SEMINAR REPORT

Metallophytes, a unique biological resource: its molecular mechanisms and applications

By

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CERTIFICATE

This is to certify that the seminar report entitled **“Metallophytes, a unique biological resource: its molecular mechanisms and applications”** has been solely prepared by **Sharat Prabhakaran (2018-11-165)**, under my guidance and has not been copied from seminar reports of any seniors, juniors or fellow students.

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DECLARATION

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1. Introduction

1.1 Heavy metal pollution:

The environment and its compartments have been severely polluted by heavy metals. This has compromised the ability of the environment to foster life and render its intrinsic values. Heavy metals are known to be naturally occurring compounds, but anthropogenic activities introduce them in large quantities in different environmental compartments. This leads to the environment's ability to foster life being reduced as human, animal, and plant health become threatened (Wuana and Okieimen, 2011). This occurs due to bioaccumulation in the food chains as a result of the nondegradable state of the heavy metals. Developed physical and chemical heavy metal remediation technologies are demanding costs which are not feasible, time-consuming, and release additional waste to environment (Kumar *et al.*, 2019).

From a data published by Central Water Commission in 2018 (Table 1), it was understood that about 42 rivers in India were contaminated by at least 2 heavy metals. Our national river Ganga around who's basin flourished agriculture at one point of time in history is contaminated by five toxic heavy metals – chromium, copper, nickel, lead and iron. These river basins are no longer fit for cultivation.

Table 1: **No. of rivers polluted with unacceptable levels of heavy metals**

Contaminant	**Permissible limit (mgL^{-1})**	**No. of rivers**
Lead	**10**	**69**
Nickel	**20**	**25**
Iron	**300**	**137**
Copper	**50**	**10**
Chromium	**50**	**21**
Cadmium	**3**	**25**

1.2 Mode of action of heavy metal toxicity:

The toxicity of heavy metals (HM) is manifested in many ways when plant cells accumulate them at high levels. HMs can be divided into two groups: redox active (Fe, Cu, Cr, Co) and redox inactive (Cd, Zn, Ni, Al, etc.). The redox active HMs are directly involved in the redox reaction in cells and result in the formation of $O_2^{\bullet -}$ and subsequently in H_2O_2 and •OH production via the Haber-Weiss and Fenton reactions. Exposure of plants to redox inactive HMs also results in oxidative stress through indirect mechanisms such as interaction with the antioxidant defense system, disruption of the electron transport chain, or induction of lipid peroxidation (Emamverdian *et al.,* 2015). The latter can be due to an HM-induced increase in lipoxygenase (LOX) activity. Another important mechanism of HM toxicity is the ability of HMs to bind strongly to oxygen, nitrogen, and sulphur atoms. This binding affinity is related to free enthalpy of the formation of the product of the HM and ligand with low solubility of these products. Because of these features, HMs can inactivate enzymes by binding to cysteine residues. For example, Cd binding to sulfhydryl groups of structural proteins and enzymes leads to misfolding and inhibition of activity and interference with redox-enzymatic regulation.

Many enzymes need cofactors to work properly for both HM ions (such as Fe2+, Mg 2+, Cu 2+, Ca 2+) and organic molecules (such as haem, biotin, FAD, NAD, or coenzyme A). The displacement of one HM ion by another leads to the inhibition or loss of enzyme activities. Divalent cations such as Co2+, Ni2+, and Zn2+ displace Mg2+ in ribulose-1,5bisphosphate-carboxylase/oxygenase (RuBisCO) and result in a loss of activity. Displacement of Ca^{2+} by Cd^{2+} in calmodulin, an important protein in cellular signalling, led to the inhibition of calmodulin-dependent phosphodiesterase activity in radish. Additionally, HMs cause membrane damage through various mechanisms, including the oxidation of and cross-linking with protein thiols, inhibition of key membrane protein such as H+-ATPase, or causing changes in the composition and fluidity of membrane lipids. Accumulation of methyl glyoxal, a cytotoxic compound, was found to increase in response to HM stress in plants due to impairment of the glyoxalase system.

2. Metallophytes:

Metallophytes are endemic plant species of natural mineralized soils, hence have developed physiological mechanisms of resistance and tolerance to survive on substrates with high metal levels.

Metalliferous soils provide very restrictive habitats for plants due to phytotoxicity, resulting in severe selection pressures. Species comprising heavy-metal plant communities are genetically altered ecotypes with specific tolerances to, e.g., cadmium, copper, lead, nickel, zinc and arsenic, adapted through microevolutionary processes. Evolution of metal tolerance takes place at each specific site.

A high degree of metal tolerance depends on the bioavailable fraction of the metal or metalloids in the soil and the type of mineralization. At extremely high soil metal concentrations, especially on polymetallic soils, even metal-tolerant genotypes are not able to evolve extreme tolerances to several heavy metals simultaneously. Adapted genotypes are the result of the Darwinian natural selection of metal-tolerant individuals selected from surrounding non-metalliferous populations. Such selection can lead ultimately to speciation and the evolution of endemic taxa.

Heavy-metal tolerance was first reported by Prat (1934) in *Silene dioica* and further demonstrated experimentally in grasses by Bradshaw and co-workers in *Agrostis spp*., by Wilkins in *Festuca ovina* in the late 1950s and 1960s. Followed by Baumeister and co-worker in the herb *Silene vulgaris*.

3. Classification of metallophytes

Baker and Walker (1990), categorized plants into three groups according to their strategies for adaptation (Fig. 1):

- **Metal excluders** - Metal excluders are plants which effectively limit the levels of heavy metal translocation within them and maintain relatively low levels in their shoot over a wide range of soil levels; however, they can still contain large amounts of metals in their roots.

- **Indicators** - Metal indicators are plants that accumulate metals in their above-ground tissues and the metal levels in the tissues of these plants generally reflect metal levels in the soil (Baker and Walker 1990). However, under continued uptake of heavy metals this plant species die-off. Heavy metal indicator plants render biological and ecological functions in that they are possible indicators of pollution and useful in absorption of pollutant.

- **Hyperaccumulators** - are plant species that concentrate metals in their above-ground tissues to levels far exceeding those present in the soil or in the non-accumulating species growing nearby. These plants are capable of extracting heavy metals from soils and concentrate them in their shoots, and they are widely used in phytoremediation. Accumulated heavy metals have been reported to play physiological and ecological functions, for example in prevention of bacterial and fungal diseases. Some species can hyperaccumulate one particular metal each, while others can hyperaccumulate more than one metal each. Cd > 100 mg/kg; Pb > 1000 mg/kg; Zn > 10 000 mg/kg.

Metal-tolerant plants avoid intoxication by an excess of heavy metals by means of special cellular and molecular mechanisms, as long as the soil metal levels do not exceed the levels of metal tolerance. They can thus thrive on soils that are too toxic for non-adapted species and ecotypes. Hyperaccumulators have huge potential in phytoremediation and phytomining of heavy metals.

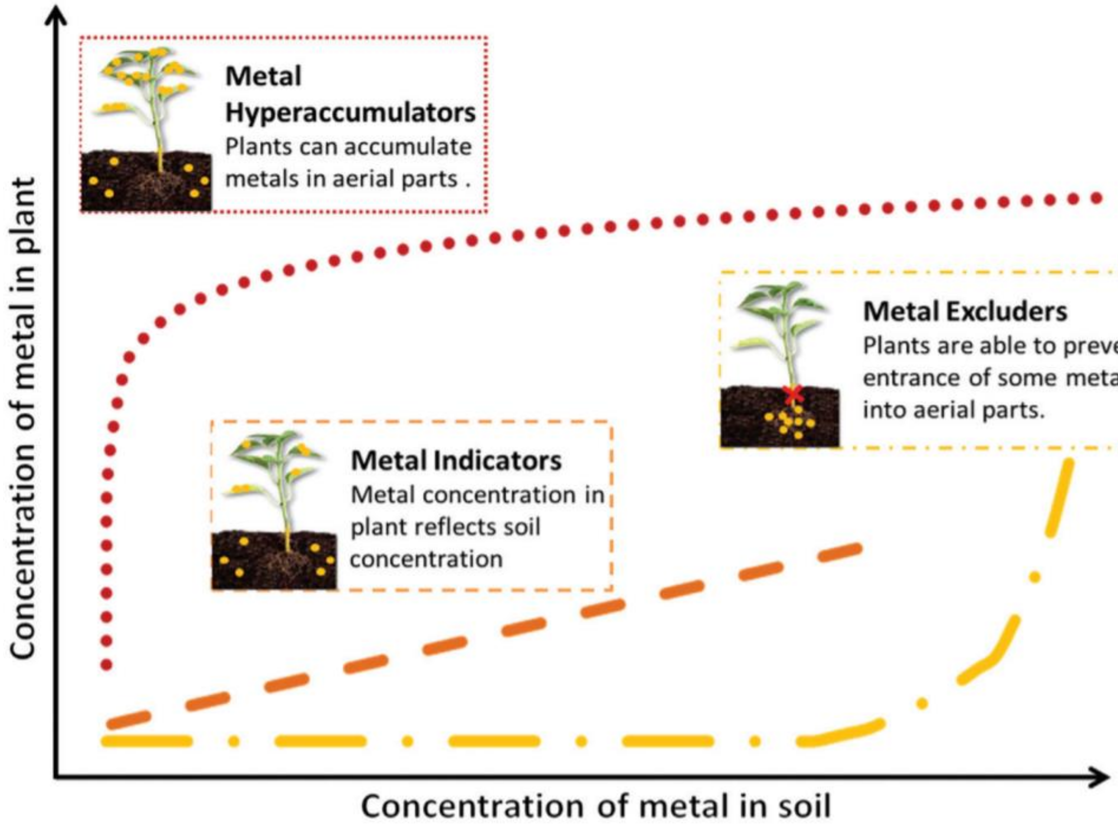

Fig. 1: Classification of metallophytes based on Heavy metal uptake

4. Mechanisms of hyperaccumulation:

4.1 Heavy Metal Uptake:

Hyperaccumulators have an extraordinary ability to absorb heavy metals from the soil under varying concentration of heavy metals (Ma et al., 2001; Yang et al., 2002). Although heavy metals are taken up by hyperaccumulators, their uptake is affected by several factors such as pH, water content, organic substances, etc. Moreover, heavy metal uptake requires a suitable transporting system to enter the plant. Several researchers have reported that pH affects (i) proton secretion by roots that further acidify rhizosphere, thus enhancing metal dissolution, and (ii) the growth of metal-accumulating plant species. Apart from pH, organic substances released from the roots affect growth in hyperaccumulating plants. have reported that organic acids released influence Cd solubility by forming Cd complexes (Fig. 2). Therefore, pH and organic substances released from the rhizosphere of a hyperaccumulator mobilize heavy metal and enhance absorption.

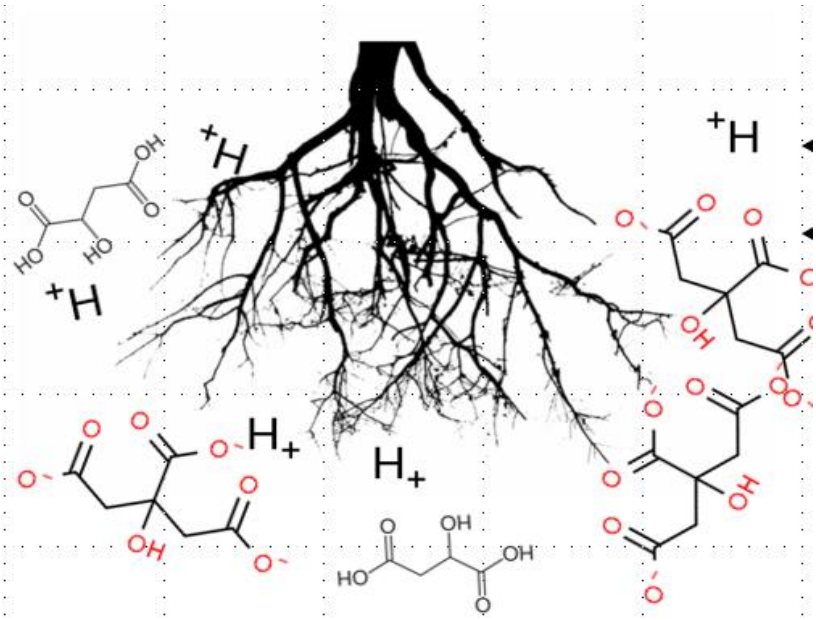

Fig. 2: Protons and organic acids in heavy metal uptake

4.2 Overexpression of transporter proteins:

Constitutive overexpression of genes also attributes to enhanced heavy metal uptake. To pinpoint the genes involved in overexpression, several comparative studies have been performed in hyperaccumulating *Arabidopsis helleri* and *Thlapsi caerulescens* with that of non-hyperaccumulating species. Studies on *T. caerulescens* and *A. halleri* have revealed that increased Zn uptake is due to overexpression of genes belonging to the ZIP (Zinc regulated transporter Iron-regulated transporter proteins) family encoding plasma membrane located transporters (Assunção et al., 2001): ZTN1 and ZTN2 in *T. caerulescens* and ZIP6 and ZIP9 in *A. halleri*. The decreased uptake of Cd under increasing Zn concentration was noticed in both genera.

Major transporter protein families:

- **ABC -** ATP-binding cassette
- **CDF -** Cation diffusion factor
- **HMA -** Heavy metal ATPase
- **NRAMP -** Natural resistance associated macrophage protein
- **MATE -** Multidrug and toxin extrusion
- **CAX -** Calcium exchange protein

Gao *et al.* (2013) conducted a transcriptomic analysis of cadmium stress response in the hyperaccumulator. The *Sedum alfredii* Hance hyperaccumulating ecotype (HE) has the ability to hyperaccumulate cadmium (Cd), as well as zinc (Zn) and lead (Pb) in above-ground tissues. employed RNA-seq to explore the transcriptome of S. alfredii Hance and to identify transcriptional changes in response to high Cd accumulation in shoots. Grew *S. alfredii* in two conditions - normal soil (0 mM $CdCl_2$) and contaminated soil (100 mM $Cdcl_2$). The plants were grown for two weeks. There were no significant toxicity symptoms in the cadmium treated plants (Fig. 3).

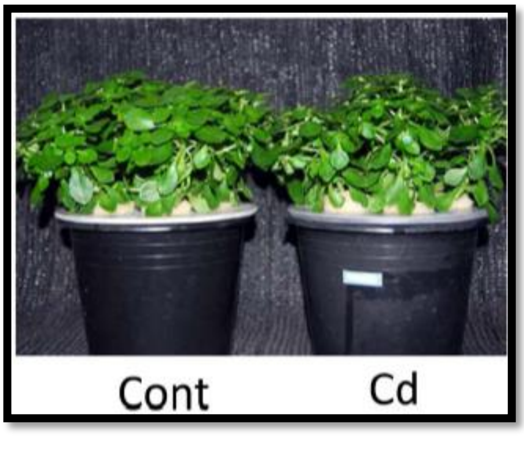

Fig. 3: Control vs Cadmium treatments

From the elemental composition analysis, it was found out that the plant could accumulate more 5000 mgkg^{-1} of cadmium in its leaf tissue (Fig. 4) without any signs of toxicity.

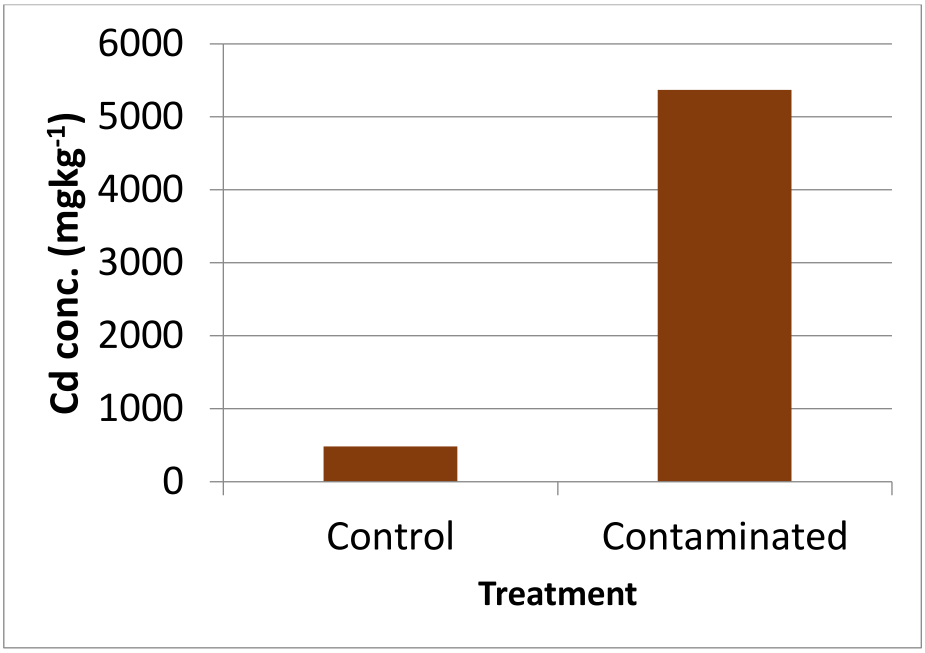

Fig. 4: Cadmium accumulation in shoots

From their transcriptomal study, it was revealed that a number of transporter genes had been over expressed when grown in high Cd concentrations (Table 2).

Table 2: Transporter genes over expressed under Cadmium stress

Gene	**Control**	**Cd treat.**	**Fold change**
Nramp 4	24	135.4	5.6
Nramp2	20.1	90.8	4.5
Nramp3	30.9	135	4.4

4.3 Complexation of heavy metals with chelating agents

4.3.1 Formation of Metal Complex by Phytochelatins:

Chelation of HMs in the cytosol by high affinity ligands is potentially a very important mechanism of HM detoxification and tolerance in plants under HM stress. Plants make two types of peptide metal binding ligands: phytochelatins (PCs) and metallothioneins (MTs). Recent advances in the understanding of different aspects of biosynthesis and function of PCs are derived predominantly from molecular genetic approaches using model organisms. PCs are a family of Cys-rich polypeptides with the general structure (γ-GluCys) n-X, in which X is Gly, γ-Ala, Ser, Gln, or Glu and n = 2–11 depending on the organism, although the most common forms have 2–4 peptides. PCs are synthesized from GSH; the metal binds to the constitutively expressed enzyme γ-glutamyl cysteinyl dipeptidyl transpeptidase (PC synthase), thereby activating it to catalyze the conversion of GSH to phytochelatin (Das and Jayalakshmy, 2015). The biosynthesis of PCs is induced by many HMs, including Cd, Hg, Ag, Cu, Ni, Au, Pb, As, and Zn; however, Cd is by far the strongest inducer. PCs complex Cd ions through the thiolic group (–SH) of Cys and the PC-Cd complexes are accumulated in vacuole through the activity of ABC transporters, thus limiting the circulation of free Cd^{2+} inside the cytosol (Fig. 5).

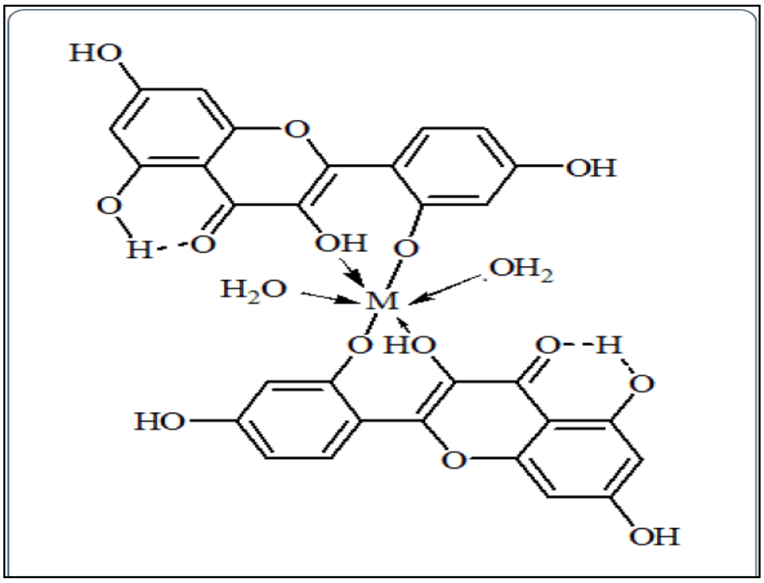

Fig. 5: Chelation of heavy metal ions by phytochelatins

4.3.2 Complexing by metallothioneins:

MTs are low molecular weight (4–8kDa), Cys-rich, HM-binding, gene-encoded polypeptides that can bind HMs via the thiol groups of their Cys residues. Although the precise physiological function of MTs has not yet been fully elucidated, proposed roles include:

(a) participation in maintaining the homeostasis of essential transition HMs,

(b) sequestration of toxic HMs,

(c) protection against intracellular oxidative damage.

4.3.3 Metal Chelation by Organic Acids, Amino Acids, and Phosphate Derivatives:

Mechanism of HM tolerance and detoxification in plants can be divided into two categories: external exclusion and internal tolerance. In the external detoxification process, organic acids excreted from plant roots may form stable HM-ligand complexes with HM ions and change their mobility and bioavailability, thus preventing the HM ions from entering plants or avoiding their accumulation in the sensitive sites of roots (Fig. 6). In internal HM detoxification, organic acids may chelate with HM in the cytosol, where the ions can be transformed into a nontoxic or less toxic form.

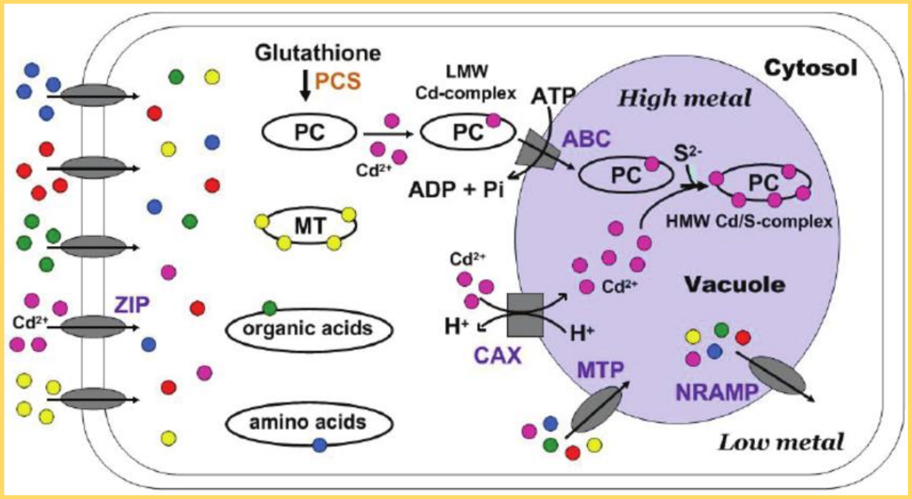

Fig. 6: Chelating agents and various transporter involved is sequestration

4.4 Modulation of Transcription Factor (TF):

Transcription factor are considered to be the key regulators of gene expression (Fig. 7). Metal response element binding transcription factor 1, also called metal responsive transcription factor 1 (MTF-1), plays an important role in the cellular response and tolerance to HM stress by triggering the activation of genes responsible for HM uptake, transport, and detoxification. TFs involved in HM stress response and tolerance have already been identified in different plant species. Importantly, Cd-responsive TFs share the same signal transduction pathway with other stress-related TFs. TFs belonging to different families, such as WRKY, basic leucine zipper (bZIP), ethylene-responsive factor (ERF), and myeloblastosis protein (MYB), play a significant role in controlling the expression of specific stress-related genes in response to Cd stress.

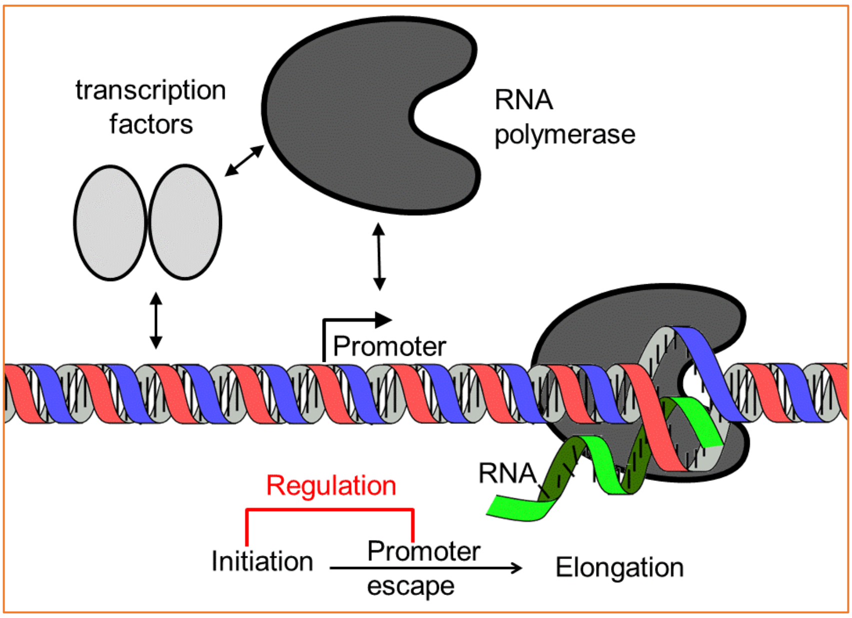

Fig. 7: Modulation of transcription factors

A number of transcription factors have been reported to play a key role in heavy metal tolerance (Table 3). CAPF1 is known to enhance the production of the production of enzymatic anti-oxidants ascorbate peroxidase, super oxide dismutase, glutathione reductase.

Table 3: Transcription factors reported in heavy metal tolerance

Name of TF	**Family**	**Response**	**Reference**
CaPF1	AP2/EREBP	Enhanced APX, GR, SOD production	Tang *et al*. (2005)
Hsfs	HSF	Up regulation of metallothionein gene	Shim *et al*. (2009)
WRKY 22, 25	WRKY	MAPK and oxyplin signalling	Opdenakker *et al*. (2012)
ZIP39	bZIP	Regulates endoplasmic reticulum stress response	Takahashi *et al*. (2012)
WRKY 6	WRKY	Transposon silencing	Castrillo *et al*. (2013)

4.5 Induction of Antioxidant Defense and Glyoxalase System:

HM stress invariably induces oxidative stress and antioxidative defence systems, composed of free radical scavenging molecules such as AsA and GSH, and the enzymes involved in their biosynthesis and reduction (Hossain *et al*., 2012). Additionally, methyl glyoxal, a cytotoxic compound, was found to increase in response to various abiotic stresses, including HM stress, which is mainly detoxified by the glyoxalase system in plants and enhanced oxidative stress tolerance (Fig. 8).

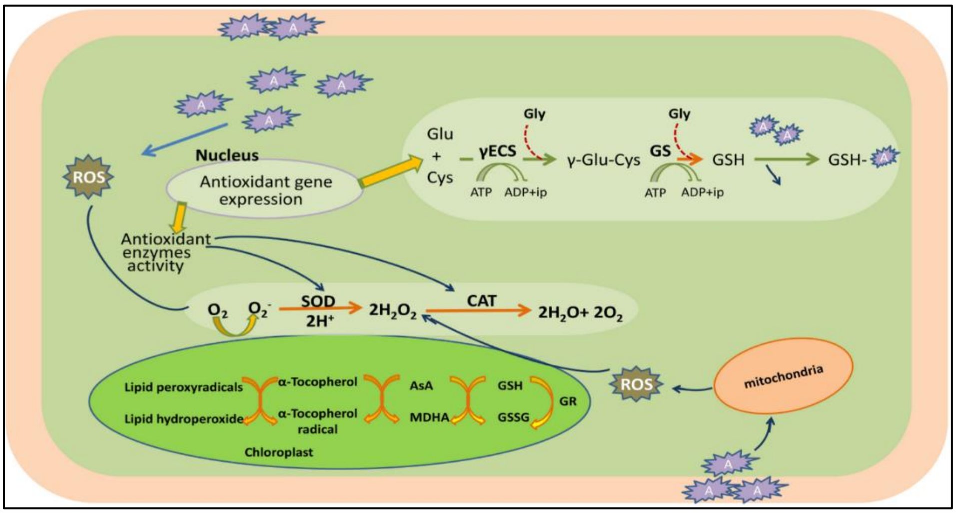

Fig. 8: Anti-oxidant and glyoxalase systems in a cell

An analysis of the changes that occur in the leaf proteome was studied by Zhao *et al.* (2011). They grew three week old *P. americana plants* in different concentration of $CdCl_2$ for

48 hrs – 0 mM and 800 mM. They observed no significant change in the phenotype. The proteomal analysis was done using 2- dimensional electrophoresis, and the found oud that number of protiens involved in the glyoxal pathway (Table 4) as well as the antioxidant pathway (Table 5) were over expressed.

Table 4: Fold changes in the enzymes involved in glyoxal pathway

Enzymes	**Fold change**
Adenosyl S homocystein synthase	5.25
Cobalamine independent methionine synthase	3.03
Glutathione s - transferase	4.61

Table 5: Fold changes in the enzymes involved in antioxidant pathway

Enzymes	**Fold change**
2 -Cys peroxiredoxin precursor	2.04
Benzoquinone reductase	2.02

4.6 Synthesis of various metabolites:

4.6.1 Synthesis of Salicylic Acid:

Salicylic acid (SA) is a natural signal molecule which plays an important role in regulating a number of physiological and biochemical process making plants resistant to biotic and abiotic stresses.

4.6.2 Synthesis of Proline:

Accumulation of proline in response to HM stress has also been widely reported. Moreover, many HM-tolerant plants have also been reported to possess substantially elevated constitutive proline levels in the absence of excess HM ions when compared with their nontolerant relatives.

4.6.3 Polyamines:

Polyamines (PAs) are low molecular weight organic cations and are ubiquitous in all living organisms (Ono *et al.*, 2012). The common PAs in plants are spermidine (Spd), spermine (Spm), and their diamine precursor, putrescine (Put). They influence a variety of growth, and development processes in plants and have been suggested to be a class of plant growth regulators and to act as second messengers.

4.6.4 Synthesis of Nitric Oxide and heavy metal tolerance:

Nitric oxide (NO), an ubiquitous bioactive signalling molecule, plays an important role in a broad spectrum of multiple physiological processes in plants by regulating the level and toxicity of ROS and hormones and by inducing transcriptional changes that permit the identification of genes involved in different functional processes such as signal transduction, defense and cell death, transport, basic metabolism, and ROS production and degradation. NO protects plants from oxidation damage by regulating general mechanisms for cellular redox homeostasis.

5. Application of metallophytes:

The major applications of metallophytes are phytoremediation and phytomining.

5.1 Phytoremediation:

The use of metallophytes and the associated microbes, along with proper soil amendments and agronomic techniques to either contain, remove or render toxic environmental contaminants harmless. In other words, phytoremediation is basically a solar-powered pollutant removal system. The modern technologies of phytoremediation are based on different uptake mechanisms which include phytoextraction, phytostabilization, phytoevaporation, rhizofiltration and rhizodegradation.

Different approaches of phytoremediation:

5.1.1 Phytoextraction

Phyto-extraction, also known as phyto-accumulation, phyto-absorption or phyto-sequestration is the uptake of contaminants from soil or water by plant roots and their translocation and accumulation in aboveground biomass (Fig. 9). Metal translocation to shoots is a crucial biochemical process desirable for an effective phytoextraction because the harvest of root biomass is generally not feasible (Bhargava *et al.*,2012).

5.1.2 Phytostabilization

Phytostabilization deals with the decrease in the bioavailability and mobility of heavy metals in soils due to their stabilization from off-site transport with the help of plants (Pulford and Watson, 2003). In recent years, this method which is based on the ability to accumulate specific heavy metal in plants and in the root zone was also called phyto-deposition or phyto-sequestration (Fig. 9).

5.1.3 Phytoevaporation

Phyto-evaporation is the uptake of pollutants from soil by plants, and their conversion to volatile form and subsequent release into the atmosphere (Fig. 9). This can be applied for organic pollutants and some heavy metals i.e. Se and Hg. The use is restricted by the fact that the process does not fix the contaminant completely; only transfers pollutants from soil to atmosphere from where it can be redeposited.

5.1.4 Rhizofiltration:

Rhizofiltration is similar to phyto-accumulation, but the plants used for cleanup are raised in greenhouses with their roots in water. This system can be used for ex-situ groundwater treatment. That is, groundwater is pumped to the surface to irrigate these plants. Typically, hydroponic systems utilize an artificial soil medium, such as sand mixed with perlite or vermiculite (Fig. 9). As the roots become saturated with contaminants, they are harvested and disposed.

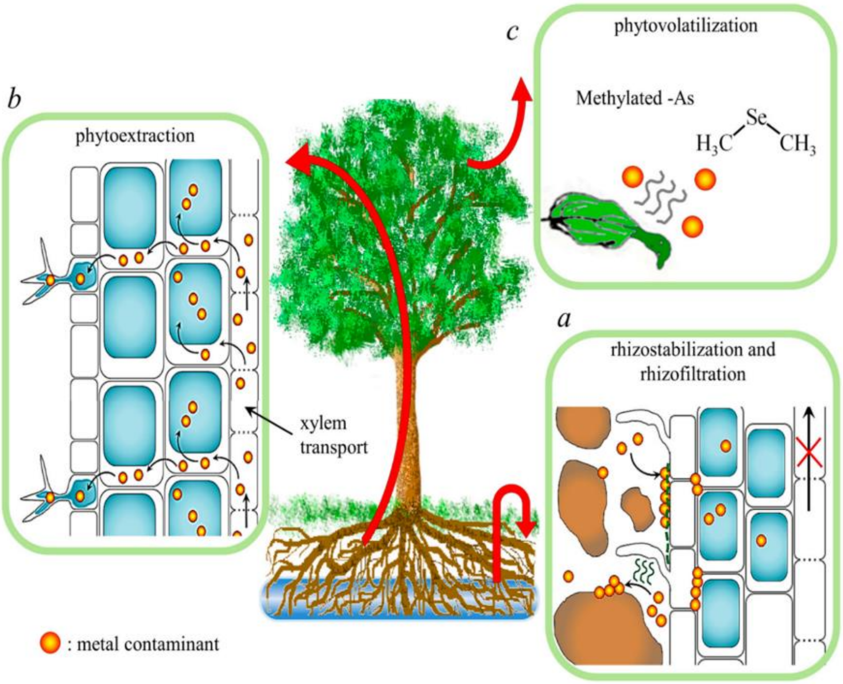

Fig. 9: Various approaches in phytoremediation

5.2 Phytomining:

Phytomining technology employs hyperaccumulator plants to take up metal in harvestable plant biomass. Harvesting, drying and incineration of the biomass generates a high-grade bio-ore (Fig. 10). However, two decades after its inception and numerous successful experiments, commercial phytomining has not yet become a reality. To build the case for the minerals industry, a large-scale demonstration is needed to identify operational risks and provide "real-life" evidence for profitability (Sheoran *et al.*, 2013).

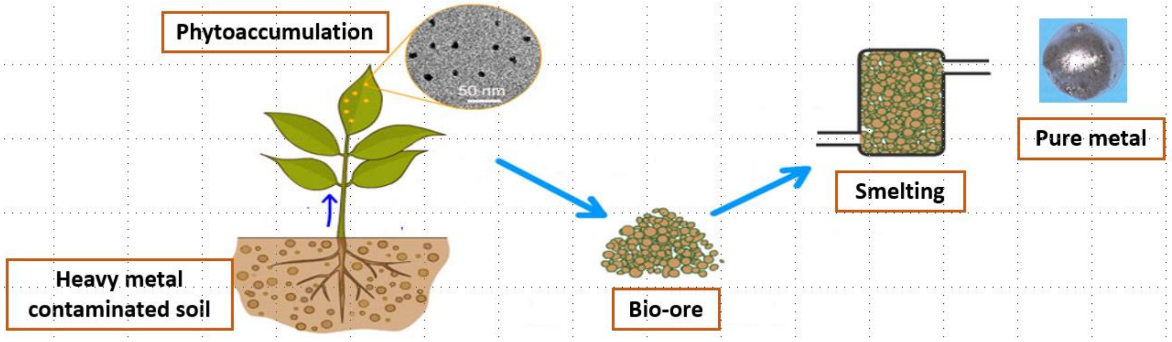

Fig. 10: Process of phytomining

Phytomining may in principle be undertaken to produce As, Se, Cd, Cu, Co, La, Mn, Ni, Pb, Tl, and Zn, as hyperaccumulator plants are known for all of these elements. However, Cu, Co, La, and Pb hyperaccumulators have poor accumulation characteristics and are therefore not (currently) considered for phytomining (Van der Ent *et al.*, 2015).

Rosenkranz and co-workers conducted a nickel phtomining field trial using *Odontarrhera chalcidica* and *Noccaea goesingenis* on Austrian serpentine soils. The phytoming efficiency of both the species were compared in high density planting of 110 plants per square meter. It was found out that *O. chalcidica* managed to accumulate more concentration of Ni in its shoots (Fig.11). The overall nickel mining efficiency was highest in *O. chalcidica*. It managed to yield about 53.6 kg of pure nickel per hectare, whereas *N. goesingenis managed to yield* 34.2 kg of pure nickel per hectare (Fig.12).

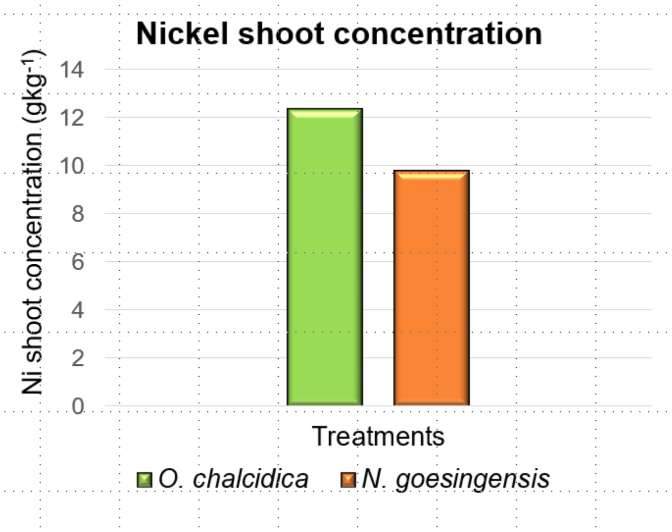

Fig. 11: Concentration of nickel in shoots

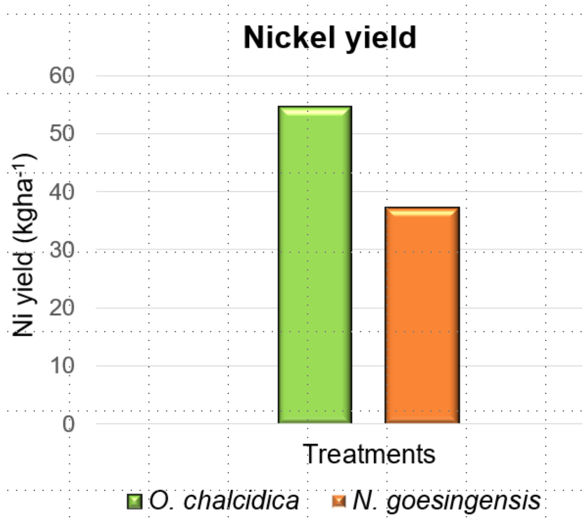

Fig. 12: Nickel yield per hectare

Economic feasibility depends on the element market price, the annual yield per unit area (biomass produced and contained amount of target element), and the availability of surface areas enriched in this element. Current (2015) prices per metric ton are high for Ni (US$15,000), Se (US$52,000), and Tl (US$60,000), but low for As (US$1550), Mn (US $2350), Cd (US$1750), and Zn (US$2100). Therefore, phytomining may be feasible for Ni, Se, and Tl, but of these elements, large surface areas with enrichment exist only for Ni and Se. Two exceptions can be made for instances where the actual metal/metalloid-rich biomass has a value in itself apart from the sole metal value: (i) Zn or Mn-based catalysts prepared from hyperaccumulator biomass and (ii) organic micronutrient fertilizers made from hyperaccumulator biomass rich in either Zn, Ni, Mn, or Se.

6. Major barriers in phytoremediation and phytomining:

Phytoremediation though much spoken about from a very long time, however is not being followed (Mahar *et al.,* 2016). This is primarily due to some of the barriers.

- Lack of genotypes suitable for the locality
- Time consuming process
- Selectivity in remediation
- Limited to shallow soils

Phytoremediation though much spoken about from a very long time, however is not being followed. This is primarily due to the fact that there aren't many genotypes identified which can tackle this problem (Robinson *et al.,* 2015). The recent omics wave, have resulted in the development of an ocean of techniques that is helpful in dissecting the phenotype and looking into the functional network of genes, transcripts and proteins in an organism. Identification of the candidate genes or regulators using various omics tools such as, genomics, transcriptomics, proteomics, metabolomics can help to overcome these barriers by engineering local genotypes into highly efficient accumulators and thus make this long-awaited technology a success (Pilon Smits and Pilon, 2002).

This recombinant DNA technology using the heterologous expression of genes in plants is a relatively new and evolving discipline that enhances plant tolerance to toxic pollutants. The idea behind introduction of a new trait to the plant is not only to provide advantages against abiotic stresses, like HMs, but also to revamp the shelf life, yield, and resistance of the plant (Chaney *et al.,* 2018). Recent research outcomes, including introduction of a foreign gene and/or overexpression of gene(s) that are involved in better metal uptake, transport, and their sequestration, and/or their products like enzymes required in the breakdown of toxic pollutants, have gained popularity and also present challenging possibilities for efficient phytoremediation.

Shim *et al.* (2013) engineered a gene – yeast cadmium factor 1 (*YCF 1*) into poplar tree. Number of transgenic lines were developed and their tolerance were compared. Lines 7 and 11 showed no symptoms, whereas line 16 showed little. The control and line 4 showed severe necrotic spots (Fig. 13).

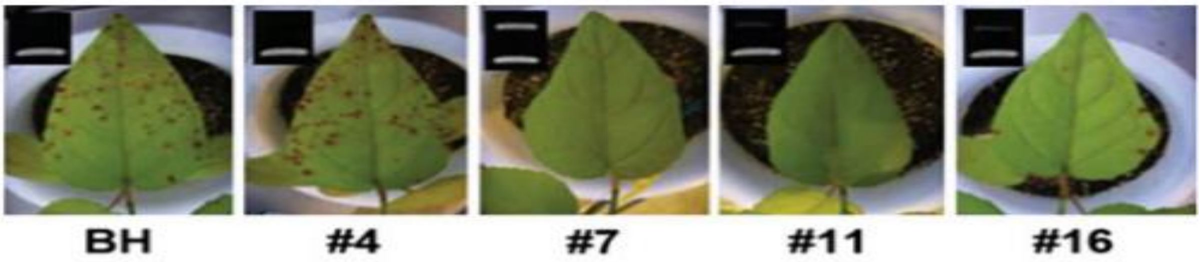

Fig. 13: Comparison of toxicity symptoms in the leaf of various line

Level of expression of the gene was analysed using real time reverse transcriptase PCR. In support of the toxicity symptoms observed line 4 expressed the highest level of transcripts followed by 11. Then 16 the remaining lines had no expression. As expected, the control also did not express (Fig. 14).

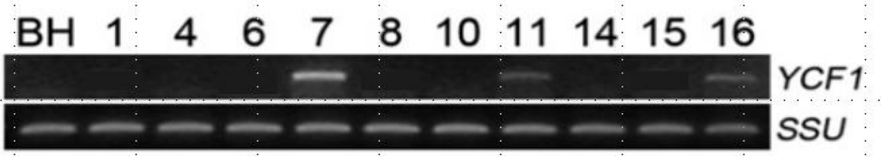

Fig. 14: Expression levels of *YCF1*

Cadmium accumulation rates of all the lines were compared ny growing the lines in a hydroponic medium contaminated 800 μM of $CdCl_2$. It was evident that line 7 accumulated the highest concentration of cadmium among the lines (Fig 15).

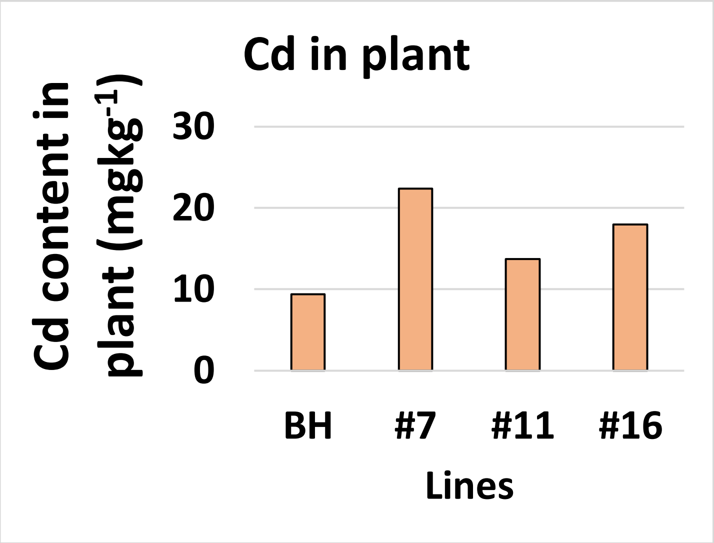

Fig. 15: Cadmium concentration in the lines (mgkg^{-1})

7. Future thrust:

- Tapping of the unexplored metallophyte resources can be carried out and a greater number of hyperaccumulators be identified.
- Using various omics tools, which have become very much affordable in the present days, a better understanding of molecular mechanism, genes and transcripts that play a major role in heavy metal tolerance and the hyper accumulation process of heavy metals can be identified.
- Development of super extractors – genotypes having better growth and biomass, lower specificity can be engineered using genetic engineering in local genotypes using various genetic engineering tools such as trangenesis, cisgenesis, gene stacking, pathway engineering and genome editing using tools such as CRISPR.
- Phytomining is a method of income generating phytoremediation, where the phyto-remediation process is carried out, the elements are recovered and income can be generated in the process.
- Phytomining can also be carried out for precious metals such as gold, platinum and other metals.

8. Summary:

- ➢ Metallophytes are a unique biological resource, which are very less explored and lesser exploited.
- ➢ Molecular mechanisms involved in the hyperaccumulation process that distinguishes the hyperaccumulators from the normal plants.
- ➢ How hyperaccumulators can be used as potential tool for phytoremediation oh heavy metal contaminant in the environment.
- ➢ Phytoremediation though much spoken of from a very long time, however not being followed due to certain barriers.
- ➢ An understanding of the various genes and transcripts involved in hyperaccumulation mechanism can be achieved using the various omics tools.
- ➢ Once the mechanisms and the genes and transcripts involved are identified they can be then further used to incorporate in the local genotypes using various genetic engineering

tools such as trangenesis, cisgenesis, gene stacking, pathway engineering and genome editing using tools such as CRISPR.

9. Conclusion:

To prevent and treat hazardous pollution, wide range of physical techniques for heavy metal removal has been developed. However, theoretically they seem as amazing technologies but practically not so. None of these techniques are seen to be put into use for the remediation of contaminated lands. This is due to their high costs, complexity of the technology involved and the impact on physical, chemical and biological properties of the soil. Phytoremediation is by far, the more feasible technique available.

Phytoremediation is a technology that is yet to reach its true potential. Better understanding and identification of the molecular mechanisms for hyperaccumulation and tolerance using various omics tools coupled with use of genetic engineering to incorporate the various genes that into our local genotypes, makes the long-awaited technology a success.

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Discussions:

1. **Among the various phytoremediation techniques, why is phtoextraction preferred over phytostabilization and phytovolatilization?**

Ans. Phytostabilzation is a technique where in the heavy metals are temporarily stabilized or immobilized within the soil itself. This is achieved by converting it from one form to another. This could again revert back into the toxic form in the future. In phytovolatilization, plants are used to take up heavy metals from the soil and release them into the atmosphere. They could further result in air pollution or may also eventually fall back into to the soil. Both these approaches are not a permanent solution to our problem in hand. Where as in phytoaccumulation hyper accumulator plants are used, to take up heavy metals present in the soil and store them in their above ground parts in a non-toxic form. Once they are mature, they can be harvested from the site and be taken of from the site itself.

2. **Are there any commercially available varieties available in India?**

Ans. The concept though very old hasn’t had much progress. This mainly due to lack of genotypes identified, poor adaptability of these genotypes, slower rate of cleansing process due to the slow growth and low biomass of metallophytes. Plant breeders haven’t been much interested in such areas of crop improvement. This technology is still in a primitive stage. Breeders are still working on improving the accumulation efficiency and adaptability of hyperaccumulators. Scientists are conducting experiments on identification of the various mechanisms and the genes involved in the process. Using this information genetic engineering is being used to improgress these traits into our local genotypes and increasing their efficiency. In this seminar we had seen one such experiment where they had developed a transgenic hyperaccumulating poplar.

3. **Would it affect the human beings and other animals?**

Ans. These plants are grown with the sole intention of being grown and removed from the field once they are mature and not for human consumption of any manner. Generally, these plants are considered by herbivores as less attractive and deter them because these plants are toxic to them. To be on the safer side, the remediated site should be considered as hazardous zones, be isolated and phytoremediation be conducted on them and the harvested produce be treated properly prior to their safe disposal.

4. **Are there any species of hyperaccumulators that have been reported to accumulate gold?**

Ans. Yes, metallophytes have been reported to accumulate a number of precious metals such as silver, gold and platinum.

Gold – *Artemisia sp., Pseudotsuga menziesi, Lonicera sp.*

Platinum - Bherkhya *codii, Sinapis alba, Alysum bertalonii.*

Silver – *Salix miyabaena*, *Brassica juncea.*

Precious heavy metals like gold, don't easily dissolve in water so plants have a hard time in taking the particles in through their roots and hence the accumulation rates are low. By altering the chemical conditions of the rhizosphere, accumulation efficiency can be increased.

5. **Isn't this technology only feasible for cleaning the surface soils or top soils?**

Ans. To prevent and treat hazardous pollution, wide range of physical techniques for heavy metal removal has been developed. However, theoretically they seem as amazing technologies but practically not so. None of these techniques are seen to be put into use for the remediation of contaminated lands. This is due to their high costs, complexity of the technology involved and the impact on physical, chemical and biological properties of the soil. The depth is a limitation in case of these physical and chemical techniques too. Hence, in all way's, phytoremediation is the more feasible technique. Scientists are working on reaching farther depths of the soil through genetic engineering of tree crops for hyperaccumulation. Furthermore, the heavy metal pollution at the rhizosphere level is our primary concern because this from where the heavy metals enter into our food chain.

6. **Are there any metallophytes reported in India?**

Ans. Several plant species of Barak Valley, South Assam, have been reported to possess substantial hyperaccumulating potential that can be used for Cu phytoremediation from soil and water.

Examples: *Calandula officianalis, Pityrogramma calomelanos.*

Others**:** Brassica juncea**,** Salix *species Populus deltoides Sorghastrum nutans.*

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MBB 591: Master's Seminar

Name	: Sharat Prabhakaran	Venue	: Seminar hall
Admission no.	: 2018-11-165	Date	: 09-01-2020
Major Advisor	: Dr. Minimol J. S.	Time	: 10.45 am

Metallophytes, a unique biological resource: its molecular mechanisms and applications

Abstract

Heavy metal pollution has severely compromised the ability of the environment to foster life and render its intrinsic values. Anthropogenic activities such as mining, agriculture, industrialization have introduced heavy metals in large quantities into most of the soils, including agricultural lands, rendering them unfit for cultivation. Remediation of heavy metals requires special attention to protect soil, air and water quality and thus the human health. Physical and chemical remediation methods already developed are cost demanding, time-consuming and release additional waste into the environment. Hence, the use of plants for cleaning the soil, offers a cost effective, renewable and a promising method of remediation.

Metallophytes are endemic plant species of natural mineralized soils, hence they have developed physiological mechanisms of resistance and tolerance to survive on substrates with high metal levels (Baker and Walker, 1990). These plants have immense potential in phytoremediation and phytomining. However, only few metallophytes have been identified (250 species) till date, and their molecular mechanisms have to be fully understood. However, in general, molecular mechanisms that contribute to heavy metal hyperaccumulation in plants include, heavy metal uptake, complexation of heavy metals within plant cell, compartmentation of heavy metals within the cell, antioxidant defence and glyoxalase systems, modulation of transcription factors and synthesis of stress related metabolites (Sunitha *et al.*, 2013). A proteomic study conducted in *Phytolacca americana* revealed the roles of antioxidants and glutathione in Cd stress tolerance (Zhao *et al.*, 2011).

Phytoremediation is the use of metellophytes, along with proper soil amendments and agronomic techniques, to either contain, remove or render toxic environmental contaminants harmless (Schwitzguebel, 2000). Hyperaccumulator *Sedum alfredii* when exposed to high concentration of cadmium could accumulate 5367 mgkg^{-1} of cadmium in its shoots (Gao *et al.*, 2013). Instead of just cleaning the environment without any returns, some of the economically important elements can be recovered and income can be generated from it. The process of growing and harvesting selected

hyperaccumulator plants on an agronomical scale followed by metallurgical processing of the bio-ore to produce economically valuable trace elements is called phytomining (Van der Ent *et al.*, 1998). A comparative phytomining yield trial using hyperacumulators *Odontarrhena chalcidica* and *Noccaea goesingensis* yielded 53.6 kgha^{-1} and 34.2 kgha^{-1} of nickel respectively, in high density plantation (Rosenkranz *et al.*, 2019).

Phytoremediation, though much spoken about, is not widely followed due to the lack of local genotypes. The recent omics wave has resulted in the development of an ocean of techniques that are helpful in dissecting the phenotype and looking into the functional network of genes, transcripts and proteins in an organism. Identification of the candidate genes or regulators, can help to overcome these barriers by engineering local genotypes into highly efficient accumulators and thus make this long-awaited technology a success.

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