To Quantify the Heavy Metals Uptake from Soil using Phytoremediation Process for Different Contaminant Concentrations

Waqas Umar

Civil (Environmental) Engineering Department, University of Engineering and Technology Peshawar, Pakistan 2021

Abstract: *Heavy metals contamination affects a large proportion of the world population, mostly in developing countries where environmental policies are either non - existent or rarely enforced due to which several health - related problems 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 heavy metals, daily for a period of ninety days. Atomic absorption spectrometry was used to determine the amounts of the 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. From the previous studies it is concluded that the heavy metals concentration has impact on the removal efficiency of heavy metals from the soil using thypa latifolia specie. But no specific attention was drawn towards the removal efficiency compared with the increase or decrease of the heavy metal's concentration. Our study focused on relation between concentration of heavy metals and removal efficiency. It was concluded from statistical analysis and graphical representation that direct relation is exist between the removal efficiency and heavy metals concentrations for the said study. We also made the comparison amongst the heavy metal's remediation using thypa latifolia species based on which we suggest the use of thypa latifolia for maximum remediated heavy metal. Further research is required to determine when the relation of concentration of heavy metals and removal efficiency will reverse. In addition, utilizing several other plants species and heavy metals will further strengthen the result authentication statistically.*

1. 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 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 byRufus 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

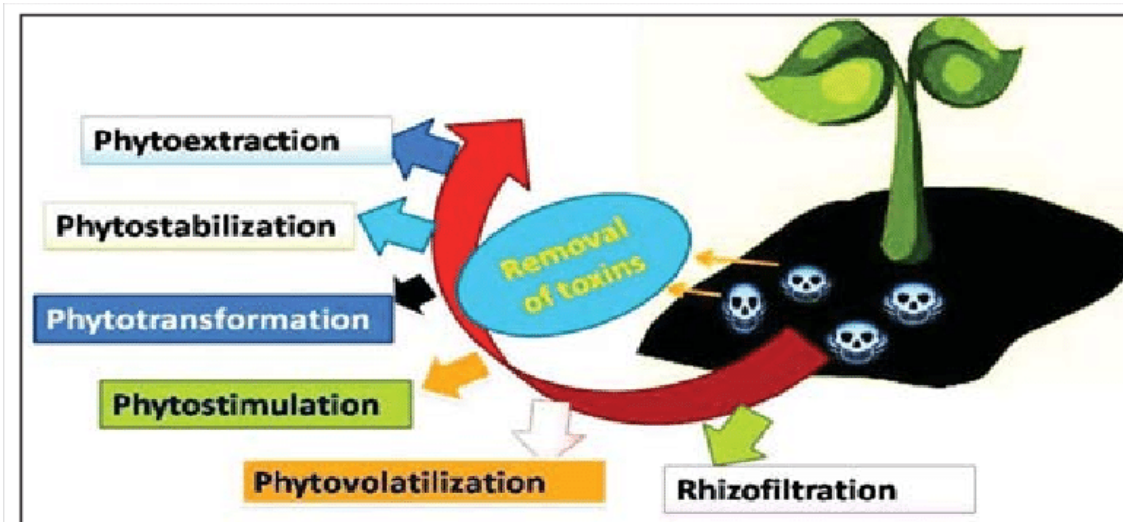low concentration when it come to the human environment specially food chain and drinkable water sources (13) .

Figure 1: Showing sub processes in phytoremediation

2. Literature Review

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 (21).

Problem Statement

Research have been carried out to study the effects of phytoremediation technique for heavy metal removal from soil using typha latifolia specie (22) (23). 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.

3. 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 10x10 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 soil and water

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	1.10

Plantation:
Four groups were created namely C1, C2, C3 andC4. further Each group contains 5 pots namely C1a, C1b, C1c, C1d, C1e and same for other three groups. Total 20 pots were planted using typha latifolia specie.

Figure 2*: Showing pots planted with typha latifolia

Solution preparation
Different concentration of solutions were prepared accordingly for each group. Each pot in C1 group required 90mg/90 days daily irrigation. so in total 450 mg of each

heavy metal was required for C1 group. For C2 900mg, for C3 1350 mg and for C4 1800mg for 90 days was required. As shown in figure.

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 C1 get 2 mg/L daily. in C2 each pot get 4 mg/L, in C3 each pot get 6 mg/L and in C4 each pot get 8 mg/L of solution.

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

Concentration of heavy metals added to water and soil				
Daily addition in water (mg/L)				
Heavy metals	Concentration in C1	Concentration in C2	Concentration in C3	Concentration in C4
Nickel	1	2	3	4
Chromium	1	2	3	4
One time addition to soil (mg/L)				
Heavy metals	Concentration in C1	Concentration in C2	Concentration in C3	Concentration in C4
Nickel	10	20	40	80
Chromium	10	20	40	80

Harvesting:

After 90 days the specie was detached from the soil pots and theremaining's were burnt to ashes and then disposed off 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 figure.

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. 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 - 2.896. Now we will reject the null hypothesis if our calculated value of T - statistics is less then - 1.96. and if our value comes greater then - 2.896, we will have no evidenceto reject null hypothesis.

Step 1: State null and alternate hypothesis:
Null hypothesis (Ho): C1=C2
Alternate hypothesis (H1): C1<C2

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 below.

Table 3: Shows the analysis comparison between C1 and C2 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	9.24	1.29	2.86	- 10.66	- 2.98	$3.1*10^{-5}$	Reject null
C2		76.92	2.67	7.17						

Table 4: Shows the analysis comparison between C1 and C3 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 5: Shows the analysis comparison between C1 and C4 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	1.90	2.65	- 17.93	- 2.98	$3.8*10^{-5}$	Reject null
C4		87.72	2.20	4.82						

Table 6: Shows the analysis comparison between C1 and C2 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	1.03	2.97	- 12.25	- 2.98	$1.0*10^{-5}$	Reject null
C2		72.83	2.94	8.64						

Table 7: Shows the analysis comparison between C1 and C3 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	0.50	3.65	- 11.11	- 2.98	$5.9*10^{-5}$	Reject null
C3		75.51	4.2	17.64						

Table 8: Shows the analysis comparison between C1 and C4 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						
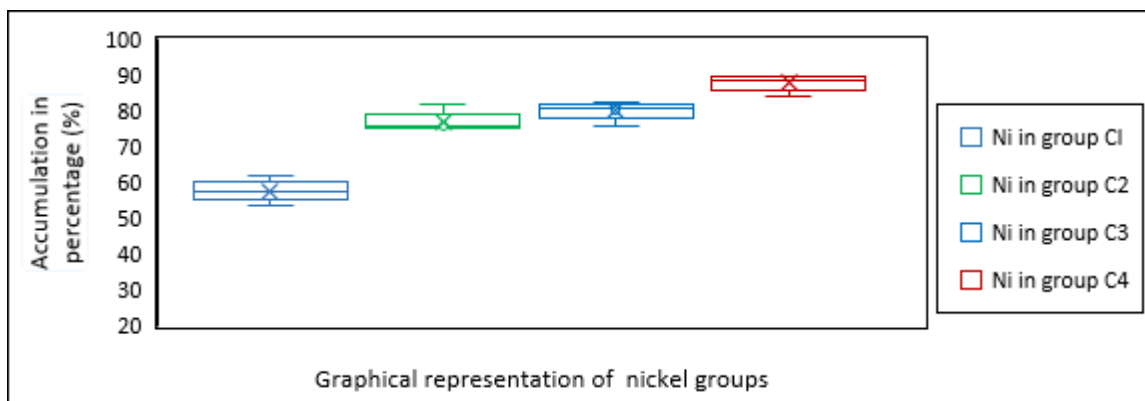
Figure 6: Box and whiskers chart showing percent accumulation for Nickel groups
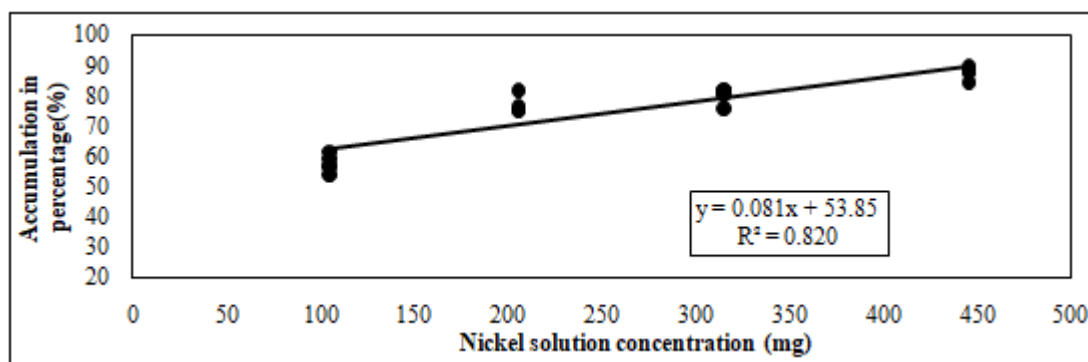
Figure 7: Showing R^2 value for Nickel accumulation
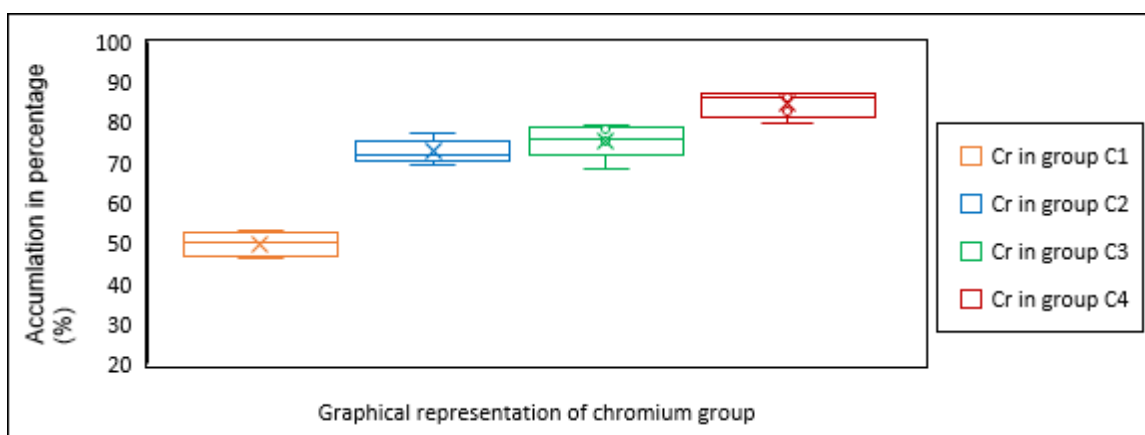
Figure 8: Box and whiskers chart showing percent accumulation for Chromium groups
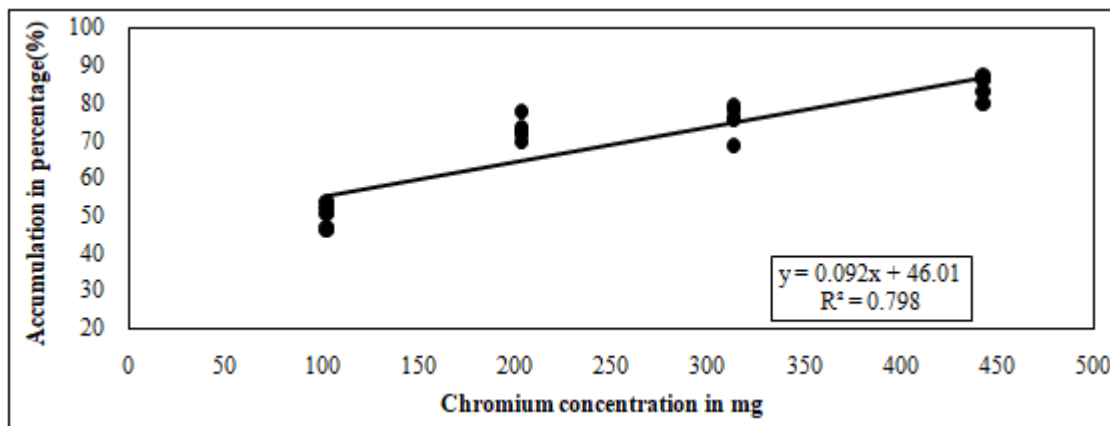
Figure 9: Showing R^2 value for Chromium accumulation

4. Final Result

The table listed below shows the overall summary of the statistical analysis.

Table 9: Showing overall summary of the results

Summary of heavy metals analysis							
Heavy metal	Ho	Ha	Level of confidence	T - critical	T - statistics	P - value	Reject/ Accept
Ni	C1=C2	CI< C2	0.99	- 2.98	- 10.66	0.000311	Null rejected
Ni	C1=C3	CI< C3	0.99	- 2.98	- 12.36	0.000055	Null rejected
Ni	C1=C4	CI< C4	0.99	- 2.98	- 17.93	0.000038	Null rejected
Cr	C1=C2	CI< C2	0.99	- 2.98	- 12.25	0.000010	Null rejected
Cr	C1=C3	CI< C3	0.99	- 2.98	- 11.11	0.000596	Null rejected
Cr	C1=C4	CI< C4	0.99	- 2.98	- 17.54	0.000018	Null rejected

5. Conclusion

1) It is concluded from the study that the variation in concentration has impact on the removal efficiency of the phytoremediation technique.
2) The statistical analysis and graphical representation evident that increasing the concentration also increase the uptake capacity of typha latifolia.
3) The removal efficiency for each heavy metal is different however the overall removal capacity is more than 50% for every heavy metals.
4) No result was obtained which shows the inverse relation between concentration and removal efficiency.
5) Best result was obtained for nickel followed by chromium with more than 50% removal overall
6) Also, from the practical use it can concluded that field study has more significant result as compared with laboratory scale study.
7) R² values shows that the data fits the trend line significantly as it is nearer to 1. So, the data sets are reliable.

6. Future Recommendation

1) Same procedure can be applied on different heavy metals and contaminants, also other species can be introduced for betterment.
2) With different concentration and in more controlled conditions this study can be carried out.
3) Biomass analysis can also be carried out rather than analyzing the soil parameters.
4) The more you make groups and samples the more accurate the result will be.

5) Need budget for large scale studies and application on commercial basis to acknowledge the beneficial use of plant species.
6) Field application is required to check the credibility of the research on practical grounds.

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