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To Measure the Absorption of Metals, from Soil through the Process of Phytoremediation at Varying Levels of Concentrations

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Abstract

The contamination of metals has an impact, on a considerable number of people worldwide particularly in developing nations. This occurs primarily because environmental policies are either lacking or rarely enforced leading to health related issues are abundant. To reduce the contamination levels in soils, various chemical, physical, and biological methods are utilized. Among biological methods a popular method is phytoremediation in which plants species are utilized that can absorb heavy metals from soil as part of the nutrition Intake. A recent study indicated that the removal capability of *Typha latifolia*, a plant species commonly used for phytoremediation, is related with concentration of heavy metals in the soil and/or irrigation water, but the study remained inconclusive regarding precise characterization of this effect, due to small sample size and lack of control on the contamination of heavy metals. In this study, the effect of concentration of lead, chromium, nickel, and copper on the extraction efficiency of *Typha latifolia* is characterized, following a careful regimen of irrigation to supply exact quantities of heavy metals. Several specimens of *Typha latifolia* were grown in pots under laboratory conditions and irrigated using predetermined quantity of clean water with added known concentrations of the four heavy metals, daily for a period of ninety days. Atomic absorption spectrometry was used to determine the amounts of the four heavy metals before and after the irrigation period to estimate the amounts of heavy metals absorbed by the plants. Concentration increased heavy metals extraction efficiency from the soil using *Thypa latifolia* specie. Also result shows a trend line between different heavy metals removal by plant species as we increased concentration the efficiency also increased. By finding the value of P our results were more than 99% in confidence interval and no result was obtained which proves our hypothesis incorrect. The equations and specifically the R^2 value further strengthen our findings as indicator used for authenticity of calculated results. Overall result obtained for heavy metal removal was above 50%for low concentration and increased up to 80% with the increase in concentration of heavy metal in irrigation water.

Keywords: Concentration • NEQS and EPA • Heavy metals • Phytoremediation

Introduction

Environment plays a key role in the survival of life on earth. All human activities directly or indirectly affect the environment in positive or negative way. If we take example of rapid growing population across the world, it vanishing rapidly the resources on the earth and as well as inside the earth crust [1]. For overcoming the food shortage, the modern agricultural techniques will be applied to increase the production of food from the agricultural lands [2]. With all these processes the natural environment is being contaminated at a rapid rate then the standards limits imposed by NEQS and EPA [3]. Chemicals, including metals, are responsible for significant mortality and morbidity [4]. WHO estimates that "more than 25% of the total disease burden is due to environmental factors, including exposure to toxic chemicals" [5]. Heavy metals which contaminate environment through different industrial processes and from natural sources like tsunamis and floods carrying metals staying from ore to the water and soil. The soils contaminated with heavy metals are very harmful for the health of crops as well as for the health of human and animals when it comes in the food chain. This contamination can be minimized by certain technique which includes physiochemical and biological methods [6]. Among this phytoremediation technique is developing and environment friendly technology to minimize the quantity of heavy metals within the soil by uplifting to the body of plant species. In this technique, hyper accumulator plants, which have

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high accumulation for heavy metals in their tissues are planted on soil and water bodies [7].

Phytoremediation is the use of plants and associated soil microbes to reduce the concentrations or toxic effects of contaminants in the environments [8]. It is a relatively recent technology and is perceived as cost-effective, efficient, novel, eco-friendly, and solar-driven technology with good public acceptance. This concept of phytoremediation was earlier put forward by Rufus Chaney in 1983 and then gained public exposure in 1990 [9]. And has increasingly been examined as a potential practical and more cost effective technology than the soil replacement, solidification and washing strategies presently used [10]. Phytoremediation is the use of plants to remediate contaminants of heavy metal pollutants in soil. The process of phytoremediation includes transpiration and root growth minimizing leaching, control eroding, introducing a favorable environment in the root area, and adding organics to the substrate [11]. Heavy metals occur in the environment both naturally and due to human activities. Those metals which are heavy in density and having atomic number greater than 20 referred to as heavy metals [12]. Heavy metals are very toxic and carcinogenic even at very low concentration when it come to the human environment specially food chain and drinkable water sources (Figure 1) [13].

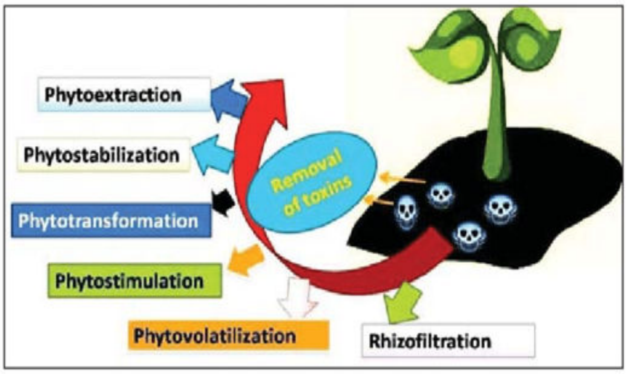

Figure 1. Showing sub processes in phytoremediation.

Wetland plants such as *Typha latifolia*, *Phragmites*, *Scarps*, *Persia*, *Juncus*, and *Spartina* have been shown in tests to reduce heavy metal levels in polluted water. *Typha latifolia* is a marshy and wetland plant that can be seen growing among emergent wetland plants. Plants grow to be around 2-3 meters tall, with a characteristic fruiting spike and tall sword-shaped leaves [14]. This study decided on statistical techniques had been used to decide the heavy metal accumulation and its controlling aspect and to discover the foundation of those metals in soil samples (forty) gathered from sites alongside the Khoshk riverbanks, Shiraz, Iran. The levels of Pb, Zn, Cr, Cd, Ni, and Cu had been decided in every sample. Discriminant evaluation discovered that investigated sites are different in phrases of heavy metal accumulation. From the ANOVA and correlation evaluation, it turned into determined that soil natural remember is the maximum essential issue controlling the distribution of heavy metals. It ought to be mentioned that parametric statistical checks require the records to be typically distributed [15]. This study recognized the concentration of serious metal like contaminants in mining areas using X-ray emission technique. A complete of thirty soil samples are gathered indiscriminately from 3 mining spots within the Osun state. Twelve samples were taken from Igun and Ijana-Gada areas, and 6 from Igbadae site. These samples were analyzed for heavy metals. Result shows metal contamination was significant in Igun, Ijana-Gada, and Igbadae mine tailings form moderate to severe. The correlation between Mn and Ti was 82%, showing a strong linear relationship at the 0.01 significance point and a conventional source of those metals [16]. This study examined the concentrations of heavy metal in soils and plants at the side of the complex. Samples are gathered in difficult and sedimentary rocks of Zhob and Loralai valleys. Heavy metals that represent manganese, lead, chromium, iron, copper, nickel, cobalt, and cadmium concentrations have been tested in soils and plants using atomic absorption spectrometry. Results found higher concentration in these sites. Statistical analyses confirmed that the geogenic supply became chiefly liable for significant metals infection. ANOVA test found as compared to the locality these sites have higher concentrations at ($p<0.05$) [17]. In this study nine pots of *Typha latifolia* were planted under precise setting. Plants were irrigated with wastewater containing heavy metals with different dilutions. The soil was examined before and after the plantation to find out the uptake capacity of plant species under different concentrations. X-ray fluorescence spectrometry was carried out for this study. The uptake of heavy metals by species was acknowledged, however due to the small range of samples the relationship between removal efficiency and heavy metals concentrations cannot be established. Bio-mass analysis was expensive, so this alternate process was followed for final result [18]. This study was carried out for the treatment of dairy farm effluents. Group investigation was done using totally different dilutions (0 to 100%) of effluents for *A. pinnata* species. The discoveries revealed that after fourteen days of phytoremediation experiments, the probability was $P<0>0.9533$ and there was minimum difference between experimental and model predicted results. The outlined level of statistical significance was 95% confidence interval [19]. The *Pistia stratiotes* was tested for phytoremediation potential in water. Five groups with four samples each were evaluated. This study indicates that Pistia Stratiotes is an effective phytoremediation agent for the weedkiller clomazone in water. One-way analysis of variance was used to compare means among groups that were considered different when $P<0.05$ using Graph Pad. The plant battled foliar modifications at concentrations 100 times higher than those proposed for the use of Clomazone on crops. Clomazone deposits in water were lowered 90% during phytoremediation tests with *Pistia stratiotes*, suggesting that the plant can be used for the intemperance of this weedkiller in water reserves [20]. This study examined the status, development, and contests of phytoremediation in African. The natural impact of the pollution, phytoremediation techniques and the potential specie. With the aid of using soil fauna and flora, the result of harvested biomass and its prospects are also discussed. The power of metallic accumulation by plants from media could also be expected the ingestion of translocation component and bio-concentration component. TF and BCF illustrates the ratio of metallic concentration within the root and soil. Relatively, BCF indicates pollution absorbed, withdrawn, and combined within the root region. For a plant to be considered as an indicator, excluder or hyperaccomulators, BCF and TF screening should be done.

Problem statement

Research have been carried out to study the effects of phytoremediation technique for heavy metal removal from soil using *Typha latifolia* specie. However, these studies have some laps to fully understand methodology. In this work, we will try to cover these laps and use new methodology to explore this technique in depth. So, the statement on which this research is based on "Phytoremediation is an effective technique for improving contaminated soils". However, the effect of varying concentrations on the plant's ability to remove heavy metals from soils needs further investigation. Phytoremediation is an advanced technique and needs further research to understand the effect of various parameters such as number of samples, type of specie and quantity of heavy metals in the soil on the workability of plants.

Materials and Methods

Material includes

Soil, tap water, plantation pots, plastic shed, plant species, and graduated cylinder, trowel, and heavy metal chemicals.

Method

The procedure for the performing research study are carried out on the following way.

Sampling

Water samples were collected from tap water source in a sealed bottle. The water samples were tested for preexisting contaminants under consideration. The obtained results are accounted for calculating final result. The soil sample was collected from 10 feet deep source having 10 × 10 area. Four samples from the sides and one sample from the center are collected and mixed thoroughly to get a uniform sample and then sealed in a plastic bag to be tested in laboratory for the heavy metal contaminations.

Initial laboratory results

Soil and water samples were tested in laboratory and the following results obtained showing the amount of existing heavy metals. Tables shows concentration of soil and water (Table 1).

Heavy metals in water		Heavy metals in soil	
Heavy metals	Concentration (mg/L)	Heavy metals	Concentration (mg/L)
Nickel	0.07	Nickel	2.19
Chromium	0.04	Chromium	1.1
Lead	0.19	Lead	1.16
Copper	0	Copper	1.14

Table 1. Heavy metals in soil and water.

Plantation: Four groups were created namely C_1, C_2, C_3 and C_4. Further each group contains 5 pots namely C_{1a}, C_{1b}, C_{1c}, C_{1d}, C_{1e} and same for other three groups. Total 20 pots were planted using *Typha latifolia* specie (Figure 2).

Figure 2. Showing pots planted with *Typha latifolia*.

Solution preparation

Different concentration of solutions was prepared accordingly for each group. Each pot in C_1 group required 90 mg/90 days' daily irrigations. So in total 450 mg of each heavy metal was required for C_1 group. For C_2 900 mg, for C_3 1350 mg and for C_4 1800 mg/90 days was required (Figure 3).

Figure 3. Packets showing heavy metals salts for solution.

Irrigation

Irrigation was done using the solution of heavy metals contaminants by doing this each pot in C_1 get 2 mg/L daily in C_2 each pot gets 4 mg/L, in C_3 each pot gets 6 mg/L and in C_4 each pot get 8 mg/L of solution (Table 2).

Concentration of heavy metals added to water and soil				
Daily addition in water (mg/L)				
Heavy metals	**Concentration in C_1**	**Concentration in C_2**	**Concentration in C_3**	**Concentration in C_4**
Nickel	1	2	3	4
Chromium	1	2	3	4
Lead	1	2	3	4
Copper	1	2	3	4
One time addition to soil (mg/L)				
Heavy metals	**Concentration in C_1**	**Concentration in C_2**	**Concentration in C_3**	**Concentration in C_4**
Nickel	10	20	40	80
Chromium	10	20	40	80
Lead	10	20	40	80
Copper	10	20	40	80

Table 2. Shows the concentration of solution to be added daily.

Harvesting

After 90 days the specie was detached from the soil pots and the remaining's were burnt to ashes and then disposed of properly to avoid spreading in the soil. The soil from each pot was collected separately, thoroughly mixed, and then sealed in individual plastic packing for the soil analysis. Samples were tested in laboratory for difference in the added and remaining heavy metals concentration to get the removal efficiency of *Typha latifolia* specie for targeted heavy metals. As shown in Figures 4 and 5.

Figure 4. Showing harvesting phase.

Figure 5. Packets collection for final laboratory analysis.

Statistical analysis

The principle of statistics was applied to check the authentication of the results obtained. For our project we want to compare the groups for the removal efficiency of heavy metal from the soil as our sample size is less than 30 so we applied student T-test statistics formula in our case we used pooled estimate of the common standard deviation (Sp). From the data we calculated the standard deviations and between groups we need to compare the variance ratio and if it falls between 0.5 to 2. Then we can easily find value of Sp and consequently the t-statistics value.

Results and Discussion

We need level of confidence and alpha value. We select the 99% confidence level, and the alpha value is derived from T-table with degree of freedom in our case our degree of freedom is 8 which gives the value of +2.896, -2.896. as we have left tailed test so our value of consideration is -.896 now we will reject the null hypothesis if our calculated value of T-statistics is less than 1.96 and if our value comes greater then -2.896, we will have no evidence to reject null hypothesis (Figures 6-13).

Step 1. State null and alternate hypothesis

Null hypothesis (H_0): C_1=C_2

Alternate hypothesis (H_1): C_1<C_2

Step 2. Select appropriate test statistics

As mentioned earlier we can only use T-statistics because our sample size is less than 30 and also variance of population is unknown.

Step 3. Set up decision rule

Decision is based on the value of T-table against the degree of freedom, which is 8, for 95% confidence interval in (lower) tailed test. So, the value in table is -1.96.

We will reject Ho if value of calculation occurs less then table value and if occurs greater then table value then we will fail to reject Ho.

Step 4. Compute the value of Sp (pooled estimate of common standard deviations) Sp value is dependent on the variance ratio between the comparing groups. If the ratio comes between (0.5-2). Then we will proceed to calculate Sp value for each category.

Step 5. Conclusion

From our calculations we find the removal efficiency for each group. Also, we applied statistical analysis for comparing two groups. The findings are listed in the Tables 3-11.

Sample	Heavy metal	Mean	St. dev.	Variance	Var; ratio	Sp	T-statistic	T-critical	P-value	Result
C1	Ni	57.64	3.03	9.24	1.29	2.86	-10.66	-2.98	3.1×10^{-5}	Reject null
C2		76.92	2.67	7.17						

Table 3. Shows the analysis comparison between C_1 and C_2 for Nickel.

Sample	Heavy metal	Mean	St. dev.	Variance	Var; ratio	Sp	T-statistic	T-critical	P-value	Result
C1	Ni	57.64	3.03	9.18	1.27	2.86	-12.36	-2.98	5×10^{-5}	Reject null
C3		80.07	2.69	7.23						

Table 4. Shows the analysis comparison between C_1 and C_3 for Nickel.

Sample	Heavy metal	Mean	St. dev.	Variance	Var; ratio	Sp	T-statistic	T-critical	P-value	Result
C1	Ni	57.64	3.03	9.18	1.9	2.65	-17.93	-2.98	3.8×10^{-5}	Reject null
C4		87.72	2.2	4.82						

Table 5. Shows the analysis comparison between C_1 and C_4 for Nickel.

Sample	Heavy metal	Mean	St. dev.	Variance	Var; ratio	Sp	T-statistic	T-critical	P-value	Result
C1	Cr	49.86	2.98	8.88	1.03	2.97	-12.25	-2.98	1.0×10^{-5}	Reject null
C2		72.83	2.94	8.64						

Table 6. Shows the analysis comparison between C_1 and C_2 for Chromium.

Sample	Heavy metal	Mean	St. dev.	Variance	Var; ratio	Sp	T-statistic	T-critical	P-value	Result
C1	Cr	49.86	2.98	8.88	0.5	3.65	-11.11	-2.98	5.9×10^{-5}	Reject null
C3		75.51	4.2	17.64						

Table 7. Shows the analysis comparison between C_1 and C_3 for chromium.

Sample	Heavy metal	Mean	St. dev.	Variance	Var; ratio	Sp	T-statistic	T-critical	P-value	Result
C1	Cr	49.86	2.98	8.88	0.83	3.18	-17.54	-2.98	1.8×10^{-5}	Reject null
C4		84.62	3.27	10.6						

Table 8. Shows the analysis comparison between C_1 and C_4 for chromium.

Sample	Heavy metal	Mean	St. dev.	Varian ce	Var; ratio	Sp	T-statistic	T-critical	P-value	Result
C1	Pb	54.52	2.93	8.58	0.56	3.45	-9.91	-2.98	4.7×10^{-5}	Reject null
C2		76.2	3.91	15.3						

Table 9. Shows the analysis comparison between C_1 and C_2 for lead.

Sample	Heavy metal	Mean	St. dev.	Variance	Var; ratio	Sp	T-statistic	T-critical	P-value	Result
C1	Pb	54.52	2.93	8.58	0.85	3.06	-10.07	-2.986	5.7×10^{-5}	Reject null
C3		75.25	3.18	10.11						

Table 10. Shows the analysis comparison between C_1 and C_3 for lead.

Sample	Heavy metal	Mean	St. dev.	Varianc e	Var; ratio	Sp	T-statistic	T-critical	P-value	Result
C1	Pb	54.52	2.93	8.58	0.68	3.26	-15.42	-2.98	1×10^{-4}	Reject null
C4		86.38	3.56	12.67						

Table 11. Shows the analysis comparison between C_1 and C_4 for lead.

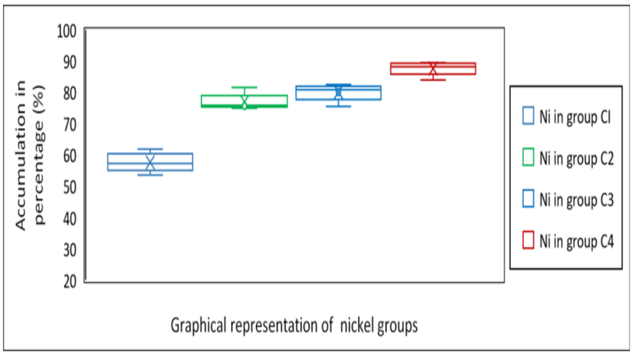

Figure 6. Box and whiskers chart showing percent accumulation for Nickel groups.

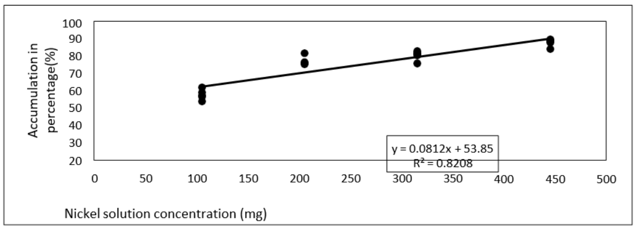

Figure 7. Showing R^2 value for Nickel accumulation.

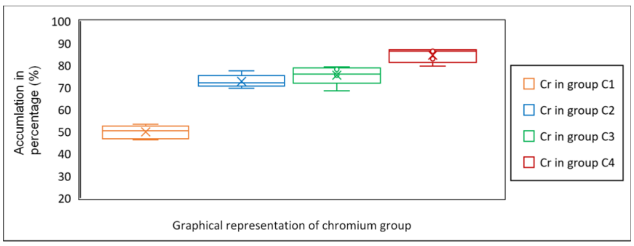

Figure 8. Box and whiskers chart showing percent accumulation for chromium groups.

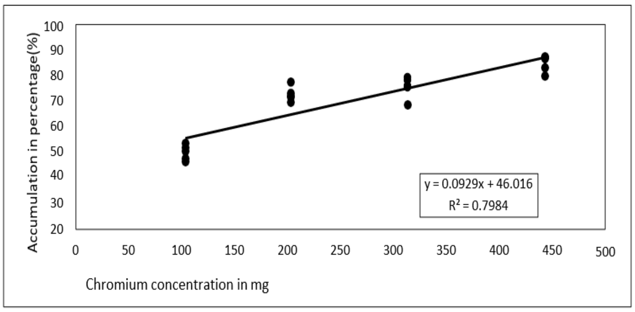

Figure 9. Showing R^2 value for chromium accumulation.

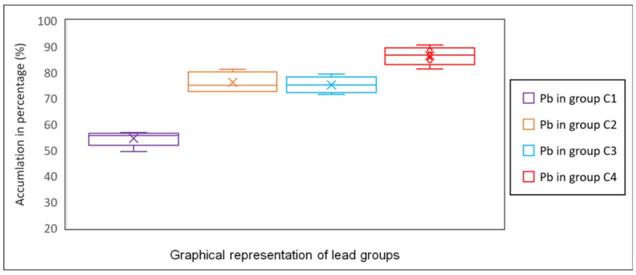

Figure 10. Box and whiskers chart showing percent accumulation for lead groups.

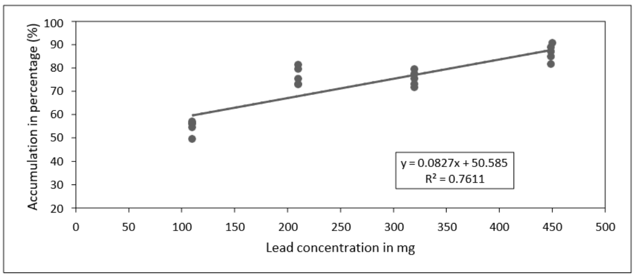

Figure 11. Showing R^2 value for lead accumulation.

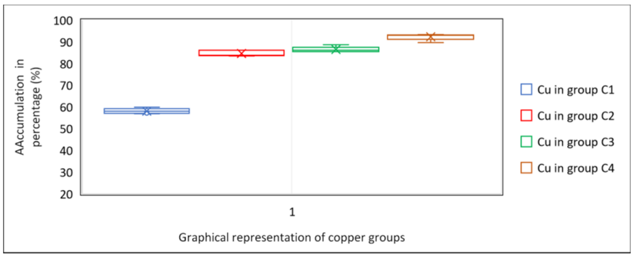

Figure 12. Box and whiskers chart showing percent accumulation for copper groups.

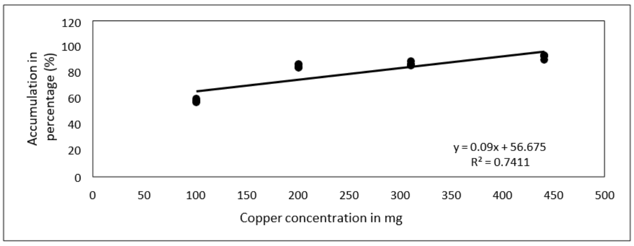

Figure 13. Showing R^2 value for copper accumulation.

Final result: The Table 12 listed below shows the overall summary of the statistical analysis.

Summary of heavy metals analysis

Ho	Ha	Level of confidence	T-critical	T-statistics	P-value	Reject/accept
$C_1=C_2$	$Cl<C_2$	0.99	-2.98	-10.66	0.000311	Null rejected
$C_1=C_3$	$Cl<C_3$	0.99	-2.98	-12.36	0.000055	Null rejected
$C_1=C_4$	$Cl<C_4$	0.99	-2.98	-17.93	0.000038	Null rejected
$C_1=C_2$	$Cl<C_2$	0.99	-2.98	-12.25	0.00001	Null rejected
$C_1=C_3$	$Cl<C_3$	0.99	-2.98	-11.11	0.000596	Null rejected
$C_1=C_4$	$Cl<C_4$	0.99	-2.98	-17.54	0.000018	Null rejected
$C_1=C_2$	$Cl<C_2$	0.99	-2.98	-9.91	0.000472	Null rejected
$C_1=C_3$	$Cl<C_3$	0.99	-2.98	-10.7	0.000575	Null rejected
$C_1=C_4$	$Cl<C_4$	0.99	-2.98	-15.42	0.000101	Null rejected
$C_1=C_2$	$Cl<C_2$	0.99	-2.98	-33.28	0.000002	Null rejected
$C_1=C_3$	$Cl<C_3$	0.99	-2.98	-27.6	0.000003	Null rejected
$C_1=C_4$	$Cl<C_4$	0.99	-2.98	-32.46	0.000004	Null rejected

Table 12. Showing overall summary of the results.

Conclusion

- It is concluded from the study that the variation in concentration has impact on the removal efficiency of the phytoremediation technique.
- The statistical analysis and graphical representation evident that increasing the concentration also increase the uptake capacity of *Typha latifolia*.
- The removal efficiency for each heavy metal is different however the overall removal capacity is more than 50% for every heavy metals.
- No result was obtained which shows the inverse relation between concentration and removal efficiency.
- Best result was obtained for copper followed by nickel, lead and chromium with more than 50% removal overall
- Also, from the practical use it can concluded that field study has more significant result as compared with laboratory scale study.
- R^2 values shows that the data fits the trend line significantly as it is nearer to 1. So, the data sets are reliable.

Future Recommendation

- Same procedure can be applied on different heavy metals and contaminants; also other species can be introduced for betterment.
- With different concentration and in more controlled conditions this study can be carried out.
- Biomass analysis can also be carried out rather than analyzing the soil parameters.
- The more you make groups and samples the more accurate the result will be.
- Need budget for large scale studies and application on commercial basis to acknowledge the beneficial use of plant species.
- Field application is required to check the credibility of the research on practical grounds.

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