Asian Plant Research Journal

2(2): 1-14, 2019; Article no.APRJ.46767

Phytoremediation of Heavy Metals from Water of Yamuna River by *Tagetes patula*, *Bassica scoparia*, *Portulaca grandiflora*

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Authors' contributions

This work was carried out as a final year project of M.Sc student at Dept. of Biotechnolology, MRIIRS. Author AG worked as a supervisor for this project work, she designed the study, performed the statistical analysis and wrote the drafts of the manuscript. Author NM M.Sc final year student managed the analyses of the study and literature searches. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/APRJ/2019/v2i230042
Editor(s):
(1) Dr. Nesreen Houssien Abou- Baker, Associate Professor, Department of Soil and Water Use, Division of Agricultural and Biological Research, National Research Centre, Giza, Egypt.
Reviewers:
(1) Shabir Hussain Wani Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, J&K, India.
(2) Dr. S. Murugesan, University of Madras, India.
Complete Peer review History: http://www.sdiarticle3.com/review-history/46767

Original Research Article

Received 13 October 2018
Accepted 01 February 2019
Published 04 March 2019

ABSTRACT

Heavy metal contamination is a worldwide problem, causing many serious diseases and the levels of contamination varied from place to place. Heavy metals like cadmium (Cd), mercury (Hg), zinc (Zn), chromium (Cr), and lead (Pb) etc. are very injurious even at low concentration and are present in Yamuna river water. Phytoremediation has great potential as an efficient cleanup technology for contaminated soils, groundwater, and wastewater. It is a cheap and very efficient technique for metal removal. A study had been carried out to detect the efficiency of phytoremediation technique for removal of heavy toxic metals from water of Yamuna river. This study also focused on the phytoremediation capacity of all of three selected plants: *Tagetes patula, Bassica scoparia,* and *Portulaca grandiflora.* Bioaccumulation of heavy metals in various parts of plants has also been checked.

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Keywords: Yamuna River; Tagetes patula; Bassica scoparia; Portulaca grandiflora; heavy metals; phytoremediation.

1. INTRODUCTION

Yamuna River originates from Yamunotri glaciers of Himalayas. It is the largest tributary of river Ganga. It is around 1370 kilometers in length. It flows across the states of Haryana, Delhi, Uttar Pradesh. It merges into Ganga River in Allahabad. Big cities like Mathura, Agra, Delhi lie on the Yamuna river bank. It is classified into five segments like Delhi segment, Upper segment, Himalayan segment, Eutrophicated segment, Diluted segment depend on the basis of ecological and hydrological conditions. The quality of water, river water in Himalayan segment is very good and also meets all the standards within this segment. Yamuna river water is trapped by a Wazirabad barrage for the purpose of domestic supply of water in Delhi. The Okhla barrage of Delhi receives the water of seventeen drain sewage, Najafgarh drain. It is the most polluted segment of river Yamuna. Today it has become the most polluted and dirtiest river of the country and was once described as the lifeline of Delhi city [1] and [2].

It has been given the grade “E” by the Central Pollution Control Board (CPCB), which means it is only good for recreation and industrial cooling. No underwater life found in this segment of the river. The domestic discharges from Delhi, Faridabad, Noida, Ghaziabad, Mathura, Agra, Haryana, has rendered the river unfit for any use.

Even taking a dip in river water can cause various health and skin regarding issues. One of the major contaminants present in river water is toxic heavy metals. Presence of toxic heavy metals is an issue of major concern because of bio-accumulative nature of metals. These metals have geological origin, but entering into the river water can be by erosion, weathering and anthropogenic activities of human beings like agricultural runoff, industrial processing, sewage disposal etc. Environmental related exposure of these heavy metals are like lead paint, household dust, silver foil in food, surface soil, batteries, peeling paints, sewage wastes, plumbing system etc. Use of fertilizers and pesticides is also a great source of heavy metals like Cd, As. Some of these metals are essential for human beings, but in very low concentration, such as Ca, Cu, Fe, Cr, Mg, K, Zn, Ni, Mn, Co and Na are essential for normal growth of plants and living organisms. Cd, Ag, Al, Pb are some non essential metals and are very toxic.

High uptake and slow elimination of Heavy metals cause harm to the aquatic life. As the heavy metals get settled down in the sediment and uptake by the plants or aquatic organisms, drink by the animal and this will ultimately harm the life of organisms [3,4,5]. Human by many ways are highly exposed to heavy metals as they are also the part of the food chain. Table 1 shows the permissible limit of heavy metals (Ad, Zn, Cr, Pb, Hg) prescribed by WHO.

High uptake of lead causes changes in the gill, kidney and liver of fish. Intestine and gills are the major site of metal accumulation in fishes. It causes variation in the lipids of aquatic organisms. Lead cause swelling in the gills and jaws of fishes. Nausea, anemia and vomiting, etc problems are the side effects of lead exposure in humans.

Zinc accumulates in the gills of fish, this indicate a depressive effect in tissue respiration cause hypoxia or death of the fish. Zinc also causes a decrease in total white blood cells. Zinc cause changes in heart physiology and also cause toxic changes in ventilatory System. Headache, fever, vomiting, chest tightness, aches, chills, metallic taste in the mouth And cough are the side effects of acute exposure to zinc. Chronic exposure causes problems like cancer, kidney and lung failure.

Table 1. Maximum permissible value of heavy metals by WHO

Metals	Water (L/kg^{-1})	Sediment (µg/kg$^{-1)}$
Cadmium	0.003	6
Zinc	3	123
Chromium	0.05	25
Lead	0.01	----
Mercury	1.3	0.3

Cadmium mostly accumulates in the gills, intestine and stomach of fishes. It causes changes in enzymatic activities in marine animals and also changes in oxygen consumption. High concentration of cadmium also affects the osmotic-regulation activity in fishes. Cadmium also causes reduction in red blood cells in the fishes. Exposure to heavy metals causes various serious diseases in human beings. Cadmium exposure cause lung inflammation and lung cancer as cigarette smoking is the largest source of cadmium in humans. Osteomalacia and proteinuria are the kind of problems occur in humans due to cadmium.

Chromium cause acute and chronic effects on fishes. High chromium Uptake causes changes in metallo-enzymatic activity. Chromium gets accumulated in the gills of aquatic biota. High chromium concentration cause altered blood chemistry, osmoregulatory changes, behavioral modifications and in severe conditions hypoxia. Acute renal failure, hemolysis and gastrointestinal hamorrhage are the problems occur in humans at acute exposure to chromium. At chronic exposure to Chromium lungs cancer and pulmonary fibrosis diseases will take place.

Mercury is highly toxic to aquatic animals. It shows variable effects on oxygen consumption, osmoregulation, and enzyme activity of marine life. It also shows several effects on blood circulation system and cause reduction in RBC count. Diarrheoa, fever and vomiting are the side effects of acute mercury exposure. Nausea, nephrotic syndrome, pink disease, stomatitis, neurotic disorders and tremor diseases are the side effects of cadmium at chronic exposure as mercury is highly toxic.

Various techniques are available for remediation of contaminants. Which are chemical, physical and biological methods. The chemical method involves the use of several harsh chemicals like leaching of metals by chelating agents and chemical wash. Physical methods are very expensive and cause labour demand. That's why researchers have developed highly efficient, cost effective, eco-friendly remediation techniques, in which organic waste are biologically degraded into an innocuous state.

Removal of heavy metals with the help of microorganisms is a very efficient method, but it is confined to water system only. Some other remediation methods are bio augmentation, land farming, bio leaching, rhizofiltration, biostimulation, composting, bioreactor, and phytoremediation. Phytoremediation is a technique that uses plants for degradation or accumulation of toxic contaminants present in environment [6,7,8,9,10,11]. It involves the use of living organisms, especially plants and microorganisms to eliminate the effects of contaminants present in air, water, soil [12,13,14,15,16,17,18].

Phytoextraction of heavy metals by the hyperaccumulator plants from both soil and water is also a key area of search. This study was also focused on the phytoremediation capacity of all of three selected plants *Tagetes patula, Bassica scoparia, Portulaca grandiflora.*

1.1 Objectives

i. Determination of heavy metal content in Yamuna river water sample
ii. Removal of contaminants from river water sample with the help of Hyper - accumulator plants
iii. Evaluation of Bio-accumulation capacity of all of three selected plants

2. MATERIALS AND METHODS

2.1 Waste Water Collection

Water sample was collected from Yamuna river enrooted Delhi-Agra via Haryana, near Palwal District. Water sample was preserved in a can at freezing temperature (6-8°C). This water was further used for phytoremediation study.

2.2 Plants Used

Three different plants (*Tagetes patula, Bassica scoparia, Portulaca grandiflora*) were used for the study. The seeds of the plants were collected from a local nursery at Delhi-NCR. The plant classifications have been listed in Table 2 (Sources Wikipedia).

T. patula grown and harvested annually and flowers are yellow and red in colour, reaching 0.3 m to 0.5 m in size. The plant size varies from 0.1 to 2.2 m tall. They have fibrous roots. In India it grows from October to April. The plants common name is called "Marygold". The leaves of the plants include oil glands and the oils are pungent. It can grow in any sort of soil. *T. patula* is widely cultivated in India it also have various uses in medicines.

Table 2. Taxonomical classification of the experimental plants

Classification	*Tagetes patula*	*Bassica scoparia*	*Portulaca grandiflora*
Kingdom	Plantae	Plantae	Plantae
Order	Asterales	Caryophyllales	Caryophyllales
Family	Asteraceae	Amaeanthaceae	Portulacaceae
Genus	Tagetes	Bassia	Portulaca
Species	*T. patula*	*B.scoparia*	*P. grandiflora*

The main reason for selecting this plant for phytoremediation is its ability of resisting adverse condition like pests, salinity, drought etc. *T. patula* is good for phytoextraction of heavy metals like arsenic, Mercury etc.

It is a small but fast growing annual plant as it has grown 30 cm tall. The leaves of the plant are thick and fleshy, up to 2.5 cm long arranged in a cluster like structure. The flowers are 2.5-3 cm diameter with five petals. The colour of flowers varied from red, pink, white, orange and yellow. In India it is called **"9 o clock"** flower because it blooms at 9 a.m. It generally requires no attention as it gets spread very easily by itself. This plant can easily grow in adverse conditions like pesticides, high heavy metal concentration, chemicals etc. This plant consumption known to reduce the risk of cancer and heart diseases [19,20,21,22,23].

It is a large annual herb. The plant is helpful in controlling soil erosion. This plant is suggested as an agent for phytoremediation technique because it is hyperaccumulator of cadmium, zinc, mercury, chromium. It is an evergreen foliage plant. The seeds of the plant help in regulation of hypertension and obesity etc.

2.3 Procedure

2.3.1 Model set up

i. Six plastic boxes were taken.
ii. Two boxes for each plant.
iii. For setting up the model, one plastic box was placed on another.
iv. Small holes were induced in the centre of each plastic box for the passage of plant roots as shown by the pictures below in Figs. 1-5.
v. After germination of seeds in soil, small plants were transplanted. From the soil in the upper plastic box which was already filled with garden soil.
vi. Roots of the plants were allowed to reach the lower plastic box. Already filled with contaminated water sample of Yamuna river through induced wholes.

Fig. 1. Set up for plant

Fig. 2. Set up of different plants

2.3.2 Growth period

i. Plants were allowed to grow in that setup for eight weeks.
ii. During these eight weeks, generally called "Growth period", proper attention to the plants was given just to make sure. That none of the plant will die.

iii. Fertilizers such as cow dung was mixed into the soil.
iv. Plants were placed beneath a tree, because much, sunlight exposure can cause browning of plants.

2.3.3 Change in size parameters

Growth in the length of the plants was measured. After completion of fourth and eighth week by a centimetre scale.

2.3.4 Lab work

After 8 weeks, all of the three plants were harvested and the water samples (initial untreated and final treated) from all the three plants were taken and stored in three different plastic bottles with proper labelling.

Fig. 3. Set up for *P. grandiflora*

Fig. 4. Picture of *B. scoparia*

Fig. 5. Picture of *T. patula*

2.3.5 Acid Digestion

Acid digestion method was used for preparing the water and tissue samples. It is done by adding a considerable amount of acids and heating, until the solution gets completely decompose and release metals.

a. For acid digestion of water samples

The water samples were autoclaved and added in the glass beakers.

As nitric acid can never use alone, so it was combined with sulphuric acid.

To the water samples, first added 5 ml of concentrated HNO_3 and 10 ml of concentrated H_2SO_4, boil on a hot plate at 90°C for evaporation, until dense fumes of dense SO_3 appears.

After clearing of the solution, no brownish fume appears, then distilled water was added to make solution dilute and heated.

Then the solution was centrifuged at 3000 rpm for 25 min and the pellet was discarded, supernatant was taken and stored in test tubes with proper labelling.

b. For acid digestion of plant tissues

Plants were first wiped with 0.01N HCl followed by rinsing with distilled water, then the plants were separated into different parts viz. roots, stems, leaves. And let them dry in oven for 15 min or less. All the parts were ground into grinder and 2 g of sample were taken in the glass beaker after weighing For digestion, HNO_3 And $HCLO_4$ acids was used to the sample, first 5 ml of HNO_3 added and heated on a Hot plate at temperature 100°C for 30 to 35 min, then 2.5 ml of $HCLO_4$ added to the mixture and boiled, white fumes appeared, later 5 ml of dilute water added to the mixture and again boiled until the fumes were totally released.

Detection of heavy metals present in all the samples was done by AAS technique.

3. RESULTS AND DISCUSSION

Final growth in the length of plants is given in the table below and also shown in the picture given below.

The amount of heavy metals present in the water sample and in the plant tissue sample were analyzed by a technique called "Atomic absorption spectrometry". The amount of heavy metals such as Cd, Hg, Zn, Cr, Pb in the initial untreated water sample and also in final treated Water samples are given in the table below.

In the present study, cadmium was undetectable in the water sample of *B. scoparia* and *T. patula* absorbed greater amount of Cd as compared to *T. patula*. The chromium concentration found very less in the treated water sample by *T. patula* and it was highest in *P. grandiflora*. Zinc level highest in *P. grandiflora* and lowest in *T. patula*. The Hg concentration found highest in *P. grandiflora* and there is approximately no difference in the results of *T. patula* and *B. scoparia*. Pb concentration has been found in this decreasing order *P. grandiflora*> *B. scoparia* > *T. patula*. So according to this result *T. patula* is good for treatment of chromium, zinc, mercury, lead from wastewater. *B. scoparia* is good for the removal of mercury most as compared to other heavy metals from waste water and *P. grandiflora* is proved to be a good remediation agent for cadmium etc mostly as compared to other heavy metals from contaminated water.

Bioaccumulation of heavy metals by plants: Plants also have the ability to accumulate the metals, were checked with the help of AAS technique, after the acid digestion process of samples. The results of AAs are given in the Table 6.

According to the above result, accumulation of zinc, mercury and chromium was highest in the roots of *T. patula*. Lead and cadmium accumulation was highest in the roots of *P. grandiflora*.

Table 4. Change in length (cm) of the plants

Plants	Zero day	After four weeks	After eight weeks
T. patula	5 cm	9.5 cm	19 cm
B. scoparia	6 cm	8.5 cm	11.5 cm
P. grandiflora	3.5 cm	7 cm	13 cm

Table 5. Presence of heavy metals (mg/L) in water sample

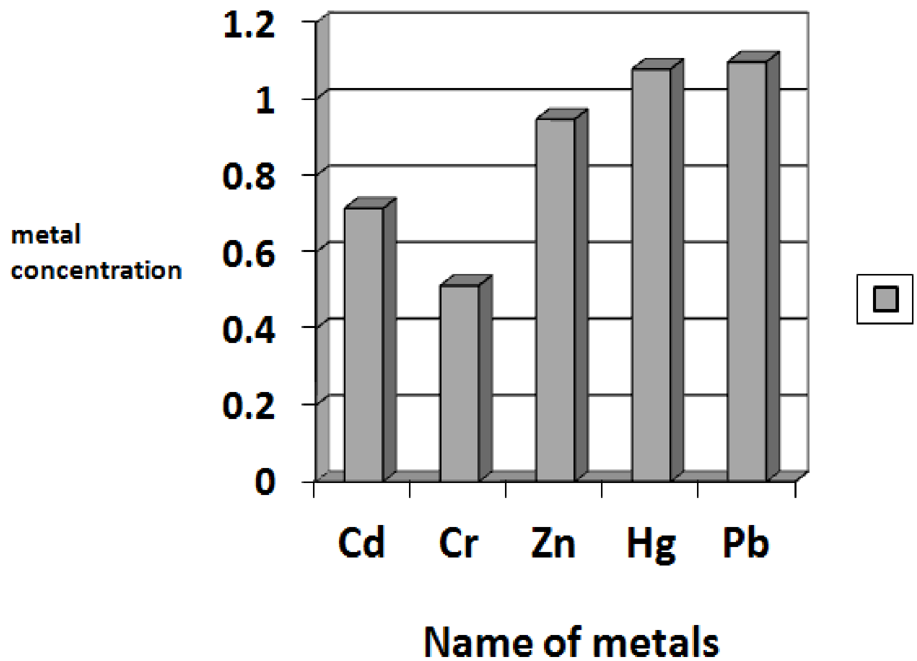
Metals	Initial water sample	*Tagetes patula*	*Portulaca prandiflora*	*Bassia scoparia*
Cd	0.715	0.489	0.315	0
Cr	0.513	0.269	0.418	0.379
Zn	0.948	0.533	0.697	0.705
Hg	1.079	0.782	0.969	0.783
Pb	1.098	0.055	0.079	0.069

Table 6. Presence of heavy metals in the roots (mg/kg^{-1}) of plants

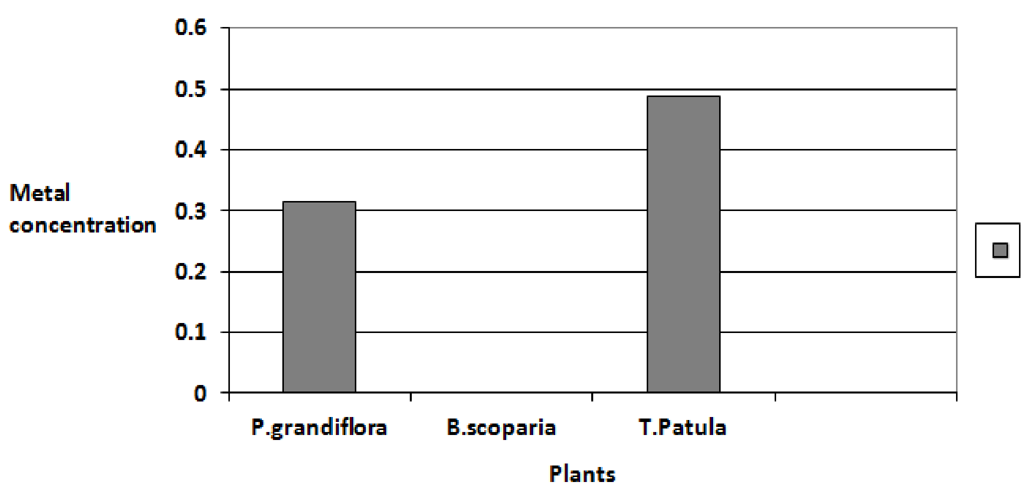
Metals	*Tagetes patula*	*Portulaca grandiflora*	*Bassia scoparia*
Cd	19	22	8
Cr	12	7.9	6.3
Zn	5.9	4	2.6
Hg	27	25.2	15.9
Pb	33	38	13.7

Table 7. Presence of heavy metals in the stems (mg/kg^{-1}) of plants

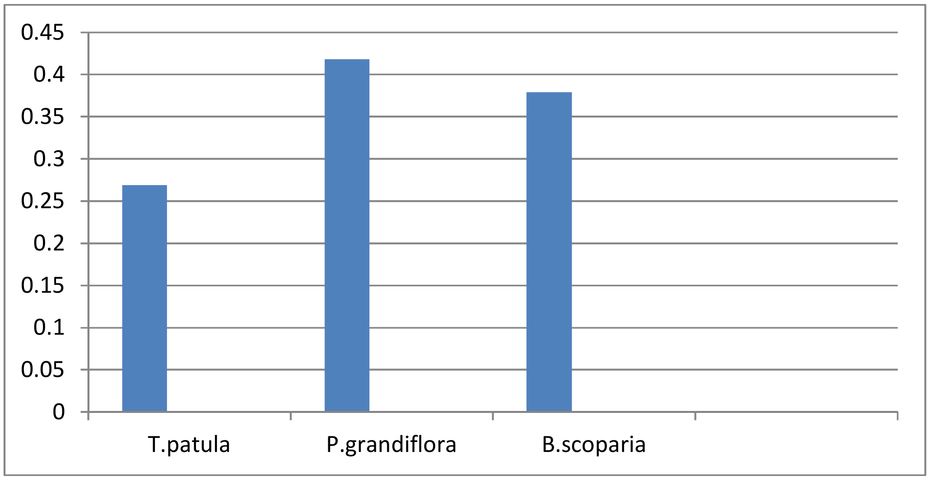
Metals	*Tagetes patula*	*Portulaca grandiflora*	*Bassia scoparia*
Cd	21.9	18.8	6.9
Cr	32.2	30.1	4
Zn	19.7	17	3.1
Hg	25	8.6	21
Pb	16.02	11.7	7



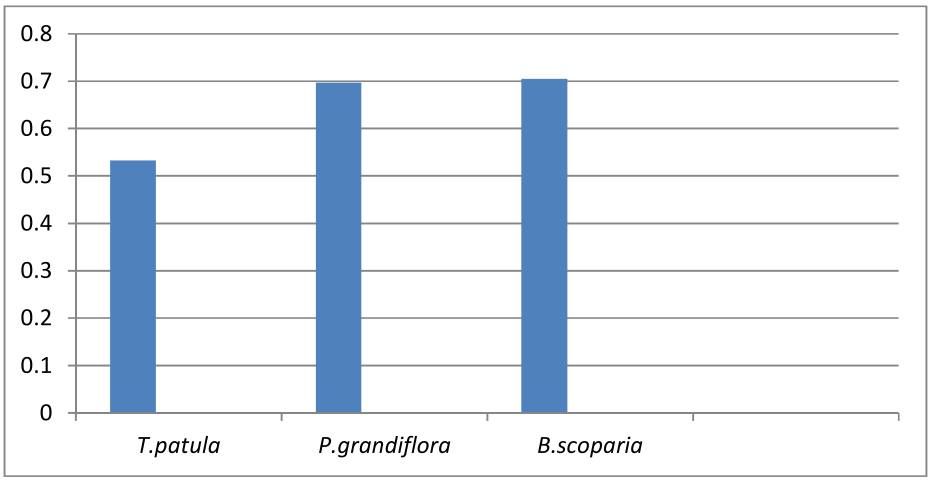
Graph 3.1. Graphically representation of concentration of heavy metals in untreated initial water sample



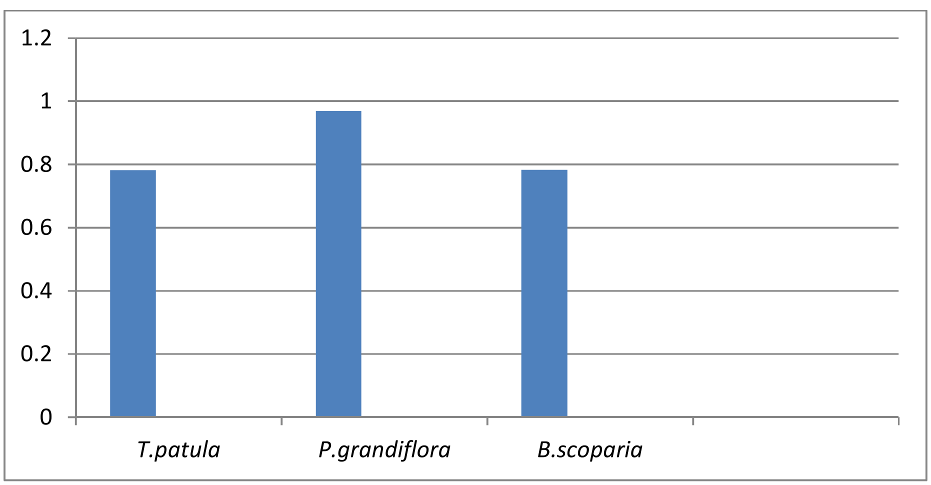
Graph 3.2. Cadmium concentration left in treated water sample after eight weeks



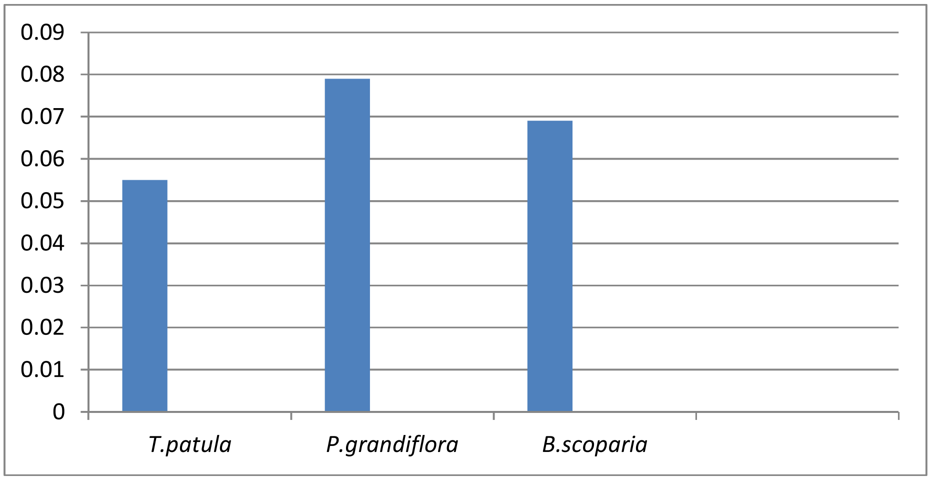
Graph 3.3. Chromium concentration left in water samples after eight weeks



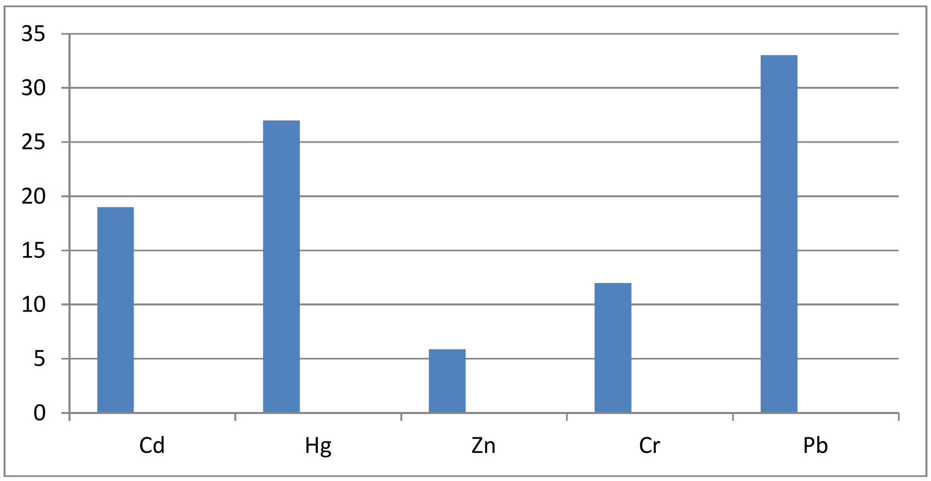
Graph 3.4. Zinc concentration left in water sample after eight weeks



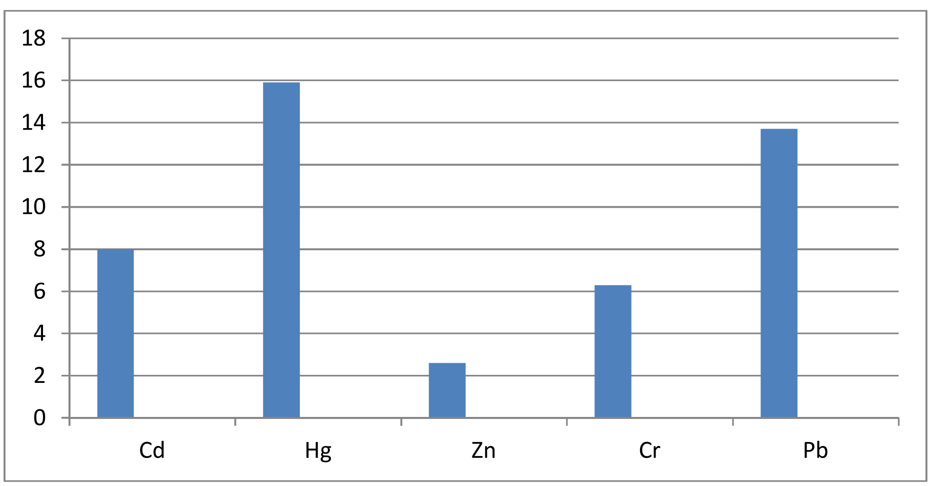
Graph 3.5. Mercury concentration left in water after eight weeks



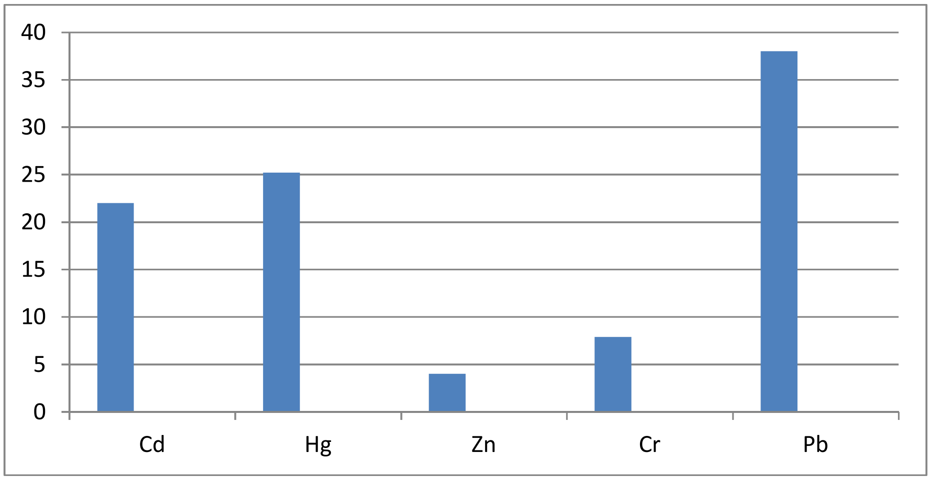
Graph 3.6. Lead concentration left in water sample after eight weeks



Graph 3.7. Heavy metal concentration in roots of *T. patula*



Graph 3.8. Heavy metal concentration in roots of *B. scoparia*



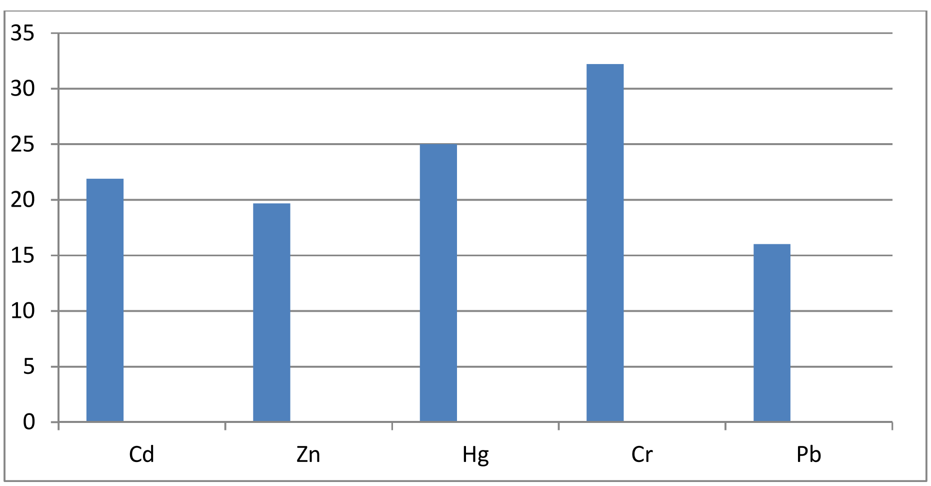
Graph 3.9. Heavy metal concentration in roots of *P. grandiflora*

According to the result given in Table 7, stems of *T. patula* has the highest efficiency for accumulating all the above heavy metals, even *P. grandiflora* and *T. patula* shows approximately the same results for accumulation of heavy metals in their stems.

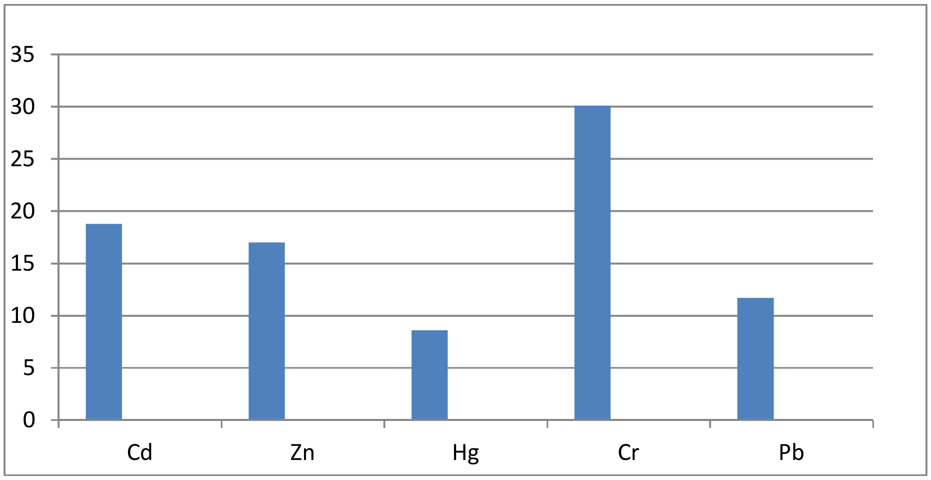
According to the Table 8, *T. patula* accumulated highest amount of heavy metals in its leaves and *P. grandiflora* and *B. scoparia* accumulated a great amount of cadmium in their leaves. *P. grandiflora* has also accumulated a significant level of chromium in its leaves.

Table 8. Presence of heavy metals in the leaves (mg/kg^{-1}) of plants

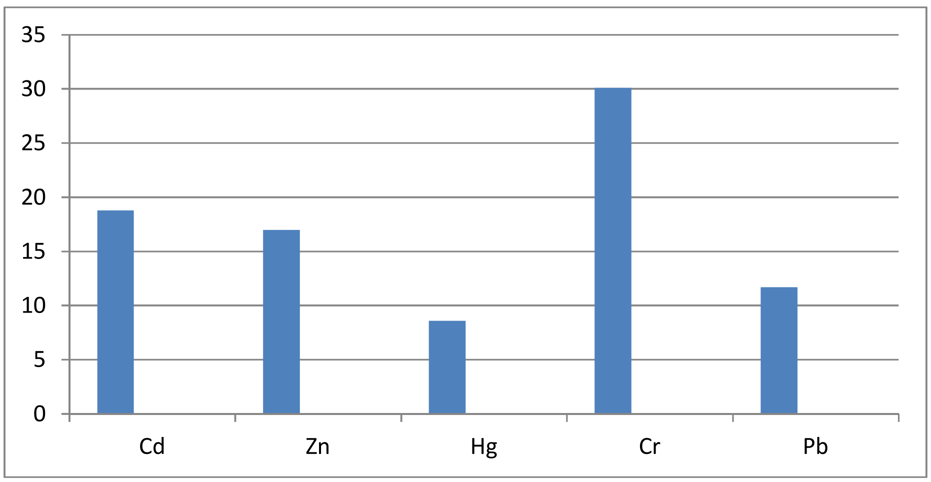
Metals	*Tagetes patula*	*Portulaca grandiflora*	*Bassia scoparia*
Cd	61	36.1	27.9
Cr	47.7	20.8	7.2
Zn	21.9	2.3	2.6
Hg	13.11	4.6	1
Pb	31.9	4.6	5.8



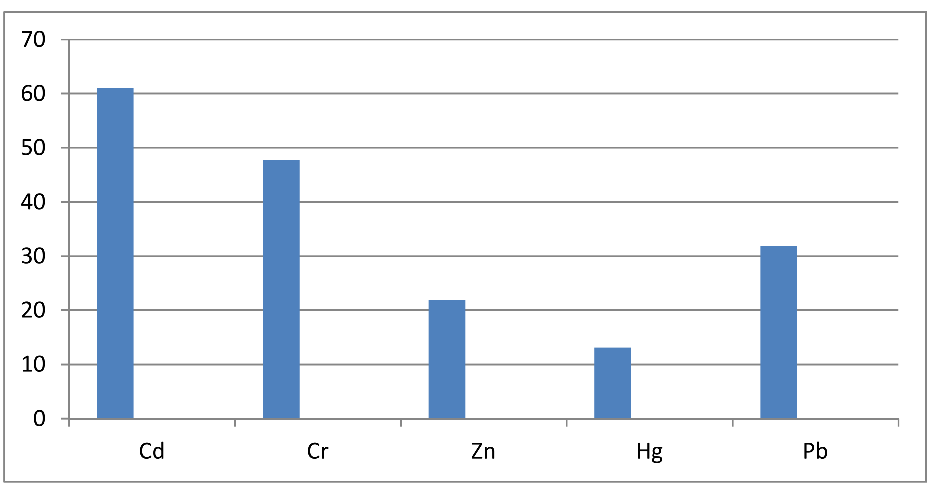
Graph 3.10. Heavy metal concentration in Stems of *T. patula*



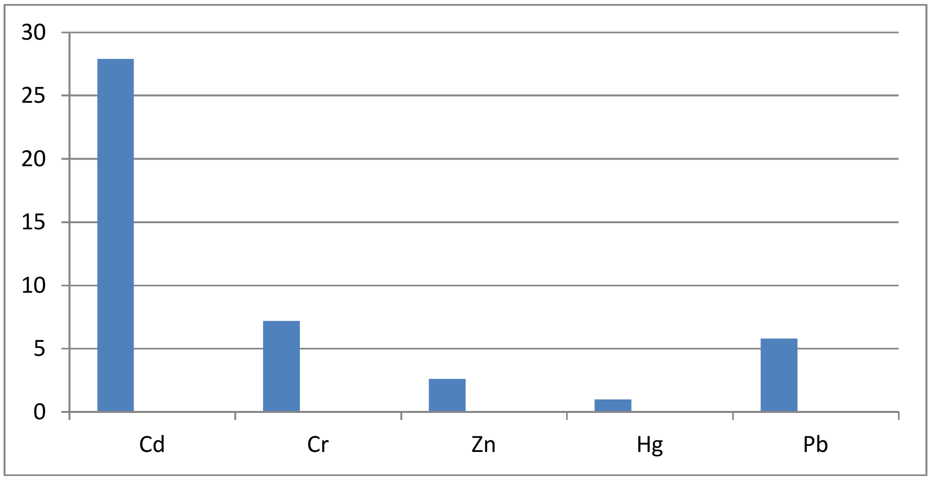
Graph 3.11. Heavy metal concentration in Stems of *B. scoparia*



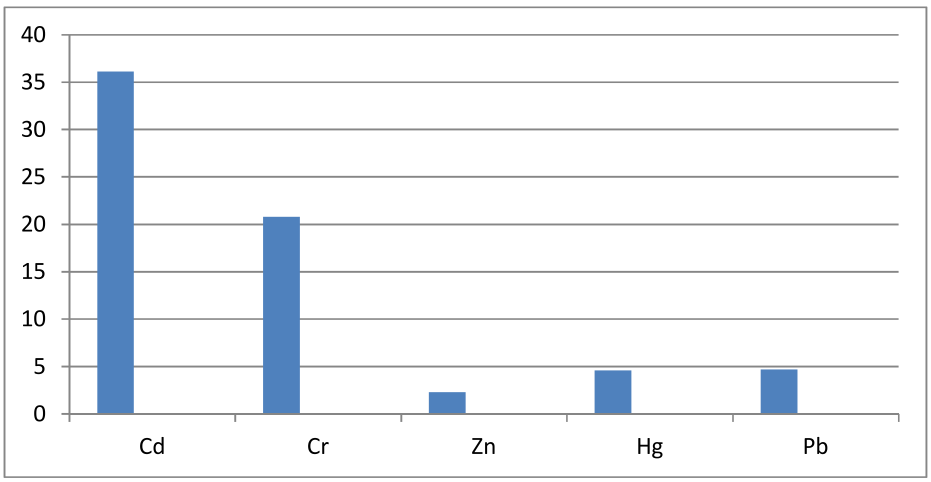
Graph 3.12. Heavy metal concentration in Stems of *P. grandiflora*



Graph 3.13. Heavy metal concentration in Leaves of *T. patula*



Graph 3.14. Heavy metal concentration in Leaves of *B. scoparia*



Graph 3.15. Heavy metal concentration in leaves of *P. grandiflora*

4. CONCLUSION

Phytoremediation is an effective, cheap or low maintenance technique for removal of heavy metals from environment. Out of all the three plants, *T. patula* shows a better growth in size and also shows the highest bio accumulating capacity for heavy metals. It can be concluded from the above study that the water quality of Yamuna river is good before entering national capital Delhi. The main disastrous impact is from Najafhgarh drains. From the above experiment, it can be said that phytoremediation, phytoextraction technique can be used for making Yamuna river pollution free, but we have to stop mixing untreated sewage water in Yamuna river. This project is a little attempt towards the big problem of Yamuna river pollution. This study showed the phytoremediation capacity of all of three selected plants: *Tagetes patula, Bassica scoparia,* and *Portulaca grandiflora.* Bioaccumulation of heavy metals in various parts of plants has also been analysed. The study concludes that the non-edible plants can be used for treatment of wastewater and contaminated soil in in-situ techniques. Further, Phytomining can be done to recover and reuse the heavy metals from plant tiisues after phytoremediation.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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