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Study of Effectiveness of Phytoremediation at Different Contamination Level of Wastewater

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Abstract

Plant species such as *Thlaspi caerulescens Viola calaminaria, Euphorbia prostrata, Arundo donax, Brassica juncea, Helianthus annuus, Festuca arundinacea, Populus* species etc. are long known for their ability to extract heavy metals from soil and waste water. Various attempts at increasing efficiency of extraction by phytoremediation include adding micro- organisms, co-cropping various species and using genetically engineered species. So far, the effect of levels of concentration of heavy metals on extraction efficiency of these plants has not been investigated. The purpose of this study is to test for the hypothesis that the concentration of heavy metals in soils affects the efficiency of phytoremediation. Nine samples of *Typha latifolia* were planted under controlled environment. Plants were irrigated by wastewater of industrial effluents diluted with different amounts of clean water, while providing protection from rainwater. The soil in the planters was tested for heavy metals to obtain levels of contamination already present in the soil and after plants was fully grown, using X-Ray Fluorescence Spectrometry, while the wastewater used for irrigation was tested using Atomic Absorption Spectrometry. The amount of heavy metals already present in the soil and that introduced by irrigation was compared to the amount left behind in the soil after maturity of plants, were compared to test the hypothesis. The results suggested that rate of extraction of heavy metals using phytoremediation was indeed affected by the level of concentration of heavy metals in the soil and irrigation water, however, owing to the small number of samples, the relationship between extraction rate and concentration levels could not be established. The study provides enough evidence to support the stated hypothesis and opens a new avenue of research to optimize the extraction efficiency of *Typha latifolia* and other species.

Keywords: Phytoremediation; Heavy metals; Wastewater of varying concentration; Hypothesis testing

Introduction

Heavy metals released to environment through different sources finally become part of the soil. Heavy metals are not biodegradable and remain in environment for long times having adverse effects on biotic and abiotic (water, soil, air) life. Heavy metals are present in soil in various forms [1] such as:

a) Oxides, carbonates and hydro carbonates.

b) Constitutes of silicate minerals structures.

c) Free metals ions or combined with soil organic matter.

d) Soluble metals complex.

Various conventional technologies, such as land filling, soil washing, solubilizing agents, vitrification of polluted soil at high temperature, solidification, using stabilizing agents, electrochemical process, and green plants are used to remove heavy metals from soil; along with high cost equipment, chemicals used in conventional technologies cause environment pollution and changes in the soil properties [2]. Phytoremediation (using green plants to extract contaminants from soil) is environmentally friendly and is very economical compared to traditional techniques [3,4]. Efficiency of phytoremediation has been found to depend on nature of contaminants, soil properties, plant type, and bioavailability etc. [5]. High bio mass plants extract a larger quantity of heavy metals but are relatively uneconomical owing to higher labor costs [3,6]. Disadvantages of phytoremediation include longer process, roots left behind in the treated area, and incompatibility of several species with local climate and level of contamination [7]. Phytoremediation was used for reclamation of soil contaminated by radioactive materials in Ukraine [8], in Japan after the Fukushima Nuclear power plant disaster [9], and in Spain used to remove mine wastes [10]. Any type of waste discharged outdoor specially when waste contain chemicals and heavy metals has bad effects on living things such as human beings, animals and plants [11,12]. Anything which is disposed to environment at last become the part of soil which gets contaminated [13] pollutants present in the environment become the part of human body through various routes such as food chain, inhalation of particulate matter, ingestion and through direct contact [14]. Environment can be protected from waste and their bad affects by recycling of waste, using sustainable techniques to reduce volume of waste. Techniques used to remediate soil are expensive and not environment friendly as compared to phytoremediation [2]. List of contaminants can be removed from soil using plants. Using plants for remediation purposes don't affect badly surrounding and is not expensive because it requires little labor work and no chemicals [3,4].

Heavy metals are natural constitute of soil but increase in use of heavy metals such as Lead, Copper, Cobalt, Chromium, Zinc, Manganese, Nickel and metalloid Arsenic in industries with increasing population for various products which finally become the part of soil and ground water which is becoming alarming situation regard health problems [15-17]. Vehicular traffic, petrochemical and tanning industries are responsible of high concentration of Lead, Copper,

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Cobalt, Chromium, Zinc, Manganese, Nickel and metalloid Arsenic in soil and water [18,19]. Bioremediation is a technique to remediate soil and water using living organism, various bioremediation techniques are natural attenuation, Biougmentation, Biostimulation, Bioleachon, Biofilters, Biopiling, Biosorption, Bioleaching, Biovoting, Composting, Phytoremediation, Land farming and Rhizoremediation [20-32].

Problem Statement

Since traditional phytoremediation method poses some limitations regarding their applications at large scale, so there is a dreadful requirement to amend this approach using up-to-date chemical, biological and genetic engineering apparatuses. To improve efficiency of phytoremediation various plants species are used for removal of heavy metals from soil and wastewater at different conditions. So far, the effect of levels of concentration of heavy metals on extraction efficiency of plants has never been investigated using *Typha latifolia* plant.

Hypothesis/Solution

The purpose of this study is to test for the hypothesis that the concentrations of heavy metals in soils affect the efficiency of phytoremediation using *Typha latifolia* plant. Our null hypothesis is different concentration level does not affect extraction rate, and alternate hypothesis is that different concentration level affect extraction rate. Considering the need of phytoremediation in this research work we have checked the effects of various contamination levels of heavy metals using *Typha latifolia* plant grown in controlled environment.

Research Methodology

Nine samples of *Typha latifolia* were planted under controlled environment. Plants were irrigated by wastewater of industrial effluents diluted with different amounts of clean water, while providing protection from rainwater. The soils in the flower pot was tested for heavy metals to obtain levels of contamination already present in the soil result is shown in Table 1. And after plants were fully grown, using X-ray fluorescence spectrometry, while the wastewater used for irrigation was tested using Atomic Absorption Spectrometry. The amount of heavy metals already present in the soil and the amount introduced by irrigation was compared to the amount left behind in the soil after maturity of plants were compared to test the hypothesis.

In this research work we used *Typha latifolia* plant. Instead of bio-mass analysis, soil analysis was done which was comparatively economical. Despite being economical, the cost of tests dictated the number of samples. In controlled environment, soil analysis gives more accurate analysis of extraction rate (biomass analysis cannot account for Phytovolatization). Over all methodology is shown in Figure 1.

Wastewater used for irrigation purposes was collected from industrial effluents at two points which are then mixed to form composite sample. Sample taken from composite sample was analyzed for heavy metals Lead, Chromium, Copper, Zinc, Manganese, Cobalt and Cadmium using atomic absorption spectrometry. Concentration of heavy metals in wastewater is shown in Figure 2.

Soil of known contamination was poured in nine pots. In each pot *Typha latifolia* was planted shown in Figure 3 and the flowerpots were protected from rain water and other contaminates added to it from environment by providing ventilated and transparent plastic sheet shown in Figure 4. Lawn division is done on the base of various contamination level of wastewater provided to each three of them. Three type of pots are:

Heavy metals	ppm
Zinc	296
Copper	444
Chromium	753
Manganese	2263

Table 1: Concentration of heavy metals in soil before plantation.

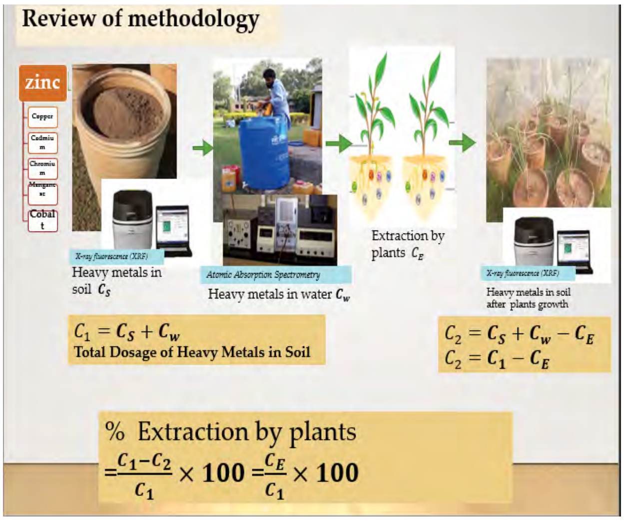
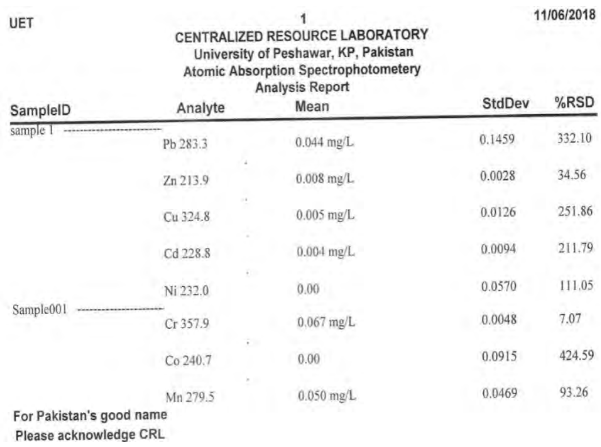


Figure 1: Review of methodology.



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Atomic Absorption Spectrophotometery
Analysis Report

SampleID	Analyte	Mean	StdDev	%RSD
sample 1	Pb 283.3	0.044 mg/L	0.1459	332.10
	Zn 213.9	0.008 mg/L	0.0028	34.56
	Cu 324.8	0.005 mg/L	0.0126	251.86
	Cd 228.8	0.004 mg/L	0.0094	211.79
	Ni 232.0	0.00	0.0570	111.05
Sample001	Cr 357.9	0.067 mg/L	0.0048	7.07
	Co 240.7	0.00	0.0915	424.59
	Mn 279.5	0.050 mg/L	0.0469	93.26

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Figure 2: Concentration of heavy metals in water used for irrigation.

a) **Labelled as lawn A:** Irrigated by industrial wastewater which is diluted ten times by domestic water.

b) **Labelled as lawn B:** Irrigated by industrial wastewater which is diluted five times by domestic water.

c) **Labelled as lawn C:** irrigated by undiluted industrial wastewater.

To make ten times diluted industrial wastewater solution ten liter of domestic water were added to one liter industrial wastewater of known amount of heavy metals. After we mixing equal amount of ten times diluted wastewater is provided to each three pots of lawn A, similarly lawn B was irrigated by five time diluted industrial wastewater and in lawn C each pot was irrigated by equal amount of undiluted industrial

Figure 3: Plantation of *Typha latifolia* in nine flower pots.

Figure 4: Shed is provided to protect from rain water.

wastewater. During growth process of *Typha latifolia* is protected from rain water and under controlled environment only industrial wastewater of known concentration and known amount were supplied. *Typha latifolia* reached to maturity in winter season from October to January 2019.

Laboratory Analysis and Discussion

Before and after plantation of *Typha latifolia* soil is analyzed in laboratory for heavy metals detection using X-Ray fluorescence spectrometry and atomic absorption spectrometry for industrial wastewater before plantation only. Heavy metals concentration in soil plus provided in form of various contamination level of wastewater during irrigation process up to maturity of plant was monitored. After nineteen days *Typha latifolia* reached to maturity. Roots and aerial parts of plants are removed from each flowerpot; soil is dried and well mixed in pot which is then analyzed in laboratory using X-ray fluorescence spectrometry. Concentration of heavy metals in soil after *Typha latifolia* get mature was found using X-ray fluorescence spectrometry shown in Table 2.

Amount of heavy metals provided and percentage extraction by plants was calculated using following formula, which is shown in Table 3.

Heavy metals in soil = C_s

Heavy metals in wastewater used for irrigation = C_w

Heavy metals extracted by plants= C_E

Heavy metals in soil after plants growth $C_2=C_s+C_w-C_E$ Total dosage in of heavy metals in soil $C_1 = C_s+C_w$

$$\% \text{ Extraction by plants}= \frac{C_1 - C_2}{C_1}\times 100 = \frac{C_E}{C_1}\times 10$$

Analysis is made using testing hypothesis. Following test are performed to test hypothesis.

1. State the null and alternate hypothesis, H_0 and Ha.
2. Select the decision criteria α (“or level of significance”).
3. Establish the critical values.
4. Draw a random sample from the data and calculate the mean of that sample.
5. Calculate the standard deviation (S).
6. Calculate the values of test statistic t.
7. Compare the calculate value of t with the critical value of t, and then accept or reject null hypothesis.

Null Hypothesis and Alternate Hypothesis

Null hypothesis is claim about data collected which can be tested.

Concentration level	Lawn	Zinc	Chromium	Manganese
Ten-time diluted Wastewater	A1	292	621	1851
	A2	288	671	1906
	A3	273	597	1961
Five-time diluted Wastewater	B1	295	737	2017
	B2	296	740	1975
	B3	295	596	2046
Undiluted wastewater	C1	278	687	1984
	C2	286	697	2022
	C3	293	702	2114

Table 2: Heavy metals concentration in soil after *Typha latifolia* growth.

Variables	Zinc		Chromium		Manganese	
	Concentration	Extracted	Concentration	Extracted	Concentration	Extracted
A_1	298.13	2%	755.44	18%	2270.5862	18%
A_2	298.13	3%	755.44	11%	2270.5862	16%
A_3	298.13	8%	755.44	21%	2270.5862	14%
B_1	299.74	2%	757.94	3%	2278.2848	11%
B_2	299.74	1%	757.94	2%	2278.2848	13%
B_3	299.74	2%	757.94	2%	2278.2848	10%
C_1	312.912	11%	778.4	12%	2341.184	15%
C_2	312.912	8%	778.4	10%	2341.184	14%
C_3	312.912	6%	778.4	10%	2341.184	10%

Table 3: Heavy metals provided and extracted by plants irrigated by various contamination level of wastewater.

	Heavy Metals	Pooled St- Dev.	T-Statistic	P-value
Test: 1 H_o: $\lvert\mu_o-\mu_5\%\rvert = 0$ H_a: $\lvert\mu_o-\mu_5\%\rvert > 0$	Zinc	3.33	4.47	0.005
	Chromium	57.83	0.33	0.381
	Manganese	4.67	0.95	0.199
Test: 2 H_o: $\lvert\mu_o-\mu_{10}\%\rvert = 0$ H_a: $\lvert\mu_o-\mu_{10}\%\rvert > 0$	Zinc	2.5	3.09	0.018
	Chromium	13.83	-2.80	0.024
	Manganese	5.50	-1.57	0.096

Table 4: Statistic calculation for heavy metals.

In our research work null hypothesis is that difference contamination level does not affect mean extraction rate of phytoremediation for *Typha latifolia* plants. After testing if this hypothesis is rejected as false then there is an alternate (or experimental) hypothesis H_A that is logically must be accepted.

In case of our research work we performed two tests.

Test 1

Null Hypothesis H_0: $|\mu_o - \mu_5\%| = 0$ for null hypothesis probability $\alpha = 0.05$

Alternate Hypothesis: H_a: $|\mu_o - \mu_5\%| > 0$

Test 2

Null Hypothesis H_0: $|\mu_o - \mu_{10}\%| = 0$ for null hypothesis probability $\alpha = 0.05$

Alternate Hypothesis: H_a: $|\mu_o - \mu_{10}\%| > 0$

Statistic calculation for heavy metals Zinc, Chromium and Manganese at five time diluted industrial wastewater and ten time diluted wastewater is shown in Table 4.

Conclusions

Following conclusions can be drawn from the above study:

1. In case of Zinc, we can see that null hypothesis is rejected with very low p-values (0.5% and 1.8%), therefore, we can strongly conclude that concentration of zinc does affect the extraction efficiency of *Typha latifolia*.
2. In case of Chromium we observe that null hypothesis is rejected for 10 times dilution with a very low p value of 2.4%, while in case of 5 times dilution, the null hypothesis could not be rejected, however, this may be attributed to the outlier in the data.
3. In case of Manganese, the null hypothesis could not be rejected and may require an increased sample size for stronger conclusions.
4. The study provides enough evidence to support the hypothesis that concentration of heavy metals in the soil (and irrigation water) does affect the phytoremediation efficiency for *Typha latifolia*.
5. Further studies with higher sample sizes, higher variations in concentration, and different plant species are recommended.
6. Lead, Copper, Chromium and Cobalt provided during irrigation process were totally extracted by *Typha latifolia* during ninety days growth process.

Recommendations

Phytoremediation is environmental friendly technique and is need of the industrial period. Therefore it should be to improve it efficiency.

Following recommendations are made for future studies:

1. To check efficiency of plants using maximum number of various contamination level.
2. Climatic variation.
3. Efficiency of Hyper accumulator and low accumulator plant at different concentration level of wastewater.

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