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RESEARCH ARTICLE

PHYTOREMEDIATION POTENTIAL OF *ALTERNANTHERA SESSILIS L.* GROWING IN INDUSTRIALLY CONTAMINATED ENVIRONMENT

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ABSTRACT

Phytoremediation potential of *Alternanthera sessilis L.* for the heavy metals chromium (Cr), Iron (Fe), Nickel (Ni), Copper (Cu) and Zinc (Zn) was studied. The samples of water, soil and plant material were collected from heavily polluted Thane-Belapur Industrial Area, Navi Mumbai, India. The collected samples of water and soil were analyzed for physico-chemical parameters as well as heavy metals while plant samples were analyzed for heavy metals using ICP-AES. The results for physico-chemical parameters for water and soil showed all the values were above the permissible limits. Highest concentration of these metals was reported in soil than wastewater. These metals were reported in the sequence of Fe>Cu>Cr> Zn>Ni. *Alternanthera sessilis L.* showed high bioaccumulation of these heavy metals Roots have higher concentration of heavy metals as compared to shoots. The bioaccumulation of heavy metals have sequence of Fe>Zn>Cr>Cu>Ni. From the experiment it is clear that *Alternanthera sessilis* L. have ability to withstand such high concentration of heavy metals and prove to be potential species for phytoremediation.

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INTRODUCTION

Industrial and agricultural activities in the past century have led to discharge of a wide range of contaminants natural and synthetic compounds, over great areas of land and water. Physico-chemical parameters play a major role in ascertaining the distribution pattern and quantitative abundance of organisms inhibiting a particular aquatic ecosystem (Singh *et al.*, 1999). Though, the hazardous wastes generated by most of the industries has different kind of toxic substances which are of inorganic as well as organic in nature, almost all of them are differentially toxic to the various components such as plants, animals and the human being (Branzini *et al.*, 2012). The major pollutants of the industrial origin which are of much concern to the environmental scientists are toxic heavy metals. They are natural components of the Earth's crust, but in many ecosystems the concentration of several heavy metals has reached toxic levels (Abii, 2012). Heavy metals are defined as the group of elements whose densities are higher than 5 g cm^{-3} and recognized as ubiquitous environmental contaminants (Massa *et al.*, 2010). High concentration of heavy metals in soil can negatively affect plant growth as these metals interfere with metabolic function of plants, including physiological and biochemical processes, inhibition of photosynthesis, and respiration and degeneration of main cell organelles, even leading to death of plants (Schmidt, 2003). Most contaminated soil can be remediated by chemical, physical and biological techniques. The chemical and physical treatments irreversibly affect the properties of contaminant in water and soil, destroy biodiversity and may render useless for plant growth. Phytoremediation offers sustainable remediation technique by overcoming the conventional chemical and physical technologies (Salt *et al.*, 1995; Cunningham *et al.* 1995; Chaney *et al.*, 1995). The idea of using plants for phytoremediation to remove metals from soil comes from the discovery of different wild plants, often endemic to naturally mineralized soils that accumulate high concentration of metals in their foliage (Baker, 1987; Raskin *et al.*, 1997). Natural occurring plant species are capable of accumulating extraordinarily high metal levels and makes the investigation of this phytoremediation particularly interesting (Bradshaw, 1983). Therefore, naturally occurring *Alternanthera sessilis* L. were selected for phytoremediation of Cr, Fe, Ni, Cu and Zn from the heavily polluted Thane-Belapur Industrial area. An empirical approach such as bioaccumulation factor was determined to find out accumulation of heavy metals from soil to plant body (Ghosh and Singh, 2005). On other hand, the translocation factor was determined to study the translocation of heavy metals from roots to aerial parts that is shoots (Marchiol *et al.*, 2004).

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MATERIALS AND METHODS

Area of study

Thane-Belapur industrial area is referred in MIDC document as the TTC (Trans-Thane Creek) is one of the most industrialized area containing near about 2000 industrial units. It covers an area of 2,546 hectares at $19^0$04'22.52"N and $73^0$01'08.40"E, and lies on east of the Thane creek, Thane-Belapur road between the urban centers of Thane and Nerul. It has approximately sixteen kilometers in length and flanked by the Mumbra-Parsik hills to the east. The industrial composition of this area has been dominated by petrochemical and engineering units. These industries discharge polluted water into canals, rivers, creeks, and sea. Five stations (S1, S2, S3, S4 and S5) were selected so as to cover most part of these areas as shown in Figure 1.

Physico-chemical parameters

All glasswares were first cleaned with tape water thoroughly and finally with de-ionized water. The chemicals and reagents used for analysis were of analytical reagent (A.R.) grade. The procedure for calculating the different parameters were conducted in laboratory. All equipments were checked and calibrated according to the manufacturer's specification. Physico-chemical parameters of water and soil samples were done by using standard methods (APHA, 1998, Manivaskam, 2005 and Gupta, 2000).

Heavy Metals Analysis

10 $cm^3$of each water sample separately, was evaporated to dryness on hot plate. 10$cm^3$of concentrated HNO_3 was added and again evaporated to dryness. 4 cm^3 of 70 % perchloric acid was added and reduced to minimum value. Obtained colorless sample were then cooled and volume was made up to 100 cm^3. 0.5 gm of soil samples were taken in a Teflon beaker and 10 ml nitric acid or perchloric acid and 5 ml Hydrofluoric acid were added to it. The solution is heated on a hot plate to dryness. 10 ml of aqua-regia was added to the dry mass and heated till everything dissolves. The solution was diluted to standard volume, 100 ml with distilled water. Each sample was maintained in triplicate. The samples of water and soil were analyzed for Cr, Zn, Fe, Cu and Ni by Inductive Coupled Plasma- Atomic Emission Spectrometer (ICP-AES) model ARCOS.

Heavy Metals analysis of plant samples

Alternanthera sessilis L plants were collected from the same sites as water and soil and were washed thoroughly with the tap water, cleaned with distilled water and then separated into roots and shoots. All plant parts were oven dried at 72°C for 72 h and then ground to powders. For total metal concentrations in the plant components, 1.0 g of plant samples in 10 ml of Nitric Acid were heated on a hot plate and perchloric acid were added drop wise till all organic matter were destroyed, solution became clear and diluted to 100 ml with distilled water. The analysis was done by Inductive Coupled Plasma- Atomic Emission Spectrometer (ICP-AES) model ARCOS. To assess the analytical precision, three analytical replicates of each sample, an appropriate standard reference material (from Sigma-Aldrich Company) and a reagent blank were performed in each analytical batch. The readings / result obtained were multiply by suitable factors to get metal concentration in mg/kg. The Bioaccumulation factor (BCF) and Translocation factor (TF) were also calculated by using following formula.

$$\mathrm{BCF} = \frac{\text{Metal concentration in plant tissue (whole plant/portal)}}{\text{Initial concentration of metal in substrate (Soil)}}$$

$$\mathrm{TF} = \frac{\text{Metal concentration (Stem + leaves)}}{\text{Metal concentration in roots}}$$

RESULTS AND DISCUSSION

Physico-chemical parameters

Selected physico-chemical properties which can be used as indicator of wastewater and soil are presented in Table 1 and 2 while its graphical representations are given in Figure 2(a&b) and Figure 3 respectively. The average pH value is 8.22 in water while it is 7.634 for soil indicating alkaline nature. The Average EC is found to be 2.66 for water and 2.394 for soil which indicates its ionic strength and its degree of ionic mineralization (Naudet *et al*., 2004). The average DO is 1.053 mg/L and BOD is 0.256 mg/L indicates the presence of high organic and inorganic pollutants (Suraj *et al*., 2014). Average COD reported is 92.333 mg/L while total alkalinity and total dissolved salts reported is 1875.667 mg/l and 3796.667 mg/l respectively. Total hardness of wastewater is found to be 67.0 mg/L while that of in soil is found to be 1.457 mg/L. Chloride content is 159 mg/L and 1.574 mg/L for wastewater and soil respectively. Phosphate value is reported 0.292 mg/L and 0.20 mg/l while sulphate is reported 184.333 mg/L and 0.24 mg/L for wastewater and soil respectively. Nitrate content of wastewater reported is 6.969 mg/L. the soil in the area was very poor in organic carbon and organic matter and was reported 1.344% and 1.90 % respectively. All these physico-chemical parameters indicate that the industrial effluent having towards toxicity as they are above the permissible ranges and high level of pollution in the current area (Suraj *et al*., 2014).

Heavy metals in wastewater and soil

The seasonal variation of heavy metals for wastewater and soil are given table 3 and 4 while its graphical representation are given in figure 3 and 4 respectively. The result shows that the average concentration of chromium was found to be 14.846 mg/L and 516.6667 mg/Kg in wastewater and soil respectively which is above the permissible limit. Chromium is one of a highly reactive metal used mostly in electroplating, leather tanning, metal finishing, as pigments, mordant and textile coloring processes in the industry. The three main use of chromium are in metallurgy, refractory and as chemicals (Moore and Ramamoorthy, 1984). The average Fe concentration was found to be 24.85 mg/L and 2087.763 mg/kg in wastewater and soil respectively. There is possibility of iron leached in water from iron mines which flows in the effluent. The average Ni concentration was found to be 0.857mg/L and 904 mg/kg in wastewater and soil respectively. It is used in a variety of metallurgical processes such as electroplating and alloy production as well as in nickel-cadmium batteries and this may be the reason for high concentration in present study area. The average Cu concentration was found to be 4.601 mg/L and 731.0667 mg/kg in wastewater and soil respectively. Rainfall and the subsequent leaching of organic matter and minerals influence the concentration of copper in waste water (Minaxi *et al*., 2008).

Table 1. Physico chemical Analysis for wastewater from the Thane Belapur Industrial Area

Parameters	pH	EC	DO	COD	BOD	TH	TA	TDS	Cl^-	PO_4	SO_4	NO_3
N	3	3	3	3	3	3	3	3	3	3	3	3
Min	6.9	1.16	0.4516	74	0.225	41	549	790	79	0.054	37	1.212
Max	9.4	5.62	2.258	115	1.129	81	3172	8290	292	0.753	283	17.878
Sum	24.8	7.98	3.1612	277	1.580	201	5627	11390	477	0.876	553	20.908
Mean	8.266	2.66	1.053	92.333	0.526	67	1875.66	3796.66	159	0.292	184.333	6.969
Std. error	0.731	1.480	0.602	12.032	0.301	13.012	757.346	2289.114	66.955	0.230	75.067	5.457
Variance	1.603	6.571	1.087	434.33	0.271	508	172072	1.5720	13449	0.159	16905.33	89.341
Std. dev	1.266	2.563	1.042	20.840	0.521	22.53	1311.76	3964.86	115.9698	0.399	130.020	9.452

Note: Except pH and EC (mmho cm^{-1}) all values are reported in mg/L

Table 2. Physico chemical Analysis for soil from the Thane Belapur Industrial Area

Parameters	pH	EC	OC	OM	TH	Cl	PO4	SO4
N	3	3	3	3	3	3	3	3
Min	7.1	1.66	0.03	0.02	0.65	0.42	0.05	0.21
Max	8	2.95	2.49	3.24	2.38	2.39	0.3	0.29
Sum	22.9	7.18	4.03	5.85	4.37	4.72	0.6	0.72
Mean	7.634	2.394	1.344	1.95	1.457	1.574	0.2	0.24
Std. error	0.273	0.383	0.715	0.984	0.503	0.594	0.077	0.025
Variance	0.224	0.439	1.534	2.899	0.759	1.055	0.0175	0.0019
Stand. dev	0.473	0.663	1.239	1.703	0.870	1.028	0.133	0.045

Note: Except pH, EC (mmho cm-1), OC (%) and OM (%) all values are reported in mg/L

Table 3. Metal concentration (mg/L) in water L. Collected from thane Belapur Industrial Area

Metals	Cr	Fe	Ni	Cu	Zn
N	3	3	3	3	3
Min	0.41	1.15	0.37	2.8	0.952
Max	40.12	39.15	1.45	6.718	26.4
Sum	44.54	74.55	2.57	13.803	31.557
Mean	14.847	24.85	0.857	4.601	10.519
Std. error	12.67933	11.93412	0.316298	1.142012	7.995835
Variance	482.296	427.27	0.3001333	3.912573	191.8001
Stand. dev	21.96124	20.67051	0.5478443	1.978022	13.84919

Table 4. Metal concentration (mg/kg) in soil Collected from thane Belapur Industrial Area

0	Cr	Fe	Ni	Cu	Zn
N	3	3	3	3	3
Min	234	0	177	294	604
Max	906	4591	2129	1596.2	3424
Sum	1550	6263.29	2712	2193.2	5052
Mean	516.6667	2087.763	904	731.0667	1684
Std. error	201.1876	1341.49	616.0571	432.5745	878.4077
Variance	121429.3	5398784	1138579	561362	2314800
Stand. dev	348.4671	2323.528	1067.042	749.241	1521.447

Table 5. Metal concentration (mg/kg) in *Alternanthera sessilis* L. Collected from thane Belapur Industrial Area

Metal in mg/Kg	Plant Parts		Bioaccumulation Factor (BCF)	Translocation Factor (TF)
	Root	Shoot		
Cr	40.00 ±2.00	2.9±0.68	0.083	0.072
Fe	2323±98.60	1063.4±6.59	1.622	0.457
Ni	3.7±0.90	24.6±0.51	0.033	6.648
Cu	21±3.51	9.8±0.30	0.153	2.142
Zn	161.6±20.26	101.4±1.41	0.155	0.629

The average Zn concentration was found to be 10.519 mg/L and 1684 mg/kg in wastewater and soil respectively. The possible high value of Zn is due to domestic and industrial discharge of effluent such as liquid manure, composed material, fertilizers and pesticides in the study area (Gajbhiye and Bhalerao, 2016). From the result it is clear that the values of all studied metals were above the permissible level given by WHO (Suraj *et al*., 2014). Metals concentration in soil were much higher than the wastewater due to soil act as sink for heavy metals and are usually deposited in top soils (Govil *et al*. 2001).

Metal accumulation in *Alternanthera sessilis* L.

The metal concentration accumulated in roots and shoots by *Alternanthera sessilis* L. from the study area are given in table 4 along with bioaccumulation factor and translocation factor while its graphical representation is given in figure 5 and 6. Total Cr concentration in roots is 40.00 ±2.00 mg/ kg while that of in shoots is 2.9±0.68 mg/ kg. It was found that roots accumulate more Cr than the shoots and indicates less translocation of Cr in upper part of the plants. Bioaccumulation factor (0.083) and translocation factor (0.072) is less than one.

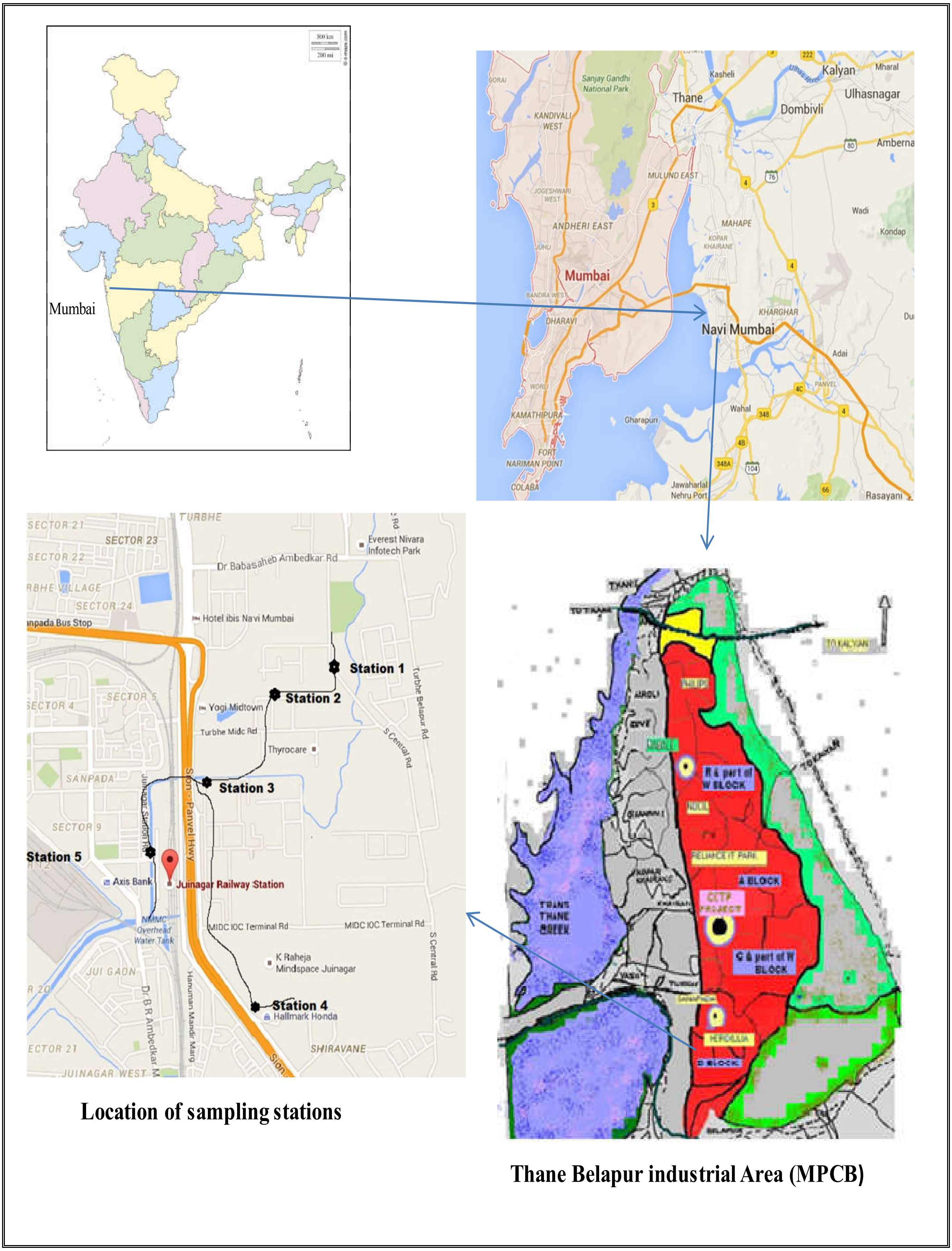

Figure 1. The geographical location of Thane- Belapur Industrial area

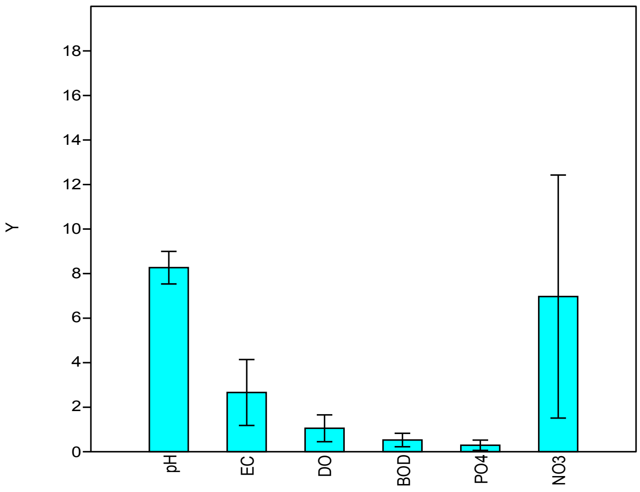

Figure 2 a. Graphical representation of physico-chemical parameter of wastewater sample

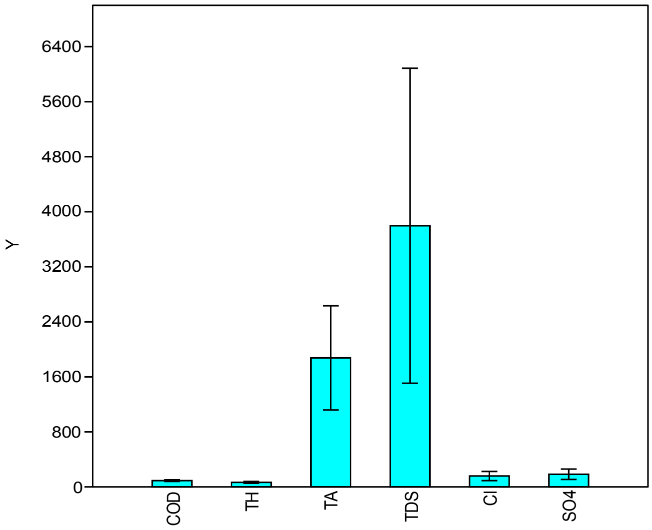

Figure 2 b. Graphical representation of physico-chemical parameter of water sample

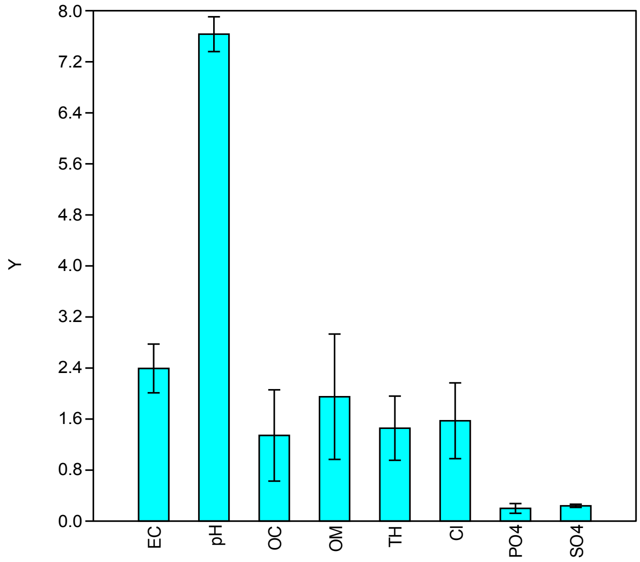

Figure 2 b. Graphical representation of physico-chemical parameter of soil samples

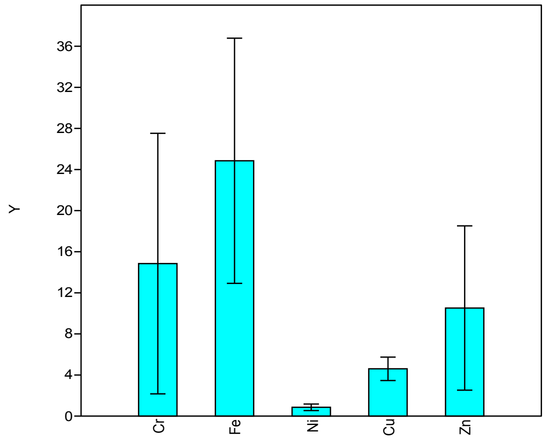

Figure 3. Graphical representation of heavy metals in wastewater

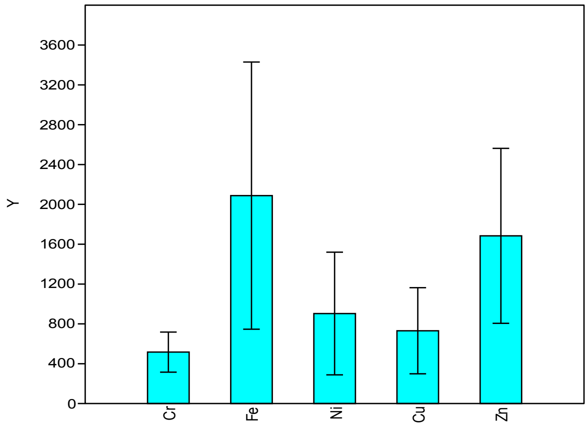

Figure 4. Graphical representation of heavy metals in soil

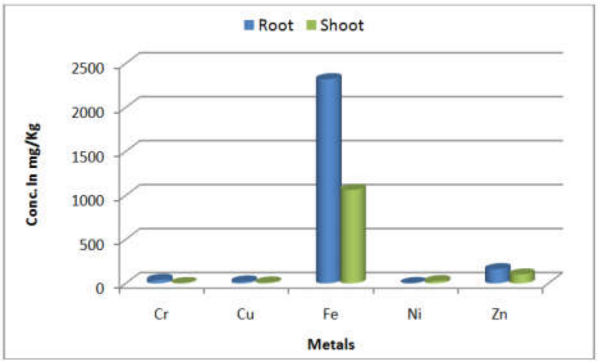

Figure 5. Graphical representation of heavy metals bioaccumulation in roots and shoots by *Alternanthera sessilis L.*

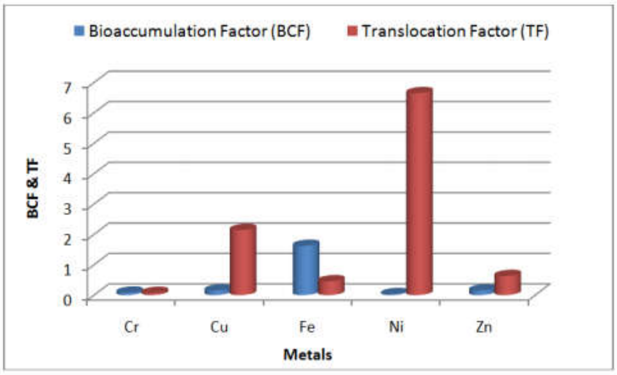

Figure 6. Graphical representation of Bioaccumulation Factor (BCF) and Translocation Factor (TF) For heavy metals by *Alternanthera sessilis L.*

These plants can be utilized for rhizofiltration of these heavy metals. This is an agreement with earlier reports on many plants in which significant accumulation of Cr metal in roots was observed (Nematian and Kazemeini, 2013). This could be because of Cr is immobilized in the vacuoles of the root cells and showed less translocation, thus rendering it less toxic. Cr is a non essential and toxic metal to plant growth and it may be possible that plants do not have any specific mechanism of transport of Cr (Shankar *et al*., 2005). Iron (Fe) is an essential micronutrient for plants and animals (Kunze *et al*., 2001). However, excessive Fe uptake can produce toxic effects. Total Fe concentration in roots is 2323±98.60 mg/ kg while that of in shoots is 1063.4±6.59mg/ kg. Results obtained from plant analysis asserted that roots of *A. sessilis* are found to be highly capable of Fe accumulation. Bioaccumulation factor is 1.622 which is greater than 1 but translocation factor is 0.457 which is less than 1 indicates less translocation to aerial part. Plants with BCF greater than one and TF less than one (BCF> 1 and TF< 1) have the potential for phytostabilization (Yoon *et al*., 2006). Badr *et al*. (2012) reported Fe accumulation in *Rhazia stricta* (291.60 μg/g) are in agreement of present study. Total Ni concentration in roots is 3.7±0.90 mg/ kg while that of in shoots is 24.6±0.51 mg/ kg. . Nickel (Ni) is considered to be among non essential elements needed for the healthy growth of plants, animals and soil microbes (Khan and Moheman, 2006)). However, study suggests that nickel is an essential element in many species of plants and animals. It can interact with iron found in haemoglobin and helps in oxygen transport, stimulate the metabolism as well as being regarded as a key metal in several plants and animals enzyme systems. However, at higher concentrations Ni can be toxic (Jadia and Fulekar, 2009). This observation found true in studied plant as translocation factor (6.648) is much more greater than one and the concentration of nickel is higher in shoots than roots. Total Cu concentration in roots is 21±3.51mg/kg while in shoots is 9.8±0.30 mg/kg. Metal content of all the studied plants in roots are much higher than the shoots. Within roots, Cu is associated mainly with cell walls and is largely immobile. A metal-rich rhizo-concentrations composed of iron hydroxides and other metals that are mobilized and precipitated on the root surface (Sundby *et al*., 1998). Plants with both BCFs and TFs greater than one (TF and BCF> 1) have the potential to be used in phytoextraction The process of phytoextraction generally requires the translocation of HMs to the easily harvestable plant parts, that is, shoots (Yoon *et al*., 2006). Translocation factor (TF) for Cu is 2.142 indicate that the plant can be used for phytoextraction. Total Zn concentration in roots is 161.6±20.26 mg/kg while in shoots is 101.4±1.41 mg/kg. Zn is an essential trace element for organisms which serves as structural ions in transcription factors and transferred in metallothionein. It is the only metal represented in all six enzymes classes. 100 mg/kg of Zn in plant are toxic (Nematian and Kazemeini, 2013). In the present study, quite high amount of Zn accumulates in both roots and shoots which were higher than toxic levels. Bioaccumulation Factor (0.155) and translocation factor (0.629) indicates grater accumulation but no translocation of Zn in aerial parts.

Conclusion

From the present study, it could be concluded that heavy metals are potentially hazardous to plant, animal and human beings if they exceeded the threshold levels. Physico-chemical parameters of wastewater and soil indicate that all reported values were not according to permissible level given by WHO and high pollution level. Heavy metals were also reported high in both wastewater and soil but concentration were high in soil than wastewater. *Alternanthera sessilis L.* showed high bioaccumulation of these heavy metals. Roots have higher concentration of heavy metals as compared to shoots except Ni. The bioaccumulation of heavy metals have sequence of Fe>Zn>Cr>Cu>Ni. From the study, it is clear that *Alternanthera sessilis* L. have ability to withstand such high concentration of heavy metals and prove to be potential species for phytoremediation.

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