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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**

7
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 flows across the states of Haryana, Delhi, Uttar
27 Pradesh. It merges into Ganga river in Allahabad. Big cities like Mathura, Agra, Delhi lie on

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
32 Wazirabad barrage for the purpose of domestic supply of water in delhi. The Okhla barrage of
33 Delhi receive water of seventeen drain sewage, Najafgarh drain. It is the most polluted segment
34 of river Yamuna. Today it has become the most polluted and dirtiest river of the country and was
35 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 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 into 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 in 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 theses heavy metals are like lead paint, house
46 hold dust, silver foil in food, surface soil, batteries, peeling paints, sewage wastes, plumbing
47 system etc. use of fertilizers and pesticides is also a great source of heavy metals like Cd, As.
48 Some of these metals are essential for human beings but in very low concentration such as Ca,
49 Cu, Fe, 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. Humans by many ways are highly
54 exposed to heavy metals as they are also the part of food chain. Table 1 shows the permissible

55 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 ($\mu g/kg^{-1}$)
Cadmium	0.003	6
Zinc	3	123
Chromium	0.05	25
Lead	0.01	----
Mercury	1.3	0.3

58

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

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

75 Chromium cause acute and chronic effects on fishes. High chromium Uptake causes changes in

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

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

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

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

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

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103 **1.2 OBJECTIVES**

- 104
- 105 i. Determination of heavy metal content in Yamuna river water sample
 - 106 ii. Removal of contaminants from river water sample with the help of Hyper - accumulator
 - 107 plants
 - 108 iii. Evaluation of Bio-accumulation capacity of all of three selected plants
- 109

110 **2. Material and Methods**

111 **2.1 Solution:** Water sample from Yamuna river was collected and preserved in a can at freezing

112 temperature.

113 **2.2 Plants used:-** Three different plants (*Tagetes patula*, *Bassica scoparia*, *Portulaca*

114 *grandiflora*) were used for the study. The seeds of the plants were collected from local

115 nursery at Delhi-NCR. The plants classifications have been listed in table 2.

116 Table 2:

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

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118 *T.patula* grown and harvested annually and flowers are yellow and red in colour, reaching 0.3 m

119 to 0.5 m in size.the plant size vary from 0.1 to 2.2 m tall. They have fibrous roots.In India it

120 grows from October to April. Plants common name is called “Marygold” . the leaves of plant

121 include oil glands and the oils are pungent. It can grow in any sort of soil. T.patula is widely

122 cultivated in India it also have various use in medicines.

123 The main reason for selecting this plant for phytoremediation is its ability of resist adverse

124 condition like pests, salinity, drought etc. *T.patula* is good for phytoextraction of heavy metals
125 like arsenic, Mercury etc.

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

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

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138 **2.3 Procedure**

139 **2.3.1 Water Collection:** Water sample was collected from Yamuna river enrooted Delhi-Agra
140 via Haryana, near near Palwal district, as shown in picture below.

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142 **2.3.2 Model set up:**

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

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contaminated water sample of Yamuna river through induced wholes.



Figure a: Set up for plant

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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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165 **2.3.3 Growth period:**

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

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173 **2.3.4 Change in size parameters:** Growth in the length of the plants was measured After

174 completion of fourth and eighth week by a centimeter scale.

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176 **2.3.5 Lab work:** After 8 weeks, all of the three plants were harvested and the water Samples

177 initial untreated and final treated, from all the three plants Were taken and stored in three

178 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 considerable amount of acids and heating, Until the matrix get 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 used alone, so it was combined with sulfuric acid. To the water samples, first added 5ml of concentrated HNO₃ and 10ml of concentrated H₂SO₄, boil on a hot plate at 90° temperature for evaporation, until dense fumes of dense SO₃ appears. After clearing of the solution, no brownish fume appear, then Distilled water was added to make solution dilute and heated.

Then solution was centrifuged at 3000 rpm for 25 min and 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 grind into grinder and 2g of sample were Taken in the glass beaker after weighing For digestion, HNO₃ And HClO₄ acids was used To the sample first 5ml of HNO₃ added and heated on a Hot plate at temperature 100° for 30 to 35 min, then 2.5ml Of HClO₄ added to the mixture and boiled. white fumes Appeared, later 5ml of dilute water added to mixture and again boiled until the fumes were totally released.

Detection of present heavy metals 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

206 and also shown in the picture given below.

207 **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

209

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

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

Metals	Initial water sample	<i>Tagetes Patula</i>	<i>Portulaca GrandiFlora</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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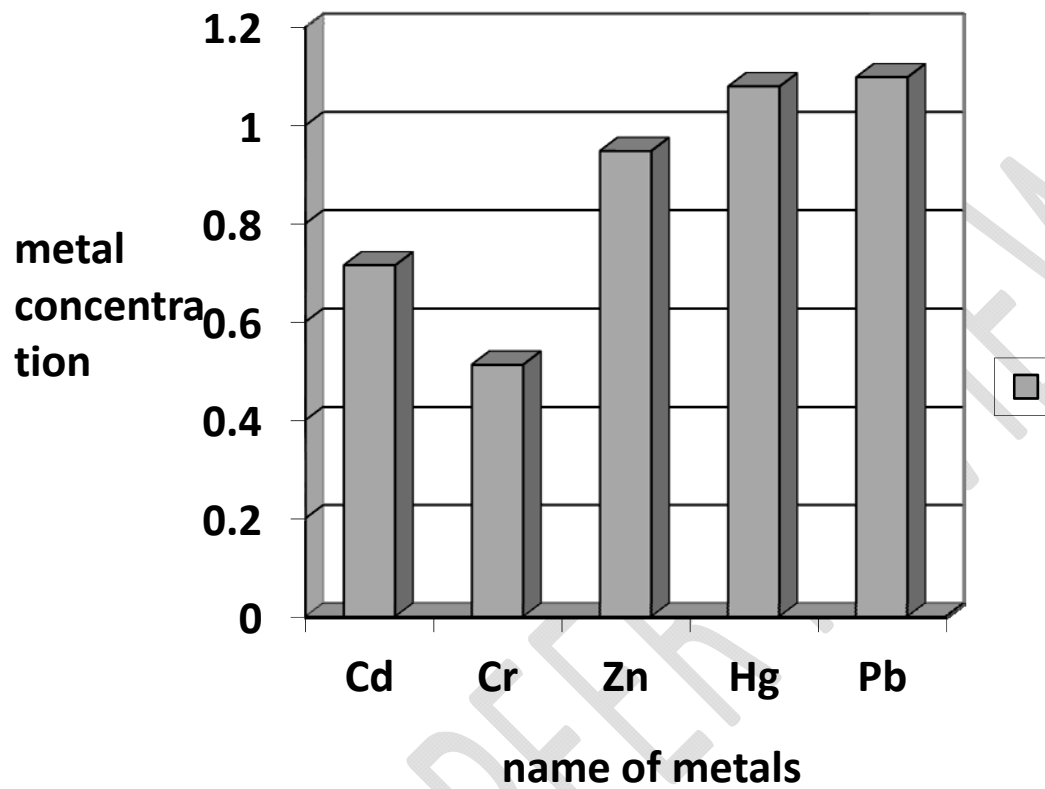
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218 According to the results, Cadmium was undetectable in the water sample of *B.scoparia* and

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

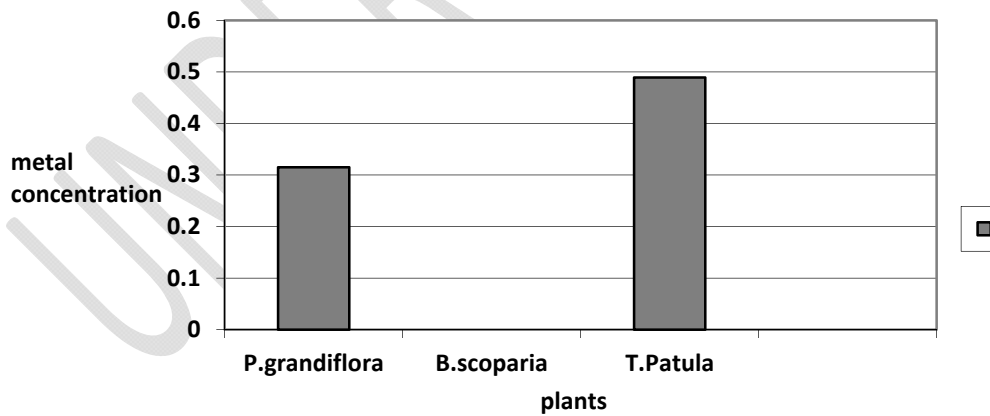
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229 **Graph3. 1: Graphically representation of concentration of heavy metals in untreated initial**
230 **water sample**

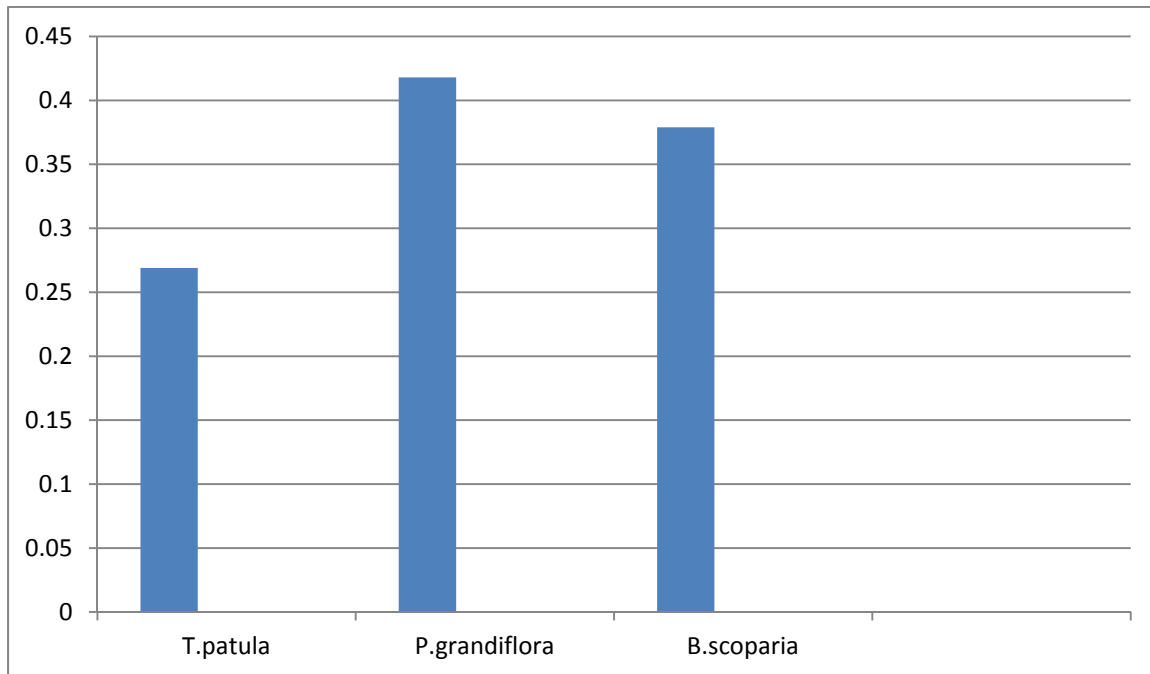


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232 **Graph 3.2: Cadmium concentration left in treated water sample after eight weeks**



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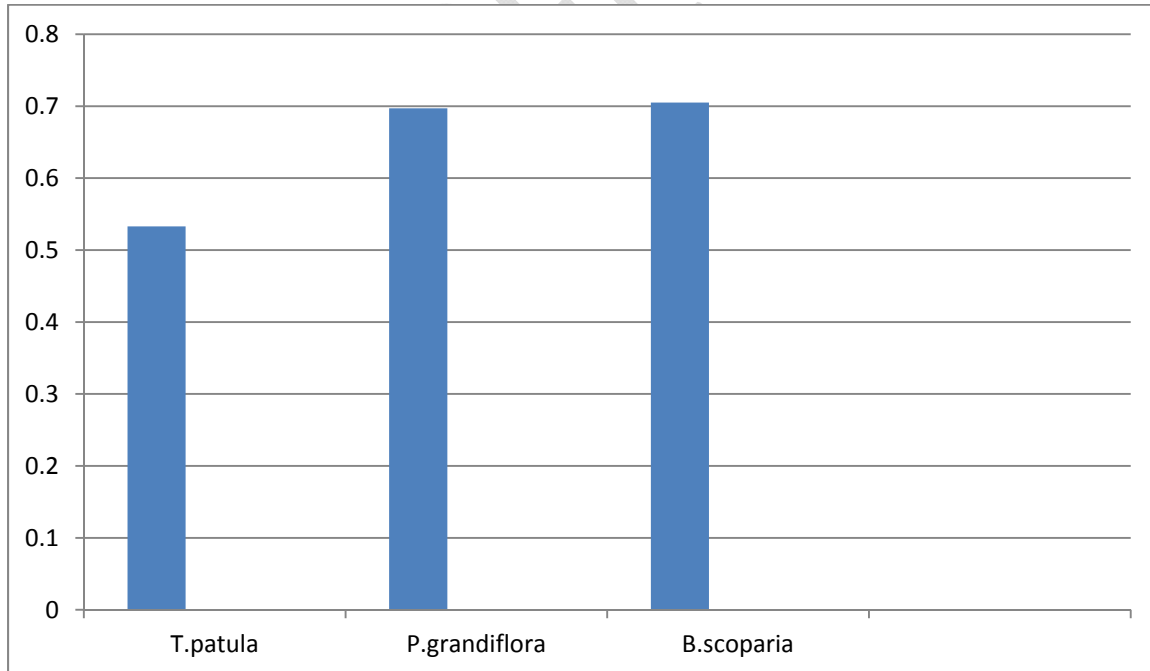


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Graph 3.3: Chromium concentration left in water samples after eight weeks

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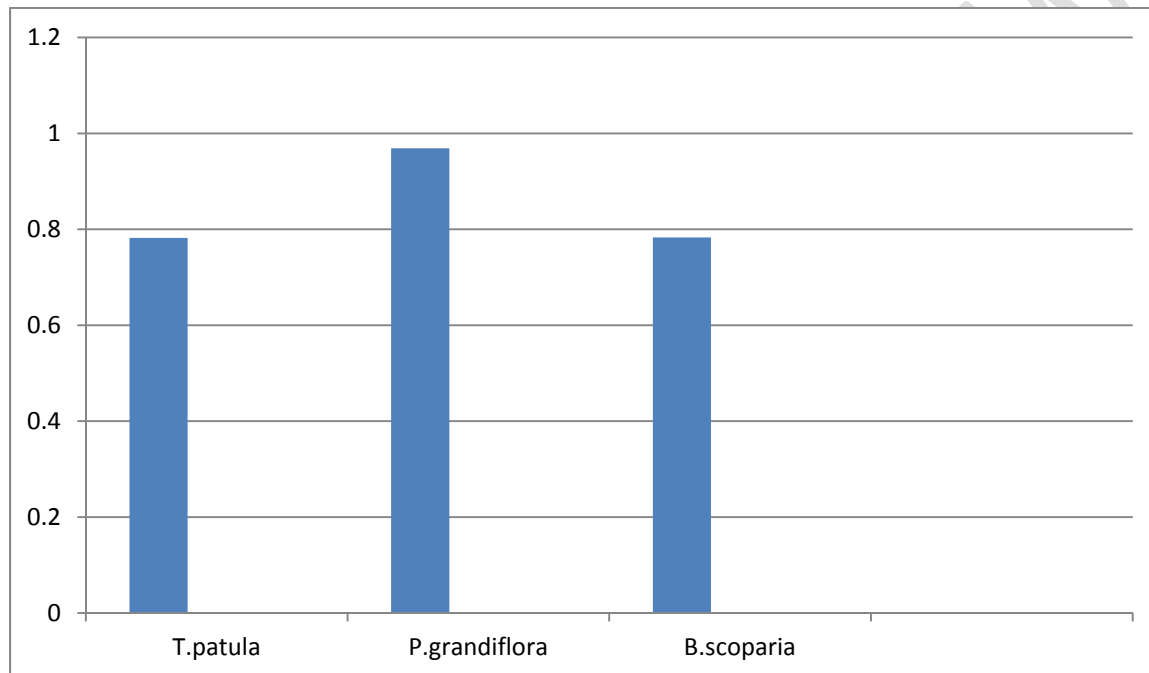
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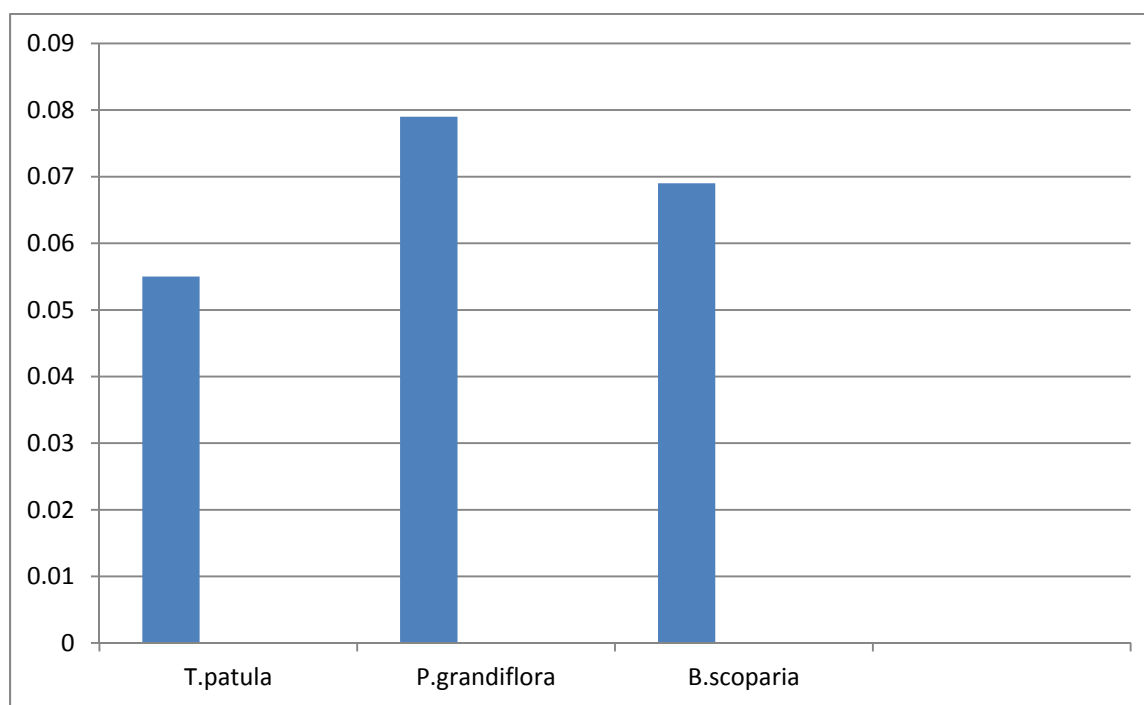
Graph 3.4 :Zinc concentration left in water sample after eight weeks



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

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

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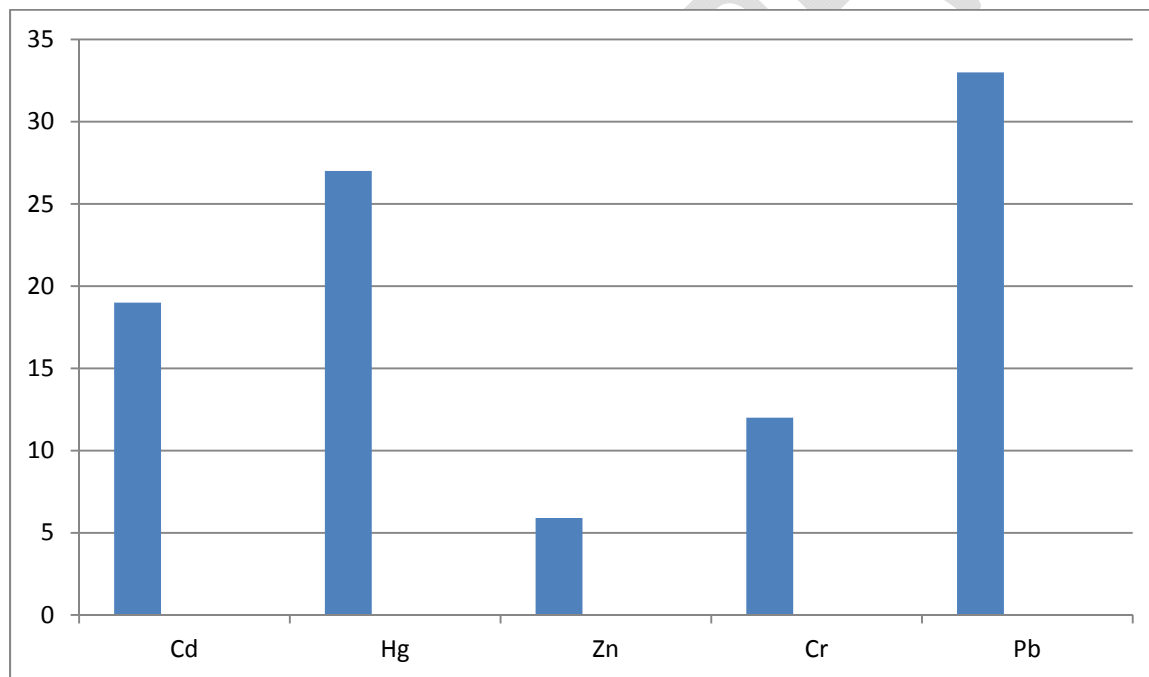
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Metals	<i>Tagetes Patula</i>	<i>Portulaca Grandiflora</i>	<i>Bassia Scoparia</i>
Cd	19	22	8
Cr	12	7.9	6.3
Zn	5.9	4	2.6
Hg	27	25.2	15.9
Pb	33	38	13.7

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

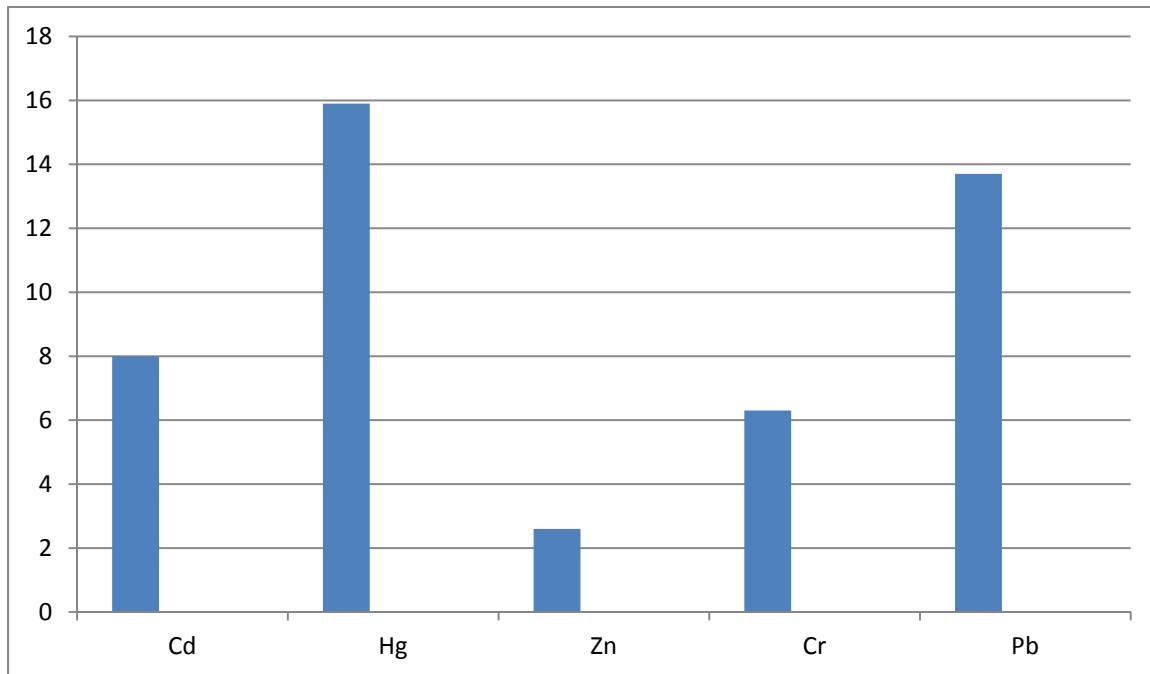
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Graph 3.7: Heavy metal concentration in roots of *T.patula*



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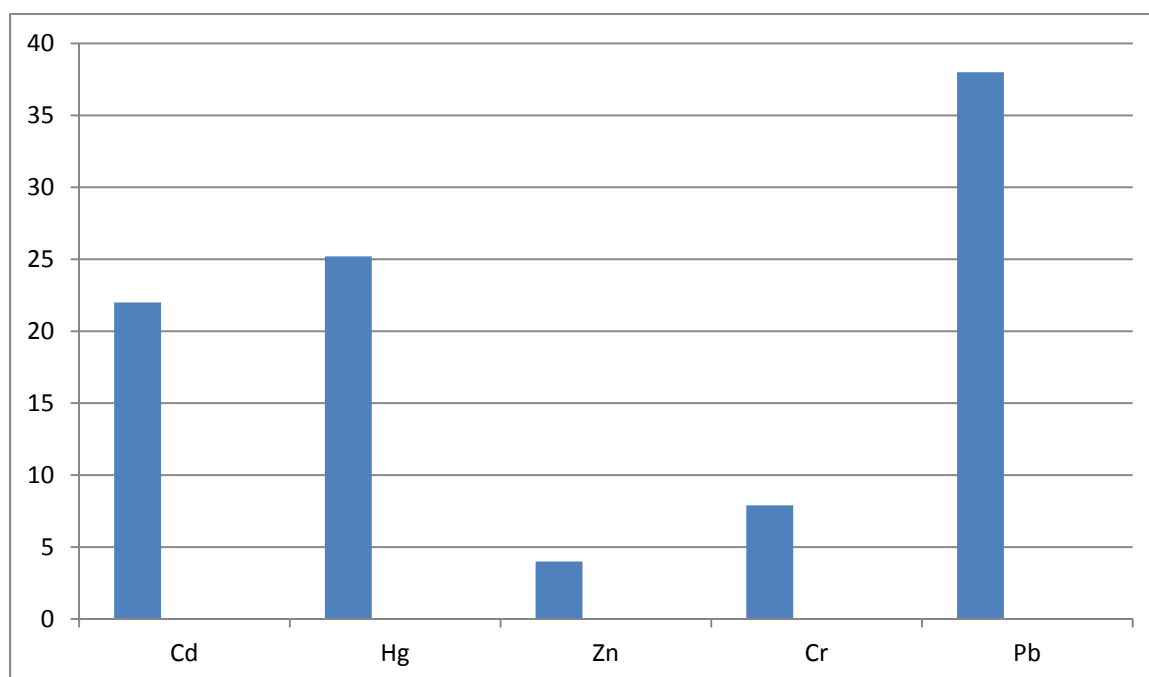
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Graph 3.8: Heavy metal concentration in roots of *B.scoparia*

UNDER PEER REVIEW



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

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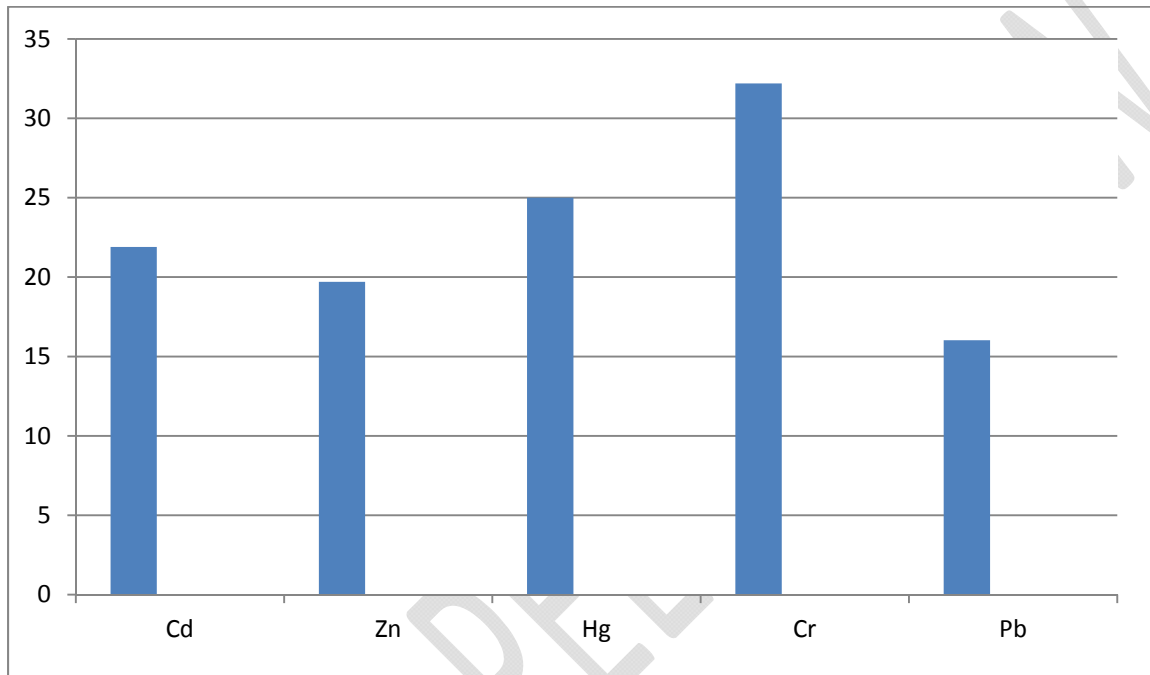
Table 7: Presence of heavy metals in the Stems (mg/kg⁻¹) of plants

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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

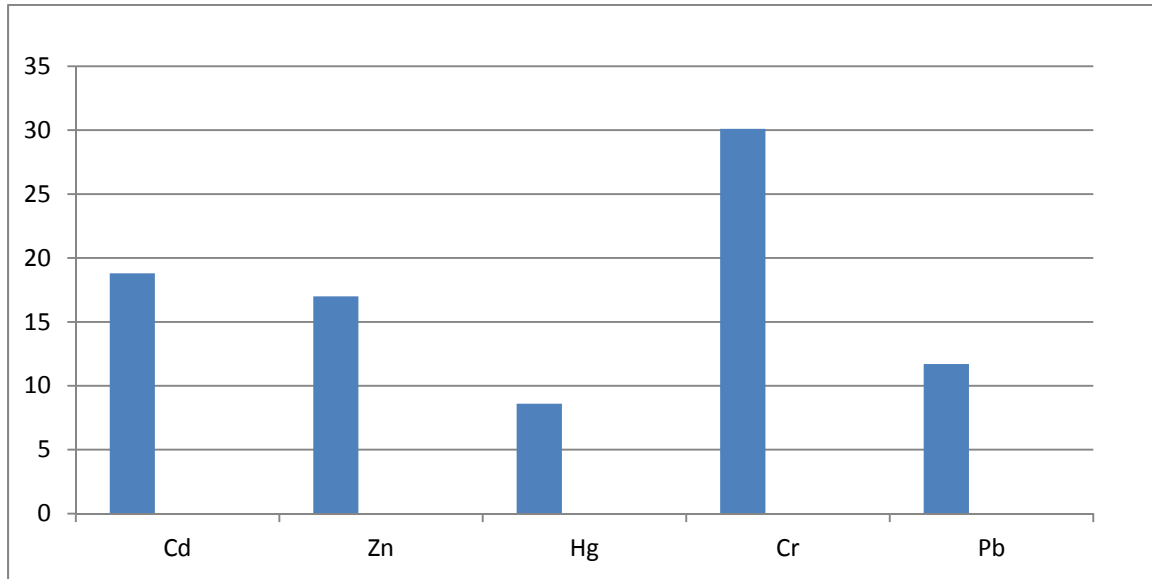
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287 According to the result given in above table, stems of *T.patula* has the highest efficiency for
288 accumulating all the above heavy metals, even *P.grandiflora* and *T.patula* shows approximately
289 same results for accumulation of heavy metals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



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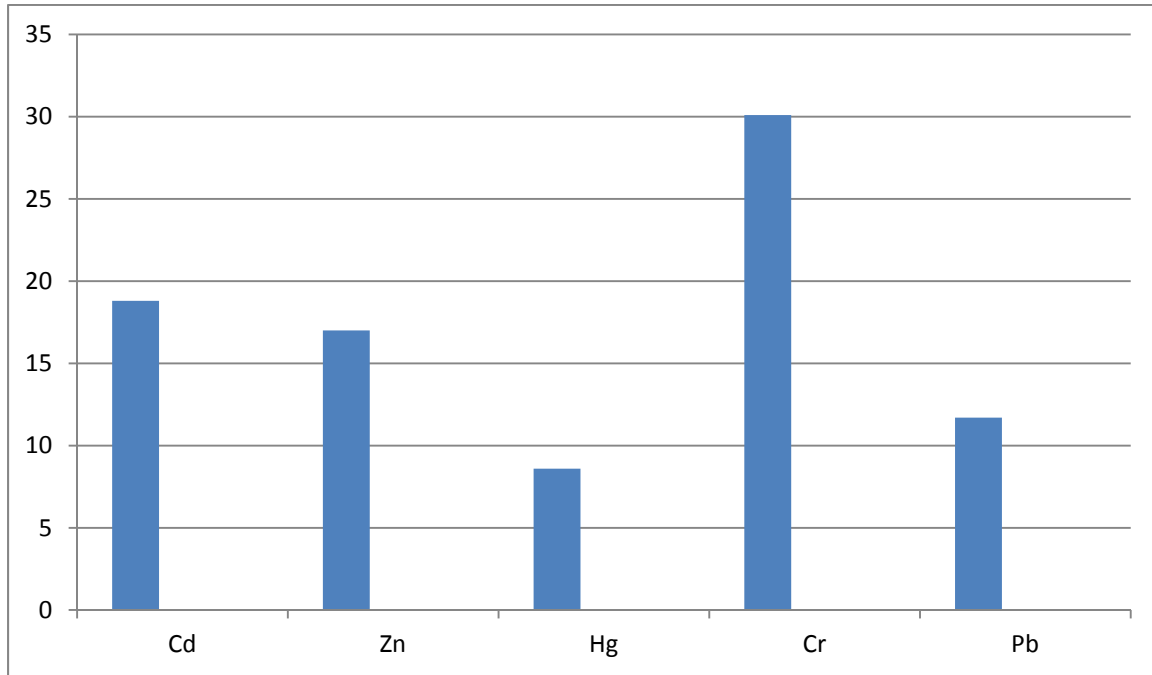
298 **Graph 3.11: Heavy metal concentration in Stems of *B.scoparia***

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UNDER PEER REVIEW



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

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

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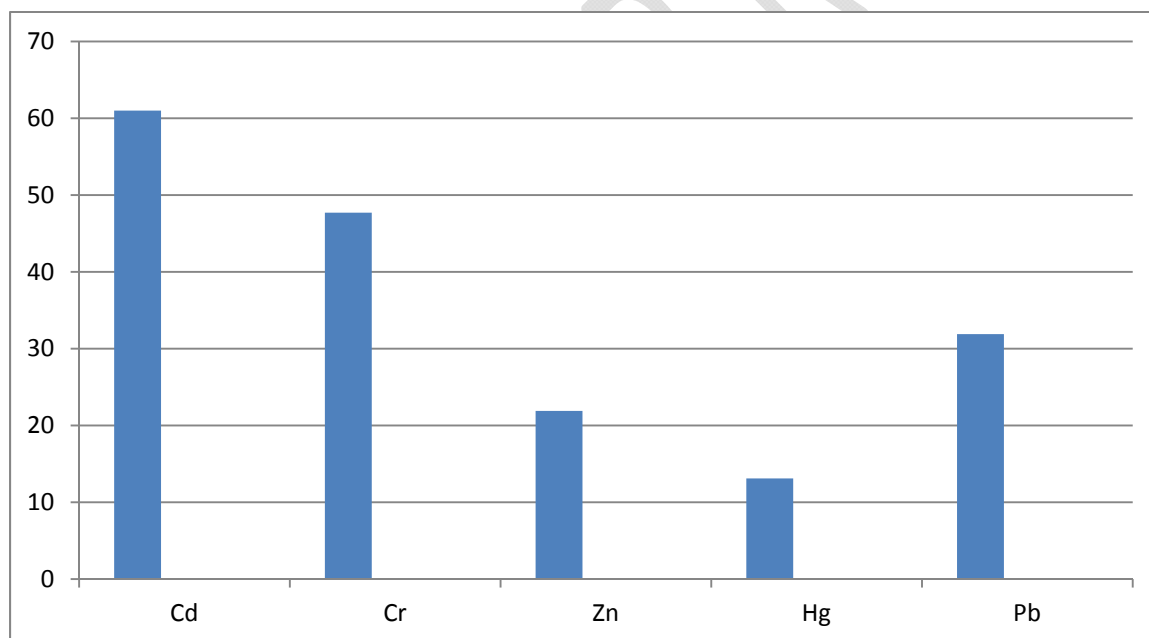
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Metals	<i>Tagetes Patula</i>	<i>Portulaca Grandiflora</i>	<i>Bassia Scoparia</i>
Cd	61	36.1	27.9
Cr	47.7	20.8	7.2
Zn	21.9	2.3	2.6
Hg	13.11	4.6	1
Pb	31.9	4.6	5.8

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

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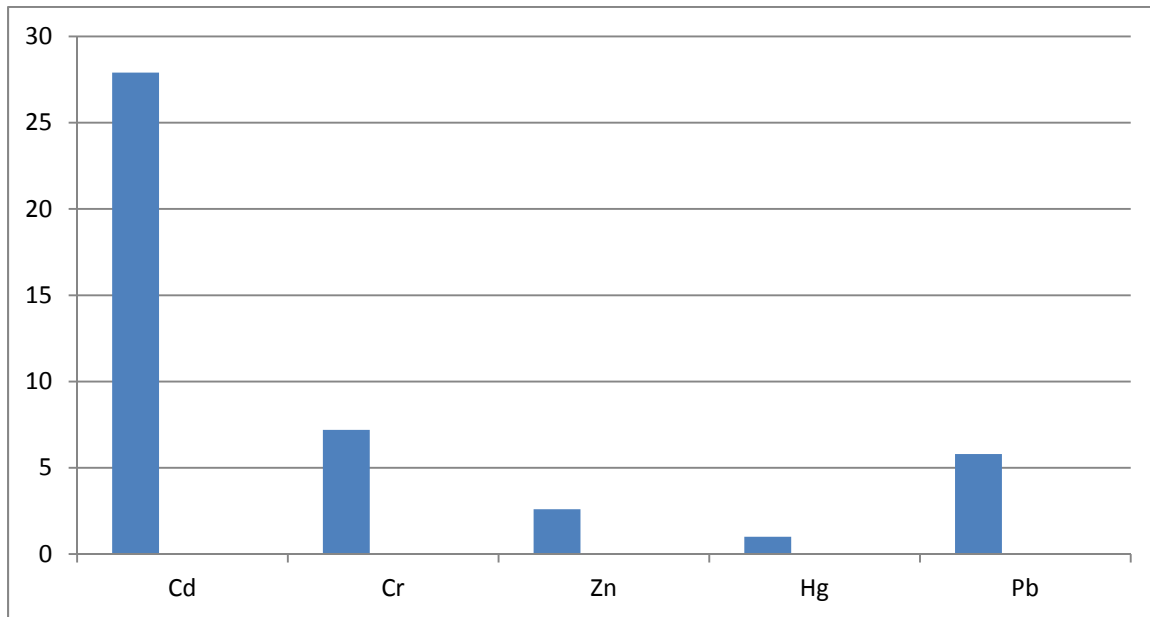


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317 **Graph 3.13: Heavy metal concentration in Leaves of *T.patula***

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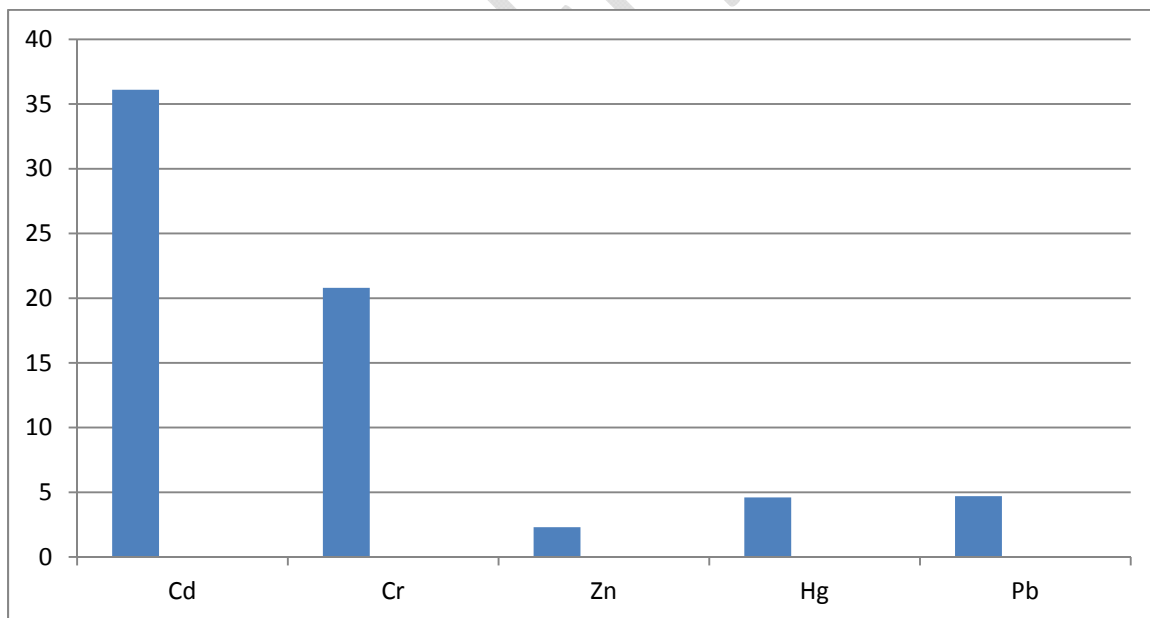
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Graph 3.14: Heavy metal concentration in Leaves of *B.scoparia*

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

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327 **4. Conclusion:** Phytoremediation is an effective, cheap or low maintenance technique for
328 removal of heavy metals from environment. Out of all the three plants, T.patula shows a
329 better growth in size and also shows the highest bio accumulating capacity for heavy
330 metals. it can be concluded from the above study that the water quality of Yamuna river
331 is good before entering national capital delhi. The main disastrous impact is from
332 najafgharh drains. From the above experiment it can be said that phytoremediation ,
333 phytoextraction technique can be used for making Yamuna river pollution free but we
334 have to stop mixing untreated sewage water in Yamuna river.this project is a little
335 attempt towards the big problem of Yamuna river pollution.

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