Journal of Bioremediation & Biodegradation

Jahnavi P et al., J Bioremediat Biodegrad 2020, 11:7

Review Article Open Access

Accumulation of Heavy Metals in Plant Parts during Phytoremediation

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Abstract

The primary source of metal pollution are the burning of fossil fuels, mining and smelting of metallic resources, downwash from power lines, municipal wastes, fertilizer, pesticides and sewage. Although adverse health effects of heavy metals have been known for a long time, exposure to heavy metal continues and is even increasing in some areas for example; mercury is still used in gold mining in many parts of Latin America. Arsenic is still common in wood preservatives and tetra-ethayl lead remains a common additive to petrol, although this use has decreased dramatically in the developed countries. Since the middle of the 19th century, production of heavy metals increased steeply for more than 1000 years, with concomitant emission to the environment.

Keywords: Bioremediation; Phytoremediation; Heavy metals; Polluted soils; Cadmium

Introduction

Heavy metals are commonly defined as those having a specific density of more than 5 g/cm3 and metallic elements with atomic number >20. The main threats to human health from heavy metals are associated with exposure to lead, cadmium, mercury and arsenic (arsenic is a metalloid, but is usually classified as a heavy metal). Heavy metals have been used in many different areas for thousands of years. Lead has been used for at least 5000 years, early applications including building materials, pigments for glazing ceramics and pipes for transporting water. In ancient Rome, lead acetate was used to sweeten old wine and some Romans might have consumed as much as a gram of lead a day. Claude Monet used cadmium pigments extensively in the mid 1800's, but the scarcity of the metal limited the use in artists' materials until the early 1900's.

Heavy metals contaminants that are commonly found in the environment are cadmium (Cd), chromium (Cr), copper (Cu), mercury (Hg), lead (Pb), nickel (Ni), and zinc (Zn). No doubt some of these are necessary for plant growth and are known as micronutrients such as Zn, Cu, Mn, Ni and Co, while others Cd, Pb, Hg have unknown biological function. Biological systems are affected by the metals and do not undergo biodegradation but can be accumulated in different organism thus causing various diseases and disorder even in relatively lower concentration.

Heavy metals are reported as priority pollutants due to their mobility in natural water ecosystem and due to their toxicity. Heavy metals cannot be destroyed biologically but only transformed from one oxidation state or organic complex to another as a result of the alteration of its oxidation state,

The metal may become either,

1) More water soluble and is removed by leaching.

2) Inherently less toxic.

3) Less water soluble so that it precipitates and then becomes less bioavailable or removed from the contaminated site, or

4) Volatilized and removed from the polluted area.

The primary source of metal pollution are the burning of fossil fuels, mining and smelting of metallic resources, downwash from power lines, municipal wastes, fertilizer, pesticides and sewage. Although adverse health effects of heavy metals have been known for a long time, exposure to heavy metal continues and is even increasing in some areas for example; mercury is still used in gold mining in many parts of Latin America. Arsenic is still common in wood preservatives and tetra-ethyl lead remains a common additive to petrol, although this use has decreased dramatically in the developed countries. Since the middle of the 19^{th} century, production of heavy metals increased steeply for more than 1000 years, with concomitant emission to the environment.

Emissions of heavy metal to the environment occur via a wide range of processes and pathways, including to the air (eg. During combustion, extraction and processing), to surface waters (via runoff and releases from storage and transport) and to the soil (hence into ground waters and crops). Atmospheric emissions tend to be of greatest concern in terms of human health, both because of the quantities involved and the widespread dispersion and potential for exposure that often ensues. The spatial distributions of cadmium, lead and mercury emissions to the atmosphere in Europe can be found in the meteorological synthesizing Centre–East website (http://www.msceast.org/hms/emission.html#spatial). Lead emissions are mainly related to road transport and thus most uniformly distributed over space. Cadmium emissions are primarily associated with non-ferrous metallurgy and fuel combustion, whereas the spatial distribution of anthropogenic mercury emission reflects mainly the level of coal consumption in different regions.

Datura stramonium L. and *Lantana camara* L. significance as phytoremediant for the contaminated soil with heavy metal

Datura stramonium could be good hyper accumulator for cadmium (II). *Datura stramonium* is widely distributed, reproduced easily by its seed and have strong ecological adaptability.

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Received date: October 27, 2020; **Accepted date:** November 10, 2020; **Published date:** November 17, 2020

Hence *Datura stramonium* has great potential for remediation and could be considered as hyper accumulator for cadmium (II) and chromium (VI) contaminated soils. Plant tolerance for cadmium (II) and chromium (VI) was evident in form of increase in shoot height and shoot dry matter of the plant. However, root biomass was significantly reduced in soils with increase cadmium (II) and chromium (VI). Accumulation of metals cadmium (II) and chromium (VI) in *Datura stramonium* induces stress and causes growth reduction [1].

Native plant species used for the phytoremediation process must generate large biomass and they can capable of accumulating high concentration of the toxic metal [2]. There were only few studies about *in vitro* culture of *Datura* and *Lantana*, but these plants are having enormous potential of heavy metal accumulation. Therefore, taking into consideration, the phytoremediation importance, it is necessary to study the effective tissue culture protocols for it. *Datura* constitutes a genus of nine species of flowering plants of the Solanaceae family. Its common name was Angel's Trumpets. Other plant *Lantana camara* is a native of West Africa and tropical Americas. It is commonly known as red sage, yellow sage, and *Lantana*. *Lantana* is an aromatic shrub having quadrangular stems and prickles on it.

Lantana camara L. which is a new plant owing to its remarkable capacity to extract lead and cadmium from polluted soils in Vietnam has been suggested as a model species for research on phytoextraction of metals. Moreover, this plant has a rapidly growing and developing very fast. They can also grow in extreme conditions and are able to endure long periods of drought or heavy rains. Finally, its multicolored flowers allow integrations into a floral arrangement of a landscaping project [3]. *Lantana camara* L. has capacity to grow well in soil contaminated with a high lead concentration. Efficient phytoextraction needs hyper extracting plants to remove heavy metals from polluted soils but also a high shoot to root for accumulating metals in the harvestable parts [4]. The remediation efficiency also depends on the amount of aboveground biomass and the bioavailability of metals [5].

Material and Methods

Quantitative estimation of cadmium and lead in the experimental soil (AOAC, 1990)

Prior to any experimental work, the soil to be used for the experimental purpose was analyzed for the content of Cd and Pb to assess the concentration of Cd and Pb already existing in the soil. Soil samples were dried in hot air oven at 80 ^{o}C for 48 hours or until the constant dry weight was attained. Dried soil was ground and powdered thoroughly and was used for analysis. 1 g dried soil sample was weighed and taken into a conical flask. 10 ml concentrated HNO_3 was added into it. The mixture was boiled at a constant temperature for about 45 mins. After cooling, 5 ml of 70% $HClO_4$ was added and the mixture was further boiled until the release of dense white fumes. After cooling, 20 ml distilled water was added and heated until a clear solution was obtained. At room temperature, the mixture was filtered through Whatman no. 44 filter paper and transferred quantitatively to a 50 ml volumetric flask by adding de-ionized and double-distilled water. Samples were analyzed through Inductively Coupled Plasma Mass Spectrometry (ICP-MS) for quantification of Cd and Pb. Results were expressed as mg/kg metal content in dry material of respective plant part. Three replicates were analyzed and mean was calculated. Results were expressed as mean ± S.D. (Standard Deviation). [Reference method of ICP-MS was APHA ICP 23rd Edition (section-3120 B)].

Quantitative estimation of cadmium (Cd) and lead (Pb) contents in plant parts (AOAC, 1990)

At maturity all plants were uprooted from the pots. Plants grown in each concentration were separated into root, stem and leaves and were dried in hot air oven at 80 ^{o} C for 48 hours or until the constant dry weight was attained. Each dried plant part of each concentration was ground and powdered thoroughly and used for analysis. 1 g dry powder of each sample was weighed and taken into a conical flask and 10 ml concentrated HNO_3 was added. The mixture was boiled at a constant temperature for about 45 mins. After cooling, 5 ml of 70% $HClO_4$ was added and the mixture was further boiled until the release of dense white fumes. After cooling, 20 ml distilled water was added and heated until a clear solution was obtained. At room temperature, the mixture was filtered through Whatman no. 44 filter paper and transferred quantitatively to a 50 ml volumetric flask by adding de-ionized and double-distilled water. Samples were analysed through Inductively Coupled Plasma Mass Spectrometry (ICP-MS) for quantification of Cd and Pb. Results were expressed as mg/kg metal content in dry material of respective plant part. [Reference method of ICP-MS was APHA ICP 23rd Edition (section-3120 B)].

Results and Discussion

Soil analysis reveal that parameters which are present in soil are suitable for growth of plants and it also reveals that Pb and Cd heavy metal are absent in selected soil. From the soil test we found presence of micronutrient which is Nitrogen, Phosphorus, Potassium, Boron, Sulphur and Manganese Table 1.

Soil sample	N(mg)	P(mg)	K(mg)	EC	pH	B(mg)	S(mg)	Mn(mg)	Pb(mg)	Cd(mg)
Control	0.7	0.2	0.14	0.35	7.35	0.15	0.12	0.1	Absent	Absent

Table 1: Assessment of Soil Analysis before the Experiment

Soil sample treated with heavy metal in which *Datura stramonium* L. were grown was analyzed for heavy metal accumulation Table 2.

Treatment	Pb and Cd in Soil (mg/kg)	
	65 DAS	95 DAS
Pb 600	3.589	2.157
Pb 700	3.524	1.986
Cd 15	3.628	1.872
Cd 20	0.398	0.218

Table 2: Lead and Cadmium Accumulation in Soil Sample with *Datura stramonium* L. after 65 DAS and 95 DAS

Soil sample treated with heavy metal in which *Lantana camara* L. were grown was analyzed for heavy metal accumulation Table 3.

Treatment	Pb and Cd in Soil (mg/kg)	
	65 DAS	95 DAS
Pb 600	3.589	2.157
Pb 700	3.524	1.986
Cd 15	3.628	1.872
Cd 20	0.398	0.218

Table 3: Lead and Cadmium Accumulation in Soil Sample with *Lantana camara* L. after 65 DAS and 95 DAS

Pb and Cd in Plant Part/Treatment	Pb and Cd in Leaves (mg)	Pb and Cd in Shoot (mg)
Pb 600	1.396	2.895
Pb 700	1.886	3.154
Cd 20	1.645	0.187

Table 4: Lead and Cadmium Accumulation in Stem and Leaves of *Datura stramonium* L. after 65 DAS

Pb and Cd in Plant Part/Treatment	Pb and Cd in Leaves (mg)	Pb and Cd in Shoot (mg)
Pb 600	2.359	4.220
Pb 700	2.416	4.316
Cd 20	2.156	0.340

Table 5: Lead and Cadmium Accumulation in Stem and Leaves of *Datura stramonium* L. after 95 DAS

Pb and Cd in plant part/Treatment	Pb and Cd in leaves (mg)	Pb and Cd in shoot (mg)
Pb 600	1.504	0.428
Pb 700	1.761	0.647
Cd 15	0.241	0.073

Table 6: Lead and Cadmium Accumulation in Stem and Leaves of *Lantana camara* L. after 65 DAS

Pb and Cd in plant part/Treatment	Pb and Cd in leaves (mg)	Pb and Cd in shoot (mg)
Pb 600	2.594	0.869
Pb 700	1.621	1.352
Cd 15	0.357	0.089

Table 7: Lead and Cadmium Accumulation in Stem and Leaves of *Lantana camara* L. after 95 DAS

Accumulation of lead and cadmium in soil gradually decreasing as increasing days of plant. After 65 DAS uptake of different concentration of lead heavy metal shows more result and cadmium 15 mg/kg concentration shows more result in compare to other concentration of cadmium. After 95 DAS uptake of heavy metal by soil was less in compare to 65 DAS. All concentration of lead and cd 15 mg/kg concentration shows more result in compare to cd 20 mg/kg. From the result we interpret that deterioration of heavy metal was started in soil as this heavy metal was uptakes by plants. In general, the plant showed very good capability of accumulating Pb and Cd from the soil. This indicating that soil had accumulated heavy metal in good amount and did not show any negative effect on growth of plant.

Our results are in confirmation with that the shoot metal concentration of plants can partially reflects the efficiency of plants on the remediation of soil heavy metals. Thus, the ratio of shoot metal concentration to total soil metal concentrations can also partially reflect the ability of plants to absorb soil heavy metals and transport them to shoots was reported by [6,7].

The data correspond with those by [8] who reported that excessive amounts of toxic elements in contaminated soil inhibited plant growth and development due to their phytotoxicity. Reduced growth observed at contaminated treatments may be partly due to lower net photosynthetic rate, but not exclusively, since it was argued that the reduced growth might be also due to increased tissue permeability. It might also result from inhibition of cell division was reported by [9]. Reduction in growth can be linked to the high trace elements accumulation, as in this case plants have to spend extra energy to cope with the high trace element concentrations in the tissues was reported by [10].

Study of the Accumulation of Heavy Metal in Plant Parts

Cd and Pb accumulation in *Datura stramonium* L

Accumulation of lead and cadmium content in plant parts gradually increased with the increasing concentration in soil. The results related to the uptake of Pb and Cd after 65 Day after sowing (DAS) and 95 Day after sowing (DAS) in this study suggest that shoot of *Datura stramonium* L. are efficient barriers to Pb translocation. Shoots were storing highest amount of Pb followed by leaves. The result related to the uptake of Cd translocation in this study suggest that leaves of *Datura stramonium* L. are efficient barriers to Cd translocation. Leaves were storing highest amount of Cd followed by shoot. Stem and leaves have been found to accumulate Pb and Cd almost equivalent to its concentration in the soil in all concentration under study. In general, the plant showed very good capability of accumulating Pb and

Cd from the soil. Table 4 and Table 5 represent the accumulation of Pb and Cd in shoot and leaves of *Datura stramonium* L. during plant growth.

Accumulation of lead and cadmium content in plant parts gradually increased with the increasing concentrations in soil. The results related to the uptake of Pb after 65 Day after sowing (DAS) in this study suggest that shoot of *Datura stramonium* L. are efficient barriers to Pb translocation. All treated concentration shows accumulation of heavy metal in leaves and shoot. Shoot had more heavy metal accumulation in compare to leaves however in cadmium treatment leaves shows more accumulation of heavy metal in compare to shoot. Where shoot accumulation was more in compare to leaves. The result related to the uptake of Cd and Pb translocation in this study suggest that leaves and shoot of *Datura stramonium* L. are efficient barriers to Cd and Pb translocation. Shoots were storing highest amount of Pb followed by leaves and leaves were storing highest amount of Cd followed by shoot. This indicates that *Datura* plants have a great potential to bioaccumulate the heavy metal and can be used to remediate polluted soils.

Accumulation of lead and cadmium content in plant parts gradually increased with the increasing plant growth. The results related to the uptake of Pb after 95 Day after sowing (DAS) in this study suggest that shoot of *Datura stramonium* L. are efficient barriers to Pb and Cd translocation. After 65 DAS accumulation of heavy metal by leaves and shoot were less in compare to 95 DAS. As plant growth is increasing accumulation capacity of plant is also increasing. All treated concentration shows accumulation of heavy metal in leaves and shoot. Shoot had more accumulation of heavy metal in compare to leaves. However, in cadmium treatment leaves shows more amount of heavy metal accumulation in compare to shoot. The result related to the uptake of Cd and Pb translocation in this study suggest that leaves of *Datura stramonium* L. are efficient barriers to Cd and Pb translocation. Shoots were storing highest amount of Pb followed by leaves and leaves were storing highest amount of Cd followed by shoot and root.

Cd and Pb accumulation in *Lantana camara* L

Accumulation of Lead and Cadmium content in plant parts gradually increased with the increasing concentration in soil. The results related to the uptake of Pb and Cd after 65 Day after sowing (DAS) and 95 Day after sowing (DAS) in this study suggest that leaves of *Lantana camara* L. are efficient barriers to Pb and Cd translocation to the above ground plant parts. Leaves were storing highest amount of Pb and Cd followed by shoot respectively. Stem and leaves have been found to accumulate Pb and Cd almost equivalent to its concentration in the soil in all concentration under study. In general, the plant showed very good capability of accumulating Pb and Cd from the soil. Table 6 and Table 7 represent the accumulation of Pb and Cd in shoot and leaves of *Lantana camara* L during plant growth.

Accumulation of lead and cadmium content in plant parts gradually increased with the increasing concentrations in soil. The results related to the uptake of Pb and Cd after 65 Day after showing (DAS) and 95 Day after showing (DAS) in this study suggest that *Lantana camara* L. is efficient barriers to Pb and Cd translocation to leaves and shoot part. After 65 DAS accumulation of heavy metal by leaves and shoot were less in compare to 95 DAS. As plant growth is increasing accumulation capacity of plant is also increasing. From the above result we conclude that leaves of *Lantana* plant accumulate maximum amount of heavy metal. However, shoots were also storing heavy metal but in compare to leaves the result were minimum. Leaves were storing highest amount of Pb and Cd followed by shoot respectively. Stem and leaves have been found to accumulate Pb and Cd almost equivalent to its concentration in the soil in all concentrations under study. In general, the plant showed very good capability of accumulating Pb and Cd from the soil. This indicates that both plants have a great potential to bio accumulate the heavy metal and can be used to remediate polluted soils.

Our results are in confirmation that shoot metal concentration of plants can partially reflect the efficiency of plants on the remediation of soil heavy metals. Thus, the ratio of shoot metal concentration to total soil metal concentrations can also partially reflect the ability of plants to absorb soil heavy metals and transport them to shoots. [6,7].

The primary effect of heavy metal toxicity in plants is a rapid inhibition of root growth, probably due to inhibition of cell division in the root tip as suggested by [11]. Plant age generally affects the physiology of roots and roots of a young plant have a greater potential to absorb ions than those of an older plant as observed by [12,13] Reported that the root growth, root cell viability in rice seedlings were affected by heavy metals and root cell death was remarkably increased with heavy metal concentrations. Low amounts of heavy metals caused significant reduction in growth of lettuce and carrot roots [14]. Inhibitory effects of heavy metals on growth biomass production may possibly derive from effects on metabolic plant processes.

Metals disturb plant development and metabolism at different stages of growth. An essential metal can affect plants when present in either deficient or excess amounts. With most of the elements, plant growth and yields are enhanced as the metal uptake is increased; metal uptake beyond a certain level however may either have no effect or induce toxic effects that lead to decreased growth and yields. In the case of nonessential elements, deficiency-toxicity symptoms are not observed. The initial supply of these metals does not affect plant growth, but above a certain concentration, the plant can exhibit toxic symptoms [15].

Plants capable of accumulating extraordinarily high levels of metals are referred to as hyperaccumulators [16]. Among various plants, *Alyssum*, *Brassica*, and *Thlaspi* have been found to be the most effective and dominant metal-tolerant and accumulating plants, although only a few species of each genus have been confirmed as hyperaccumulators [17]. Hyperaccumulators tend to accumulate either Ni, Cu/Co, or Cd/Pb/Zn [18]. Some metals are more amenable to hyper accumulation, due to their availability in soil. Hyperaccumulators may play some roles in the phytoremediation of metals by phytoextraction [19-21]. Hyper accumulating plant species are often capable of survival in soils where other crops may fail, as they can tolerate abnormally high levels of metal in soils and tissue.

Plant species have variety of mechanisms for removing and accumulating heavy metals and some species can take much heavier metals than others. The two basic strategies of metals uptake related to tolerance in plants involve the excluder strategies in which the concentration of metal is maintained at a constant low level and the accumulator strategies in which metals are actively concentrated within the plant tissues [3]. The most of the heavy metal taken up by plants is restricted to root and very small amount is transported to the shoots, however, plant leaves show obvious symptoms of Hg and Pb toxicity through reduction in their number, size, fresh and dry weights, as well as changes in photosynthetic pigments. It can also affect the leaves via changes in other morphological parameters like leaf length and width as well as thickness of leaf blade, cuticle, spongy and palisade mesophyll cells, and changes in shape, number and size of stomata, etc. Heavy metals are also known to affect photosynthesis by inhibiting activities. Water imbalance, alterations in membrane permeably and disturbs mineral nutrition [22].

[23] Reported that pollutants may gain entrance into the leaf via the stomata. [24] Had earlier reported that atmospheric deposition is one of the major pathways by which metal enters tree leaves. This study showed that the root might have acted as a storage organ of these minerals after uptake from the soil before being transferred to other parts of the plant. This was especially true for all the metals since higher concentration of trace metals was recorded from the root and lower concentrations from either the stem or leaf [25,26]. The high concentration of the metals in the roots of both plants may not pose any serious damage because only the leaves and stems of both plants are either consumed or used as medicinal herbs. However, periodic monitoring programme should be encouraged especially when collecting the plants from areas that are close to pollution since pollutants such as trace metals may enter the leaves via the stomata.

Conclusion

Our results are similar with Clemens who reported that, Heavy metals are not retained in the root, but they are translocated to the shoots and accumulated in aboveground organs, especially in leaves, at concentrations 100-1000-fold higher than those found in non-hyper accumulating species. After the uptake of heavy metals by plant roots, their translocation to shoots and detoxification within the storage sites are two critical steps. This is achieved by chelation, transport, trafficking, and sequestration by organo ligands at a subcellular level.

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