



Original Article

Phytoremediation of Lead Contaminated Soil with the Help of *Bambusa vulgaris*

Pooja Agrahari¹, Navneet Kumar², Neeru Pandey³, Sushma Sinku³, Shahnaz Khan³,
Ankita Sahu³, V.K. Singh², D.K. Singh*²

¹Department of Zoology, C.M. Science College, Darbhanga, Bihar, 846004, India. Email- pagrahari1234@gmail.com,

²Malacology Laboratory, Department of Zoology, D.D.U. Gorakhpur University, Gorakhpur, U.P. 273009, India.

³Department of Environmental Science, D.D.U. Gorakhpur University, Gorakhpur, U.P. 273009, India,

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ABSTRACT

Phytoremediation is a green emerging technology used to remove and degrade pollutants from soil and water. In this study, plants of *B. vulgaris* were grown in the soils, which are artificially contaminated by lead below the WHO level (12 mg/kg) and above the WHO level (72 mg/kg). After 3 months, accumulations of the lead metal were analysed in roots, leaves, and stem with the help of Atomic Absorption Spectroscopy (Perkin Elmer analyst 400). Significant decrease in level of Pb has been noted in both the treatments. Decrease in Pb level was 3.6% and 18.7% of control (0.09 and 0.43 mg/kg). It was concluded that the plant *B. vulgaris* is a very good phytoremediation tool to remove Pb metal from the soil.

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1. Introduction

Heavy metal refers to any metallic chemical element that has a relatively high density and is toxic or poisonous at low concentrations. These are not the essential elements in biological systems and additionally most of them are toxic in fairly low concentrations [1]. They adversely affect the productivity of soils, plants, animals and the entire environment if exceed certain limits [2]. Soil is the primary recipient of these heavy metals and then through plants, they transported to food chain and food web [3,4]. The Soil is the dynamic component of nature. Complex system of soil involves chemical, biological, physical, and geological, reactions simultaneously, which vary with time and space [5]. Nowadays soil pollution is getting considerable public attention among other pollution since the magnitude of heavy metal problem is growing rapidly [6].

Sources of heavy metal pollution in environment are mainly derived from anthropogenic activities such as vehicle and aircrafts exhaust, burning of fossil fuels,

industries (mainly non-ferrous industries, power plants, iron steel and chemical industries), agriculture (irrigation with polluted waters), sewage sludge, fertilizers (especially phosphates), contaminated manure, pesticide containing heavy metals, waste incineration, road traffic, and mining etc. Due to the tendency to bioaccumulation and magnification in tropic levels, heavy metals are the most dangerous substances in environment [6].

Pb occurs naturally in the soil [7] but it is found in much higher concentrations in urban and industrial areas [8]. Pb is a soft, malleable, bluish-gray naturally occurring metal found as a mineral combined with other elements, such as Sulphur (PbS, PbSO₄ [anglesite]), or oxygen (PbCO₃ [Cerussite]) [9] and it is extracted together with these metals. Pb pollution is a result of the human activity and involves the use of Pb in gasoline additives, paints, batteries, and pesticides. Pb is known to affect heme synthesis, the nervous system, and blood pressure [10]. It is

* Corresponding author : Prof. D.K. Singh Tel.: 91-9454211574

E-mail address: dk Singh_gpu@yahoo.co.in

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also believed that Pb is mistaken by the body as calcium and lead both metals form an ion due to the similar charge of 2^+ [11].

Pb is known not only for its toxicity but also because it interferes with the metabolism of some essential metals. While Pb poisoning can affect anyone of any age it is most prevalent in children, especially those living in inner cities where there is a higher occurrence of houses painted with Pb based paint and more traffic [12]. Pb has high affinity for soils and the soil retention time of 150-5,000 years and was reported to maintain a high concentration for as long as 150 years to the soil [13,14,15]. Pb can adversely affect plants and invertebrates at concentrations of 500 to 1000 ppm allowing more Pb-tolerant populations of the same or different species to take their place and this will change the type of ecosystem present [16].

In recent years, it has been shown that Pb levels in soil and vegetation have increased considerably due to traffic pollution, especially from usage of leaded petrol and exhaust combustion [17,18,19]. The ability of soil to absorb Pb increases with increase in pH, cation exchange capacity, organic carbon content, soil or water redox potential and phosphate level. The concentration of Pb in soil and dust increases as particle size decreases [20]. Pb ranks fifth behind Fe, Cu, Al, and Zn in industrial production of metals [21]. The uses of Pb for roofing and the production of ammunition have increased from previous years. Pb has been widely used in the form of Tetra Ethyl Lead (TEL) and Tetra Methyl Lead (TML) as 'antiknock' and lubricating agents in petrol, although the majority of Pb is emitted from vehicles in the form of inorganic particles.

The long-term occupational exposure to Pb may contribute to the development of cancer (the International Agency for Research on Cancer 'IARC'). Total Pb concentrations above 100 ppm (mg/kg) in soil are regarded as toxic for plants [14]. Soil containing high Pb concentration is unsuitable for plant growth and destroys the soil biodiversity. 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 [22], 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 [23] and can intensify the processes of reactive oxygen species (ROS) production leading to oxidative stress [24,25]. 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

[26]. Because of its broad range of toxicity, it is very important to extract out this metal from the soil.

Phytoremediation (Green Remediation, Botanoremediation, Agroremediation) 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) [27,28,29]. It is an emerging technology that can be applied to both organic and inorganic pollutants present in environment [30]. 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". In phytoremediation (up take), the root of established plants use solar energy to extract metals from the soil and translocate them to above - ground shoots where they accumulate or to convert them to a less toxic form. After sufficient plant growth and metal accumulation, the above ground portions of the plant are harvested and removed, resulting in the permanent removal of metals from the site. 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 [31,32]. Phytoremediation is energy efficient, aesthetically pleasing approach most suitable for developing countries. It is a "Green Revolution" in the field of innovative cleanup technologies. There are of different categories of phytoremediation including Phytoextraction, Phytodegradation, Phytovolatilization, Phytostabilization, Rhizodegradation, Phytofiltration (Rhizofiltration), Phytodesalination.

B. vulgaris belongs to the family of grasses, Gramineae (Poaceae) native to Indochina. It is also widely cultivated and is naturalized in subtropical and tropical areas of the world. Bamboos have been part of human daily lives since time immemorial. It prefers lowland humid habitats but tolerates a wide range of climatic conditions and soil types [33]. They can be characterized by having woody, usually hollow clumps, complex rhizome and branch systems, petiolate leaf blades and prominent sheathing organs. 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, and essentially contribute to the purification and regulation of the environment. Furthermore, it is used to remediate heavy metal contaminated soil especially Pb contamination. 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 [34], with some species achieving the phenomenal growth rate of one meter /day

2. Materials and Methods

2.1. Apparatus

Perkin Elmer analyst 400 atomic absorption spectrophotometer, Hot plate, Oven, Porcelain Pestle and mortar, Glassware, Whatman type filter paper 60, Micropipette.

2.2. Chemicals Used

Concentrated Nitric acid (HNO₃) (Merck, *pro analysi*), Millipore water and HCl. Standard of Pb was purchased from Perkin Elmer, 710 Bridgeport Avenue (USA).

2.3. Soil and plant collection

The soil sample used for this work was collected from DDU Gorakhpur University, Gorakhpur Campus. The soil sample was collected from the depth of 0-6 cm. *B. vulgaris* plant were collected from a park of Gorakhpur.

2.4. 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 2 mm 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 deionized water. Then different parts of plants (leaf, stem and root) were collected in Petri plates. 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.

2.5. Digestion procedure

All samples were accurately weighed (1.0 g each) and placed in a 100 ml beaker. 10 ml concentrate HNO₃ was added in each beaker and kept overnight. Residues were dissolved again with 10 ml concentrate HNO₃ followed by 10 ml of 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 borosilicate beakers. The final volumes of samples were made up to 25 ml with de-ionized double distilled water.

2.6. 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 (Perkin Elmer analyst 400) by the method of Agrahari et al. [35].

3. Results and Discussion

Bambusa vulgaris was grown in greenhouse condition to evaluate its potential to bioaccumulate, translocate and

remediate lead (Pb) from lead contaminated soil. Pb concentration in the soil of university campus was very low (2.32 mg/kg). Concentration of Pb in root, stem and leaf of *B. vulgaris* was 0.23, 0.28 and 0.27 mg/kg, respectively. Then, this plant was grown in soil containing 12 mg/kg and 72 mg/kg Pb for 3 months. After 3 months, concentration of Pb in the soil, root, stem and leaf was again measured. It was observed that Pb concentration in root and shoot were significantly increased with respect to control group of plants (Table-1).

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

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

There was a significant decrease of 3.6% and 18.7% Pb level in soil.

Lead (Pb) concentrations in different parts 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 concentration was measured in *B. vulgaris* stem (181.2% of control).

After the remediation of soil, it has noted that in both the treatments, there was significant decrease in concentration of Pb level in treated soil. There was a significant decrease of 18.7% and 3.6% Pb level 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 by Agrahari et al., [36]. The highest value of Pb deposition was 14.1 mg/kg dry weight of *F. religiosa* leaf, which was much higher than the limits recommended by World Health Organization (WHO) [36].

Agrahari et al. [35] reported the Pb concentration in different water bodies of Gorakhpur district and it was

0.03, 0.05 and 0.05 mg/L in different sites. Pb level in water bodies of Gorakhpur was near to the WHO level (0.05 mg/L), whereas Agrahari et al. [36] reported that in air it was higher than WHO level (2 mg/kg dry wt.).

Soil is precious natural assets used for sustainable agriculture since a long time. It is very difficult to define soil quality as soil's composition can vary from place to place. Intensive human activities have severely degraded or contaminated the soil's 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 [37]. 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 metals have 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 circles responsible for their accumulation in the biosphere. Heavy metals can occur in different valence states, so that one element may be 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 [38].

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 because 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 continued to experience high passenger growth, and if the trend continues it could rank among the top three aviation market in the world by 2020, it is said in the report titled 'Indian Aviation: spreading its Wings'. 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, not 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 9th 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 increased concentration of Pb in ecological system. Earlier it was also reported by Agrahari et al. [36] that the traffic density plays an important role in the lead pollution in plants. 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%. 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 uses in marriage ceremony as one of the plants 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 metals in atmosphere and soil as oxide, 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 as a Bioremediator. Level of lead (Pb) in soil can be remediated to prevent its movement to different strata of ecological chain. After remediation, isolation of Pb can be done by any safe methods to check its distribution in environment.

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 [39].

4. Conclusion

The overall objective of any soil remediation approach is to create a final solution that is protective of human health and environment. The project illustrated that *B. vulgaris* can tolerate Pb contamination without displaying any visible characteristic symptoms of metal toxicity (Chlorosis, Necrosis, Lesion and wilting) and does not significantly decrease the growth of the Bamboo plant. This study clearly demonstrated that *B. vulgaris* can remediate significant amount of Pb from the artificially contaminated soil.

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Conflict Of Interest

The authors declare that they have no conflict of interest.

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