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

5
6 **Abstract**

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

18
19 **Keywords** : Yamuna river, *Tagetes patula*, *Bassica scoparia*, *Portulaca grandiflora*, Heavy
20 metals, Phytoremediation.

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23 **1. INTRODUCTION**

24
25 Yamuna river originates from Yamunotri glaciers of Himalayas. It is the largest tributary of river
26 Ganga. It is around 1370 kilometers in length. It flow across the states of Haryana, Delhi, Uttar
27 Pradesh. It merges into Ganga river in Allahabad. Big cities like Mathura, Agra, Delhi lie on the

28 Yamuna river bank. It is classified into five segments like Delhi segment, Upper segment,
29 Himalayan segment, Eutrophicated segment, Diluted segment depend on the basis of ecological
30 and hydrological conditions. The quality of water, river water in Himalayan segment is very
31 good and also meets all the standards within this segment. Yamuna river water is trapped by a
32 Wazirabad barrage for the purpose of domestic supply of water in Delhi. The Okhla barrage of
33 Delhi receives the water of seventeen drain sewage, Najafgarh drain. It is the most polluted
34 segment of river Yamuna. Today it has become the most polluted and dirtiest river of the country
35 and was once described as the lifeline of Delhi city.

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

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

51 High uptake and slow elimination of Heavy metals cause harm to the aquatic life. As the heavy
52 metals get settled down in the sediment and uptake by the plants or aquatic organisms, drink by
53 the animal and this will ultimately harm the life of organisms. Human by many ways are highly
54 exposed to heavy metals as they are also the part of the food chain. Table 1 shows the

55 permissible limit of heavy metals (Ad, Zn, Cr, Pb, Hg) prescribed by WHO.

56

57 **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

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59 High uptake of lead causes changes in the gill, kidney and liver of fish (Mohamed and Gad,
60 2005). Intestine and gills are the major site of metal accumulation in fishes. It causes variation in
61 the lipids of aquatic organisms. Lead cause swelling in the gills and jaws of fishes. Nausea,
62 anemia and vomiting, etc problems are the side effects of lead exposure in humans.

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

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

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

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

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

94 Removal of heavy metals with the help of microorganisms is a very efficient method, but it is
95 confined to water system only. Some other remediation methods are bio augmentation, land
96 farming, bio leaching, rhizofiltration, biostimulation, composting, bioreactor, and
97 phytoremediation. Phytoremediation is a technique that uses plants for degradation of toxic
98 contaminants present in environment. It involves the use of living organisms, especially plants
99 and microorganisms to eliminate the effects of contaminants present in air, water, soil.

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

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104 1.2 OBJECTIVES

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

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111 2. Material and Methods

112 **2.1 Solution:** Water sample from Yamuna river was collected and preserved in a can at freezing
113 temperature.

114 **2.2 Plants used:-** Three different plants (*Tagetes patula*, *Bassica scoparia*, *Portulaca*
115 *grandiflora*) were used for the study. The seeds of the plants were collected from a local
116 nursery at Delhi-NCR. The plant classifications have been listed in Table 2.

117 Table 2:

Classification	<i>Tagetes patula</i>	<i>Bassica scoparia</i>	<i>Portulaca grandiflora</i>
Kingdom	Plantae	Plantae	Plantae
Order	Asterales	Caryophyllales	Caryophyllales
Family	Asteraceae	Amaceanthaceae	Portulacaceae
Genus	Tagetes	Bassia	Portulaca
Species	<i>T.patula</i>	<i>B.scoparia</i>	<i>P.grandiflora</i>

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119 *T.patula* grown and harvested annually and flowers are yellow and red in colour, reaching 0.3 m
120 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
121 grows from October to April. The plants common name is called “Marygold”. The leaves of the
122 plants include oil glands and the oils are pungent. It can grow in any sort of soil. *T.patula* is
123 widely cultivated in India it also have various uses in medicines.

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

127 It is a small but fast growing annual plant as it **has** grown 30 cm tall. The leaves of the plant are
128 thick and fleshy, up to 2.5 cm long arranged in a cluster like structure. the flowers are 2.5-3 cm
129 diameter with five petals. The colour of flowers varied from red, pink, white, orange and yellow.
130 In India it is called “**9 o clock**” flower because it blooms at 9 a.m. It generally **requires** no
131 attention as it gets spread very easily by itself. This plant can easily grow in adverse conditions
132 like pesticides, high heavy metal concentration, chemicals etc. This plant consumption known to
133 reduce the risk of cancer and heart diseases (**Thangavel** et . al ., 1999) .

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

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139 **2.3 Procedure**

140 **2.3.1 Water Collection:** Water sample was collected from Yamuna river enrooted Delhi-Agra
141 via Haryana, near Palwal District, as shown in picture below.

142

143 **2.3.2 Model set up:**

- 144 i. Six plastic boxes were taken.
- 145 ii. Two **boxes** for each plant.
- 146 iii. For setting up the model, one plastic box was placed on **another**.
- 147 iv. Small holes were induced **d** in the centre of each plastic box for the passage of plant roots
148 as shown by the pictures below in figure a, b, c, d, e.
- 149 v. After germination of seeds in soil, small plants **were** transplanted. From the soil **in** the
150 upper plastic box which was already filled with garden soil.

151 vi. Roots of the plants were allowed to reach the lower plastic box. Already filled with
152 contaminated water sample of Yamuna river through induced wholes.



153 **Figure a: Set up for plant**

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156 **Figure b: Set up of different plants**

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Figure c: Set up for *P.grandiflora*



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Figure d: Picture of *B.scoparia*



Figure e: Picture of *T.patula*

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166 **2.3.3 Growth period:**

- 167 i. Plants were allowed to grow in that setup for eight weeks.
- 168 ii. During these eight weeks, generally called “Growth period”, proper attention to the plants
- 169 was given just to make sure. That none of the plant will die.
- 170 iii. Fertilizers such as cow dung was mixed into the soil.
- 171 iv. Plants were placed beneath a tree, because much, sunlight exposure can cause browning
- 172 of plants.

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174 **2.3.4 Change in size parameters:** Growth in the length of the plants was measured. After

175 completion of fourth and eighth week by a centimetre scale.

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177 **2.3.5 Lab work:** After 8 weeks, all of the three plants were harvested and the water. Samples

178 initial untreated and final treated, from all the three plants were taken and stored in three

179 different plastic bottles with proper labeling.

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2.3.6 Acid Digestion: Acid digestion a method for dissolving samples into a solution. It is done by adding a considerable amount of acids and heating, until the matrix 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 labeling.

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

207 and also shown in the picture given below.

208 **Table 4: Change in length (cm) of the Plants**

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

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

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216 **Table 5: Presence of heavy metals (mg/L) in water sample**

Metals	Initial water sample	<i>Tagetes patula</i>	<i>Portulaca prandiflora</i>	<i>Bassia scoparia</i>
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

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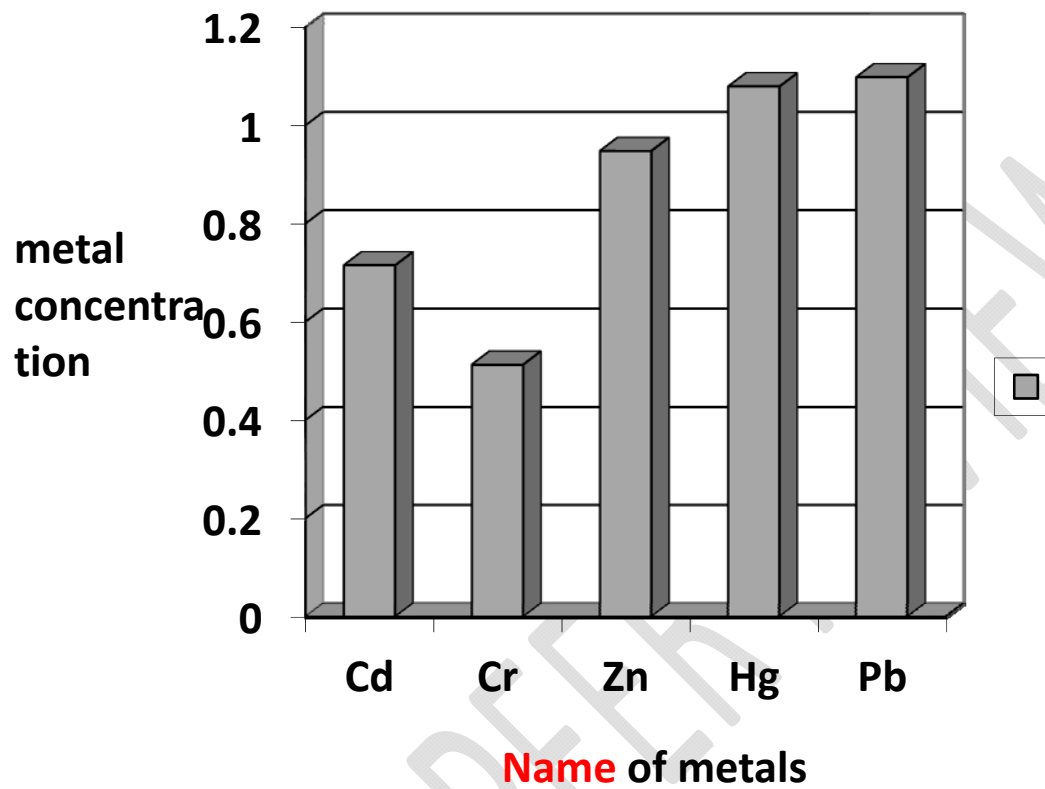
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219 In the present study, cadmium was undetectable in the water sample of *B.scoparia* and *T.patula*

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

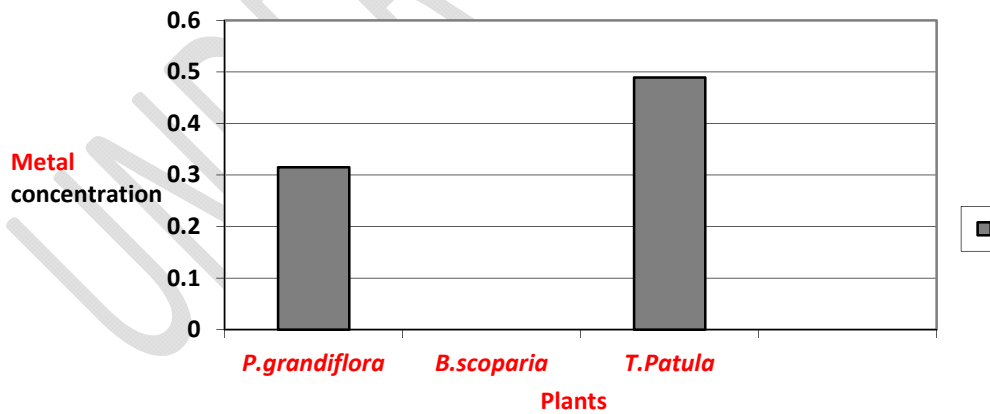
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230 **Graph3. 1: Graphically representation of concentration of heavy metals in untreated initial**
231 **water sample**

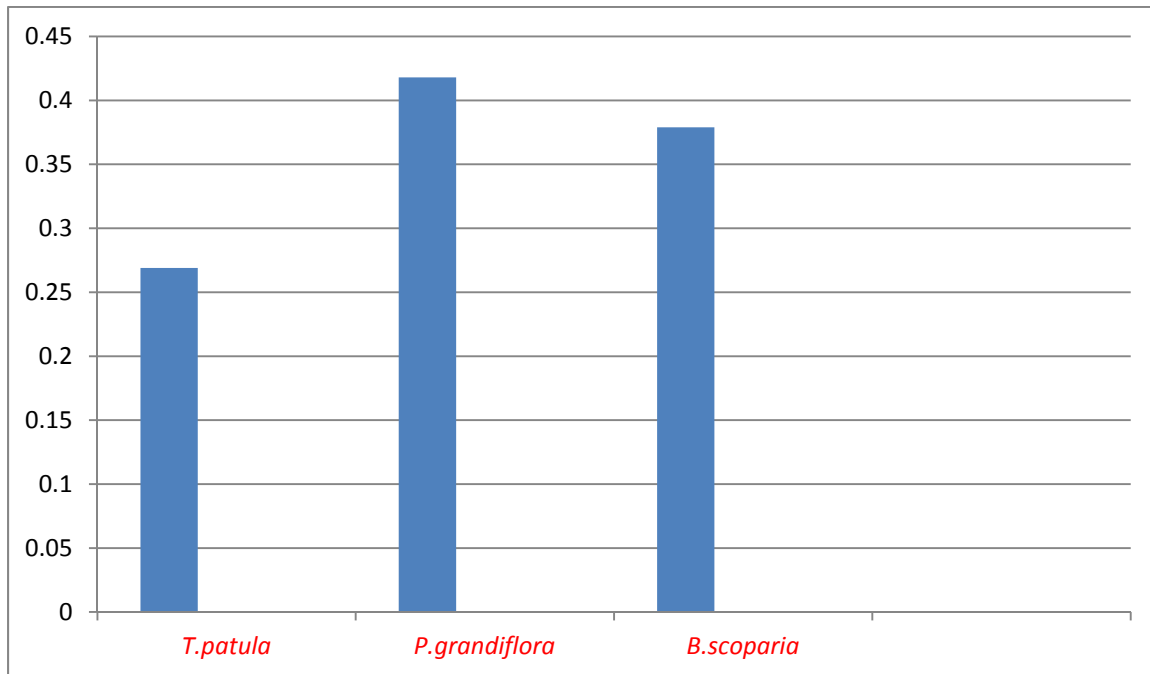


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233 **Graph 3.2: Cadmium concentration left in treated water sample after eight weeks**



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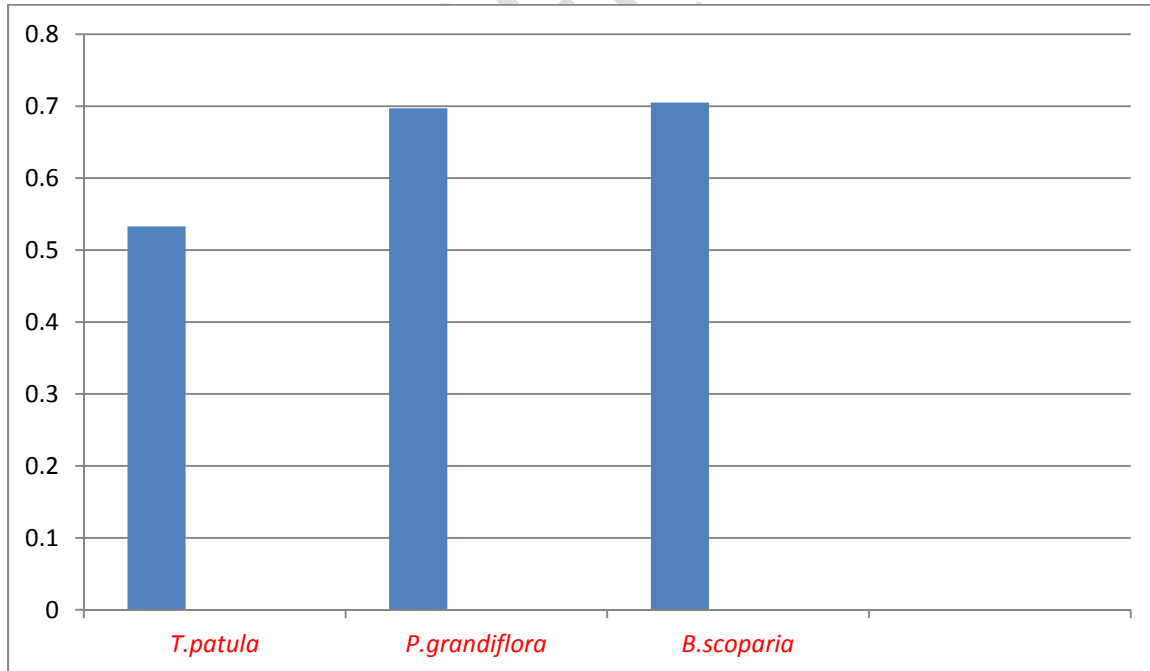


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Graph 3.3: Chromium concentration left in water samples after eight weeks

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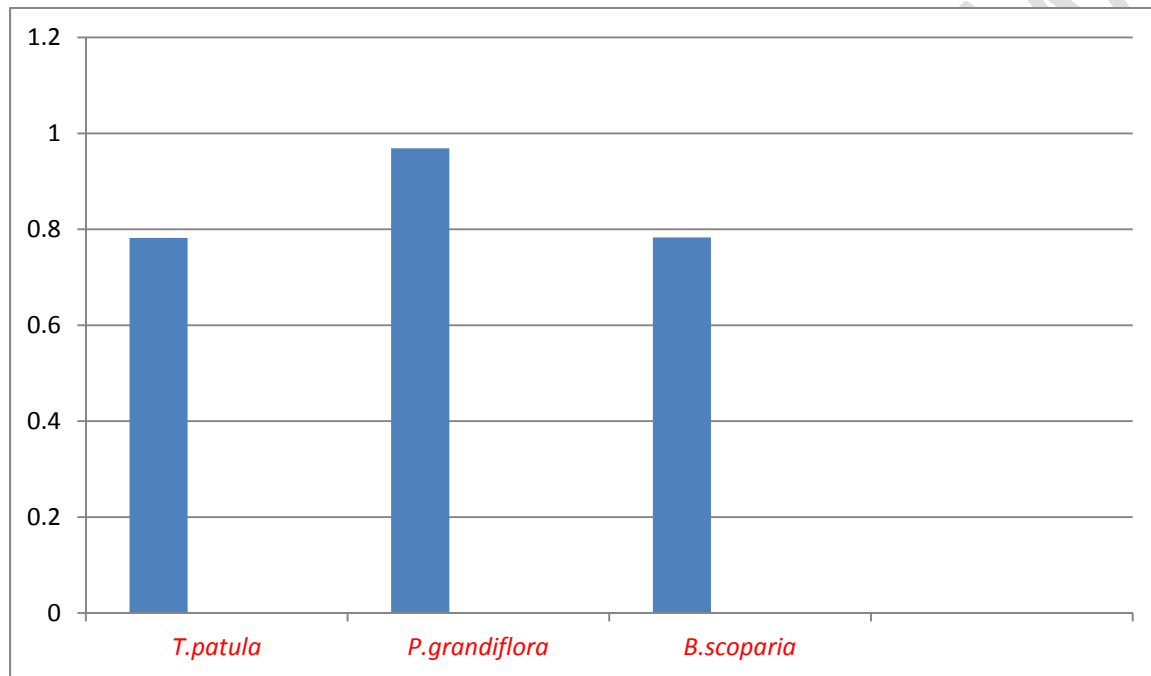
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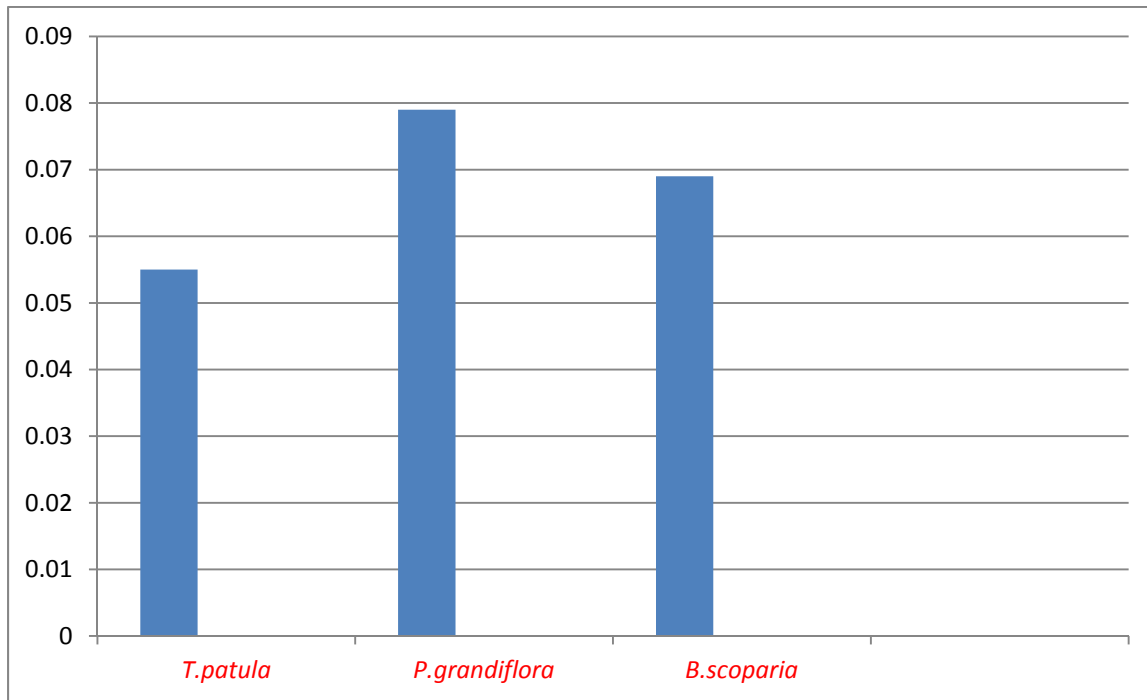
Graph 3.4 :Zinc concentration left in water sample after eight weeks



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Graph3. 5: Mercury concentration left in water after eight weeks



Graph3. 6: Lead concentration left in water sample after eight weeks

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249 Bioaccumulation of heavy metals by plants: Plants also have the ability to accumulate the Abeen
 250 checked with the help of AAS technique, after the acid digestion process of samples. The results
 251 of AAs are given in the table below.

252 **Table 6: Presence of heavy metals in the Roots (mg/kg⁻¹) of plants**

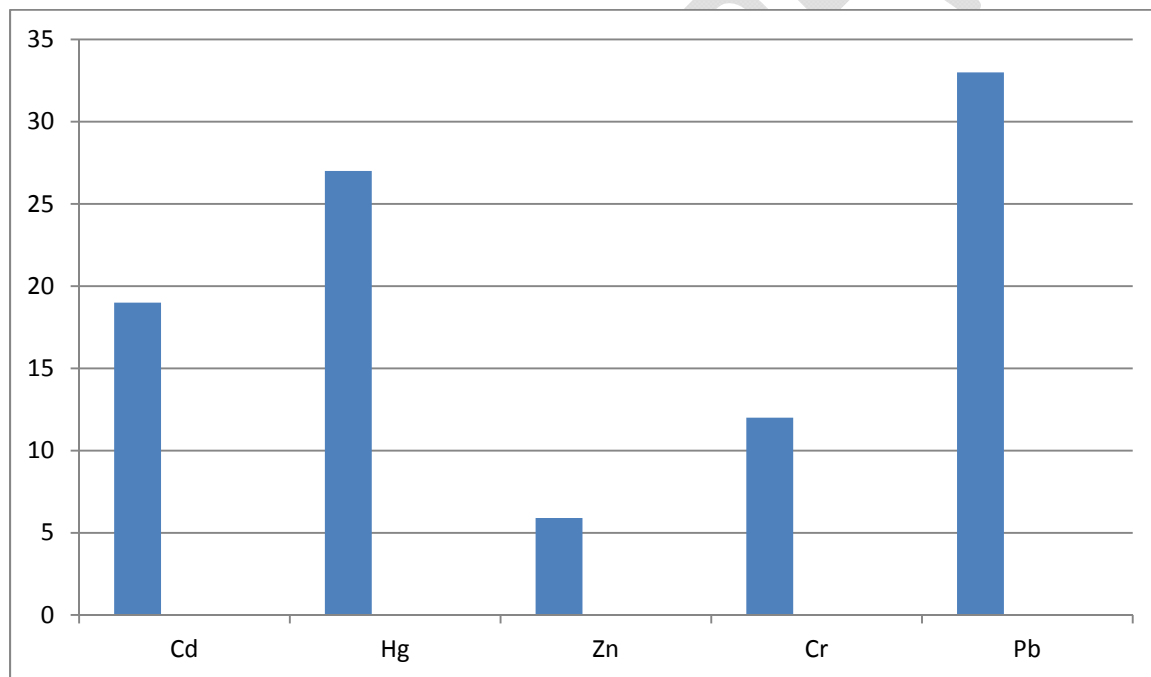
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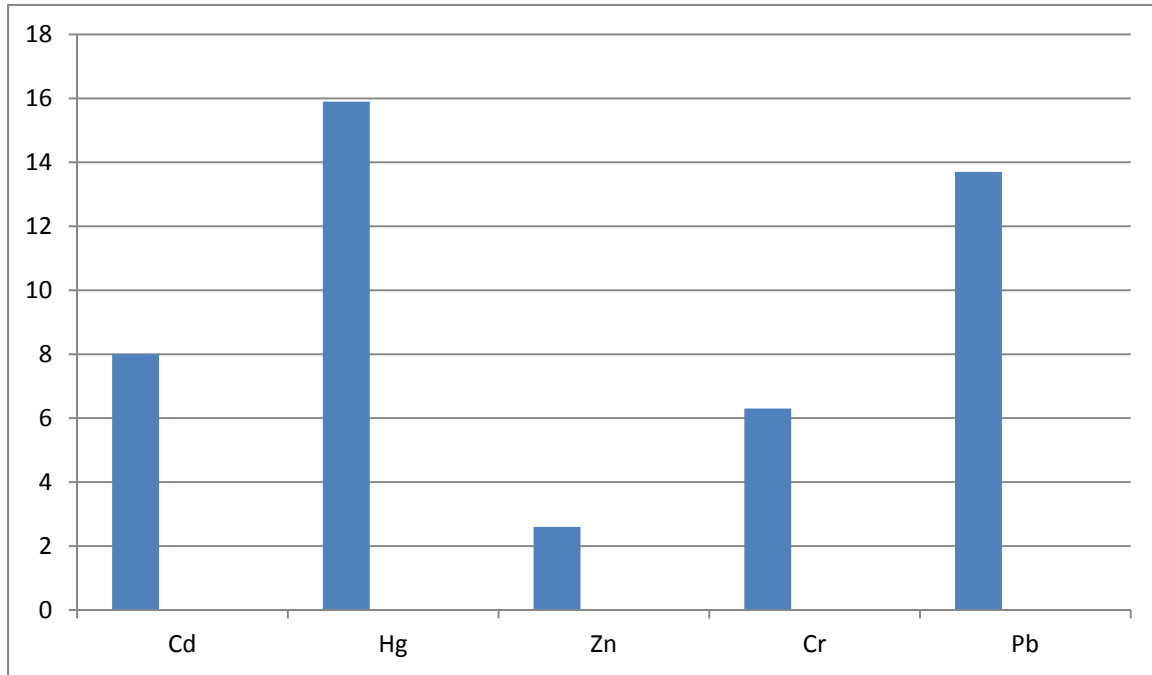
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Metals	<i>Tagetes patula</i>	<i>Portulaca grandiflora</i>	<i>Bassia scoparia</i>
Cd	19	22	8
Cr	12	7.9	6.3
Zn	5.9	4	2.6
Hg	27	25.2	15.9
Pb	33	38	13.7

263 According to the above result, accumulation of zinc, mercury and chromium was highest in the
 264 roots of *T.patula*. Lead and cadmium accumulation was highest in the roots of *P.grandiflora*.
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266 **Graph 3.7: Heavy metal concentration in roots of *T.patula***
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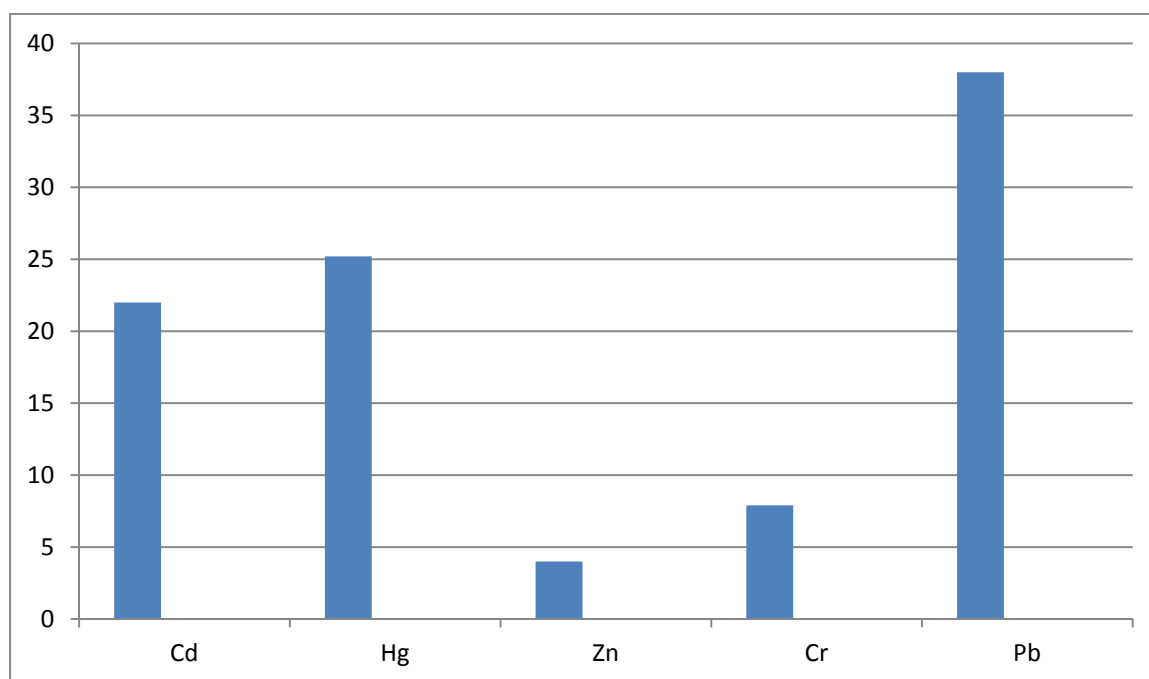
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Graph 3.8: Heavy metal concentration in roots of *B.scoparia*

UNDER PEER REVIEW



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

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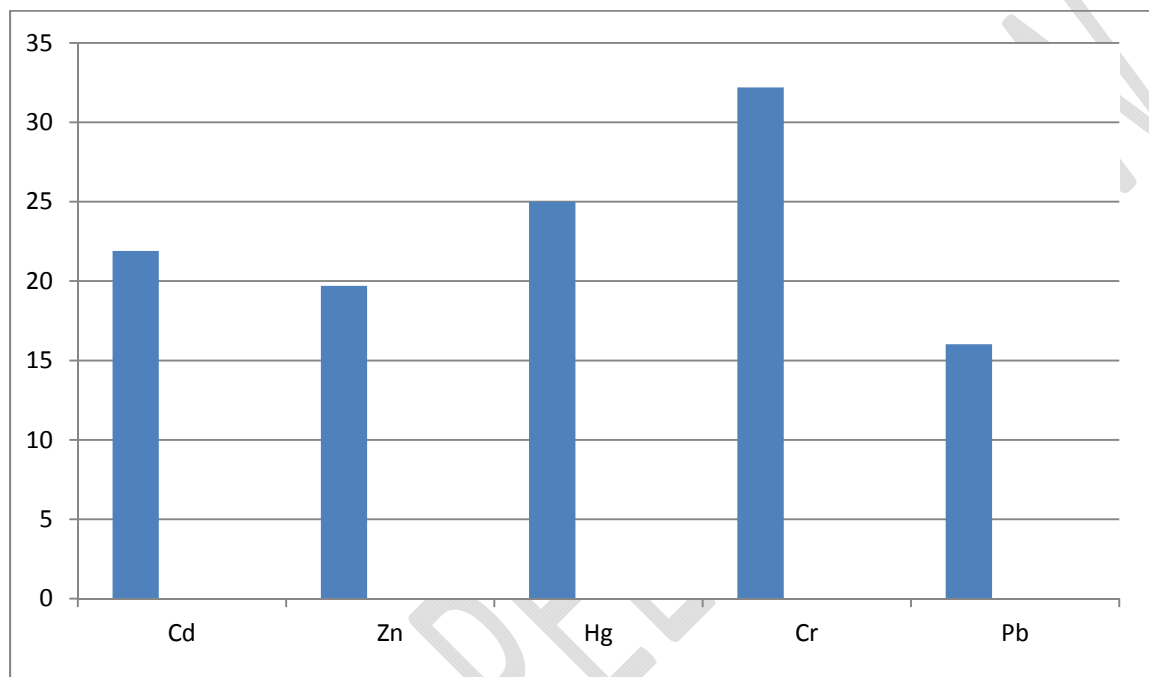
Table 7: Presence of heavy metals in the Stems (mg/kg⁻¹) of plants

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Metals	<i>Tagetes patula</i>	<i>Portulaca grandiflora</i>	<i>Bassia scoparia</i>
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

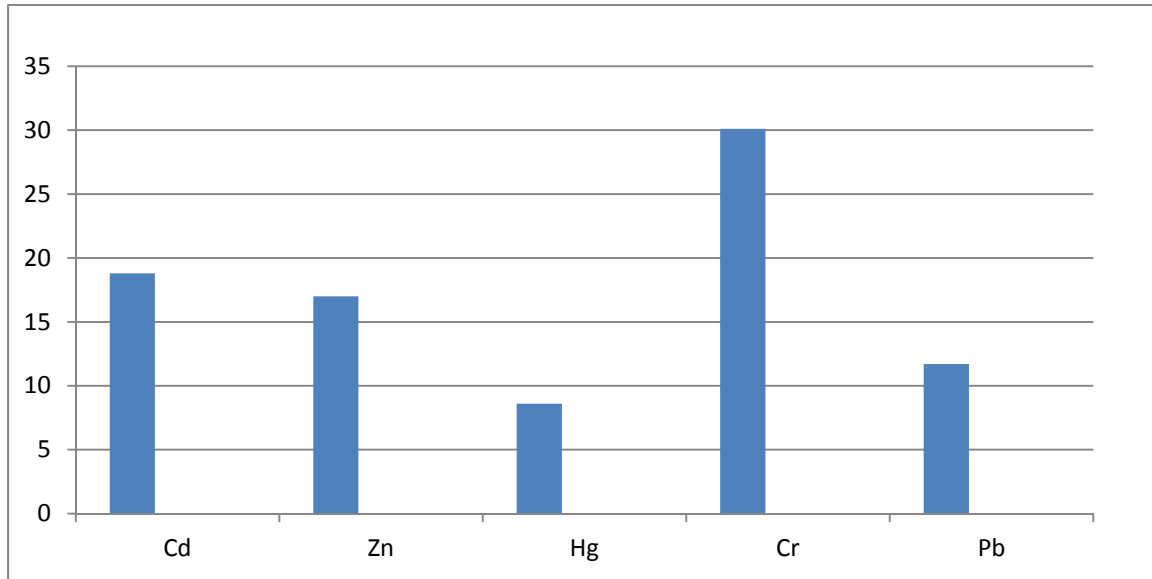
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288 According to the result given in above Table, stems of *T.patula* has the highest efficiency for
289 accumulating all the above heavy metals, even *P.grandiflora* and *T.patula* shows approximately
290 the same results for accumulation of heavy metals in their stems.
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Graph 3.10: Heavy metal concentration in Stems of *T.patula*



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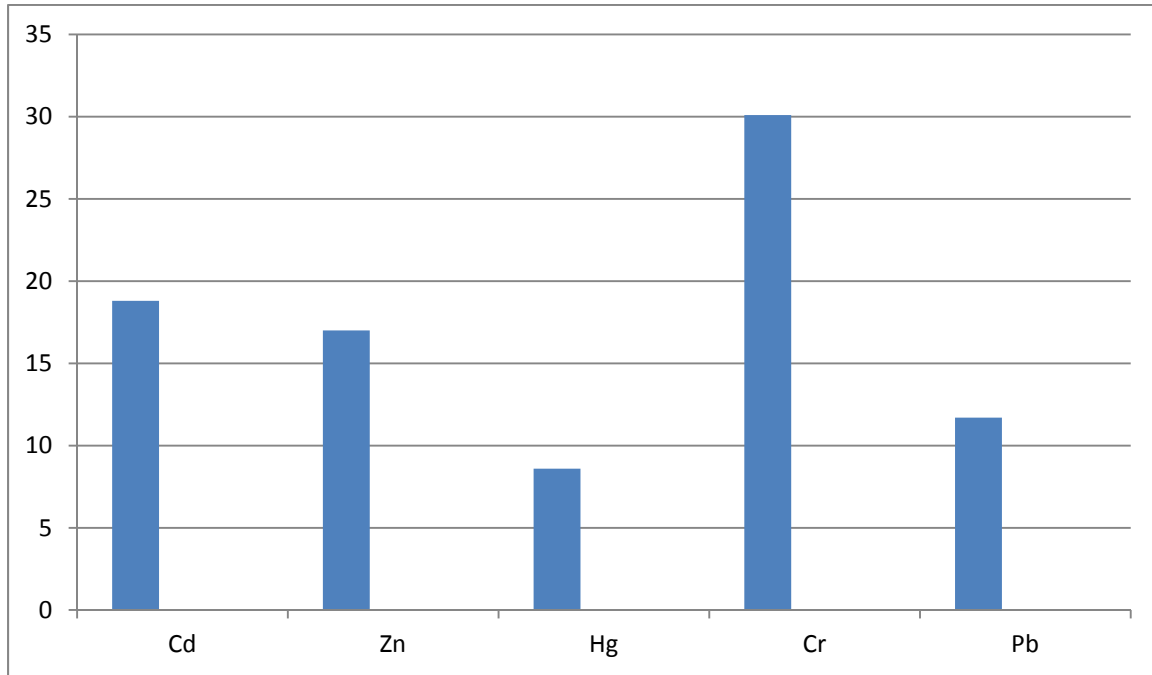
299 **Graph 3.11: Heavy metal concentration in Stems of *B.scoparia***

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UNDER PEER REVIEW



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

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307 **Table 8: Presence of heavy metals in the Leaves (mg/kg⁻¹) of plants**

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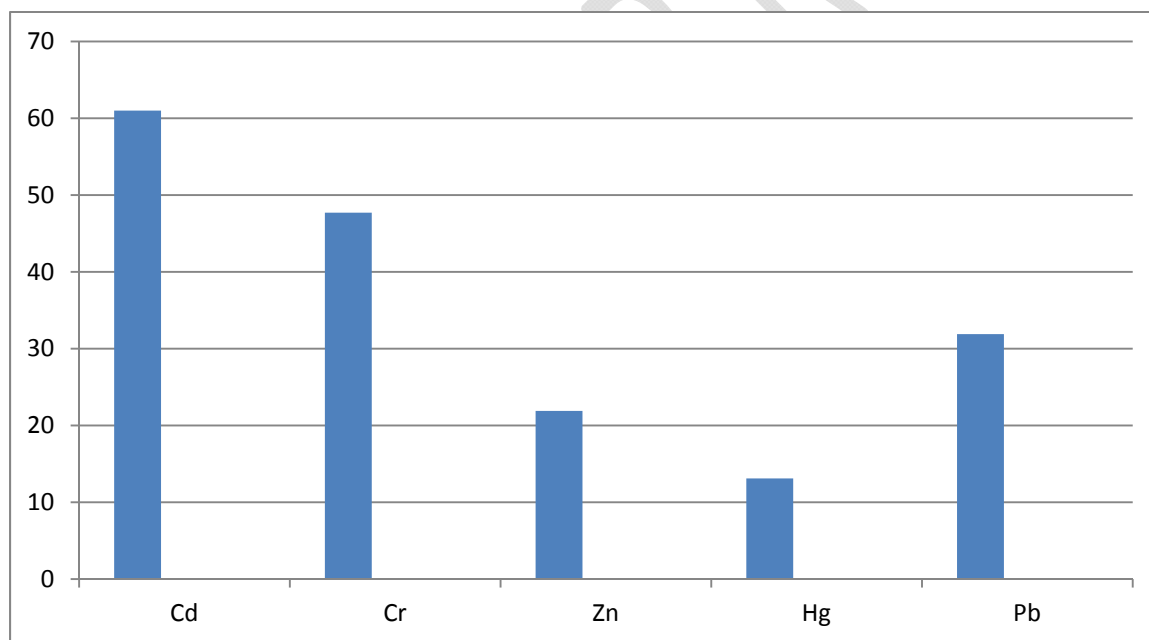
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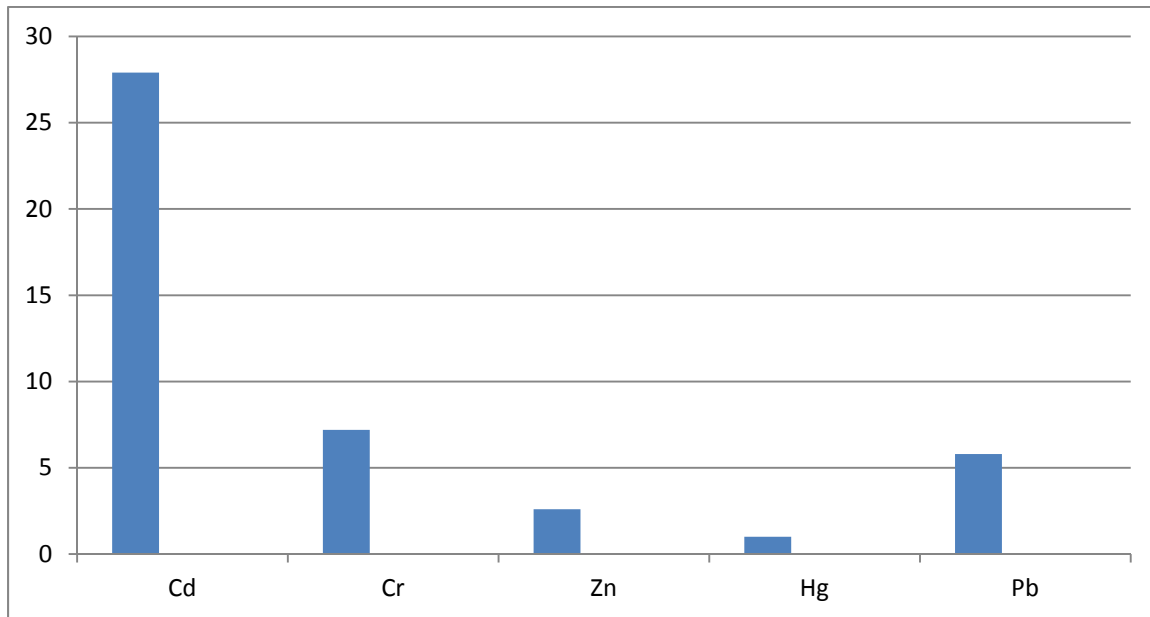
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Metals	<i>Tagetes patula</i>	<i>Portulaca grandiflora</i>	<i>Bassia scoparia</i>
Cd	61	36.1	27.9
Cr	47.7	20.8	7.2
Zn	21.9	2.3	2.6
Hg	13.11	4.6	1
Pb	31.9	4.6	5.8

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 312 According to the above table, *T.patula* accumulated highest amount of heavy metals in its leaves
 313 and *P.grandiflora* and *B.scoparia* accumulated a great amount of cadmium in their leaves.
 314 *P.grandiflora* has also accumulated a significant level of chromium in its leaves.
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 318 **Graph 3.13: Heavy metal concentration in Leaves of *T.patula***
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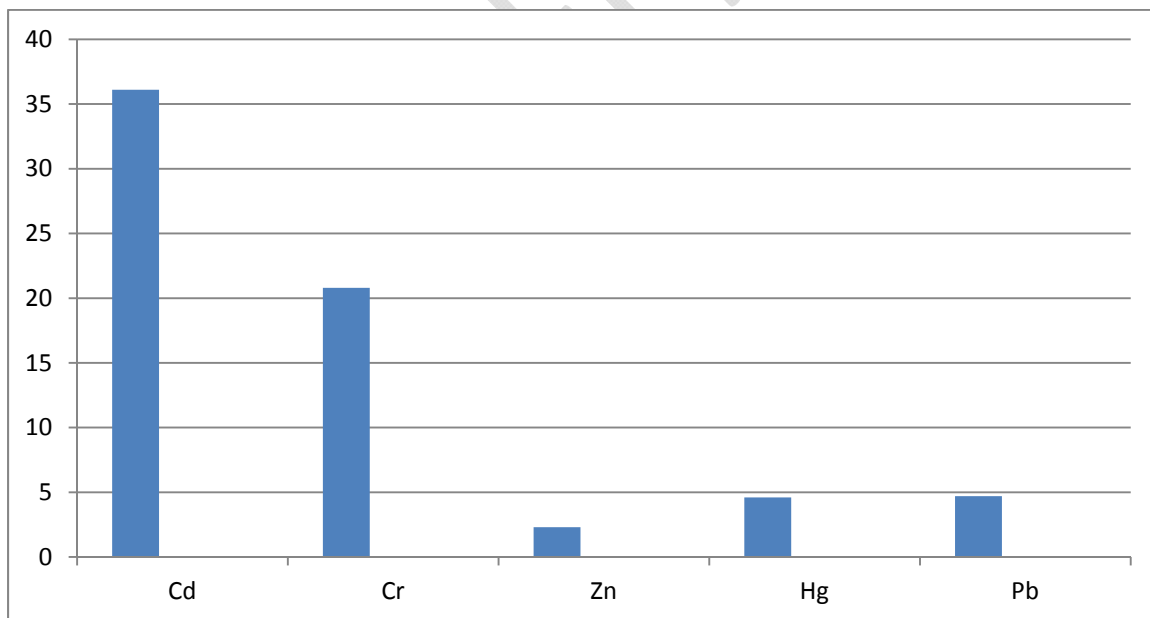
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Graph 3.14: Heavy metal concentration in Leaves of *B.scoparia*

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326 **Graph 3.15: Heavy metal concentration in Leaves of *P.grandiflora***

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328 **4. Conclusion:** Phytoremediation is an effective, cheap or low maintenance technique for

329 removal of heavy metals from environment. Out of all the three plants, *T.patula* shows a

330 better growth in size and also shows the highest bio accumulating capacity for heavy

331 metals. It can be concluded from the above study that the water quality of Yamuna river

332 is good before entering national capital Delhi. The main disastrous impact is from

333 Najafgarh drains. From the above experiment, it can be said that phytoremediation,

334 phytoextraction technique can be used for making Yamuna river pollution free, but we

335 have to stop mixing untreated sewage water in Yamuna river. This project is a little

336 attempt towards the big problem of Yamuna river pollution.

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