



## PHYTOREMEDIATION OF LEAD CONTAMINATED SOIL WITH THE HELP OF PLANT *BAMBUSA VULGARIS*

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### ABSTRACT

Heavy metals are naturally occurring elements in soil & are found throughout the Earth's crust, yet some anthropogenic activities increases the concentrations in the environment. The present study is a step to solve the problem of high level of Pb in ecological system. Plant *B.vulgaris* is a very good tool for bioremediation to remove Pb from soil. The level of Pb in different parts of *B. vulgaris* increases from 119 to 181% of control. It indicates that the plant has great potential to remediate Pb from the soil. Pb in the soil of university campus was very low (2.32 mg/kg). Level of Pb in root, stem and leaf of *B. vulgaris* was 0.23, 0.28 and 0.27 mg/kg, respectively. Now, this plant was grown in soil containing 12 mg/kg and 72 mg/kg Pb for 3 months. After 3 months, again concentration of Pb in the soil, root, stem and leaf of root was measured. It was observed that Pb concentration in root and shoot were significantly increased with respect to control group of plants. The present study also supports the fact that use of *B. vulgaris* in traditional Hindu way of life is very important. It's use in marriage ceremony as one of the plant that is planted in the worship place, as well as in any pooja ceremony.

**KEYWORDS:** Soil, Metals, Remediation, phytovolatilization.

### INTRODUCTION

Global industrialization, war, nuclear processes and modern agricultural activities accelerates the pollution of biosphere, especially the Soil. The risk to human and environmental health is increasing. Cocktail of pollutants is the chief contributor of the global epidemic of cancer, and other degenerative diseases. The Soil is the dynamic component of nature. Complex system of soil involves chemical, biological, physical, geological, reactions simultaneously, which vary with time and space. Nowadays, soil pollution is getting considerable attention of scientist, social workers and public. Soil is the primary recipient of the heavy metals, which enters in the plants and ultimately in the food chain and food web (Abii, 2012; Schickler et al., 1999). Although heavy metals are naturally occurring elements in soil & are found throughout the Earth's crust, yet some anthropogenic activities increases the concentrations in the environment. Heavy metals poses a huge threat to human health because once they enter the body the destruction that occurs is usually irreversible.

Heavy metals are the metallic elements that have specific gravity greater than 4.0. It is at least 5.0times greater than water (Akan et al., 2010) and an atomic number >20 (Jadia et al., 2008). Heavy metals percent concentration in rock composition does not exceed 0.01% (Zovko et

al., 2011). Heavy metals are also considered as trace metals because of their presence in trace concentration (in ppb) in various environmental matrixes (Friedland, 1990). They are the most dangerous substances known in environment due to their high level of durability, toxicity to the biota and their bioaccumulation and magnification in tropic levels.

They are tends to adsorb very firmly to the soil matrix and once released to the environment, they won't degrade like organic materials by microbial action through chemical oxidation. They can only transform from one oxidation state or organic complex to another. At low concentration they can cause toxic and mutagenic effects to human and other forms of life (Das, 1990).

In soils, Pb may occur as a free metal ion, complexes with inorganic constituents (e.g.,  $\text{HCO}_3^-$ ,  $\text{CO}_3^{2-}$ ,  $\text{SO}_4^{2-}$ , and  $\text{Cl}^-$ ), or may exist as organic ligands (e.g., amino acids, fulvic acids, and humic acids); alternatively Pb may be adsorbed onto particle surfaces (e.g., Fe-oxides, biological material, organic matter, and clay particles) (Uzu et al., 2009).

Pb<sup>2+</sup> (Ionic Pb), Pb-metal oxyanion, Pb oxides and hydroxides complexes are the general forms of Pb that are released into the environment. The most stable forms

of Pb are  $Pb^{2+}$  and Pb-hydroxy complexes.  $Pb^{2+}$  is the most common and reactive form of Pb, forming mononuclear and polynuclear oxides and hydroxides (GWRTAC, 1997).

The predominant insoluble Pb compounds are Pb phosphates, Pb carbonates (form when the pH is above 6) and Pb (hydr) oxides (Raskin et al., 2000). Pb sulfide ( $PbS$ ) is the most stable solid form within the soil matrix and forms under reducing conditions when increased concentrations of sulfide are present. Under anaerobic conditions a volatile organolead (Tetra Methyl Lead) can be formed due to microbial alkylation (GWRTAC, 1997).

Pb poisoning has been identified as “silent epidemic” (Mielke, 1999). Pb is known to induce a broad range of toxic effects to living organism, including those that are morphological, physiological and biochemical in origin. Pb exposure decreases the concentration of divalent cations ( $Zn^{2+}$ ,  $Mg^{2+}$ ,  $Ca^{2+}$ , and  $Fe^{2+}$ ) in human body. The biological half-life of Pb is about 35 days in blood, while in the brain it is about 2 years, and in bone Pb lasts for decades.

#### Effects on humans;-

Pb is a cumulative toxicant that affects multiple body systems including neurological, hematological, gastrointestinal, cardiovascular system. In the human body, the greatest percent of Pb is taken into the kidney followed by liver and then other soft tissues such as heart and brain. However the Pb in the skeleton represents the major body fraction. It stored in tooth and bones, where it accumulates over time. Pb has been listed as a potential carcinogen in the EPA Toxic Release Inventory (TRI) (Fergusson, 1986).

Adults absorb about 20% of Pb. Pb is particularly dangerous to children because their growing bodies absorb more Pb than adults do and their brains and nervous systems are more sensitive to the damaging effects of Pb. Children absorb up to 70% of Pb. There are no immediate and obvious symptoms. In pregnant women Pb can pass from mother to her unborn baby that leads to Miscarriage, Still birth, Pre mature birth and low birth weight, as well as minor malformation.

#### Acute effect

Acute adverse effect is usually observed only following short term exposure to high concentration. It may leads to gastrointestinal disturbance (anorexia, nausea, vomiting, and abdominal pain), hepatic and renal damage, hypertension and neurological effects (malaise, drowsiness, and encephalopathy) that may Pb to convulsions and death.

#### Chronic effects

Chronic effects of Pb exposure cause hematological effects (anaemia) or neurological disturbances (including headache, irritability, lethargy, convulsion, muscle

weakness, ataxia, tremors and paralysis). The long term occupational exposure to Pb may contribute to the development of cancer.

#### Effects on Plants

Total Pb concentrations above 100 ppm (mg/kg) in soil are regarded as toxic for plants (Kabata-Pendias, 1992). Pb accumulates in bodies of aquatic and soil organisms. These all will experience health effects from Pb poisoning. Soil containing high Pb concentration is unsuitable for plant growth and destroys the soil biodiversity. Pb caused phytotoxic effect including chlorosis, necrosis, stunt growth of root/shoot, and less biomass production on *Helianthus annuus*, *Nicotiana tabacum* and *Vetiveria zizanioides* (Djingova and Kuleff, 2000).

Many studies have shown that Pb inhibits metabolic processes such as nitrogen assimilation, photosynthesis (by inhibiting enzymes unique to photosynthesis), cell division, respiration, water absorption and transcription, (Boussama et al., 1999; Kurepa et al., 1997) accelerate abscission or defoliation and pigmentation, and reduces chlorophyll and adenosine triphosphate (ATP) synthesis. Pb may inactivate various enzymes by binding to their SH-groups (Rauser, 1995), and can intensify the processes of reactive oxygen species (ROS) production leading to oxidative stress (Cuyper et al., 1999; Prasad, 1999). In addition, Pb can negatively affect mitochondria structure by decreasing the number of mitochondrial cristae, which in turn can lower the capability of oxidative phosphorylation (Malecka et al., 2001).

India seems to be potential Pb consuming country as we foresee 10-12% per annum growth in lead Acid Battery market between 2012- 2020. By in 2020 India should surpass Europe and will be 3rd largest consumer of Pb with expected demand of over 2.2 million tones. Pb tends to stay in the top six inches of soil. It is a poison when ingested even in small amounts. Hence it is very important to extract out this metal from the soil by using phytoremediation technique. Pb is Bioaccumulated by terrestrial and aquatic plants and animals. However, Pb is not Biomagnified in terrestrial or aquatic food chains.

#### Metal Remediation Measures

Heavy metal contamination of soil may pose risk and hazards to humans and the ecosystem hence it is necessary to remove metals from the soil. Soil remediation is defined as ‘The return of soil to a condition of ecological stability together with the establishment of plant communities it supports or supported to conditions prior to disturbance’ (Allen, 1988).

The current chemical and physical methods (like Chemical precipitation, lime coagulation, ion exchange, reverse osmosis and solvent extraction) of metal removal is very aggressive, expensive, time consuming, non-

environmental friendly reduces the soil fertility and disrupted its structure.

Hence, the biological method is the best alternative method for metal removal because of its cost effective and eco-friendly (Boonyapookana et al., 2005; Su and Wong, 2004). Biological method of soil remediation is of two types (i) bioremediation and (ii) phytoremediation

Bioremediation is the use of microorganism to remediate a contaminant into a non harmful substance. It is a natural process which relies on bacteria, and fungi to alter contaminants as these organisms carry out their normal life functions. These organisms are capable of using chemical contaminants as an energy source during their metabolic processes, rendering the contaminants harmless or less toxic products in most cases. Thus, it provides an alternative tool to destroy or render the harmful contaminants through biological activity and this method is cost effective.

Phytoremediation (green remediation, botano remediation, agro remediation) involves the use of green plants to decontaminate soils, water and air. The generic term "Phytoremediation" consists of the Greek prefix phyto (plant), attached to the Latin root remedium (to correct or remove an evil) (Cunningham et al., 1996; U. S. Environmental Protection Agency, 2000; Erakhrumen and Agbontalor, 2007).

It is an emerging technology that can be applied to both organic and inorganic pollutants present in environment. It can be defined as "The use of plants, including trees and grasses, to remove, destroy or sequester hazardous contaminants from media such as air, water, and soil".

The idea of using metal accumulating plants to remove heavy metals and other compounds was first introduced in 1983, but the concept has actually been implemented for the past 300 years on wastewater discharges (Chaney et al., 1997; Henry, 2000). Phytoremediation is energy efficient, aesthetically pleasing approach most suitable for developing countries. It is considered to be a "Green Revolution" in the field of innovative cleanup technologies.

Phytoextraction (Phytoaccumulation):- In this process contaminants are directly accumulate into plant roots and translocate them to the above ground shoot and leave. Certain plants called "Hyperaccumulators" (McIntyre, 2001) absorb unusually large amounts of metals in comparison to other plants and the ambient metal concentration without showing toxicity (Klassen et al., 2000; Bennett et al., 2003). For a plant to be classified as a hyper accumulator, it must be able to accumulate at least 1,000 mg/kg (dry weight) of a specific metal or metalloid (for some metals or metalloids, the concentration must be 10,000 mg/kg) (Baker, 1981). Some known hyper accumulators for Pb are Indian

mustard, Corn, Ragweed, Turnips, Sunflowers, Broccoli and Pennycress (Raskin, 1997).

Phytodegradation: Some plants contain enzymes that can breakdown and convert ammunition wastes, Chlorinated solvents such as trichloroethylene and other herbicides. The enzymes are usually dehalogenases, oxygenases and reductases. Hence the Phytodegradation is the breakdown of organic taken up by the plants to simpler molecules that are incorporated into the plant tissue.

Phytovolatilization: Phytovolatilization is the volatilization of contaminants from the plant either from the leaf stomata or from plant stems (Anonymous, 2009 ITRC). Phytovolatilization has been widely observed for a number of contaminants, both inorganic and organic. Volatile forms of several inorganic compounds can be volatilized from plants, including Se, As (Sakakibara et al., 2010; Jia et al., 2012) and Hg (Wang et al., 2012). It is primarily used for the removal of mercury.

Phytostabilization: It is mostly used for the remediation of soil, sediment and sludge and depends on roots ability to limit contaminant mobility and bioavailability in the soil.

It can occur through the sorption, precipitation, complex action, or metal valence reduction. Most commonly plants with fibrous root systems are used in the phytostabilization, such as many grasses, herbaceous species, and wetland species. It is useful for the treatment of Pb as well as As, Cd, Cr, Cu and Zn (United States Protection Agency, 2000).

Rhizodegradation (phytostimulation): It involves the breakdown of organics in the soil through microbial activity of the rhizosphere. It is the much slower process than phytoextraction.

Rhizofiltration: Rhizofiltration is the use of plant (terrestrial and aquatic) roots or seedlings (blastofiltration) to absorb concentrate and/or precipitate hazardous compounds from the contaminated site. Rhizofiltration can be used for Pb, Cd, Cu, Ni, Zn, and Cr, which are primarily retained within the roots (United States Protection Agency, 2000).

All Phytoremediation technologies are not exclusive and may be used simultaneously. The plants act both as "accumulators" and "excluders". Accumulators survive despite concentrating contaminants in their aerial tissues. They are biodegrade or biotransform contaminants into inert forms in their tissue. The excluders restrict contaminant uptake into their biomass (Sinha et al., 2004).

#### Sample collection

Plant collection: Plants used in the project was *Bambusa vulgaris* and were collected from a park.

Description of experimental plant: - *Bambusa vulgaris* (Bamboo) belongs to the family of grasses, Gramineae (Poaceae) native to Indo-China. It is also widely cultivated and is naturalized in subtropical and tropical areas of the world. It prefers lowland humid habitats, but tolerates a wide range of climatic conditions and soil types (United Nations, 1972). For a successful remediation plant must grow quickly to accumulate more metal faster accumulating a decent amount of metal for each harvest.

There is an estimated 1000 sp. of Bamboo belonging to about 80 genera in the world. Bamboo has many environmental benefits such as - Function as carbon sinks, Control soil erosion, Provide organic matter, Regulate water levels in watersheds, Conserve biodiversity, Beautify the landscape, Essentially contribute to the purification and regulation of the environment.

Furthermore, it is used to remediate heavy metal contaminated soil especially Pb contamination. In ancient time people didn't burn bamboo woods because of its phytoextraction property of Pb. Bamboo grows 1.6 feet a day and able to remove high amount of Pb from the soil comparison to other phytoremediation plant. Bamboo is the strongest and fastest growing woody plant on earth, with some species achieving the phenomenal growth rate of one meter /day (Chin, 2005). A number of factors influence the concentration of heavy metals on and within plants. These factors include climate, atmospheric deposition, the nature of the soil on which the plant is grown and the degree of maturity of the plant at the time of harvesting.

Soil collection: - The soil sample used for this study was collected from Deen Dayal Upadhyaya campus, Gorakhpur outside the research building in September 1, 2017. The soil sample was collected from the depth of 0 – 6 cm.

Preliminary soil and plant sample preparation: The soil sample were spread on glass plate and air dried for 5-6 days in sunlight and mixed thoroughly to achieve homogeneity. Then all the soils were sieved through a 2mm stainless steel sieve and stored in a plastic bag at room temperature until use. All the Bamboo plants were washed thoroughly with tap water followed by de-ionized water. Then different parts of plants (leaf, shoot and root) were collected in petriplates. 3 gram soil and plant parts were oven dried at 90°C for 24 hours. Dried samples of plants were gently grounded using acid washed porcelain pestle and mortar.

Analysis / Digestion procedure: All samples were accurately weighed (1.0g each) and placed in a 100 mL beaker. 10 mL concentrate HNO<sub>3</sub> was added in each beaker and kept overnight. Residues were dissolved again with 10 mL concentrate HNO<sub>3</sub> followed by 10 mL 2M HCl and heated on hotplate till dryness. Left residues were warmed in 20 mL of 2M HCl to re-dissolve the metal salts.

On completion of digestion, the samples were cooled and filtered through 60 Whatman filter papers in borosilicate funnels into 150 mL boro silicates beakers. The final volumes of samples were made up to 25 mL with de-ionised double distilled water.

**Table 1: Level (mg/kg) Pb in different parts of *B. vulgaris* before and after bioremediation.**

| S. No. | Sample | Before remediation (mg/kg) | After remediation (mg/kg)   |                             |
|--------|--------|----------------------------|-----------------------------|-----------------------------|
|        |        | Control (C <sub>1</sub> )  | 12 mg/kg                    | 72 mg/kg                    |
| 1.     | Soil   | 2.32                       | 0.09 C <sub>1</sub> =3.6%   | 0.43 C <sub>1</sub> =18.7%  |
| 2.     | Root   | 0.23                       | 0.40 C <sub>1</sub> =172.6% | 0.41 C <sub>1</sub> =176.0% |
| 3.     | Stem   | 0.28                       | 0.50 C <sub>1</sub> =181.2% | 0.39 C <sub>1</sub> =138.4% |
| 4.     | Leaf   | 0.27                       | 0.50 C <sub>1</sub> =180.6% | 0.24 C <sub>1</sub> =119.7% |

Standard preparation: Standard solution was prepared by dissolving known concentration (1, 2, 5 and 10 ppm) of Pb in 25 mL double distilled water. All prepared solution was then transferred to labeled beakers for Pb analysis using Atomic Absorption Spectroscopy.

*Bambusa vulgaris* was grown in greenhouse condition to evaluate its potential to bioaccumulate, translocate and remediate lead (Pb) from contaminated soil.

## RESULTS

Soil for experiment purpose was collected from DDU Gorakhpur University campus. Measurement of Pb level was done with the help of Atomic Absorption Spectroscopy (Perkin Elmer analyst 400). Pb in the soil of university campus was very low (2.32 mg/kg). Level of Pb in root, stem and leaf of *B. vulgaris* was 0.23, 0.28

and 0.27 mg/kg, respectively. Now, this plant was grown in soil containing 12 mg/kg and 72 mg/kg Pb for 3 months. After 3 months, again concentration of Pb in the soil, root, stem and leaf of root was measured. It was observed that Pb concentration in root and shoot were significantly increased with respect to control group of plants (Table).

Lead (Pb) concentrations in different tissues of *B. vulgaris* grown in soil containing 12 mg/kg were 0.40, 0.50 and 0.50 mg/kg and in 72 mg/kg 0.41, 0.39 and 0.33 mg/kg in root, stem and leaf, respectively. Increase in Pb level in *B. vulgaris* grown in soil containing 12 mg/kg Pb, the root, stem and leaf was 172.6%, 181.2% and 180.6% control, respectively. Whereas, increase in Pb in 72 mg/kg plant *B. vulgaris* root, stem and leaf was 176.0%, 138.4% and 119.7% of control, respectively. Maximum

Pb was measured in *B. vulgaris* stem (181.2% of control). After the remediation of soil it has noted that both the treatments, there was significant decrease in concentration of Pb level in soil of treatments. There was decrease significant 18.7 % and 3.6% Pb level was noted in soil. Earlier, it has been reported that there was a significant high level of Pb deposition on the leaf of plants grown on road side (Agarhari et al., 2017). Pb deposition on the leaf of *F. religiosa* was 10.3 and 14.1 mg/kg.

## DISCUSSION

Soil is precious natural assets used for sustainable agriculture since a long time. It is very difficult to define soil quality as soil composition can vary from place to place. Intensive human activities has severely degraded or contaminated the soil natural composition. Soil is open to inputs of heavy metals from many sources as it is at the interface between the atmosphere and the earth's crust, and the substrate for natural and agricultural ecosystems (Alloway, 1990).

Inorganic pollutants which contaminate land and water bodies include heavy metals, metalloids, fluoride and cyanide etc. Contaminated soils are a common environmental problem all over the world.

Heavy metal has metallic properties (ductility, conductivity, density, stability as cations, ligand specificity, etc.) and an atomic number greater than 20. The presence of heavy metals in the environment is of great ecological significance due to their toxicity at certain concentrations, translocation through food chains and non-biodegradable these metals can undergo global ecological cycles responsible for their accumulation in the biosphere. Heavy metals can occur in different valence states, so that one element may be more or less toxic in different states. Normally heavy metals in the environment are in low concentrations. Human activities such as fossil fuel combustion, mining, smelting, and sludge amendment to soil, fertilizer application, and agrochemical application has increase the level of heavy metals in ecological cycles.

Soil contamination with heavy metals may also cause changes in the composition of soil microbial community, and adversely affecting soil characteristics. They can also block the photosynthetic electron transport chain and thus degrade chlorophyll pigment.

In sequence to sustain good quality of soil, and keep it free from heavy metals, continuous efforts have been made to build up technologies that are easy to use, sustainable and economically reasonable.

Traditional physicochemical practices have been extensively used to remediate and recover contaminated soil especially on small scale. However, these practices are more intricacy for a large scale of remediation due to high costs and side effects. Concentration of Pb in the

soil which could be as a result of its sources from automobile exhaust fumes as well as dry cell batteries, leaded gasoline, sewage effluents, runoff of wastes and atmospheric depositions could cause its bioaccumulation in plant via uptake from the soil and eventual entry into the food chain.

Pb was long ago phased-out of the automobile gasoline, but it is still in aviation fuel and is now the largest source of lead emission in India. India civil aviation sector continue to experience high passenger growth and if the trend continue it could rank among the top three aviation market in the world by 2020. Once the Pb was phased - out of automotive gasoline beginning in the 1970s. "Aviation fuel is a specialized type of petroleum (leaded) based fuel used to power piston engine aircraft".

Octane is a measure of the performance of a fuel as it burns in an engine combustion chamber. It is a measure of a gasoline's ability to resist detonation, or knock. Operating aircraft or automotive piston engines on fuels with lower octane than they require may result in damage from knock, but it is generally safe to operate piston engines on fuels of a higher octane rating than their minimum requirement. In other words it is safe to go up in octane but not down.

The various grades of avgas are identified using the Motor Octane Number (MON) combined with the following alpha - designations to indicate Pb content; Low lead (LL), Very low lead (VLL), and Unleaded (UL). Aircraft manufactures, the petroleum industry, and the FAA (Federal Aviation Administration) have worked for over a decade to find alternative fuels that meet the octane requirement of the Piston engine aircraft fleet without the additive TEL.

However, no operationally safe, suitable replacement for leaded fuel has yet been found to meet the needs of the entire Piston -engine aircraft fleet. Pollution load index of the airport site was 1.34-3 times lighter than the background site.

At present, India is the world's ninth largest aviation market with more than 80 operational airports with 17 airports having international operations, more than 700 aircraft, 14 scheduled airlines and nearly 120 non-scheduled operators.

The present study is a step to solve the problem of high level of Pb in ecological system. Plant *B. vulgaris* is a very good tool for bioremediation to remove Pb from soil. The level of Pb in different parts of *B. vulgaris* increases from 119 to 181% of control. It indicates that the plant has great potential to remediate Pb from the soil. The present study also supports the fact that use of *B. vulgaris* in traditional Hindu way of life is very important. It's use in marriage ceremony as one of the plant that is planted in the worship place, as well as in any pooja ceremony.

Burning of *B. vulgaris* is not recommended in Hindu way of life is due to avoid the release of these heavy metal oxides in atmosphere and soil, which are toxic and fatal to human health as well as environment. The present study clearly indicates that this plant *B. vulgaris* can be used in the places where Pb concentration is high in soil. Level of lead (Pb) in soil can be remediated to prevent its movement in different strata of ecological chain. After remediation, isolation of Pb can be done by any safe methods to check its distribution in the environment. Significant decrease (18.7% and 3.6%) in level of lead (Pb) concentration in soil is indication of *B. vulgaris* uses as bioremediator.

The use of different plant species for cleaning contaminated soil named as phytoremediation has gained increasing awareness since last decade, as an emerging cheaper technology. Phytoremediation is amenable to a variety of organic and inorganic compounds and may be applied either in situ or ex situ. Heavy metal absorption by plants generally increases with decreasing pH, due to dissolution of metal-carbonate complexes, releasing free metal ions into solution.

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