



Phytoremediation of heavy metal contaminated soil using *Ficus religiosa* (L.)

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ARTICLE INFO	ABSTRACT
<p>Research Article Received on August 16, 2023 Revised on September 06, 2023 Accepted on September 13, 2023 Published on October 16, 2023</p> <p>Article Authors Talad Khan, Harihar, Samuel Gorden Singh</p> <p>Corresponding Author Email taladkhan9557@gmail.com</p>	<p>ABSTRACT Intensity of toxic metal pollution in the biosphere has been increasing since the starting of industrial revolution, posing major environmental threats and human health problems. Controlled and uncontrolled disposal of waste, accidental and process spillage, mining and smelting metalliferous ores, application of sewage sludge to agricultural soil are responsible for the migration of contaminants into non-contaminated sites as dust or leachate and contribute towards contamination of our ecosystem. These contaminants include heavy metals, combustible and putrescible substances, hazardous waste, explosive and petroleum products which cover a wide range of organic and inorganic compounds. Out of all these contaminants heavy metals pose threats than organic contaminants to our ecosystem. Phytoremediation approaches to utilize a particular group of plants, known as hyper-accumulators, to extract and concentrate particular heavy metal elements from the environment. In this study heavy metal (Zn, Pb, Ni, Cd) uptakes from contaminated site of Yamuna River by <i>Ficus religiosa</i> plant, it is locally available hyperaccumulators, were assessed. It was observed that these plants accumulated these heavy metals in different parts of the plant from the contaminated site and were able to maintain a growth rate.</p>
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Environmental pollution has become a major source of health risk problems and causes several serious diseases throughout the world (Al Nagger, *et al.*, 2018). One of the serious environmental pollutions is of heavy metals from various sources. The rapid development of the industrial sector has increased the heavy metal pollution issue recently, as the need for various heavy metals is increasing for manufacturing purposes (Awa and Hadibarata, 2020). Metallic and non-metallic pollutants (Almaroai *et al.*, 2012) have increasingly contaminated the continuous increasing urbanization, industrialization and agricultural lands. This affects the ecosystems, surface, subsurface, ground water, food quality and health (Alkorta *et al.*, 2004) (Muthusaravanan *et al.*, 2018).

Heavy metals are major pollutants of the environment in air, water and soil (Pandey *at el.*, 2008, Bharath *et al.*, 2019) cause human health to risk (Zhang *et al.*, 2013, Zhang *et al.*, 2019). Heavy metal contamination cause abiotic stresses that cause environmental pollution in recent decades (Osman *et al.*, 2017). Although heavy metals are naturally occurring in the soil and some of them are essential to the life of living beings, but geologic and anthropogenic activities increase the concentration of them may be harmful for both plants and animals, which disturbs their physiology and development (Bettaieb and Arbaoui, 2018). The development of remediation techniques, several chemicals and physical methods have been used provide the way heavy meals to remediate the contaminated soil (Pandey *et al.*, 2008).

Biological remediation techniques have been used for minimizing the heavy metals such as include bioremediation, phytoremediation, bioventing, bioleaching, land forming, bioreactors, composting, bioaugmentation and biostimulation etc., but among phytoremediation technique is useful, cheap and sustainable (Ullah *et al.*, 2015, Wang *et al.*, 2020, Ali *et al.*, 2018). A large number accumulator plant species have been reported such as Chinese cabbage (Wang *et al.*, 2018b), *Brassica napus* (Rossi *et al.*, 2019), Indian mustard (Duquene *et al.*, 2009), *Pteris vittata* (Zeng *et al.*, 2019), *Helianthus annuus* (Farid *et al.*, 2018a), *Brassica campestris*, *Solanum nigrum* (Luo *et al.*, 2011, Yang *et al.*, 2011). The some important steps in the phytoremediation are phytoextraction, phytostabilization, phytovolatilation, phytodegradation, phytodesalination, rhizofiltration, rhizodegradation and phytoevaporation.

Materials and Methods

Soil Sampling and Heavy Metal Analysis

To evaluate the status of heavy metal contaminants in the soil nearby Yamuna River, soil sampling was done from Agra near Kailash temple. Soil samples were taken from two depths (0-10 and 10-20 cm). Samples of soil were collected and then transported to laboratory for further heavy metal analysis. For heavy metal (Pb, Ni, Zn and Cd) analysis, 0.5 g soil sample was digested in 10 ml of Nitric acid: Perchloric acid, 3:1. The digested samples were filtered through Whatman filter paper no. 42 and diluted with deionized water. Digested soil samples were analyzed at Dayalbagh Educational Institute (DEI), Agra using Inductively Coupled Plasma Optical Emission spectroscopy (ICP-OES).

Pot Experiment

The seeds of selected plant *Ficus religiosa* were grown in earthen pots. The pots contained 5 seeds each and were kept under natural photo radiation. The experiment was done in triplicates for replication. The seeds were supplemented with the water once in two days. To perform the pot experiment, the pots were filled with the 5 kg test soil (collected from Kailash temple).

Experimental set up were as follows:

Control = Garden soil (collected from St. John's College, Agra)

T₁ (Treatment 1) = Polluted soil (Collected from Yamuna River)

T₂ (Treatment 2) = Polluted soil + 25% vermicompost

T₃ (Treatment 3) = Polluted soil + 1 gm EDTA

After 15, 30 and 40 days intervals the plants were uprooted carefully, shoots and roots were washed with distilled water and then grinded using mortar and pestle for physiological and biochemical studies.

Chlorophyll Estimation

The Chlorophyll content was estimated according to the method of (Arnon, 1949). The plants when grown in different treatments as discussed above were taken and the leaves of plants were used for chlorophyll estimation.

Plant Length

The aboveground part of plant was taken to measure plant length. Plant length was measured in first and fourth week in control and all treated plants.

Total Biomass (Dry Weight)

Plants were removed from the soil and washed. Plants were dried in oven by setting at low heat (100°C) over night and then cooled in a dry environment and their weight was noted using weighing balance.

Sample Preparation for Inductively Coupled Plasma Optical Emission Spectroscopy

After 45 days the plants were taken out from the pots, and were washed with deionized water and oven-dried at 70°C for 48 hours, then ground into fine powder with mortar and pestle. The dried plant samples were weighed and ground for analysis of metals concentration.



Fig 1. Pot Experiment (*Ficus religiosa*) on 15, 30 and 45 days of treatments

Quantification of Phytoremediation Efficiency of Plant

Phytoextraction potential is estimated by calculating Bioconcentration Factor (BCF) and Translocation Factor (TF):

$$\text{Bioaccumulation Factor (BAF)} = \frac{\text{Average Metal Con. in Whole Plant Tissue}}{\text{Metal Con. in Soil}}$$

$$\text{Translocation Factor (TF)} = \frac{\text{Average Metal Con. in Root Part}}{\text{Metal Con. in Shoot Part}}$$

Result and Discussion

Pot experiments were conducted to evaluate the effect of treatment vermicompost and EDTA in the soil on the growth and metal uptake capacity of *Ficus religiosa*. It was observed that the plant height and dry biomass decreased in T₁, T₂ and T₃ compared to control probably due to the toxicity of heavy metals. The highest length in T₃ plant was observed comparison to T₁ and T₂. The highest dry biomass was also observed in EDTA amended soil (T₃) is comparison to without amendment (control and T₁). Photosynthetic pigment (Chlorophyll) in plants indicates the quantum efficiency of photosynthesis (Liao *et al.*, 2016).

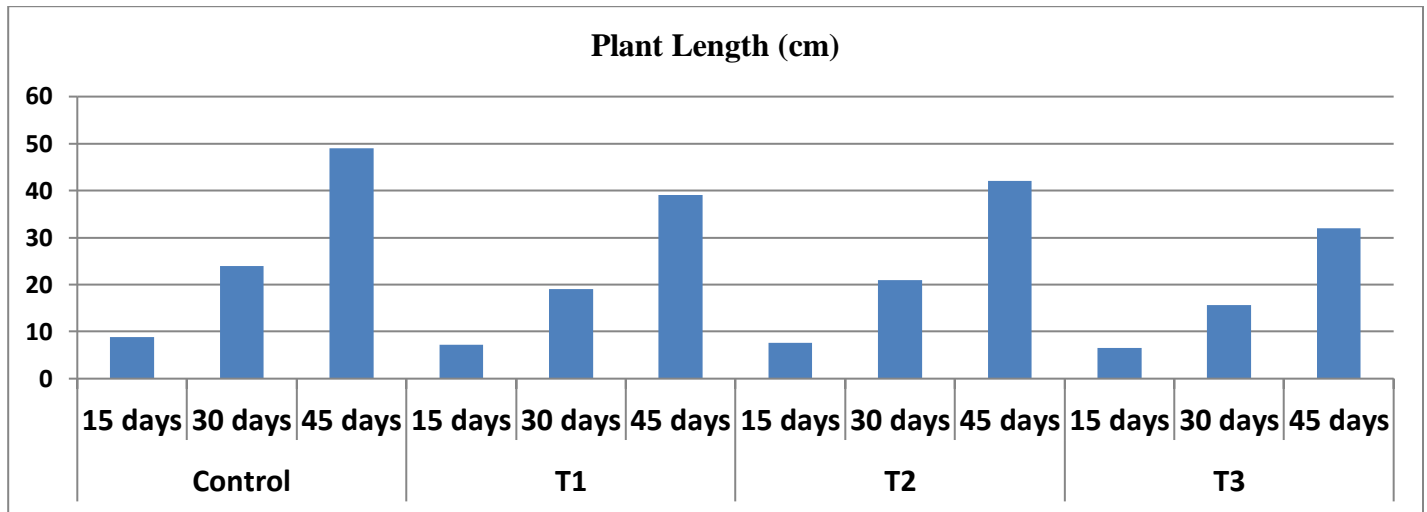


Fig 2. Length of control and treated plant on 15, 30 and 45 days of experiment

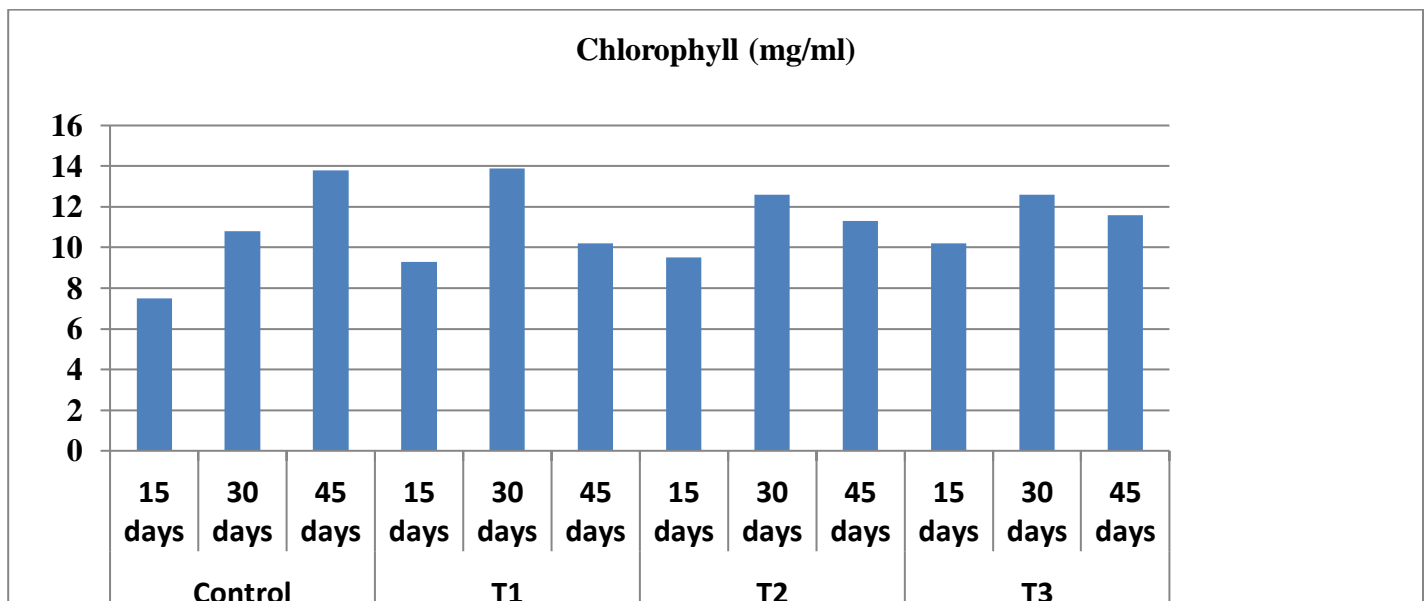


Fig 3. Chlorophyll content in control and treated plant on 15, 30 and 45 days of treatment

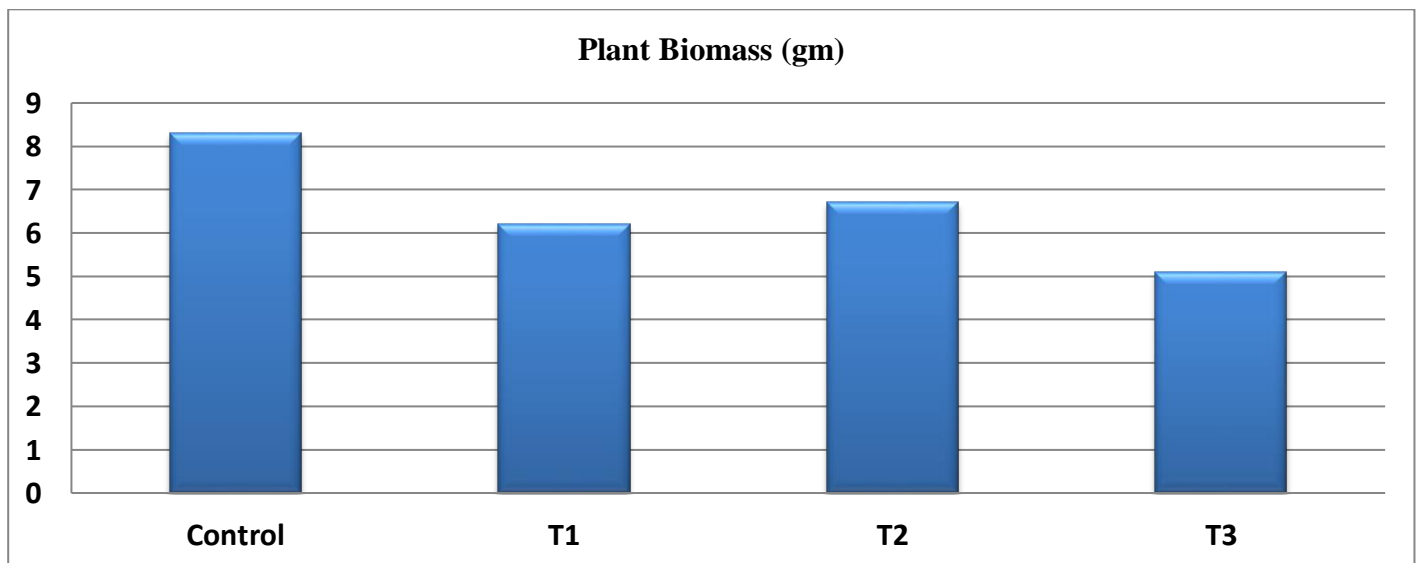


Fig 4. Total biomass (gm) of control and treated plants

Table 1. Metal concentrations (mg/kg) in initial, control and treated soil

Metal	Initial	Control			T ₁			T ₂			T ₃		
		15 days	30 days	45 days	15 days	30 days	45 days	15 days	30 days	45 days	15 days	30 days	45 days
Zn	129	0.2	0.1	0.01	84.5	66.3	41.7	80.6	59	38	76	43	28
Pb	138	0.2	0.01	0.001	93	74	54	87	68	48	81	52	25
Ni	90	0.4	0.02	0.001	76	64	43	70.4	57	37	63	49	24
Cd	60.8	0	0	0	54	38	21	52	34	20	51	33	21

Table 2. Metal uptake and accumulation concentrations (mg/kg) by *Ficus religiosa*

Metal (mg/kg)	Control		T ₁		T ₂		T ₃	
	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
Zn	0.1	0.007	72	66	78	66	74	49
Pb	0.06	0.021	86	57	98.4	43	88	57
Ni	0.01	0.001	52	40	58	49.4	72	62
Cd	0	0	36	28	34	20	48	32

Therefore, total chlorophyll in the leaves of *Ficus religiosa* was analyzed for control, T₁, T₂, T₃ set of experiment. The results of chlorophyll content of plant in different set of experiment are presented in (figure 2) which indicates a significant difference in all the treatment.

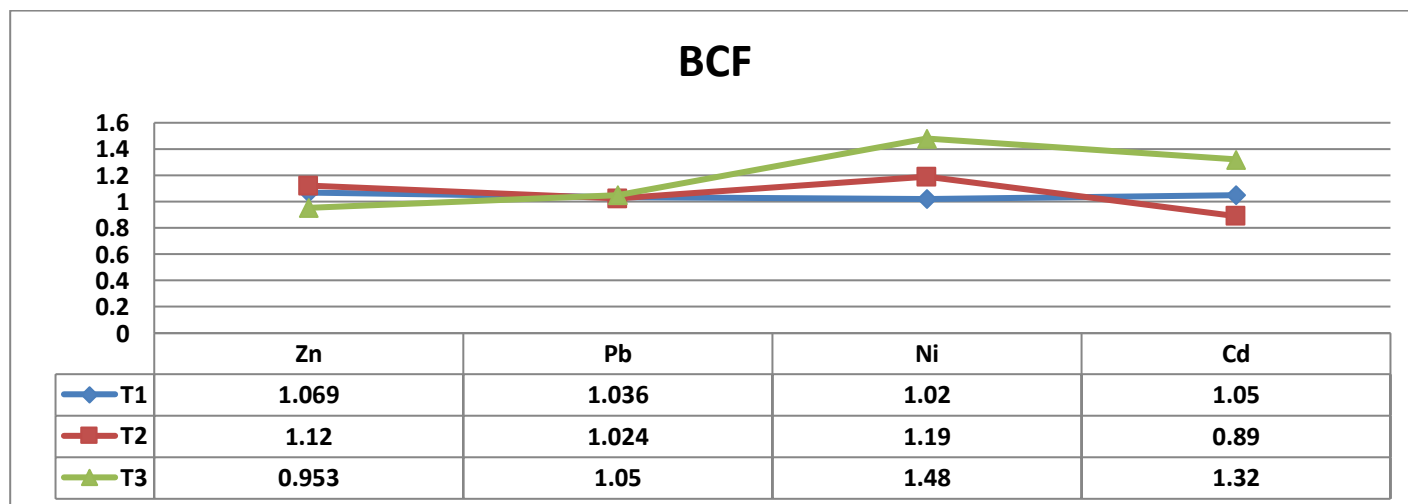
Effect of Vermicompost and EDTA on Heavy Metal uptake by *Ficus religiosa*

The heavy metal viz. zinc, lead, nickel, cadmium concentration in root and shoot and accumulation in the plant are shown in (table 2). The highest zinc accumulation by root and shoot (78 mg/kg and 66 mg/kg) were found in T₂. The highest accumulation of Cd and Ni were observed in root with EDTA (T₃).

Uptake and accumulation of heavy metals in T₃ were found in the following order Pb > Ni > Zn > Cd similarly the metal accumulation with 25% vermicompost (T₂) were found in Pb > Zn > Ni > Cd by *Ficus religiosa*.

BCF and TF

In the translocation and Bioconcentration factor are considered as the indicator for phytoremediation efficiency. The Bioconcentration factor value >1 indicates higher uptake of heavy metals in plant root and shoot then soil whereas a Bioconcentration factor of less than 1 suggested that the soil has a larger concentration of heavy metal then the plant.

**Fig 5. Bioconcentration Factor for different treatment in *Ficus religiosa***

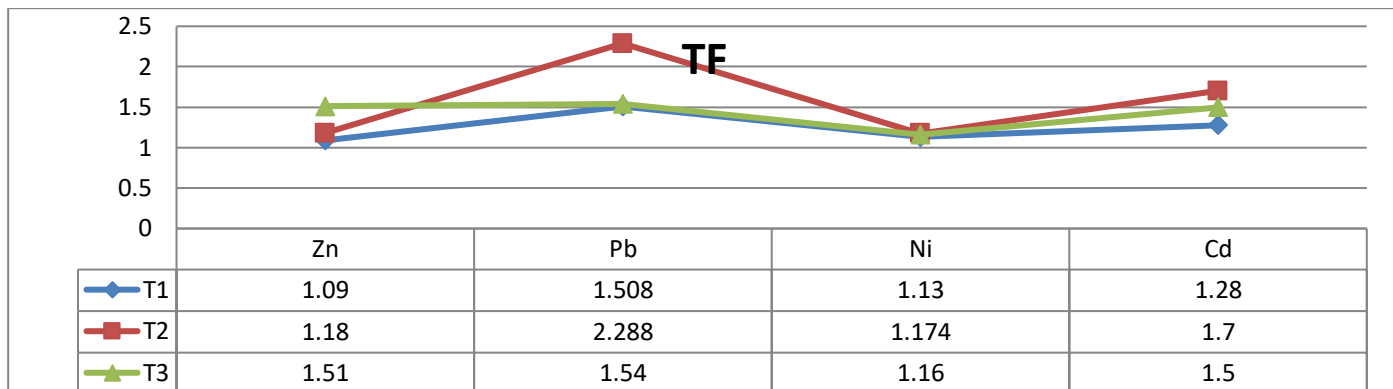


Fig 6. Translocation Factor for different treatment in *Ficus religiosa*

The value of BCF > 1 indicates a potential heavy metal hyperaccumulator species. The bio concentration factor value for *Ficus religiosa* plant is depicted in figure 1.5. In this study the value of translocation factor > 1 in all the treatments suggested that the plant (*Ficus religiosa*) is hyper accumulator. The value of translocation factor for lead and nickel were higher than other metal in all treatment, which indicates that the *Ficus religiosa* is Pb and Ni accumulator plant. In present study, it was observed that concentration of EDTA was more effective in cadmium (80 mg/ kg) and nickel (134 mg/kg) uptake as well as accumulation in *Ficus religiosa* similarly the percentage of vermicompost (25%) was more effective in Pb (141.4 mg/kg) and zinc (144 mg/kg) uptake and accumulation in this plant. However, the gradual decrease in the concentration of all heavy metals in Yamuna soil were also observed in the entire experiment (table 1).

Conclusion

The problem of soil pollution because of contamination with heavy metals is constantly increasing especially in developing countries because there are limited financial and technological resources to remediate polluted soil sources. In this situation, the use of trees to remediate polluted soil is considered as the new emerging technology, which is relatively cheaper, sustainable and inexpensive alternatives to conventional remediation methods and will be most suitable for a developing country like India. The technology of Phytoremediation offers viable solution to soil and water pollution. The study has revealed that the selected plant *Ficus religiosa* was able to reduce the concentration of the heavy metals like Zn, Pb, Ni and Cd in soil from Yamuna River.

In this study root of the selected plant showed comparatively greater uptake for all the heavy metals than shoot. The accumulation of heavy metals was more in the root than in shoot. The addition of EDTA and vermicompost to the treatment increased plant uptake of heavy metals especially lead. In summary, the addition of EDTA and vermicompost to contaminated Yamuna soil were enhance the heavy metal uptake and reduced all the heavy metals in soil around Yamuna River.

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