ubiquitin, mitogen-activated protein kinases (MAPK), extracellular regulated kinase, c-jun terminal kinases (JNK) and p38. Via JNK and p38 it activates c-fos, c-jun and egr-1 which are usually activated by growth factors and cytokines. The effects are largely dependent on the dosing regime and may be as well inversed. As shown by some experiments reviewed by Del Razo, ROS induced by low levels of inorganic arsenic increase the transcription and the activity of the activator protein 1 (AP-1) and the nuclear factor-κB (NF-κB) (maybe enhanced by elevated MAPK levels), which results in c-fos/c-jun activation, over-secretion of pro-inflammatory and growth promoting cytokines stimulating cell proliferation. Germolec found an increased cytokine expression and cell proliferation in skin biopsies from individuals chronically exposed to arsenic-contaminated drinking water. Increased AP-1 and NF-κB obviously also result in an up-regulation of mdm2 protein, which decreases p53 protein levels. Thus, taking into account p53's function, a lack of it could cause a faster accumulation of mutations contributing to carcinogenesis. However, high levels of inorganic arsenic inhibit NF-κB activation and cell proliferation. An experiment of Hu et al. demonstrated increased binding activity of AP-1 and NF-κB after acute (24 hrs) exposure to +3 sodium arsenite, whereas long-term exposure (10-12 weeks) yielded the opposite result. The authors conclude that the former may be interpreted as a defense response while the latter could lead to carcinogenesis. As the contradicting findings and connected mechanistic hypotheses indicate. there is a difference in acute and chronic effects of arsenic on signal transduction which is not clearly understood yet.⁷ Studies have demonstrated that the oxidative stress generated by arsenic may disrupt the signal transduction pathways of the nuclear transcriptional factors peroxisome proliferator-activated receptor (PPAR's), AP-1, and NF-κB, as well as the pro-inflammatory cytokines interleukin-8 (IL-8) and Tumor necrosis factor-alpha (TNF-α). The interference of oxidative stress with signal transduction pathways may affect physiological processes associated with cell growth, metabolic syndrome X, glucose homeostasis, lipid metabolism, obesity, insulin resistance, inflammation, and diabetes-2. Recent scientific evidence

has elucidated the physiological roles of the PPAR's in the ω-hydroxylation of fatty acids and the inhibition of pro-inflammatory transcription factors (NF-κB and AP-1), pro-inflammatory cytokines (IL-1, -6, -8, -12, and TNF-α), cell4 adhesion molecules (intercellular adhesion molecule-1 and vascular cell adhesion molecule-1), inducible NO synthase, proinflammatory NO, and antiapoptotic factors. Epidemiological studies have suggested a correlation between chronic consumption of drinking water contaminated with arsenic and the incidence of Type 2-diabetes. The human liver after exposure to therapeutic drugs may exhibit hepatic non-cirrhotic portal hypertension, fibrosis, and cirrhosis. However, the literature provides insufficient scientific evidence to show cause and effect between arsenic and the onset of diabetes mellitus Type 2.8

TOXICOLOGICAL EFFECTS IN ANIMALS

Acute toxic effects seen in animals after oral exposure are similar to effects seen in humans. The signs of acute arsenic poisoning in humans include intense abdominal pains, staggering, weakness, trembling, salivation, gastrointestinal effects such as vomiting and diarrhoea, fast feeble pulse, prostration, hypothermia, collapse and death. Arsenite has been shown to induce oxidative DNA damage in human vascular smooth muscle cells *in vitro*. Oral LD50 values for various arsenic compounds range from 15 to 293 mg/kg in rats and from 10 to 150 mg/kg in other animals lists oral LD50 for inorganic As ranging from 15 to 175 mg/kg for rats and 26 to 39 mg/kg for mice. Most deaths occurred within 1-day of exposure. Studies have shown that MMAIII is more toxic than arsenite in cultured human cells *in vitro*.

The ones most affected by arsenic are those involved in the absorption, accumulation, and/or excretion, i.e., the gastrointestinal tract, circulatory system, skin, liver, and kidney. However, other organs or systems that are particularly sensitive to the effects of arsenic, such as the nervous system, and those that are affected secondarily, such as the heart, are also affected. Histopathological changes have been observed in liver tissue as a result of arsenic exposure. Arsenic impaired mitochondrial respiration in rats injected with arsenite.9 Food chain represents an important pathway for arsenic exposure to the consumers. Food constituents the largest source of As intake. Daily intake of total as from the consumption of food and beverages lies between 20 and 30 µg/day. A study conducted in Mexico revealed that 30% of inorganic As came from intake of this element in food.

The plants raised over soils contaminated with As are known to accumulate this element thus exposing the consumer to its ill effects. In Bangladesh and West Bengal (India) rice has become an important dietary source of As for the people.

Various traditional medicinal concoctions have also been identified as a source of dietary arsenic.¹⁰

TOXICOLOGICAL EFFECTS ON HUMANS

Arsenic toxicity (Arsenicosis) is manifested through symptoms ranging from non-specific abdominal pain, diarrhea, nausea to multiorgan disorders and more serious consequences as malignancy. Arsenic is specifically associated with skin cancer, hyperkeratosis, melanosis, blackfoot disease, damage to blood vessels and sensation of pins and needles in hands and feet reported to be diabetogenic. Cases among pregnant women are available where arsenite (As₂O₃) readily passes through the placenta. Several studies have reported association between prolonged low-dose As exposure, reports of arsenic foetotoxicity and adverse pregnancy outcomes in terms of live birth, stillbirths, spontaneous abortion, fetel loss, premature delivery, and preterm birth are also available.¹¹

GENETIC TOXICITY

The earlier genetic toxicity data on arsenic are summarized in the Genetic Activity Profiles database for short-term tests based on data of the United States Environmental Protection Agency (US EPA) and the IARC monographs. For trivalent arsenic (AsIII), the GERMCELL and IARC databases list 11 positive findings in 25 non-human animal, plant, or microbial test systems. These include chromosomal aberrations in vitro and in vivo, micronuclei induction in mice in vivo, sister chromatid exchange (SCEs) in mammalian cells, and cell transformation in vitro. For As(V), the IARC database lists 6/13 positive findings: chromosome aberrations in vitro; SCEs in vitro; and cell transformation in vitro. In general, the lowest effective doses (LEDs) for As(III) in vitro were in the 1-10 µM range, whereas for As(V) the LEDs were usually 10-50 μM. Jacobson-Kram and Montalbano, and Basu et al. have published comprehensive reviews of arsenic genetic toxicity. Studies assessing the ability of arsenic to induce gene mutations have largely produced negative results. The results of earlier studies suggested positive mutagenic activity. Yamanaka et al. reported the mutagenicity of DMA in Escherichia coli B tester strains. The study by Nakamuro and Sayato evaluated the genotoxic activity of six arsenic compounds and found the following order of potency (from highest to lowest) was observed: As₂O₃ > AsCl₃, NaAsO₅ $> Na_2HAsO_4 > H_3AsO_4$, As_2O_5 .

Crossen indicated that arsenic is only clastogenic when present during the cell phase of DNA replication (i.e. the S-phase). Wang and Huang observed that active oxygen species are involved in the induction of micronuclei by arsenite in XRS-5 cells, an X-ray sensitive Chinese hamster ovary cell line. Zhao et al. demonstrated an association of arsenic-induced malignant transformation with DNA

hypomethylation and aberrant gene expression in the rat liver epithelial cell line. Exposure to arsenic trioxide increased the frequency of chromosomal aberrations in the peripheral lymphocytes of smelter workers. There is no conclusive evidence that arsenic causes point mutations in any cellular system and arsenic is mutagenic have shown that arsenite causes inhibition of DNA repair after the incision step in Chinese hamster V79 cells. 12 Arsenic has been known to cause chromosomal damage, but most investigators have been unable to induce direct gene mutation. Arsenic promotes genetic damage in large part by inhibiting DNA repair. The comparisons of chromosome aberration frequencies induced by trivalent and pentavalent arsenic have indicated that the trivalent forms are far more potent and genotoxic than the pentavalent forms. Epidemiological studies have demonstrated an evident causal relationship between environmental, occupational and medical exposure of man to inorganic arsenic and cancer of the skin and lungs. Most animal experiments, however, were not able to demonstrate concinogenicity, except for very few observations of increased incidence of leukemia and lung cancer. Epidemiological studies in Argentina, Chile, Canada, and Taiwan suggest correlations between drinking water arsenic and Blackfoot disease, Bowen's disease, and skin cancer. The transfer of arsenic from soil to plant is low for most plant species. This is probably due to the restricted uptake of arsenic by plant roots, the limited translocation of arsenic from root to shoot, arsenic phytotoxicity and the low bioavailability of arsenic in soil. Identification of candidates for removal of arsenic by phytoremediation is still at its preliminary stage. No such work is available from the polluted areas of Punjab. Keeping in view the presence of arsenic in the soils of the study area (Talwandi Sabo Block of Bathinda, Punjab) the present problem was taken up to identify the species suitable for remediation of the soil.13

PHYTOREMEDIATION

Phytoremediation (from Ancient Greek $\varphi v \tau o$ (phyto), meaning "plant," and Latin remedium, meaning "restoring balance") describes the treatment of environmental problems (bioremediation) through the use of plants that mitigate the environmental problem without the need to excavate the contaminant material and dispose of it elsewhere. Phytoremediation consists of mitigating pollutant concentrations in contaminated soils, water, or air, with plants able to contain, degrade, or eliminate metals, pesticides, solvents, explosives, crude oil and its derivatives, and various other contaminants from the media that contain them. ¹⁴

Application

Phytoremediation may be applied wherever the soil or static water environment has become polluted or is suffering ongoing chronic pollution. Examples where phytoremediation has been used successfully include the restoration of abandoned metal mine workings, reducing the impact taminants in soils, water, or air. Contaminants such as metals, pesticides, solvents, explosives, and crude oil and its derivatives, have been mitigated in phytoremediation projects worldwide. Many plants such as mustard plants, alpine pennycress, hemp, and pigweed have proven to be successful at hyperaccumulating contaminants at toxic waste sites. Over the past 20 years, this technology has become increasingly popular and has been employed at sites with soils contaminated with lead, uranium, and arsenic. While it has the advantage that environmental concerns may be treated in situ; one major disadvantage of phytoremediation is that it requires a long-term commitment, as the process is dependent on a plant's ability to grow and thrive in an environment that is not ideal for normal plant growth. Phytoremediation may be applied wherever the soil or static water environment has become polluted or is suffering ongoing chronic pollution. Examples where phytoremediation has been used successfully include the restoration of abandoned metal mine workings, reducing the impact of sites where polychlorinated biphenyls have been dumped during manufacture and mitigation of on-going coal mine discharges.15

PHYTOEXTRACTION

Phytoextraction (or phytoaccumulation) uses plants or algae to remove contaminants from soils, sediments or water into harvestable plant biomass (organisms that take larger-than-normal amounts of contaminants from the soil are called hyperaccumulators). Phytoextraction has been growing rapidly in popularity worldwide for the last twenty years or so. In general, this process has been tried more often for extracting heavy metals than for organics. The plants absorb contaminants through the root system and store them in the root biomass and/or transport them up into the stems and/or leaves. A living plant may continue to absorb contaminants until it is harvested. After harvest, a lower level of the contaminant will remain in the soil, so the growth/harvest cycle must usually be repeated through several crops to achieve a significant cleanup. After the process, the cleaned soil can support other vegetation. The main advantage of phytoextraction is environmental friendliness. Traditional methods that are used for cleaning up heavy metal-contaminated soil disrupt soil structure and reduce soil productivity, whereas phytoextraction can clean up the soil without causing any kind of harm to soil quality. Another benefit of phytoextraction is that it is less expensive than any other clean-up process. As this process is controlled by plants, it takes more time than anthropogenic soil cleanup methods.16

PHYTOTRANSFORMATION

In the case of organic pollutants, such as pesticides, explosives, solvents, industrial chemicals, and other

xenobiotic substances, certain plants, such as Cannas, render these substances non-toxic by their metabolism. In other cases, microorganisms living in association with plant roots may metabolize these substances in soil or water. These complex and recalcitrant compounds cannot be broken down to basic molecules (water, carbon dioxide, etc.) by plant molecules, and, hence, the term phytotransformation represents a change in chemical structure without complete breakdown of the compound. The term "Green Liver Model" is used to describe phytotransformation, as plants behave analogously to the human liver when dealing with these xenobiotic compounds (foreign compound/pollutant). After uptake of the xenobiotics, plant enzymes increase the polarity of the xenobiotics by adding functional groups such as hydroxyl groups (-OH). This is known as Phase I metabolism, similar to the way that the human liver increases the polarity of drugs and foreign compounds (Drug Metabolism). Whereas in the human liver enzymes such as cytochrome P450s are responsible for the initial reactions, in plants enzymes such as nitroreductases carry out the same role. In the second stage of phytotransformation, known as Phase II metabolism, plant biomolecules such as glucose and amino acids are added to the polarized xenobiotic to further increase the polarity (known as conjugation). This is again similar to the processes occurring in the human liver where glucuronidation (addition of glucose molecules by the UDP-glucuronosyltransferase [UGT, e.g. UGT1A1] class of enzymes) and GSH addition reactions occur on reactive centers of the xenobiotic. Phase I and II reactions serve to increase the polarity and reduce the toxicity of the compounds, although many exceptions to the rule are seen. The increased polarity also allows for easy transport of the xenobiotic along aqueous channels. In the final stage of phytotransformation (Phase III metabolism), a sequestration of the xenobiotic occurs within the plant. The xenobiotics polymerize in a lignin-like manner and develop a complex structure that is sequestered in the plant. This ensures that the xenobiotic is safely stored and does not affect the functioning of the plant. However, preliminary studies have shown that these plants can be toxic to small animals (such as snails). and, hence, plants involved in phytotransformation may need to be maintained in a closed enclosure. Hence, the plants reduce toxicity (with exceptions) and sequester the xenobiotics in phytotransformation. Trinitrotoluene (TNT) phytotransformation has been extensively researched and a transformation pathway has been proposed.¹⁷

ROLE OF GENETICS

Breeding programs and genetic engineering are powerful methods for enhancing natural phytoremediation capabilities, or for introducing new capabilities into plants. Genes for phytoremediation may originate from a micro-organism or may be transferred from one plant to another variety better adapted to the environmental conditions at the cleanup site. For example, genes encoding a nitroreductase from

a bacterium were inserted into tobacco and showed faster removal of TNT and enhanced resistance to the toxic effects of TNT. Researchers have also discovered a mechanism in plants that allows them to grow even when the pollution concentration in the soil is lethal for non-treated plants. Some natural, biodegradable compounds, such as exogenous polyamines, allow the plants to tolerate concentrations of pollutants 500 times higher than untreated plants, and to absorb more pollutants.¹⁸

HYPERACCUMULATORS AND BIOTIC INTERACTIONS

A plant is said to be a hyperaccumulator if it can concentrate the pollutants in a minimum percentage which varies according to the pollutant involved (for example: more than 1000 mg/kg of dry weight for nickel, copper, cobalt, chromium or lead; or more than 10,000 mg/kg for zinc or manganese). This capacity for accumulation is due to hyper tolerance, or phytotolerance: the result of adaptative evolution from the plants to hostile environments through many generations. A number of interactions may be affected by metal hyperaccumulation, including protection, interferences with neighbor plants of different species, mutualism (including mycorrhizae, pollen and seed dispersal), commensalism, and biofilm.¹⁹

PHYTOSCREENING

As plants are able to translocate and accumulate particular types of contaminants, plants can be used as biosensors of subsurface contamination, thereby allowing investigators to quickly delineate contaminant plumes. Chlorinated solvents, such as trichloroethylene, have been observed in tree trunks at concentrations related to groundwater concentrations. To ease field implementation of phytoscreening, standard methods have been developed to extract a section of the tree trunk for later laboratory analysis, often by using an increment borer. Phytoscreening may lead to more optimized site investigations and reduce contaminated site cleanup costs.²⁰

ARSENIC DETOXIFICATION BY PHYTOREMEDIATION

Arsenic is ubiquitous in nature, and small amount of this element can be found in every environmental compartment. Most arsenic in the environment exists in rock or soil. Arsenic is a major constituent of more than 245 minerals. Arsenic-sulphides, the most common mineral readily oxidize when exposed to air, yielding inorganic arsenic salts which are highly water-soluble. A list of some of the most common As minerals is given in Table 1. The average arsenic content of igneous rocks is 2-3 mg/kg (up to 100 mg/kg) and varies from small amounts of limestone and sandstone up to 15,000 mg/kg in some manganese ores.

Arsenic concentrations in soil depend in part on the parent materials from which the soils were derived, although they may be enriched by other sources. Natural concentrations of arsenic in the earth's crust vary but average concentrations are generally reported to range from 1.5 to 5 mg/kg. The level of arsenic in soil derived from basalts tends to be higher than in soils of gigantic origin and concentrations of 20-30 mg/kg may be found in the soils derived from sedimentary rocks. The uncontaminated soils contain between 0.1 and 40 mg As/kg (average 5-6 mg/kg). Soils overlying sulfide ore deposits may have concentrations up to 8000 mg As/kg.

Tamaki and Frankenburgur have suggested that natural arsenic emission exceeds industrial emissions while other studies maintain that industrial emissions of arsenic are significantly greater than natural emissions. ²¹ Arsenic, naturally present in most lead, copper, and gold ores, and is released during the smelting through gaseous and solid waste emission and is accumulated in the soils around the emission source. Another important source of As emission into the atmosphere is coal-burning during electrical power production and heating. Arsenic exists largely as arsenopyrite in coal and is emitted as arsenic trioxide from power plants.

Arsenic concentrations in coal from the USA, Australia, and the UK range from around 0.5 to 93 mg As/kg and fly ash particles can contain up to 1700 mg As/kg. Brown coal (from the Czechoslovakia) was found to contain up to 1500 mg As/kg. Coal from Coalfield, Northeastern India has been reported to contain 3.27 mg/kg of As with a maximum value of 40 mg/kg from. Arsenic and its compounds find a variety of industrial applications. Arsenic metal is used in the lead-acid storage batteries and formation of some copper alloys. Smelting activities generate the single largest source of anthropogenic arsenic input into the atmosphere. Agricultural uses of arsenic and arsenic compounds include pesticides, herbicides, insecticides, defoliants and soil sterilants. Pesticides are the major sources of As in agricultural soils.

Dichlorodiphenyltrichloroethane, lead arsenate (PbAsO₄), calcium arsenate (CaAsO₄), magnesium arsenate (MgAsO₄), zinc arsenate (ZnAsO₄), and Paris green (Cu[CH₂COO],Cu[AsO₂]₂) are used extensively as pesticides in agriculture. Excessive use of arsenic compounds as insecticides and defoliant has been known to increase its concentration in the respective soils. The leaching of arsenic from agricultural topsoil to subsoil has been reported by neonatal abstinence syndrome. Due to the essential role of As in animal nutrition, organic arsenicals play an important role as food additives to promote the growth of farm animals. Organic arsenicals are frequently used as desiccants and defoliants in the cotton industry and for weed control. Despite immense controversy arsenic is still used as an ingredient of wood preservatives, for debarking trees, in cattle and sheep dips and in aquatic weed control.²²

ARSENIC IN THE SOIL

Arsenic is a crystalline metalloid with three allotropic forms that are yellow, black and gray. It also exists in several forms and oxidation states (-3, 0, 3, and 5). Arsenate (V) is the stable oxidation state in aerobic conditions. In strongly reducing conditions, elemental As, As(III) and arsine (III) can exist. Arsenite(III) exists in moderately reducing conditions and is one of the most toxic arsenic compounds. Methanogenic bacteria reduce As(V) to As(III) and methylate it to methylarsinic acid. Arsenic trioxide (white arsenic) As,O₃, constitutes 97% of arsenic produced that enters end product manufactured. Arsenic trioxide is the raw material for arsenical pesticides including lead arsenate, calcium arsenate, sodium arsenite, and organic arsenicals. These compounds are used in insecticides, herbicides, fungicides, algicides, sheepdips, wood preservatives, and dyestuffs; and for eradication of tapeworm in sheep and cattle. As(III) exists in most natural water as As(OH)₃ (pKa=9.2) and is more mobile than As(V) because it is less strongly absorbed on most mineral surfaces than the negatively charged As(V) oxyanions (H₃AsO₄; pKa=2.22, 6.98, 11.53). Iron(III) oxy species have a high affinity for As(V). Arsenic and P are chemically similar. Both form insoluble compounds with

Table 1: Common As minerals: Major arsenic minerals occurring in nature.

Mineral	Composition	Occurrence
Native arsenic	As	Hydrothermal veins
Niciolite	NiAs	Vein deposits and norites
Realgar	AsS	Vein deposits, often associated with orpiment, clays and limestones, also deposits from hot springs
Orpiment	As_2S_3	Hydrothermal veins, hot springs, volcanic sublimation products
Cobaltite	CoAsS	High-temperature deposits, metamorphic rocks
Arsenopyrite	FeAsS	The most abundant As mineral, dominantly in mineral veins
Tennantite	$(Cu, Fe)_{12} As_4 S_{13}$	Hydrothermal veins
Enargite	Cu ₃ AsS ₄	Hydrothermal veins
Arsenolite	As ₂ O ₃	Secondary mineral formed by oxidation of arsenopyrite, native arsenic and other As minerals
Claudetite	As_2O_3	Secondary mineral formed by oxidation of realgar, arsenopyrite and other As minerals
Scorodite	FeAsO ₄ .2H ₂ O	Secondary mineral
Annabergite	(Ni, Co) ₃ (AsO ₄) ₂ .8H ₂ O	Secondary mineral
Hoernesite	$Mg_3(AsO_4)_2.8H_2O$	Secondary mineral, smelter wastes
Haematilite	$(Mn, Mg)_4 Al (AsO_4) (OH)_8$	
Conichalcite	CaCu (AsO ₄) (OH)	Secondary mineral
Pharmacosiderite	Fe ₃ (AsO ₄) ₂ (OH) ₃ .5H ₂ O	Oxidation product of arsenopyrite and other As minerals

Al and Fe in soils. Al-As and Fe-As are the dominant forms of arsenic in most soils though arsenic shows less affinity for Al oxides than does phosphates. As(III) seems to be adsorbed on iron(III) surfaces. Activated alumina has a 2-fold higher affinity for As(V) than for As(III) at pH 7. Kaolinite and montmorillonite have higher affinities for As(V) than for As(III). Abiotic oxidation of As(III) is enhanced in the presence of the clay minerals kaolinite and illite, a process that results in strongly-bound As(V) species. Arsenic mobility and phytotoxicity are greater in sandy than in clayey soils. The total concentration of arsenic in the soil does not reflect the amount available for plant uptake because of the adsorption of arsenic in the soil. Therefore, different methods are used to determine availability of arsenic in the soil. Various extractants that could simulate release of arsenic under different conditions in the soil have been reported to correlate significantly with plant arsenic uptake. Sequential extraction has been widely used to assess metal availability and mobility in soils. Different reagents are used to separate metals to operationally-defined chemical associations. Among all the methods, the one developed by Tessier is the most wide used to assess metal geochemistry in soils. However, for soil arsenic, due to its chemical similarity to phosphate, the method used for P fractionation has been used for arsenic fractionation. Soil arsenic is operationally separated into four fractions: water-soluble plus exchangeable arsenic (WE-As), Al-bound arsenic (Al-As), Fe-bound arsenic (Fe-As), and Ca-bound arsenic (Ca-As), using NH₄NO₂, NH₄F, NaOH, and H₂SO₄. Based on sequential extraction, information about the chemical

binding form, retention, and partitioning of metals in soils can be estimated. Even though sequential extraction suffers from the lack of specificity during chemical fractionation and resorption of dissolved metals by soils during the extraction, it is still a useful tool to evaluate metal biochemistry in soils.

In arsenic contaminated soils, soil pH is one of the major factors determining arsenic availability. Akins and Lewis examined the effects of pH (4-8) on arsenic sorption by soils using a sequential fractionation procedure. They found that, at low pH (pH=4), Fe-As is the most abundant form followed by Al-As, whereas at high pH (pH=6-8), Ca-As is more predominant. This is similar to behaviors of P in soils; that is, Fe-P and Al-P are predominant in acid soils, whereas Ca-P dominates in alkaline and calcareous soils. In addition to soil pH, arsenic partitioning and retention in soils is also influenced by the presence of organic matter, Fe, Mn, and Al oxides, and clay minerals. However, the capacity of a soil to retain arsenic mainly depends on the content of extractable hydrous oxides of Fe and Al in the soil. The efficiency of phytoremediation depends on soil characteristics and contaminant. Adsorption of arsenic in soils is influenced by a number of factors: types and amount of soil colloids (clay minerals; Fe, Al and Mn oxides; and organic matter); pH; presence of organic and inorganic ligands; and soil texture. The difference in the rate of sorption of arsenite on A and B-horizons of five West Virginia soils was explained by differences in pH, iron oxide, and organic matter content. There is a strong association between As and Fe (mainly goethite) in soils for both natural and added As. Hydroxy aluminum on the external surfaces of micaceous minerals significantly retains As. Arsenic mobility and phytotoxicity are greater in sandy soils than in clayey soils because hydrous Fe and Al oxides vary directly with clay content of the soil.²³

ARSENIC CONCENTRATIONS IN SOIL AND WATER

Arsenic is the 20th most abundant element in earth crust, making about 5 mg/kg of 16 earth's crust, with an average concentration of 2 mg/kg in igneous and sedimentary rocks 17. It is a naturally occurring element typically found in soil at background concentrations 18 ranging from 0.1 to 40 mg/kg. As is commonly associated with sulfides, 19 oxides/hydroxides of aluminum (Al), iron (Fe) and manganese (Mn), other sources are 20 volcanic eruptions and sea salt sprays. In soil, As is present in the form of oxides, 21 hydroxide, chlorides and sulfides, such as enargite (Cu,AsA₄), cobaltite (CoAsS), 22 skutterudite (CoAsS₄) and its average concentration in different regions of the world is 9.36 23 mg/kg. Heavy use of As containing pesticides is considered as the major reason for its pollution. Arsenic and P are chemically similar. Both form insoluble compounds with 1 Al and Fe in soils. In soil, Al-As and Fe-As complexes are the dominant chemical forms; while arsenic has less affinity for Al oxides than phosphates. As(III) gets adsorbed on iron 3 (III) surfaces. Kaolinite and montmorillonite have higher affinities for As(V) than for 4 As(III). Arsenic mobility and phytotoxicity are greater in sandy soils. Rising arsenic concentrations in groundwater are alarming due the health risk to plants, animals, and human health. Higher levels of arsenic were found in groundwater sources than in surface water sources. Many countries around the world (including Taiwan, Argentina, India, Bangladesh, Mexico, Hungary, and Chile) have reported extensive arsenic groundwater contamination. Use of such contaminated water for irrigation of crops may lead to arsenic contamination of agricultural soils. The presence of high As concentration in the aquifer may be due to desorption of arsenic from Fe and Mn oxides, weathering of primary silicate minerals, and apatite under high pH and alkalinity from silicate and carbonate reactions.²⁴

ARSENIC POLLUTION

More than thirty countries all over the world have been reported to be affected due to the presence of excessive As in their environment. The arsenic contaminant has reached mammoth levels in many countries namely, Afghanistan, Bangladesh, Cambodia, China, India, Lao PDR, Mongolia, Myanmar, Nepal, Pakistan, Thailand, and Vietnam. The limit for As in drinking water, set by the WHO, was 50 µg As/L (WHO, 1993) which was later revised to 10 µg As/L. In India, the presence of elevated levels of As in groundwater has been confirmed in states of West Bengal, Bihar, Uttar Pradesh, Jharkhand, the seven northeastern states, Andhra Pradesh, parts of Chhattisgarh, Haryana, Himachal Pradesh and Punjab. Chatterjee and Banerjee reported the presence of

As ranging from 17.50 ± 0.52 to 9740 ± 226 mg/kg in soil in the vicinity of lead industry in Kolkata. Arsenic contamination was reported from Chandigarh and some villages of Punjab, Haryana, and Himachal Pradesh. Hundal analyzed water samples from different regions of Punjab and reported the presence of As ranging from 0.3 to 2.89 ppb in the water samples from various canals and the groundwater of the arid south-western region of Punjab, i.e., Sangrur, Mansa, Faridkot, Muktsar, Bathinda and Ferozepur was reported to contain 11-688 µg/L As in water. Presence of As in most regions is geogenic in origin but in case of Talwandi Sabo block of Punjab, which is an important cotton producing region, the presence of As in soil and water is attributed to natural sources as well as contamination from agricultural inputs. 25

HYPERACCUMULATION OF ARSENIC

The term hyperaccumulators were first used for plants accumulating Ni and was later generalized to plants accumulating a metal more than 100-fold relative to its concentration in the soil. First arsenic hyperaccumulate discovered was Pteris vittata L.; followed Pityrogramma calomelanos L. and many other species of the Pteris genus such as Pteris cretica L., Pteris longifolia L., Pteris umbrosa L. and Pteris argyraea L. and Pteris quadriaurita L., Pteris ryiunkensis L. and Pteris biaurita. Some other plants growing on mine wastes from various sites in the United Kingdom and on smelter wastes in northeast Portugal have also been reported as arsenic hyperaccumulators (>1000 mg/kg As). Many found that *P. vittata* (brake fern) accumulated 7234 mg As/kg in the fronds. Francesconi identified another arsenic hyperaccumulator, P. calomelanos which accumulated up to 8350 mg As/kg (dry weight) in the fronds while the roots contained only 88-310 mg As/ kg. Zhao et al. reported high (27,000 mg As/kg dry wt.) accumulation of arsenic in P. vittata fronds in an 18-day hydroponic experiment.²⁶

MECHANISM OF ARSENIC UPTAKE

Arsenate, chemically very similar to phosphate, is thought to enter the root cell by the same uptake mechanism as phosphate in a variety of organisms. This mechanism seems to have, however, a lower affinity for As than for P. Phosphate is taken up as H_2PO_4 at low soil pH and at high pH as HPO_4 . Similarly arsenate is taken up at low pH as H_2ASO_4 and at high pH as $HASO_4$. The root system is the primary site of damages when As reaches phytotoxic levels. Compared to P, translocation of As to shoots is generally low. Typical As concentrations in aerial parts are <2 mg As/kg (O'Neill, 1995), and crop damage is usually expected before As reaches concentrations which are considered critical for human health.

The degree of As uptake varies widely from species to species. Roots accumulate higher concentrations than stems,

leaves or seeds. Uptake increases with increasing arsenic concentration in the soil. While As concentrations generally remain below 1 mg/kg fresh weight in food crops, grasses were found to contain up to 3460 mg As/kg (dry weight) grown on spoil with As concentrations up to 26,430 mg As/kg. Grasses on urban soils containing 20 mg As/kg were found to accumulate up to 3 mg As per kg dry weight.²⁷

PHYTOTOXICITY OF ARSENIC

Toxicity of As in plants is well documented. Injury symptoms from higher quantities of arsenic in soil were reported by Thompson and Batjer who recognized this metalloid as the reason for necrotic spots, discoloration of leaves and ultimately defoliation in young peach trees planted in soils contaminated with lead arsenate. Kabata–Pendias has also reported that wilting, violet color (anthocyanin), discoloration of roots and cell plasmolysis as the toxicity symptoms for arsenic in plants.

Symptoms of arsenic toxicity have been studied in many details and it has been established that it manifests initially as chlorosis, retarded growth, gradual browning and ultimately death of the plant occurs. Visual symptoms of phytotoxicity include leaf wilting, followed by retardation of root and shoot growth. This is often accompanied by root discoloration and necrosis of leaf tips and margins, indicating inhibition of root water uptake and ultimately resulting in death from wilting. Toxicity of arsenic in growth medium on the chlorophyll content of leaves has been reported in rice, maize, bean and tea.²⁸

Phytotoxicity of arsenic depends on the form and availability of arsenic in the soil. Organic arsenic compounds are less toxic than inorganic compounds, and the toxicity decreases in the following order: arsenite > arsenate > organic As compounds. The phytotoxicity of As in soils depends primarily on soil texture and secondly on the pH. There is an increase in As phytotoxicity as soils become more acid, particularly when the pH drops below 5 and As sorbents such as Fe- and Al-oxides are dissolved.

Sheppard concluded that phytotoxicity of inorganic As was influenced by soil type. It was reported that inorganic As was five times more toxic to plants in sand (mean=40 mg As/kg) than in clay (mean=200 mg As/kg) soils. Arsenic phytotoxicity is expected to be greater in sandy soils than in other soil types, because sandy soils generally contain low amounts of Fe and Al oxides and clay minerals. Arsenic is known to disturb uptake and transport of mineral nutrients in plants. Disturbance of plant mineral nutrition is the main cause for yield decrease, the most frequent sign of As toxicity. According to Liebig the phytotoxicity of arsenic is attributed to its ability to substitute for phosphate in enzyme-catalyzed reactions particularly to uncouple phosphorylation reactions and thus to interfere with the energy status of the plant.²⁹

ARSENIC DETOXIFICATION IN PLANTS

Hyper accumulating plants possess an efficient mechanism for detoxification of these accumulated metals. According to Salt et al. these mechanisms include chelation, compartmentalization, biotransformations and cellular repair. Carbohydrates (such as melate, oxalate, and citrate) are commonly the major charge balancing anion present in the cell vacuoles of photosynthetic tissues. Several of these carbohydrates have been associated with high metal concentrations in plants.

The tripeptide GSH is synthesized by gamma-glutamylcysteine synthetase (γ -ECS) and glutathione synthetase (GS). Increasing GSH synthesis is considered a mean to increase cellular defence against oxidation stess. Since GSH is precursor of phytochelatin (PC) overexpression of γ -ECS or GS leads to high PC accumulation under metal exposure. Glutathionine conjugates with Arsenic (As III-GS3) are transported into vacuoles of plants by ABC transports.

PC synthesis has been induced on exposure to arsenate in number of plant species. Intact PCs-arsenic complexes have been isolated from plant tissues suggesting that PCs are also involved in arsenic detoxification in plants. PC synthesis was induced on exposure to arsenate in *P. vittata*.

Plant possess arsenate reduction to reducing As(V) to As(III) showing homology with yeast arsenate reductase acr2 which seem to be essential for As(V) tolerance. Studies on the acr2 mutant showed that phenotypes are strongly dependent on plant P-status. According to Dhankher, overexpression of ECS and Ars C (arsenate reductase) substantially increased As(V) tolerance in *Arabidopsis thaliana*.

Efflux of As roots represents another potential strategy for metal detoxification. The process involves uptake of As(V) an intracellular reduction to As(III) and followed by efflux of As(III).

Methylation of As in plants is another potential detoxification mechanism because methylated arsenic are less toxic than inorganic As. This is less abundant in plants than in animals.³⁰

DETOXIFICATION MECHANISMS IN PLANTS

Hyper accumulating plants possess efficient mechanisms for detoxifying accumulated metal. These mechanisms include chelation, compartmentalization, biotransformation and cellular repair. Heavy metals are generally transported and deposited in the vacuole as metal chelates. Baker et al. explained that the solution concentration of free metal ions taken up by plants into their tissues is reduced greatly when they are chelated by specific high-affinity ligands (like oxygen donor ligands, sulfur donor ligands, and nitrogen-donor ligands). They gave examples of oxygen donor ligands as organic acids, in particular carboxylic acid anions, which are abundant in the

cells of terrestrial plants and form complexes with divalent and trivalent metal ions of reasonably high stability. Carboxylates (such as malate, aconitate, malonate, oxalate, tartrate, citrate, and isocitrate) are commonly the major charge-balancing anion present in the cell vacuoles of photosynthetic tissues. Several of these carboxylates have been associated with high metal concentrations in plants. Malate was proposed as a carrier to transport Zn²⁺ ions into the vacuole in *Thlaspi caerulescens*. Sulfur-donor ligands (like metallothioneins and PCs) form highly stable complexes with heavy metals because sulfur is a better electron donor than oxygen. Metallothioneins are gene-encoded low-molecular-weight, cysteine-rich peptides found in fungi and mammals recently shown to be induced by Cu in plants. In fungi and mammals, metallothioneins are involved in metal detoxification but their role in plants is not yet well understood.31 Recent studies show the existence of a group of organic solute transporters, belonging to the ATP binding cassette (ABC) transporters superfamily, that is directly energized by MgATP. These pumps are competent in transporting a broad range of substances including sugars, peptides, alkaloids, and inorganic anions. Belonging to the ABC family, the MRPs identified in plants are thought to participate in transporting exogenous and endogenous amphipathic anions and glutathionated compounds from the cytoplasm to the vacuole. They function in herbicide detoxification, cell pigmentation, storage of antimicrobial compounds, and alleviation of oxidative damage. Plant MRPs are also suspected to play a role in channel regulation and transporting heavy metal chelates. GSH S-conjugate and metabolite (M) transport is achieved by specific ABC transporters.

PCs are low-molecular-weight, cysteine-rich peptides that are especially produced by plants when exposed to heavy metals and are known to bind cadmium and copper in plants. The PC-metal complexes are less toxic than free metal ions to cellular plant metabolism. They have been shown to be essential for cadmium detoxification in A. thaliana and are believed to bind Pb and Hg by competing with Cd. The GSH-mediated transfer of Cd²⁺, Pb²⁺, Cu²⁺ and Hg²⁺ to PCs was demonstrated by Mehra. PC synthesis has been induced on exposure to arsenate in a number of plant species. Intact PCs-As complexes have also been isolated from plant tissues suggesting that PC are also involved in arsenic detoxification in plants. Though PC synthesis was induced on exposure to arsenate in P. vittata, only PC2 was detected in the plant. The molar ratio of PC-SH to As suggested that only a small proportion (1-3%) of the As in P. vittata can be complexed with PCs.³² Resistance to arsenic in arsenic non-hyper accumulating plants has been shown to involve a decreased uptake of arsenate due to suppression of the high-affinity phosphate uptake system. This is because the mechanisms of arsenic uptake are similar to those of phosphorus resulting in competitive uptake. Arsenic detoxification might also include methylation and biotransformation by microorganisms. Some bacteria enzymatically reduce arsenate to arsenite by ArsC and the arsenite is then pumped out by the membrane protein Ars B.33

PHYTOREMEDIATION

Current methods for remediation of arsenic-contaminated soils include soil removal and washing, physical stabilization, and/or the use of chemical amendments, all of which are expensive and disruptive, with an average cost of \$404,700 per ha. USEPA recommended excavation, capping, solidification and stabilization, verification, soil washing/acid extraction, soil flushing, phytoremediation, etc. as current remediation technologies for arsenic-contaminated soil.

Phytoremediation includes any remediation method that uses plants to either remove pollutants or render them harmless in soil and water systems, and can be applied for both organic and inorganic pollutants present in soil, water, and air. This practice is gaining popularity because of its overall cost-effectiveness. The term phytoremediation includes several strategies.³⁴

Phytoextraction

The use of pollutant - accumulating plants capable to extract and translocate pollutants to the harvestable parts. It uses tolerant plants that concentrate soil contaminant sin their above ground biomass so that the contaminant-enriched biomass can be properly disposed.

Phytostabilization

The use of pollutant-tolerant plants for mechanical stabilization of polluted land in order to prevent bulk erosion, reduce airborne transport, and leaching of pollutants. It is used to provide a cover of vegetation for a moderately to heavily contaminated site, thus preventing wind and water erosion. Plants suitable for phytostabilization have extensive root system, provide good soil cover, possess tolerance to the contaminant metals and ideally immobilize the contaminant in the rhizosphere. Arsenic-tolerant plants that can be used for phytostabilization purposes have been known for a long time.

Phytoimmobilization

The use of plants to decrease the mobility and bioavailability of pollutants by altering soil factors that lower pollutant mobility (formation of precipitates, insoluble compounds and sorption on roots). Based on the chemical similarities of arsenic and phosphorus, there may be precipitate formation of arsenic-/lead compounds as shown for phosphorus-lead precipitates in the rhizosphere of *A. capillaries*. Other plant-mediated processes of arsenic immobilization at the soil-root interface e.g. accumulation of arsenic on iron plaque in the oxidized rhizosphere of salt marsh plants.

Phytovolatilization

The use of plants to volatilize pollutants and has been demonstrated for Hg and Se. Volatilization of arsenic is also

known to occur in natural environments, but rhizosphere studies have not been reported. Available information on arsenic volatilization suggests that the volatile compounds account only for small proportions of total arsenic.

PHYTOREMEDIATION OF ARSENIC

Heavy metals pollution is amongst the commonest form of environmental pollution. These metals have accumulated over time from the smelting and mining activities of man, from poor waste disposal practices and from modernization. Of recent the impact of heavy metal pollution of the environment is stirring up serious concerns since the discovery that some edible plants accumulate these metals to a level, toxic to both themselves and to the animals that consumes them. Common features of heavily polluted soil include barrenness, desertification, erosion, and this usually result in developmental stagnation in areas with such pollution. More researches have recently been stepped up in the field of remediating soils polluted with heavy metals. Traditional method includes, excavation of the top soil, capping of the soil, stabilization of the polluting heavy metals, soil washing. In recent time, emphases have been drawn to the use of plants that has high metal accumulating and tolerating capacity to remediate metal-contaminated soil. This mini-review highlights the different conventional and recent practices in the control of heavy metal pollution.³⁵

PHYTOREMEDIATION: THE SOLUTION FOR ARSENIC CONTAMINATION

Although phytoremediation has been around since the beginning of time, only recently have scientists, engineers and business people come together to fine tune this relatively simple concept: that plants can actually clean-up toxic pollutants in soil and groundwater naturally. Interest in commercial phytoremediation only truly began in the early 1990s. A significant turning point for this technology was its use to decontaminate soil and groundwater at chernobyl, Ukraine in 1986. After phytoremediation proved successful in this instance the market for these toxin-eating plants began to grow as new applications were discovered. More recently scientists have discovered an application to clean up a hugely controversial contaminant: arsenic. This discovery may give phytoremediation the publicity it needs to move forward as an effective and reliable technology in the United States.36

With the large amount of controversy pertaining to the arsenic rule put out by the EPA and the recent reconsideration of this rule by the Bush Administration, this issue has been making headlines across the US. A large part of this controversy is due to the costly cleanup needed to meet the standards determined by the EPA. Many of the communities that the standard is directed at are small in population with less than 3,300 people, with very limited financial ability to afford compliance or lobby against the rule. The many small

communities to meet the standards, they would be forced into very expensive treatment.³⁷

A possible solution to this issue may be the consideration of phytoremediation. Phytoremediation may be the perfect solution to the controversial arsenic dilemma. For years, the site remediation industry has been searching for a costeffective, ethical method to clean up contaminated sites, and now they may have found it. Researchers from the University of Florida discovered that a common brake fern, or *P. vittata*, is the first plant found to hyperaccumulate arsenic. This plant has proven highly efficient in soaking up arsenic, showing levels as much as 200 times higher in the fern than the concentrations in the soil where it is growing. Recognizing that these ferns may potentially present a huge market for the cleanup of arsenic all around the world scientists are now fine-tuning their research to concentrate on how the plant takes up, distributes and detoxifies the arsenic. The *P. vittata* is likely to be a large player in the phytoremediation industry due to its ability to accumulate such high concentrations of the toxin. Other phytoremediation plants have not proven as successful as this one.38

Although the process of phytoremediation sounds simple, there is a bit more preparation involved than simply planting trees around a toxic waste site and letting them do their thing. Rather, a significant amount of preparation and research is involved before an engineer can actually determine which plants to use and how the plants break down the contaminants that are being targeted. For instance, there are occasions where plants may break down a product and produce even more hazardous bi-products. With this in mind, the implementation and effectiveness of phytoremediation processes is only as good as the research and preparation done beforehand. The emphasis on understanding the basics of contaminant breakdown are crucial for developing phytoremediation solutions. Other concerns when determining which plant to use might be the climate or environment in which the contaminant is being treated. For example, some plants that might flourish in Florida may not grow at all in the colder less humid climates. Additionally the depth or location of the contaminant must be considered.39

According to D. Glass Associates, the overall phytoremediation market will reach revenues between \$214 million to \$370 million by 2005. With this proven ability to clean up arsenic, it is now more likely than ever before that the phytoremediation market will become a more important part of the overall remediation market, thus revenues for this market may be higher than originally thought. It is likely that companies already involved in natural cleanup technologies will want to develop a phytoremediation component to add to their line. Phytoremediation companies are smaller regional manufacturers that will realistically have to partner with larger engineering firms to work on contaminated sites. This is mainly due to the fact that there are very few sites that can be cleaned up solely by phytoremediation. Rather, like most

cleanup technologies, they have to be coupled with others in order to be totally effective.

Therefore, small phytoremediation technology developers will likely lack the necessary resources to be successful on their own in the site cleanup industry. By partnering with large engineering firms or licensing out their technology, they will penetrate the market more easily. These companies will likely aid phytoremediation to become more widely accepted within the remediation community. As developers and end-users become more familiar with the advantages of phytoremediation, begin to understand the possibility of new applications, and discover technologies that help meet regulatory standards- this market is set to see positive growth.

Whether or not the arsenic standard is ultimately passed in the United States, there will still be a significant market for the *P. vittata* all around the world. Arsenic has become a problem in many parts of the world as a result of leaching from mine tailings and being used as a pesticide in the US, Canada, Mexico, Thailand, and Japan. Recently, Bangladesh was determined to be one of the most highly contaminated countries in the world. The United Nations states that around 25 million Bangladeshis are at risk of disability or death from arsenic poisoning from the country's drinking water. The World Health Organization claims that the level of arsenic poisoning in Bangladesh is one of the largest ever. With this massive worldwide market for the cleanup of arsenic the *P. vittata* plant is likely to drive the phytoremediation market towards a long and healthy future.⁴⁰

Arsenic cannot be destroyed in the environment; it can only change its form or become attached to or separated from particles. It may change its form by reacting with oxygen or other molecules present in air, water, or soil, or by the metabolic action of plants or animals.

Arsenic is a contaminant of concern in groundwater at many remediation sites. Because it readily changes valence states and reacts to form species with varying toxicity and mobility, effective treatment of arsenic can be challenging. Treatment of contaminated groundwater can result in residuals that, under some environmental conditions, have unstable toxicity and mobility. In addition, the revised MCL for arsenic in drinking water could result in lower treatment goals for aboveground treatment systems. A lower treatment goal may significantly affect the selection, design, cost, and operation of arsenic treatment systems. All

BIOCHEMISTRY OF ARSENIC DETOXIFICATION

All living organisms have systems for arsenic detoxification. The common themes are (a) uptake of As(V) in the form of arsenate by phosphate transporters, (b) uptake of As(III) in the form of arsenite by aquaglyceroporins, (c) reduction of

As(V) to As(III) by arsenate reductases, and (d) extrusion or sequestration of As(III). While the overall schemes for arsenic resistance are similar in prokaryotes and eukaryotes, some of the specific proteins are the products of separate evolutionary pathways.⁴²

MOLECULAR MECHANISM OF PHYTOREMEDIATION

Uncovering the underlying molecular mechanisms in plants, especially those capable of hyperaccumulation, will provide further insights for engineering plants for phytoremediation in the future. Heavy metals are the main group of pollutants and progress in the molecular mechanism of plant stress response to heavy metals has been made, especially in herbaceous plants such as *A. thaliana*, *Arabidopsis halleri*, and *T. caerulescens*. High-throughput technologies, in particular microarray, have allowed the complexity of plant stress response to be tackled. Much work has been reported recently in these filed.⁴³

A. THALIANA

A. thaliana has always played a very important role in uncovering the molecular mechanism of plant response to pollutants as its genome information is available, and it can be easily mutated. Three Arabidopsis genes, oxophytodienoate reductases 1 (OPR1), OPR2, and OPR3 were found to be up-regulated by exposure to TNT. Subsequent biochemical characterization revealed that two of the three OPR1 lines and all of the OPR2-overexpressing lines exhibited enhanced tolerance to TNT. It is identified the potential target gene in A. thaliana for phytoremediation and phytosensing of chemical contaminants, RDX and TNT, by microarray analysis. Genes that were differentially expressed included oxidoreductases, cytochrome P450s, transferases, transporters, and several unknown expressed proteins. Two transcription factors bZIP19 and bZIP23 were found to be able to regulate the adaptation to zinc deficiency and zinc homeostasis in plants. To detect the potential genes that are related to the sensing mechanism and metabolism of toluene, many scientist conducted a microarray analysis on the seedlings grown on toluene-containing media. The results show a coordinated induction and suppression of 202 and 67 toluene-responsive genes respectively, include genes encoding cytochrome P450s, glucosyltransferases, and transporters. Many scientist revealed cross-talk between Fe homeostasis and Zn tolerance in A. thaliana by analyzing natural variation at the FRD3 MATE transporter locus.44

POPULUS

Many scientist compared the physiological and molecular response to cadmium stress in two *Populus nigra* L. genotypes originating from contrasting environments in northern (genotype 58-861) and southern. Their results

showed that the later was markedly more tolerant to Cd stress and the glutathione pathway was also involved in the differential Cd tolerance of the two genotypes. Many scientist conducted the transcript analysis of *Populus* × *canescens* response to cadmium. They found that about 48% of the differentially regulated transcripts formed a coregulation network in which 43 hub genes played a central role in crosstalk among distinct biological processes. This enhanced our understanding about the molecular mechanism of woody plant response to heavy metal.

The correlation analysis of SNP diversity with the phenotypic response to exposure to cadmium in *Populus* spp. was conducted, and a positive correlation was established between genetic variation, cadmium accumulation, and its bioconcentration in the root. The quantitative trait loci (QTL) and candidate genes for cadmium tolerance in *Populus* were identified. Functional characterization of these candidate genes should enhance our understanding of Cd metabolism and transport and phytoremediation capabilities of *Populus*.⁴⁵

BRASSICA JUNCEA

B. juncea is a promising plant species that can be used for phytoremediation of heavy metals. B. juncea root proteome in response to cadmium exposure was analyzed, and the enzymes such as peptide methionine sulfoxide reductase, and 2-nitropropane dioxygenase in alternative redox-regulation mechanisms, as well as O-acetylserine sulfhydrylase, GSH-S-transferase (GST), and GSH-conjugate membrane transporter were essential players in the Cd hyperaccumation and tolerance of B. juncea. The transcripts levels of two B. juncea cation-efflux family proteins, BjCET3 and BjCET4 could be substantially increased by the introduction of Zn, Cd, NaCl or PEG, suggesting that BjCET3 and BjCET4 may play roles in those stress condition.

CRAMBE ABYSSINICA

C. abyssinica (a member of Brassicaseae), a non-food, fast growing high biomass crop, is an ideal candidate for phytoremediation of heavy metal contaminated soils. The 38 genes involving in arsenic metabolism and detoxification were isolated successfully. In response to Cr exposure in *C. abyssinica* by a PCR-select suppression subtraction hybridization approach, a total of 72 differentially expressed subtracted cDNAs were sequenced and found to represent 43 genes.

OTHER PLANTS

Elsholtzia splendens is generally considered as a Cu-tolerant and -accumulating plant species and is a likely candidate for phytoremediation of Cu-contaminated soils. Many scientist conducted proteomic analysis of copper stress response in E. splendens roots and leaves by two-dimensional gel electrophoresis and found that 45 protein spots were

significantly changed in roots, but only 6 were changed in leaves. The identified root proteins were involved in various cellular processes such as signal transduction, regulation of transcription and translation, energy metabolism, regulation of redox homeostasis, and cell defense while the leaf proteins were mainly degraded fragments of RuBisCo and antioxidative protein. Depending on Cd and Zn uptake, several antioxidant enzymes showed significantly different activities in Nicotiana tabacum. Although superoxide dismutase and catalase were usually elevated, several other enzymes and isoforms of GST were strongly inhibited. They suggested that when planning phytoremediation of sites, mixed pollution scenarios have to be anticipated and the plants should be selected according to both their stress resistance and hyperaccumulative capacity. A broccoli (Brassica oleracea var italica) cDNA encoding COQ5 methyltransferase (BoCOQ5-2) in the ubiquinone biosynthetic pathway was cloned. Transgenic Arabidopsis expressing BoCOQ5-2 volatilized three times more Se than the vector-only control plants when treated with selenite and exhibited increased tolerance to Se.

Eleven QTLs for arsenic accumulation in maize (Zea mays L.) were detected. In Portulaca oleracea, the peroxidase 2a (PoPRX2a) is potentially useful in the remediation of phenolic pollutants. In Se-hyperaccumulator Astragalus racemosus, out of the 125 Se-responsive candidate genes identified, six of them responded to both selenate and selenite treatments. A novel gene CEJ367 was highly induced by both selenate (1920-fold) and selenite (579-fold). These identified genes may allow us to create Se-enriched transgenic plants. HvHMA2, a P(1B)-ATPase from barley, is highly conserved among cereals and functions in Zn and Cd transport. Solanum nigrum was found to be a cadmium (Cd) accumulator, and the transcriptome analyses revealed higher expression of the genes that encoded several metal transporters as well as antioxidant-related genes, and several organic and amino acid biosynthesis/metabolism-related genes in Cd-treated S. nigrum, which indicated that the different responsive mechanisms of the transporter genes to Fe deficiency might be responsible for differential uptake and redistribution of metals in the two *Solanum* species. A major latex-like protein is a key factor in Cucurbitaceae family crop contamination by persistent organic pollutants. TaHMA2 is another gene from wheat (Triticum aestivum L.), which belongs to heavy metal ATPase 2 (HMA2).46

REMEDIATION TECHNOLOGIES

Contaminated soil can be remediated by chemical, physical or biological techniques. The available techniques may be grouped into two categories: (a) *ex situ* techniques which require removal of the contaminated soil for treatment on or off site, and (b) *in situ* methods, which remediate without excavation of contaminated soil. *In situ* techniques are favored over the *ex situ* techniques due to their lower cost and reduced impact on the ecosystem.⁴⁷

Excavation method

These involve the excavation and reburial of polluted soils in special landfills. This even as the commonest means of reclaiming contaminated soil does not actually remediate the soil.

Capping of the polluted soil

This involves top soiling of the polluted soils with uncontaminated soils from off site to a depth that would minimize uptake of heavy metals by vegetation.

Fixation and inactivation (stabilization) of the polluting heavy metals

This involves the conversion of the polluting heavy metals to forms that are less mobile and available for plants and micro flora.

Soil washing

This technique involves the use of acids (HCI and HNO₃), chelators (ethylenediamine tetraacetic acid, nitriloacetic acid, DTPA, etc.) and other anionic surfactant (biosurfactant) to solubulise the polluting metals.

Phytoremediation

Heavy metals cannot be destroyed biologically (no "degradation," change in the nuclear structure of the element, occurs) but are only transformed from one oxidation state or organic complex to another, remediation of heavy metal contamination in soils is more difficult.

Phytoremediation is new technology based on the use of higher plants for cleanup process of contaminated environment. Fundamental and applied researches have unequivocally demonstrated that selected plant species have potential to remove, degrade, metabolize, or immobilize a wide range of contaminants. Phytoremediation, as well as the sustainable development is based on the implementation of three fundamental components: environmental protection, economic growth and social equality. Until now, methods used for their remediation such as excavation and landfill, thermal treatment, acid leaching and electro reclamation are not suitable for practical applications, because of their high cost, low efficiency, large destruction of soil structure and fertility and high dependence on the contaminants of concern, soil properties, site conditions, and so on. Thus, the development of phytoremediation strategies for heavy metals contaminated soils is necessary.

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Some plants can accumulate remarkable levels of metals – 100-1000-fold the levels normally accumulated in most species. Metal hyperaccumulating species have been identified in at least 45 plant families and individual species (or even populations) can accumulate different metals such Zn, Cd, Cu, Co, Ni, Se, and As or particular combinations of these. There are several known plant species that have demonstrated arsenic uptake: watercress, several species of ferns, duckweed, and Indian mustard. The level of uptake by these plants varies. There are several, however, that exhibit hyperaccumulating characteristics. Current research has demonstrated that two species of ferns: P. vittata and P. calomelanos, can be considered hyperaccumulators. While phytoremediation has several advantages, it also has its own set of disadvantages. One major drawback of phytoremediation is that the contaminant is simply transferred from the soil to the plant. The contaminant taken up by the plant is not always transformed into a less toxic species either. Essentially, the burden of contamination is shifted from the soil to the plant. The storage and designation of a disposal site for biomass containing toxic contaminants needs to be well-though out and planned. According to Tu et al. as arsenic fronds age, the arsenite contained within them oxidizes back to arsenate. Leachate samples, taken from the fronds which were rinsed with acid and allowed to decompose, contained significant amounts of arsenate; it is therefore vitally important that the biomass from plants used to remediate contaminated sites be stored away from drinking water supplies in order to prevent secondary contamination. Plant biomass containing arsenic should not be burned because combustion of inorganic arsenic leads to release of toxic As₂O₃.

Phytoextraction involves the use of plants that has the ability to concentrate the heavy metal in their shoot tissue, to remediate contaminated lands. Usually, the shoot biomass is harvested for proper disposal in special site or are burnt to recover the metal. Some plant species can take up heavy

metals and concentrate them in their tissue. The plants can be harvested and the contaminated plant material disposed of safely. Sometimes soil amendments are added to the soil to increase the ability of the plants to take up the heavy metals. One type of plant used for this purpose is called Indian mustard. This plant has been used to extract lead from soil and reduce lead contamination at various contaminated sites.

Other plants that may be used for phytoextraction include alfalfa, cabbage, tall fescue, juniper, and poplar trees. Phytofiltration (rhizofilteration) involves the use of plants to absorb, concentrate or precipitate metals from aqueous waste. In this method, heavy metals are removed directly from water by plant roots. The plants are grown directly in water or in water-rich materials such as sand, using aquatic species or hydroponic methods. In field tests sunflowers on floating rafts have removed radioactive metals from water in ponds at chernobyl, and other plants removed metals from mine drainage flowing through diversion troughs. Phytostabilization is the use of plants to reduce the mobility of heavy metal through absorption and precipitation by plants, thus reducing their bioavailability.

The term phytoremediation (phyto meaning plant and the Latin suffix remedium meaning to clean or restore) refers to a diverse collection of plant-based technologies that use either naturally occurring, or genetically engineered, plants to clean contaminated environments. Phytoremediation is clean, simple, cost effective, non-environmentally disruptive green technology and most importantly, its by-products can find a range of other uses.⁴⁸

Phytoremediation is an eco-friendly approach for remediation of contaminated soil and water using plants comprised of two components, one by the root colonizing microbes and the other by plants themselves, which accumulates the toxic compounds to further non-toxic metabolites. Various compounds viz., organic synthetic compounds, xenobiotics, pesticides, hydrocarbon and heavy metals, are among the contaminants that can be effectively remediated by plants.⁴⁹

TYPES OF PHYTOREMEDIATION TECHNOLOGY

The four different plant-based technologies of phytoremediation, each having a different mechanism of action for remediating metal-polluted soil, sediment or water:

Phytoextraction

Plants absorb metals from soil through the root system and translocate them to harvestable shoots where they accumulate. Hyperaccumulators mostly used this process to extract metals from the contaminated site. The recoveries of the extracted metals are also possible through harvesting the plants appropriately.

Phytovolatilization

Plants used to extract certain metals from soil and then release them into the atmosphere by volatilization.

Phytostabilization

In this process, the plant roots and microbial interactions can immobilized organic and some inorganic contaminants by binding them to soil particles and as a result reduce migration of contaminants to grown water.

Phytofiltration

Phytofiltration is the use of plants roots (rhizofiltration) or seedlings (blastofiltration) to absorb or adsorb pollutants, mainly metals, from water and aqueous waste streams.

LIMITATIONS

The application of phytoremediation for pollution control has several limitations that require further intensive research on plants and site-specific soil conditions. It is generally slower than most other treatment viz., chemical, physical and microbiological plants with low biomass yields and reduced root systems do not support efficient phytoremediation and most likely do not prevent the leaching of contaminants into aquatic system. Environmental conditions also determine the efficiency of phytoremediation as the survival and growth of plants are adversely affected by extreme environmental conditions, toxicity and the general conditions of soil in contaminated lands. In phytoremediation technology, multiple metals contaminated soil and water requires specific metal hyperaccumulator species and therefore requires a wide range of research prior to the applications. Though the phytoremediation is cost effective, environment friendly, ability to reclaim heavy metals contaminated site, several limitations also create trouble in implementing the strategy, e.g., metal must be in bio-available form to plants; if metals is tightly bound to the organic portions of the soil, sometime it may not be available to plants. Furthermore, if the metals are water soluble, in nature it will pass by the root system without accumulation.

The phytoremediation of mixed heavy metals contaminated soil have conformant with some problem e.g., The cadmium/zinc model hyperaccumulator *T. caerulescens* is sensitive toward copper (Cu) toxicity, which is a problem in remediation of Cd/Zn from soils in the presence of Cu by application of this species. In *T. caerulescens* Cu-induced inhibition of photosynthesis followed the sun reaction type of damage, with inhibition of the photosystem II reaction center charge separation and the water-splitting complex. Despite some limitations, present day phytoremediation technology are using worldwide and various research laboratories are at present engaged to overcome the limitations. ⁵⁰

PLANTS SELECTIONS CRITERIA FOR PHYTOREMEDIATION

Plant species selection is a critical management decision for phytoremediation. Grasses are thought to be excellent candidates because their fibrous rooting systems can stabilize soil and provide a large surface area for root-soil contact. The selection of plants is possibly the single most important factor for fruitful phytoremediation strategy. The application of indigenous plant species for phytoremediation is often favored as it requires less management and acclimatizes successfully in native climate conditions and seasonal cycle. However, some exotic plant species may perform better in remediation of specific metals and can be safely used where the possibility of invasive behavior has been eliminated. Some important criteria in selecting plant species for phytoremediation are:⁵¹

- The levels of tolerance with respect to metal known to exist at the site
- The level of adequate accumulation, translocation, and uptake potential of metals
- High growth rate and biomass yield
- Tolerance to water logging and extreme drought conditions
- Availability, habitat preference, e.g., terrestrial, aquatic, semi-aquatic, etc.
- Tolerance to high pH and salinity
- Root characteristic and depth of the root zone.

METAL HYPERACCUMULATORS PLANTS/FAMILIES

Over 500 plant species comprising of 101 families have been reported, including members of the Asteraceae, Brassicaceae, Caryophyllaceae, Cyperaceae, Cunouniaceae, Fabaceae, Flacourtiaceae, Lamiaceae, Poaceae, Violaceae, and Euphobiaceae. Metal hyperaccumulation occurs in approximately 0.2% of all angiosperms and is particularly well represented in the Brassicaceae. Recently Environment Canada has released a database Phytorem which compiled a worldwide inventory of more than 750 terrestrial and aquatic plants with potential value for phytoremediation. The study of Co accumulations in 670 species of terrestrial plants showed that leaf Co concentration was in general less than 0.2 ppm, with the exception of Ericales, Euasterids and Asparagales, where 0.3-0.5 ppm of Co was measured. A list of 26 Co hyperaccumulators species belonging to the families of Lamiaceae, Scrophulariaceae, Asteraceae and Fabaceae has been reported. Zn hyperaccumulation was first discovered in 1865 in Noccaea caerulescens (syn. T. caerulescens) of Brassicaceae and Ni hyperaccumulation, which was first reported in 1948 in Alyssum bertolonii of Brassicaceae. A member of Crassulaceae Sedum alfredii is the only known Cd hyperaccumulator outside the Brassicaceae. The hyperaccumulation of arsenic (As) has been discovered in only two species of the Brassicaceae apart from a number of pteridophytes.⁵²

PHYSIOLOGY OF METAL HYPER ACCUMULATION

Metals influenced the physiology of plants by promote or inhibit the growth. Some metals that required in high concentration suggest a structural or osmotic role, while effects at low concentration may indicate a role as cofactor for specific enzymes. The summary of the beneficial effects of Al, Co, Na, Se, and Si on plants under-represented adopted from. Many plants developed Al tolerance characteristics via either apoplastic or symplastic detoxification mechanisms. In higher plants, Co has been reported to strongly bind to roots and to be mainly absorbed from the soil solution through passive transport. Co is chemically similar with nickel (Ni) and these two elements enter inside the cells through plasma membrane carriers and may be transported by IRT1. A strong adsorption of Cd on root apoplast might act as a driving force to extract the metal from the soil, compete with the symplastic absorption and contribute to the amount of metals taken up by the hyperaccumulator, at least in its roots. (Se hyperaccumulators namely Stanleya sp. and Astragalus sp. can accumulate 1000-15000 ppm (0.1-1.5% Se), even from low external concentrations and this has been enhanced by some specialized transporter. Hyperaccumulators such as Astragalus bisulcatus, Brassica oleracea have a specific selenocysteine methyltransferase, lead to accumulation of Se. Toxic metal ions Hg preferentially binds with sulfur and nitrogen rich ligands (amino acids) and entered inside the cells. Hg effect damage includes blocking functional groups of enzymes, polynucleotide's, or transport systems for nutrient ions, denaturing and inactivating enzymes and disrupting cell and organelle membrane integrity. The possible causal mechanisms of Hg toxicity are changes in the permeability of the cell membrane, reactions of sulfhydryl (-SH) groups with cations, affinity for reacting with phosphate groups and active groups of ADP or ATP and replacement of major cations.

Heavy metals like copper (Cu) and iron (Fe) can be toxic in excess amount because of their participation in redox cycles producing hydroxyl radicals which are extremely toxic to living cells. Unlike Cu and Fe, Cd is a non-redox metal that is strongly phytotoxic and caused growth inhibition and plant death. Cd-induced changes in lipid profile and by affecting the enzymatic activities associated with membranes, such as the H⁺- ATPase. Cd is also reported to damage the photosynthetic apparatus decrease chlorophyll content and inhibit the stomatal regulations. The major storage site for Zn and Cd in plants is cell wall of roots, vacuoles of epidermis and bundle sheath of leaves. The Cd influx and efflux in leaf mesophyll layer depends on the expression of plasma membrane and tonoplast transporters. Root metal uptake rates are increased, generally through an increase in V_{MAX} , without major changes in KM, of root metal uptake rates. Works on Alyssum lesbiacum has given a base of nickel uptake into vacuoles from leaf tissue and this was enhanced by the presence of Mg/ATP, presumably via energisation of the vacuolar H⁺-ATPase. The model hyperaccumulator T.

caerulescens is capable to tolerat Zn, Cd and Ni and it has been shown that part of the Ni is translocated as a stable Ni-NA complex in the xylem sap.⁵³

BIOMASS PRODUCTION

The discussion of the effect of heavy metal on biomass productions of metal hyperaccumulation is restricted here to Cd, Pb, Zn, V and Cr.

In metal hyperaccumulators the biomass production level depends on the concentration of the metals and duration of exposures, e.g., the biomass is negatively correlate with Cd concentration in *Brassica napus*, cultured in the nutrient solution and the reduction in dry weight was significantly higher for the root than the shoot. Considering the high aboveground biomass production and Cd accumulation in the shoot, *B. napus* can be a potential candidate for the phytoextraction of Cd.

Biomass production were extensively studied in split pots filled with soil spiked at 0, 3, 6, 12, 25 and 50 mg Cd/kg soil by and revealed decline in biomass production which established that cadmium is toxic for biomass production.

Suitable levels of Pb, Zn and Cd could stimulate the biomass production in A. paniculata and thus, it provides a new plant material for understanding the mechanisms of stimulatory effect and co-hyper accumulation of multiple heavy metal. However, biomass production is inhibited in Glycine max and Phaseolus vulgaris in treated with VOSO₄. Similarly in Enhydra fluctuans, biomass production was not affected up to concentration of 2.5 mg/L V₂O₅ when exposed for 7 days. However, V₂O₅ concentration >2.5 mg/L significantly reduced the biomass and increased in treatment duration enhanced the V₂O₅ toxicity. The author recorded that after 21 days exposure of 10.0 mg/L V₂O₅ to E. fluctuans approximately 42.47% biomass was decreased. Similarly in Vallisneria spiralis L., an increased in treatment duration enhanced the chromium toxicity and 0.1 µg/mL Cr caused 7% decrease in biomass after 48 h and 64% loss of biomass was recorded after 72 h exposure of 10 µg/mL Cr. The results addressed that heavy metals toxicity to biomass were concentration and duration's dependent.54

However, modern transgenic research approach at present going on for introduction of novel traits into high-biomass plants for development of effective phytoremediation technologies. A number of transgenic high biomass yield plants have been successfully generated in an attempt to modify the tolerance, uptake or homeostasis of trace elements.

BIOCHEMICAL RESPONSES

Several heavy metals such as Fe, Cu, Co, Mn, Mo, and Ni are essential elements to plant metabolism. In

higher concentrations, many heavy metals inhibit plants biochemical production and this has been extensively studied and reviewed. Photosynthetic pigments of plants belonging to different group's exhibit differential tolerance to metals. Heavy metal substituted chlorophylls and related porphyrins have been known in vitro for a long time. Many researchers examined the effect of heavy metals on photosynthesis and observed a decrease in fluorescence. Cd-induced reduction of photosynthetic pigment were recorded in two species, viz., *Myriophyllum heterophyllum* and *Potamogeton crispus*. The highest decrease in chlorophyll a was recorded in 7.34 mg/g in *M. heterophyllum* and 8.09 mg/g in *P. crispus* (at 64 mg/L and 96 hrs) have given as evidence for the Cd toxicity to chlorophyll.

Cadmium hyperaccumulator Atriplex halimus subsp. schweinfurthii was sensitive to high Cd results reduction of chlorophyll pigments, stomatal transpiration and root hydraulic conductivity. The Cr uptake by many aquatic plants influenced in biochemical process results alteration of pigments and amino acids. It has been reported that Cr(VI) causes toxicity to δ-amionolevulinic acid dehydratase (an enzyme involved in Chlorophyll biosynthesis) by impairing δ-amiono levulinic acid utilizations. Further, suggested that Cr(VI) could exchange the Mg from active site of enzyme resulting into phaeophytin and thus depleted chlorophyll contents in Cr treated plants. Chromium also inhibits chlorophyll biosynthesis by creating nutrient imbalance. It has been reported that chromium-induced degradation of carotenoid in some plants while in contrast reported that carotenoid contents were increased in Cr treated *V. spiralis*. Thus, the effects of heavy metals on carotenoid contents were plants and metal specific.55

Mercuric cations have a high affinity for sulfydryl (-SH). In almost all proteins contain sulfydryl groups or disulfide bridges, Hg could disturb the normal functions of proteins in binding in two sites of a protein molecule without deforming the chain, lead to protein precipitation.

Mercury affects both light and dark reactions in photosynthesis and caused inhibitions of electron transport activity, oxygen evolution and quenching of chlorophyll fluorescence in photosystem II (PS II). Substitution of the central atom of chlorophyll, magnesium, by mercury *in vivo* is an important damage mechanism because it prevents photosynthetic light harvesting in the affected chlorophyll molecules and results in the breakdown of photosynthesis.

ROLE OF PHYTOREMEDIATION

Numerous terms are being used simultaneously, in the literature to refer, these processes and may overlap to some extent. Phytoremediation consists of four to five different technologies, each having a different mechanism such as:

1. Phytoextraction or phytomining or phytoaccumulation: plants take up and translocate metal contaminants

- from soil to the above-ground portions, which then are harvested to remove the contaminant from the site.
- Phytodegradation or phytotransformation: plants disintegrade pollutants which may occur within the plant by the metabolic activity or breakdown of the pollutant external to the plant contributed by various organic compounds released into the rhizosphere.
- 3. Rhizofiltration: plants get rid of contaminants present in the solution surrounding the root zone by adsorption or precipitation onto their roots or absorption of contaminants into their roots from the solution. This technique is used to clean contaminated water such as groundwater or a waste stream.
- Phytostabilization: plants immobilize contaminants in the soil and groundwater through absorption and accumulation by root or precipitation within the rhizosphere.
- Phytovolatilazation: plants volatilize pollutants, they take up the pollutants from the soil or water in the transpiration stream and volatilize into the atmosphere in a modified or unmodified form.

Arsenic phytoremediation involves immobilization, fixation, and removal either as fixed in soil or accumulated in plant parts.⁵⁶

ROLE OF PLANT IN REMEDIATION OF ARSENIC

Plants require an adequate supply of all nutrients, as part of normal growth and development including arsenic, for their normal physiological and biological functions. Deficiency of specific nutrient occurs when plant cannot obtain sufficient amount as required; whereas excessive supply of the same, through contaminated soil results in toxicity to plants. Recommended soil application by US EPA for arsenic (As) is 41 mg/kg, whereas, recommended standards by WHO for drinking water and effluents to be released by industries are 1, 0.01 and <0.01 mg/L. The global input of arsenic to soils by humans in the last decade was estimated between 52,000 to 112,000 t/year. Arsenic contaminated sites can be remediated by utilizing the ex situ physical and chemical techniques. But physico-chemical remedies render the land futile for further use, during the process of decontamination, since they abolish all biological activities contributed by beneficial microorganisms, which are necessary for plant growth and development. Consequently, the ecosystems deteriorate with a decline in biodiversity. Arsenic contaminated sites usually have adverse soil conditions, i.e., poor soil structure, low 14 organic content, inadequate N and P, etc., and plants need to adapt to these hostile soil conditions as well as to the metal contamination.

Generally, prior to imposed selection, a species must be able to thrive and survive in As contaminated soil and or water, for which it must possess appropriate variances. Thus, only plants possessing tolerance show some pre-adaptation to these harsh conditions. Notable examples of such plants are Andropogon scoparius, ribwort plantain (Plantago zanceoluta L., Holcus lantus), mosses, lichens, crowberry (Empetrum nigrum L.), Tamarik (Tamarix pamiflora), Eucalyptus (Eucalyptus camaldulensis), Chinese Brake fern (P. vittata L.). Tolerance of plants to metals is under control of uptake systems which are directly related to metal concentrations in the soil solutions. Plants mostly possess two uptake systems: the highly inducible high-affinity system operational at low concentrations (such as the high-affinity phosphate uptake system under low phosphate status) and the constitutive low-affinity system that is effective at high concentrations. For uptake, arsenic needs to be bioavailable. Two mechanisms are responsible for arsenic transport from the bulk soil to plant roots, mass flow and diffusion. Thereafter, plants may utilize two separate systems to take up arsenic, (1) passive uptake through the apoplast, and (2) active uptake through the symplast. Once arsenic is taken up, it is translocated from the roots to the shoot system via the xylem and redistributed between tissues. The translocation of arsenic and other metals depends upon root pressure and leaf transpiration. Most plants take up arsenic as arsenate since arsenite is unstable as get oxidized to arsenate by biochemical processes in the soil system. Arsenate being a chemical analogue of phosphate competes with phosphate for its uptake system and is actively taken up. Once taken up, it is reduced in the cytosol to arsenite by GSH and translocated to the shoots.

Generally, only a minuscule amount of arsenic is translocated to the aboveground parts leading to little accumulation. The form in which arsenic translocated in plants was unknown until 1999. There was some evidence that arsenic transported as dimethylarsenic acid to the shoots and may be stored as an arsenite-tris-thiolate complex in tissues.⁵⁷

GENETIC AND MOLECULAR BASIS OF METAL HYPERACCUMULATION

Metal hyperaccumulation is a fascinating phenomenon, which has interested scientists for over a century. Hyperaccumulators constitute a group of exceptional plant species, and they possesses genetically inherited traits of metals hyperaccumulation and tolerance. The understanding of metal hyperaccumulation physiology has recently improved as a result of the development of molecular tools. Transgenic approaches successfully employed to promote phytoextraction of metals (Cd, Pb and Cu) and metalloids (As, Se) from contaminated soil by their accumulation in the aboveground biomass involved mainly implementation of metal transporters, improved production of enzymes of sulphur metabolism and production of metal-detoxifying chelators metallothioneins and PCs.

Recent research revealed that *A. thaliana* has eight genes encoding members of the type 1 B heavy metal transporting subfamily of the P-type ATPases. Three of these transporters,

HMA2, HMA3, and HMA4, are closely related to each other and are most similar in sequence to the divalent heavy metal cation transporters of prokaryotes.

Quantitative mRNA in situ hybridization in T. caerulescens shows that transporter gene expression changes during cadmium (Cd)/zinc (Zn) hyperaccumulations. Members of the ZIP gene family, a novel metal transporter family first time identified in plants are capable of transporting a variety of cations including Cd, Fe, Mn and Zn. The different cellular expression patterns for ZNT1 and ZNT5 were recorded by both belonging to the ZIP family of transition metal transporters. ZNT1 may function in micronutrient nutrition while ZNT5 may be involved in metal storage associated with hyperaccumulation. Cadmium-induced changes in cellular expression for ZNT1, ZNT5 and MTP1 could also be part of plants acclimatization to Cd toxicity. The integral membrane protein Thlaspi goesingense metal tolerance protein 1 (TgMTP1) has been suggested to play an important role in Zn hyperaccumulation. High levels of TgMTP1 at the vacuolar membrane in shoot tissue of the Zn hyperaccumulator T. goesingense has been played an important role in both Zn tolerance and enhanced Zn uptake and accumulation, via the activation of a systemic Zn deficiency response.

An antiporter is an integral membrane protein which is involved in secondary active transport of two or more ions across a phospolipid membrane in opposite directions. A recent genomic analysis provides a breakthrough in acquisition of zinc hypertolerance and hyperaccumulation characters via involvement of Zn²⁺/H⁺ antiporter. MTP1 is one of genes present in A. halleri which encodes a Zn²⁺/H⁺ antiporter involved in cytoplasmic zinc detoxification and developed a zinc tolerance characteristic. Plant tissue cultures such as callus, cell suspensions and hairy roots are extensively used in phytoremediation research as model plant systems. Many studies have demonstrated that plant tissue cultures are an extremely valuable tool in phytoremediation research. The results derived from tissue cultures can be used to predict the responses of plants to environmental contaminants and to improve the design and thus reduce the cost of subsequent conventional whole plant experiments.

During the 1990s, new methods have been developed to clean up heavy metal contaminated soil. The expensive process of excavating and disposing contaminated soil has been augmented with new methods that treat the soil in place. *In situ* fixation is a process that creates new chemical compounds in which heavy metals are much less available to living things. This on-site clean up is less disruptive to people. Slives and to the environment compared to excavating and disposing contaminated soils elsewhere. Phytoremediation uses plants by several methods to contain or clean up heavy metals. Phytoremediation has the benefit of being a relatively low-cost, natural solution to an environmental problem.⁵⁸

REMOVAL OF ARSENIC FROM DRINKING WATER BY WATER HYACINTHS

- In Bangladesh, people drink groundwater that has arsenic levels high enough to eventually cause death. Phytoremediation, the use of plants to remove pollutants, has been used to remove metals such as arsenic. Other scientists have tested the ability of water hyacinths (*Eichhornia crassipes*) to remove arsenic from water, with varying results. The purpose of my project was to determine if it is practical to use water hyacinths to remove arsenic from water. There were three experiments:
- First, tried to maximize the number of times the same water hyacinths could reduce the arsenic concentration in water. There grew water hyacinths in 300 ppb arseniccontaminated water in a greenhouse. There added light and heat to try to increase their arsenic removal abilities. It is found that the same water hyacinth plants could remove arsenic seven times, but only twice to the drinking water standard.
- Second, digested plant samples (for outside lab testing) to determine where within the plant arsenic is stored to help understand the removal mechanism. The results showed that the plants store the most arsenic in their bladders (which are for floatation) and the least in their roots.
- Third, digested the dead plant debris with hydrogen peroxide to attempt to remove the arsenic, to minimize the volume of waste generated. I tested the extracted liquid myself using ICP. The hydrogen peroxide digestion removed a significant portion of arsenic from the solids, which could allow most of the arsenic to be precipitated efficiently from the liquid for disposal.

The overall is to determine whether or not it is practical to use water hyacinths to remove arsenic from drinking water in Bangladesh. The first goal is to see if It could increase the number of times the same water hyacinths or could reduce the arsenic level to a safe drinking water standard by increasing light and temperature of the water hyacinths' immediate environment (in the greenhouse). The second goal is to better understand how water hyacinths remove arsenic by determining where the plants store the arsenic so that could work to optimize the plants' arsenic removal potential. The third goal is to find a disposal method for the water hyacinths by using a hydrogen peroxide digestion to get the arsenic out of the dead plant tissue.

Poisoning from arsenic in groundwater used for drinking is a very serious problem in many regions of the world. Up to 50 million people worldwide may be severely affected by arsenic in their drinking water. First they develop sores on their hands and feet, and then they eventually die, usually due to internal cancer. Arsenic occurs naturally throughout the world because arsenic is in the earth's crust; however, in some regions, such as Bangladesh, the western United States, Mexico, northern Chile, Argentina, Hungary, Romania,

Mongolia, Taiwan, Vietnam, Thailand, Nepal, and India, certain geological formations contain higher levels of arsenic and therefore cause the groundwater aquifers to have higher arsenic levels. It has been determined that in Bangladesh about 30% of the tube wells installed in shallow aquifers have arsenic levels over 50 parts per billion (ppb), which is the Bangladesh government's standard for drinking water. The World Health Organization and the US EPA have set a drinking water standard of 10 ppb. Five to ten percent of the tube wells in Bangladesh have arsenic levels over 300 ppb.

Water hyacinths are free-floating aqueous weeds that multiply very quickly. They have fibrous roots and obtain all of their nutrients from the water. Water hyacinths are common in Bangladesh, a fact that is important for this events.

Several scientists have looked at water hyacinths' ability to remove arsenic from water, with somewhat differing results. Some studies have reported water hyacinths to be very effective at removing arsenic from contaminated water. Misbahuddin and Fariduddin found that just the roots of water hyacinths removed 81% from a 400 ppb arsenic solution. The entire water hyacinth plant (roots, leaves, stems, etc.) was reported in the same study to have removed one hundred percent of the arsenic, and to have done so in only 3-6 hrs. Other scientists have reported that water hyacinths do not have very high arsenic removal capabilities. Zhu, Zayed, Qian, Souza, and Terry reported that water hyacinths do not accumulate arsenic well and that most of the arsenic they take up is stored in their roots. Zhu, Lytle, and Terry reported that water hyacinths convert a large portion of the arsenate they remove to the more toxic form of arsenic, arsenite, within the plant itself.⁵⁹

POTENTIAL USE OF WATER HYACINTHS IN BANGLADESH

Several studies suggest that it may be possible to use water hyacinths effectively to remove the arsenic from the drinking water that is poisoning the people of Bangladesh. Thus, water hyacinths may be a practical solution because using them as a treatment method has very little cost, given that water hyacinths grow naturally in the ponds in Bangladesh. It has been suggested that a treatment technique would be to have groundwater users draw the water from their tube wells into a large container, known as a Chari, that the farmers use for animal feed. The water hyacinths would then float in the water for a day and remove the arsenic before the water is drained into another vessel for use. Then, the Chari would be re-filled to treat the next day's supply of water.⁶⁰

OPTIMIZING ARSENIC REMOVAL BY WATER HYACINTHS

The first part of the experiment was to determine whether adding light and heat to the plants' environment would improve their arsenic uptake. It assessed plant health before and after arsenic exposure by looking at both the plants' visual health and their weight. It qualitatively assessed plant health on a scale of one to five: "1" indicated that all of the leaves and stems were brown and "5" reflected a condition where all of the leaves and stems were all green. It weighed the plants at the same time as it assessed their health. It was recorded as wet weight. For the control, it placed 24 identified water hyacinths (1187 total grams, wet) in a plastic tub with 20 L of tap water. The arsenic tub had twenty-one identified plants (1194 total grams, wet) in 20 L of tap water. Then it placed the tubs in the greenhouse. It hung grow lights above the tubs and used a timer to provide a 16 hrs light period each day. It installed aquarium heaters in the tubs and adjusted them so that the temperature of the tubs was about 23°C (75°F).

Using arsenate and arsenite powder It created a 300 ppm (as arsenic) arsenite solution and a 300 ppm (as arsenic) arsenate solution in distilled water. It had to add sodium hydroxide to make the arsenite powder dissolve, and then it neutralized the solution with acetic acid. For the control, it prepared a sodium hydroxide-acetic acid solution with the same concentrations that it had added to the arsenite solution. It added 10 mL of the arsenite solution and 10 mL of the arsenate solution to the arsenic tub to create 20 L of 150 ppb arsenate (as arsenic) and 150 ppb arsenate (as arsenic) for a total of 300 ppb (as arsenic) arsenic concentration. Then it stirred the water well. It added 10 mL of control sodium hydroxide-acetic acid solution to the control tub to match the sodium acetate concentration in the arsenic tub and stirred well.

It measured the arsenic levels of the water on days when I had supervised access to the lab (Tuesday through Friday), using a Hach colorimetric arsenic test kit, following the instructions. On testing days, it started the sampling process by stirring the arsenic tub. Then it took a 50 mL sample and tested the arsenic level using the colorimetric test kit. From that measurement of the arsenic concentration in the tub, it then calculated and added the amount of arsenite and arsenate solution It needed to add to raise the arsenic level back up to 300 ppb, always using equal amounts of arsenite and arsenate solution. Then it measured out the same amount of sodium hydroxide-acetic acid solution to add to the control tub as the amount of arsenite solution added to the arsenic tub. It added the solutions to the tubs and stirred well. It repeated these measurements and additions of more arsenic until the arsenic level was no longer reduced below 300 ppb after at least 24 hrs. As needed, it re-filled the tubs with water to maintain 20 L of water.

It is found that the same water hyacinths removed arsenic from water with a starting arsenic level of 300 ppb a total of seven times. The first trial for these plants was not under the experimental conditions described above, but it was during the summer when hoped to take advantage of the warmer temperatures and sunny days. Although the plants initially only reduced the arsenic level of the water to 120 ppb,

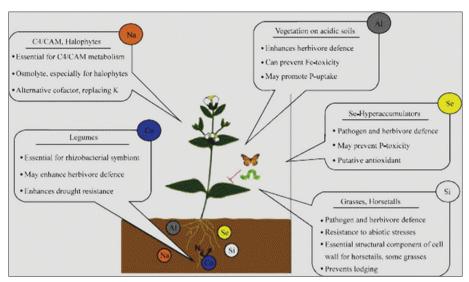


Figure 1: The mechanisms responsible for the growth promoting effects of the five beneficial elements Al, Co, Na, Se and Si.

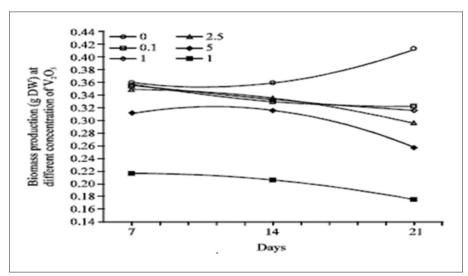


Figure 2: Biomass production of vanadium treated *E. fluctuant* was negatively correlated with concentration of vanadium in nutrient medium with increased in duration.

by two months later, the plants had reduced the arsenic concentration to 10 ppb, which meets the U.S. EPA's arsenic drinking water standard of 10 ppb.

The next trials were conducted in the greenhouse under the experimental conditions described above. The plants reduced the arsenic in the water from 300 ppb to 70 ppb (0 ppb the last time) six times. The air temperature in the greenhouse ranged from about 17-24°C. The water temperature was about 21°C most of the time. In addition to warmer temperatures, the plants had 16 hrs of direct light each day from a hanging grow light. It knew that the water hyacinths had stopped removing arsenic when the arsenic concentration of the water remained at 300 ppb after almost a week after adding arsenic.

This year the mass of water hyacinths It had was lower than last year. Last year there were 1711 g (wet) of water hyacinths

in the $20\,\mathrm{L}$ of arsenic water. This year there were only $1194\,\mathrm{g}$ (wet) of water hyacinths in $20\,\mathrm{L}$ of arsenic water. It is quite possible that, if the mass of water hyacinths had been higher this year, they would have been able to remove more arsenic, both in terms of reducing the arsenic concentration lower each trial and being effective for more trials.

When the plants stopped removing arsenic, it assessed their health. The control plants were very green. The whole top parts of the plants exposed to arsenic were beginning to die and turn brown and a paler green. Assessing with 1-5 criteria, there was a statistically difference in the health of the two sets of plants at the end of the experiment according to an ANOVA test at a 95% confidence level.

The control plants gained more weight than the arsenic plants; however, there was not a statistically significant

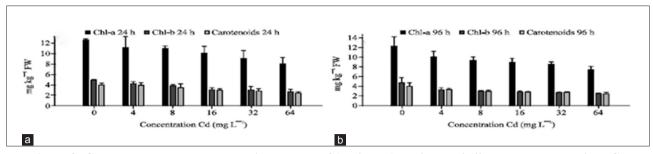


Figure 3: Chlorophyll a, b and carotenoid contents of (mg/g FW) *M. heterophyllum* exposed to various Cd concentrations for (a) 24 and (b) 96 hrs.

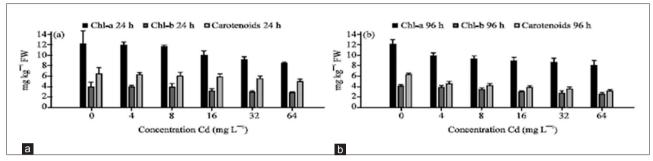


Figure 4: Chlorophyll a, b and carotenoid contents of (mg/g FW) *Potamogeton crispus* exposed to various Cd concentrations for (a) 24 and (b) 96 hrs.

difference at a 95% confidence level. In addition, it noticed that, after arsenic exposure, that most of the arsenic plants had a much smaller root mass than the control plants. A common gardening rule for fertilizer is that phosphorus is for roots. So, if the arsenic was harming the phosphate uptake system, it may have been harming the roots the most.⁶¹

CONCLUSION

Phytoremediation is initiated all over the globe and this has considered one of the low-cost novel green technologies. The physiological and molecular basis of metal hyperaccumulation in plants are still in research and development phase. This review has focused on recent evidence that identifies potential molecular mechanism that may be involved in the resistance, tolerance as well as hyperaccumulation of heavy metals. The findings suggest that in some plants ZIP family genes contribute to metal hyperaccumulation and transport, but their individual functions yet to be identified and further intensive research is needed in this concern. The identifications of individual functions of metal transporters will help to develop the knowledge of plants metal homeostasis. Results already obtained have been indicated that the physiological and biochemical responses were plants and metal-specific. Identification of novel genes with high biomass yield characteristics and the subsequent development of transgenic plants with superior remediation capacities will be encouraging further research. In depth research study is warranted to find out which plant is maximum resistant and best adapted in particular metallic environment or

region. *In situ* toxicity text could be beneficial for initial identification of particular species.

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