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### **The review of innovative approaches of bioremediation via phyto-remediation of toxic heavy metals from polluted soil**

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#### **ABSTRACT:**

Today, urbanisation and Industrialization in India raised problem of soil and water pollution in which heavy metal pollutants became eye-catching and very prominent recalcitrant compounds. Such metals if enters in food chain can cause deteriorative toxic effects and life threatening diseases in animals and humans. As a result, numbers of techniques were investigated to remediate them, one of them is bioremediation of heavy metals via plants and referred as Phyto-remediation which involves uptake of heavy metals by different plants and accumulation of metals ions within different plant parts ultimately results in removal and decrease in heavy metal content from polluted sites. Presented article contains research work in this area to identify different plants which possess potential to uptake heavy metals at higher rate and have been successfully identified and documented as potent heavy metal remediators. The different parameters which affect the rate of phyto-remediation is also reviewed successfully in presented article.

**KEYWORDS:** bioremediation, recalcitrant compounds, phyto-remediation, metal- remediators

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## INTRODUCTION:

Today, worldwide pollution problem is raised due to industrialization and dumping of heavy metal pollutants at number of sites within soil as well as water. The processes like mining, smelting and use of fertilizer, pesticides as well as release of sewage sludge also aid in deposition of toxic heavy metals<sup>1</sup>. Heavy metal contamination can be rectified by different methods including *insitu* and *exsitu* remediation. One of these methods is bioremediation which includes microorganisms and a new emerging and novel approach is phyto-remediation which is governed by plants. Various metal-ions are beneficial in trace amounts but when encountered in large quantities, impose toxic effects on cells. Such metals present within environment cannot easily be removed or degraded and called as recalcitrant compounds and thus accumulated within soil or water<sup>11</sup>.

Bioremediation by phytoremediation in case of heavy metals is an attractive alternative due to low cost and easy sequestering of metals and thus promising technique for cleaning up polluted sites. Plant based metal remediation technologies were as given below

- Phytodegradation: Organic pollutants if are degraded by plants and coupled microorganisms.
- Phytostabilisation: Plants decrease bioavailability and mobility of contaminant mainly by immobilization.
- Rhizofiltration: In this approach, metal ions are absorbed by plant roots.
- Phytovolatilisation: Plants govern volatilisation of contaminant.

The scheme of utilization of plants for remediation of heavy metals and other compounds was initially implemented in 1983 although the basic concept was evolved before 300 years<sup>26</sup>. There are many disadvantages related to physiochemical methods for soil and water remediation which are high cost and loss of fertility of polluted land are the most prominent. The phytoremediation is fascinating as it facilitates land restoration, deterioration of contaminant, conserve physical characters and biological activity of land, exclusively cheap and opportunity of metal bio-recovery is possible<sup>27</sup>.

### 1) Interaction of plants with heavy metals:

Formally metal hyperaccumulators are focused for treating deteriorated land due to heavy metal toxicants. Such plants possess inbuilt machinery to sequester definite toxic metal ions as part of their metabolism and thus become natural-remediators<sup>29</sup>. It has been reported that potential site of metal accumulation in phyto-remediators is the barks and shoots and thus more concentrated by toxicant even than roots. At some extent, such toxic metal accumulation is beneficial for plants to inhibit microbial growth and insect attack over them and their prevalence is more prominent at heavy metal polluted sites such as mining areas and industrial waste release sites<sup>26</sup>. There are diversifying modes by which a plant takes up metals such as apoplastic & symplastic pathways to arrive at transport

system such as xylem transfer which results in accumulation within different parts of plants<sup>20</sup>. Such accumulation may leads to hazardous effects because accumulated heavy metals can enter in to food chain if plant parts consumed by animals or humans. Thus, agro- invasion of such heavy metals must be avoided by keeping remediation sites distant and plants utilized must not used for intake<sup>30</sup>.The former reports revealed that the gloomy effects of heavy metals imposed not only on microbial world and animals but also on vegetation which cannot be ignorekd. Toxic metals adversely affect vegetation primarily by decrease in photosynthetic pigments and other consequences raised mainly by alteration of stomatal conductance, deterioration and decline in Xylem dimension as well as count and chloroplast number which leads to leaf weakening<sup>28</sup>.Martinez *et al.*<sup>31</sup>has documented that heavy metal exposure leads to gene expression modulation and thus metabolism is significantly altered within exposed plants. .

The heavy metals also beneficial in trace amounts because they are part of enzymatic systems in which act as cofactors, involved in electron transport and numerous regulatory actions at bimolecular level and thus categorized as Essential metals i.e. Mn, Ni, Fe, Cu and Zn. Whereas, other Nonessential metals like Cd, Pb, Hg are not possessing any identified biological actions. Although, both Essential and Non-essential heavy meals may mount ROS response by overproducing reactive oxygen species (ROS). Conversely, Nonessential metals affect adversely to cells potently by overtaking essential metals, promote alterations in biomolecules, upsetting essential enzyme complexes and mainly upset plant defense system by modulating antioxidant content as well as functions<sup>13</sup>.No doubt plants are more resistant and sturdy organism than any and thus they make their ways to withstand heavy metals mediated effects and employ diversifying strategies to adapt with heavy metal induced stress. Some of heavymetal tolerance is induced by processes like sequestration of metals, cell organelles trapping or compartmentalization of metal ions, increase of exudates secretion and inactivation<sup>13</sup>.

## 2) Heavy metal lethal effects:

The dangerous physiological effects of heavy metals have been reviewed which are as under:

- Chromium: It potential adverse effects are irritation in intestine and stomach, damage to brain and kidney cells and severe hair loss<sup>35</sup>.
- Zinc: Its significantly high exposure leads to dizziness and fatigue like symptoms.
- Cadmium: It imposed cancer inducing as well as mutagenic effects on cells. It's exposure leads to disturbance in calcium metabolism which leads to hyper calciuria. Renal dysfunction & anemic situations are also prominent effects of Cd toxicity<sup>13</sup>.

- Arsenic: Arsenite is more toxic to the cells than arsenate. Arsenate form acts as phosphate analogue and upon uptake become hurdle in ATP synthesis and process of oxidative phosphorylation.
- Nickel: It causes toxicity in various tissues like liver, brain and kidney. It targets immune system and can induce carcinoma of stomach and throat. The fumes of Ni can induce lung cancer.
- Lead: Documented consequences of lead exposure are cardiovascular complications, kidney failure, coordination complications, memory loss, decline in intelligence and learning problems in children<sup>35</sup>.

3) Plants having high potency of heavy metal remediation:

### **3.1 Approaches of phytoremediation:**

There are two classes or streams of such remediation have been analyzed which are natural and synthetic phytoextraction. Natural remediation is also called as continuous extraction and synthetic phytoextraction is governed by enhancement of remediation by different chemicals<sup>31</sup>. Former technique is very easy and governed by different exceptionally well working accumulator plants which are reviewed here. The research on different plants has governed in past to recognise such hyper accumulator plants. Such as *T. caerulescens* can remediate Zn significantly from the polluted soil samples i.e. 440 mg of Zn was reduced to 330 mg per Kg of soil<sup>4</sup>. Wie et al<sup>14</sup>. successfully reported that monocotyledon plant species are generally more forbearing to metals than dicotyledon plant species. Such potent remediator identified for Cd and Zn remediation is Maize and Indian mustard but the later one encountered more phytotoxicity due to Cd<sup>14</sup>. Other examples are Thlaspi Species (species of *Brassicaceae*) which is potent to accumulate up to 0.5% of Pb, 0.1% Cd and 3% Zn. The site where this accumulation was observed is shoots<sup>25</sup>. Brooks, 1997 reported other *Brassicaceae* species Alyssum which was capable to accumulate Ni over 1% content. Vegetative parts of trees revealed discriminated translocation of metals from root to different parts. As one study of Sycamore seedling study show stems and bark leaves contains less significant level of metals than roots. Different metals also revealed discrete sites of accumulation such as Pb which is not retained within roots was accumulated within tree barks whereas unretained Zn goes to leaves and accumulated over there<sup>32</sup>. Other researchers investigated tissues of birch and willow trees of natural inhabited at the site of industrial contamination like explosive factories and Cr processing firms. They discovered that actively diffusive tissue parts contains metal more compared to others<sup>30</sup>. Black et al.<sup>33</sup> developed poplar clones in lime changed soil and observed Zn and Cd focuses to be the most noteworthy in foliage. In four willow species developed on muck revised soil, foliage focuses were

more prominent than those in the stem for all assortments and metals<sup>33</sup>. Tripathi<sup>36</sup> saw accumulation of Cu, Pb in roots, Cr basically in the stems, while Zn, Cd and Ni were in the leaves.

Aside from amassing abnormal amounts of metal and translocating it to the harvestable portions of the plant, a plant reasonable for phytoextraction ought to develop quickly and achieve a high biomass. Cleaning destinations in a reasonable number of harvests requires plants that produce both a high biomass and gather in any event 1– 3% metal, by dry weight. There are hyperaccumulating plants that gather exceptionally large amounts of metals yet shockingly develop gradually and have a low biomass<sup>28</sup>. The metal accumulating plants can be isolated into three gatherings based on their propensity to aggregate unique metals: (1) Cu/Co; (2) Zn/Cd/Pb; and (3) Ni accumulators. Ways to deal with discover metal-tolerant hyperaccumulating plants for phytoremediation includes hunting down, and contemplating normal hyperaccumulators, or growing hereditarily built plants that have these qualities. The presentation of metal-restricting proteins and peptides into plants to upgrade metal resilience and additionally amassing is a convincing system. These metalbinding peptides or proteins ought to be specially metal explicit with the end goal that just the harmful metals are sequestered (for instance Cd, Hg and Pb) and not basic follow metals, for example, Zn<sup>36-39</sup>.

### **3.2 Phytoremediation by potent remediators:**

#### **3.2.1 Natural/ continuous Phytoextraction:**

There has been extensive research on various plant species to get better phyto extraction of heavy metals. Some of the plant species which were recognized as good remediators and were proven to act as hyper-accumulators of metals are *Asteraceae*, *Lamiaceae*, *Fabaceae*, *Euphorbiaceae*, *Scrophulariaceae* and *Brassicaceae*. Similarly, 45 discrete families of plants were identified to possess hyperaccumulation of heavy metals<sup>32</sup>. The really excellent remediation was reported in case of *T.caerulescens* which is commonly called as Alpine pennycress<sup>34</sup>. The more significance of this species is that resulted phytotoxicity is very low and accumulation up to 26 gms per kg Zn can be obtained and additional 20% Cd uptake was also revealed successfully<sup>25</sup>. The another good option explored and documented is Indian mustard (*Brassica juncea*) who has found to possess potency to uptake Lead and translocates it to the shoots. The phytoextraction coefficient of any species can be determined by taking ratio of metal content of exterior biomass of the plants and of content present within soil. Such ratio gives indication of phytotoxicity as well. For *Brassica juncea*, this ratio is 1.7. It is a good remediator of Lead and does not encounter any phytotoxicity even beyond 500 mg/l<sup>38</sup>.has documented that removal of 1.1500 kg per acre of lead can be effectively eliminated by *Brassica juncea*.The extensive remediation has been reported for high content > 1000 mg per Kg of Ni by

means of 310 species of plants. Similar reports on phytoremediation included Co (30 species), Zn (11 sps.), Mn (10 sps.), Cu (35 sps.), Pb(14 sp.) and Se (20 sps.) remediation by various species. The tolerance and threshold of such hyper-accumulators is very high and the reason behind it is that, their normal content in such plants is naturally very high whose typical range is 20 to 500 mg per kg<sup>21</sup>. Not only Aerial but aquatic plants were also been reported to have bioremediation potential. Research in this area, ended up in recognition of some potent species like water velvet (*Azollapinnata*), water hyacinth (*Eichhorniacrassipes*) and duckweed (*Lemna minor*) which were involved in phytoextraction and phytodegradation<sup>22</sup>. One of the investigation by Jin-Hong *et al.*, involved thirteen aquatic species out of which smartweed (*Polygonumhydropiperoides*) was recognized as excellent for heavy metal removal as it is fast growing as well as has very high foliage density<sup>20</sup>. Another plant species *Eichhorniacrassipe* was reported to govern removal of Platinum. Current research on Fern (*Pterisvitatta*) shown accumulation up to 14,500 mg per kg of Arsenic although didn't show toxic effect<sup>17</sup>. The process very feasible is rhizofiltration was conducted in course of bioremoval of Cr, Cu, Pd and Ni which was done by various plants like rye, Indian mustard, sunflower, tobacco, corn and spinach. Out of which, sunflower was the most potent remediator<sup>20</sup>.

### 3.2.2 Bioavailability of heavy metals and different factors influencing:

The bioavailability of heavy metals is referred as its available content present in soil and utilized to determine likelihood of risk of usage of such land. Such metals have atomic mass ranging from 64 to 200 and thus called as heavy metals which are required in very minute quantity for metabolism of cells and large quantity of them imposed gloomy effects on cell growth. Such nonessential heavy metals might be present in ionic, particulate, oxides or colloidal forms or might also remain in bound form with other compounds like oxide encrusted organic matter, organic clays and humic acids<sup>3</sup>. There are different parameters and factors which influence metal solubility within soil as well as ground water like cation substitute capacity, content of metals, carbon concentration, redoxpotential of the place, minerals oxidation status and pH<sup>7</sup>. Out of these, soil pH has been reported as an excellent influencing factor on retention or solubility of soil metal<sup>38</sup>. Higher pH reduces the solubility of the metals in soil where as in neutral to basic soil pH, cation of metals are efficiently adsorbed on the clay and such absorption can be promoted on hydrous oxides of aluminum, iron, manganese of soil minerals. If salt content raised in soil then salt ions will compete with metal ions for same binding site. Another parameter documented is that the presence of other heavy metal inhibit uptake of specific metal ions from soil as per example one research results shown

that Pb and Cu reduce the adsorption of Ni from the soil. Thus, bioavailability of different plants depends significantly on pH and presence of other toxic heavy metals present in sample<sup>7</sup>.

### **3.3 Mechanism of metal uptake and removal by plants:**

There are different means by which vegetation withstand as well as remove metals from contaminated soil. The significantly effective techniques involves metal-exclusion, metal accumulation and metal chelation.

*Metal exclusion:* Plants which apply this technique mainly restrict the metals to their roots and they keep metal from entering their aeronautical parts or keep up low and steady metal fixation over an expansive scope of metal focus in soil, they predominantly confine metal in their foundations. The plant may adjust its layer porousness, change metal official limit of cell dividers or radiate additionally chelating substances<sup>6</sup>.

*Metal accumulation:* Various plants are hyper accumulators and can concentrate it within various parts which are localized within roots, shoots and leaves. metal hyper-accumulator as plants that contain more than or up to 0.1% for example more than (1000 mg/g) of copper, chromium, cadmium, lead, nickel cobalt or 1% (>10,000 mg/g ) of zinc or manganese in the dry issue. For cadmium and other uncommon metals, it is > 0.01% by dry weight. Scientists have distinguished hyper-accumulator species by gathering plants from the regions where soil contains more prominent than normal measure of metals as if there should be an occurrence of contaminated zones or geologically wealthy in a specific component. Around 400 hyper-accumulator species from 22 families have been distinguished. The Brassicaceae family contains countless species with most extensive scope of metals, these incorporate 87 species from 11 genera (Ali et al.,2013).

*Metal indicators :*Species which effectively collect metal in their elevated tissues and by and large reflect metal level in the dirt. They endure the current fixation dimension of metals by creating intracellular metal restricting mixes (chelators), or adjust metal compartmentalization design by putting away metals in non-delicate parts<sup>1</sup>.

### **3.4 Limitations of use of plants for bioremediation of heavy metals:**

There are two major limitations of use of plants for metal remediation which are low bioavailability of metals and limited translocations of metals from root to shoots. The ideal characteristics of hyperaccumulator plants includes high resistance potential towards intense metal pollution, adjust and sustained within harsh environmental conditions at polluted sites, expanded roots, synthesis of high foliage and swift growth characteristics, agronomically favorable for further treatments, pest and disease tolerance, addible to animals and significant in other ways. Here the main important parameter is potency of translocation of metals from roots to other parts like



leaves and shoots. Such ideal characters are very hard to be found in one plant. The majority of hyper-accumulator plants which have been investigated were shown very low biomass production. Thus, trees which are swiftly grow as well as possess potent transpiration and very vast root system are considered and should be focused for significant phytoremediation. According to former reports of various researchers<sup>15-16</sup>. *Salix* spp, and *Populus* spp, were reported for extensive remediation of Cd and Zn removal from polluted soil samples (having moderate content of metal pollutant). The heavy metal polluted industrial sites also took advantage of fast growing woody plants for synthesizing renewable energy sources and phyto accumulation. The limitations of use of such woody plants are low level of accumulation by plants and weak bio-accumulation from the soil. Thus, less bioavailability of some heavy metals and less significant uptake of metals from the soil as per the example 0.04 to 0.25 % adsorption by roots of *Populus* trees are the major limitation of phytoremediation<sup>12</sup>.

### ***3.5 Recent innovations in the area of phytoremediation:***

Plants based bioremediation is depended on the utilization of natural as well as more recently genetically modified plants which can be applied to remediate heavy metal pollution via three discrete ways. First is beneficial metals can be extracted and recovered from plants, second is phytostabilization and reduction of pollution risk and third layer of use is raise in soil fertility for better crop growth of economical importance. There are many modern phytoremediation techniques are now available. The strategy of using aquatic plants in place of areal plants also provides a good option for better remediation. Other modification like application of sludge was reported beneficial during phytoremediation. One example of betterment in this area was investigated use of EDTA for Pb contaminated soil remediation as an improvisation. EDTA is an chelating agent which increase the bioavailability of Pb and also its translocation from root to shoot in various plants like pea and corn. Thus, high amount of such metals can be accumulated in other plant parts which is responsible for better remediation. Along with developing phytoremediators side by side decrement in phytotoxic effect also helps in improving better growth of biomass and ultimately following better remediation. It can be achieved by use of some mental adsorbents like beringite, a byproduct of burned coal. It immobilizes toxic metals there by reducing its phytotoxicity. The development of plants with higher potential of remediation can be achieved by various plant breeding techniques or by genetic manipulation which can be further augmented by better bioavailability and uptake of metals.



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