ZANCO Journal of Pure and Applied Sciences
The official scientific journal of Salahaddin University-Erbil
https://zancojournals.su.edu.krd/index.php/JPAS

ISSN (print):2218-0230, **ISSN (online):** 2412-3986, **DOI:** **http://dx.doi.org/10.21271/zjpas**

RESEARCH PAPER

Effect of Phosphorus Fertilization on Phytoremediation efficacy of Heavy Metals by Wheat and Bean Plants

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A B S T R A C T:

A pot experiment was con ducted at the experimental farm of Halabja Technical College of Applied Sciences in July 2017 to July 2018, 2 km north of Halabj a city in the longitude 45°59'40"E and latitude 35°10'28"N. To determine the impact of the use of phytoremedial techniques in the governorate of Halabja on contaminated soil with heavy metals. Soil samples were collected randomly from 15 sites within the solid waste dumping area and control sample, These samples were analyzed for chemical characteristics and concentration of certain heavy metals such as Lead (Pb), Cadmium (Cd), Nickel (Ni), Zinc (Zn) and Iron (Fe) using ICP-MS (inductively coupled plasma mass spectrometer. The summary of the main results was as follow maximum concentration of heavy metals from soil in bean plant were (Pb and Ni) were (55.4 and 50.1 %) for (TSP_{40}), (Fe and Cd) were (49.7 and 44.5 %) for (TSP_{160}) and uptake of Zn increased by (45.6 %) for (TSP_{120}) compared to control treatment, while wheat plant extracted heavy metals (Pb, Cd, Ni, Fe, and Zn) by (60.6, 53.4, 37.1, 37.1, and 26.6 %) respectively for (TSP_{160}) compared with control treatment. These results evidence that Phytoextraction of (Pb, Cd, Ni, Zn, and Fe) by (Bean) plant was much greater than that by (Wheat) from municipally polluted soil after adding phosphorus fertilizer to the soil. This study confirms that (Bean) as one of the plants that could be employed in phytoremediation of soil polluted by heavy metals.

KEY WORDS: Phytoremediation, Heavy metals, Soil pollution.
DOI: http://dx.doi.org/10.21271/ZJPAS.31.5.3
ZJPAS (2019) , 31(5);18-27 .

1.INTRODUCTION :

Pollution of the environment by toxic metals such as Pb, Cd, Ni, Zn, and Fe has become a worldwide crisis, affecting agriculture products and contributing to bioaccumulation and biomagnifications in the food chain. Recently, research groups have found out that certain toxic metals can remain in the environment for a long time and may eventually bioaccumulation to higher concentrations that could affect human health (Dipu *et al.*, 2011).

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Article History:
Received: 16/06/2019
Accepted: 16/07/2019
Published: 17/10 /2019

Phytoremediation is a green solution to the problem of heavy metal contamination ‘‘Phytoremediation refers to the use of plants and associated soil microbes to reduce the concentrations or toxic effects of pollutants in the environments’’ (Greipsson, 2011). However the phytoremediation can be used for reduce or eliminate of heavy metals, radionuclides as well as for organic pollutants (like polynuclear aromatic hydrocarbons, pesticides and polychlorinated biphenyls) and other chemical materials in the polluted sites. Green plants have a great ability to uptake pollutants from the contaminated environment and accomplish their detoxification by number mechanisms such as phyto-volatilization, phyto-degradation, and phytoaccumulation (also called phyto-extraction) in this mechanism; plant roots sorb the pollutants along with other nutrient elements and water.

In addition, plants can growing fast and have high-biomass product like poplar, Jatropha and willow might be used for both phytoremediation and energy production (Abhilash *et al.,* 2012). Because of the accumulation of higher levels of heavy metals in the soil may restrict the plant growth. The aim of the study was determining the efficiency of two types of crops such as Wheat (*Triticum aestivum-Aras*) and Bean (*Vicia faba-Somar*) for clean-up polluted soil by heavy metals such as Pb, Cd, Ni, Zn, and Fe or reduce the level of these elements by using phytoremediation. There for these two plants are the most strategical and economical plants.

2. MATERIALS AND METHODS

2.1 Descriptions of the study area of (MSW)

The municipal solid waste (MSW) site is located in the north west of Halabja city. It has a total area of 10000m^2 in the longitude 45°56'57.65"E and latitude 35°12'28.08"N figure (1) and (2). The disposal place used for all kinds of municipal, industrial and hospital wastes. Also, different kinds of hazardous and recyclable waste including glass, plastic, batteries, and metal waste disposed into dump site.

Fig.1. Sites of Halabja Open Dump**.**

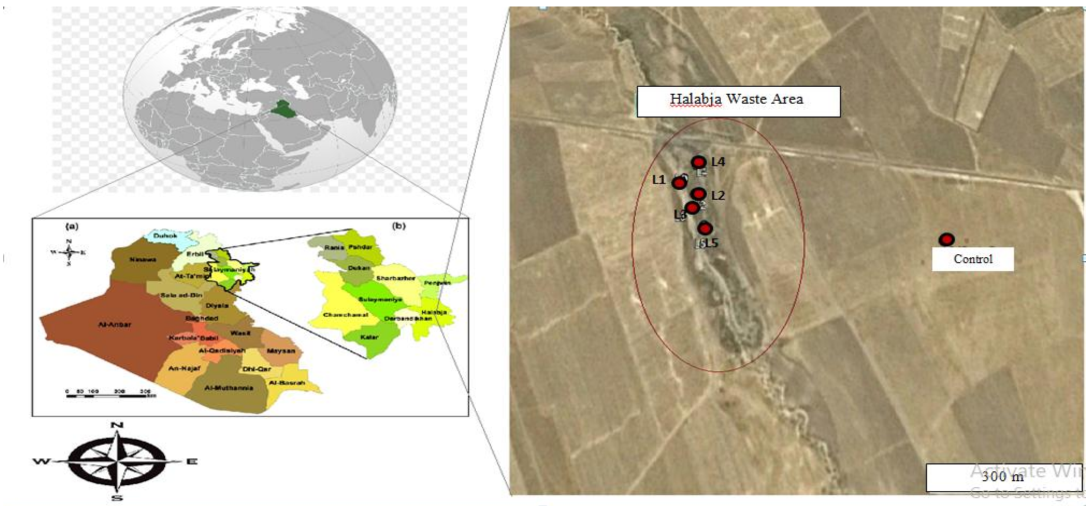

Fig. 2. Location map of the study area of Halabja Open Dump Site

2.2 Soil sampling and analysing

Soil sampling was carried randomly from 16 sites by using an auger at different directions and depths (surface layer 0-10, subsurface layer 10-20, and bottom layer 20-30) cm, within the solid waste dumping area in July 2017. Then labelled and sealed into plastic bags and transported to the lab. The samples were air dried in stainless steel trays and milled and sieved to obtain a <2 mm fraction for determine the chemical analysis then stored in polyethylene bags for laboratory analysis. The chemical analyses of the soil samples were done as shown in the table (1). These analyses performed as follows: The pH of soil extract (1:5) was measured by HANA pH-meter, Model pH211 microprocessor pH-meter using the procedure of (Estefan *et al*., 2013). The electrical conductivity was measured using EC-meter model BG43C according to (Estefan *et al*., 2013). Soil organic matter was determined by Loss On Ignition method (LOI) according to (Margesin and Fchinner, 2005). Total Calcium Carbonate ($CaCO_3$) % as described in Rowell (1996). Total heavy metal concentrations in soil and plant samples (mg kg^{-1}) were analysed by the ICP-MS method (Masson *et al.*, 2010).

Table 1: Some chemical properties of the studied soils

Location No.	**Depth (cm)**	**OM**	**Total $CaCO_3$**	**EC $mScm^{-1}$**	**pH**	**Pb**	**Cd**	**Ni**	**Zn**	**Fe**
		%				**(mg kg^{-1})**				
1	0-10	10.7	9.89	0.24	7.76	31.0	0.52	109	80.3	31372
	10-20	11.0	7.89	0.23	7.95	36.5	0.63	118	85.5	34650
	20-30	12.5	10.7	0.93	7.51	34.0	0.51	95.2	133	27808
2	0-10	9.73	10.3	0.20	8.02	18.5	0.39	102	75.0	28381
	10-20	9.53	11.4	0.18	8.12	12.9	0.41	100	63.5	28798
	20-30	8.83	9.69	0.16	8.22	11.2	0.37	102	61.8	29008
3	0-10	8.85	10.3	0.21	8.08	16.9	0.36	97.6	65.1	27715
	10-20	9.27	11.8	0.45	7.81	14.3	0.42	105	67.9	30338
	20-30	9.69	10.9	0.67	7.80	12.2	0.36	100	64.1	28409
Control sample		2.55	27.7	0.10	8.22	3.10	0.08	11.1	14.2	10221
Minimum		8.83	7.89	0.16	7.51	11.2	0.36	95.2	61.8	27715
Maximum		11.0	11.8	0.93	8.22	36.6	0.63	118	133	34650
Mean		10.0	10.3	0.36	7.92	20.9	0.44	103	77.4	29609

2.3 Pot experiments

Soil samples were collected from landfill air dried in stainless steel trays and milled using a plastic hummer and sieved to obtain a 4 mm fraction then put in to 7 kg plastic pots. Each pot (25cm height, 25cm top diameter, and 15cm bottom diameter) packed with the 6 kg of dry polluted soil. On 4/11/2017, 13 seeds of wheat

(*Triticum aestivum-Aras*) and 5 seeds of Bean (*Vicia faba-Somar*) were planted in each pot.

The experiment was designed by factorial CRD by using 5 levels of triple super phosphate 46% P_2O_5 fertilizer (0, 40, 80, 120, and 160 mg TSP kg^{-1} soil). The plant samples (Wheat and Bean) were collected on 13/5/2018 and 3/6/2018 respectively.

2.4 Plant sampling and analysing

The plant samples (Wheat and Bean) were collected on 13/5/2018 and 3/6/2018 respectively. Plant grains were harvested, and then washed with distilled water to clean up from the soil then oven dried at 65 ℃ for 72 hours. After weighing and crushed with a stainless steel mill, and protected in a polyethylene bag in a form of powder in a dry place for analysis (Mekeague, 1978). 0.3gm of powdered dried samples (seeds) digested by using (4ml HNO_3 and 2ml H_2O_2) for bean and wheat seeds and using only 6ml H_2O_2 digested grain samples then stored in labelled test tube, at room temperature until elemental analysis (Masson *et al.*, 2010).

2.5 Statistical analysis

The statistical analyses were conducted by using both statistical analysis system (SAS) and software program and the Person's correlation coefficient. Analysis of variance (ANOVA) was used, and then analysis variance means were compared by the Least Significant Differences (LSD) at ($p \leq 0.05$) level among treatments (Sas, 2002).

3. RESULTS AND DISCUSSION

3.1. Effect of different levels of (TSP) fertilizer on heavy metals concentration in wheat *(Triticum aestivum-Aras)* grains.

The application of triple supper phosphate (TSP) fertilizer has a significant effect ($P \leq 0.05$) on heavy metals (Pb, Cd, Ni, Zn and Fe) uptake in the wheat grains table (2) and figure (3). TSP supply significantly increased (Pb, Cd, Ni, Zn and Fe) uptake in the wheat grains. Generally, phosphorus fertilizers that can derived from sedimentary rocks contains the highest concentration of most of heavy metal(loid)s like Th, As, U, Zn and Cd (Alloway, 2013). The concentration of metals in soil, soil pH, organic matter, hydrous ferric oxides and density and type of soil colloids influence the heavy metal availablity (Chibuike and Obiora 2014).

The application (TSP) significantly increased Pb uptake by wheat grains was (18.71, 12.93, 7.471 and 6.265 mg kg^{-1}) in the (TSP 160, 120, 80, 40) treatments higher compared to (TSP 0.0) treatment. The highest value of Pb in wheat grains (18.71 mg kg^{-1}) was recorded in (TSP 160) treatment, while the lowest value (5.254 mg kg^{-1}) was obtained in (TSP 0.0) treatment. These may be referred to the applied P fertilizer which contain a wide range of Pb (<5.2%) (EPA, 1999). The variation of Pb content of plants tissue is affected by the presence of geochemical anomalies, genotype ability to accumulate Pb, pollution and seasonal variation (Kabata and Pendias, 2001).

Cd uptake significantly increased by the effect of (TSP) fertilizer in wheat grains compared to control sample. Cd uptake were (0.191, 0.084, 0.048 and 0.040 mg kg^{-1}) in the (TSP 160, 40, 120, 80) treatments higher compared to (TSP 0.0) treatment. The highest value of Cd in wheat grains (0.191 mg kg^{-1}) was obtained in (TSP 160) treatment, while the lowest value (0.034 mg kg^{-1}) was recorded in (TSP0.0) treatment. The P fertilizer which products naturally contain the higher amount of Cd and Zn (Alloway, 2013). Increase mobility of Cd in soil due to formation soluble complexes with organic matter (Mölders, 1999).Cadmium is a very mobile and can accumulate in plant tissues in large amount without showing any phytotoxic symptoms. Therefore it is considered one of the most dangerous heavy metals to human health (Moustakas *et al.*, 2001). The uptake of Ni significantly increased by the effect of (TSP) fertilizer in wheat compared to control sample. It means that the application of higher levels of phosphorus may cause the solubilisation of the nickel. The uptake of Zn in wheat grains ranged between (474.88-886.26 mg kg^{-1}) was higher (886.26 mg kg^{-1}) at (TSP 160) treatment, while the lowest value (474.88 mg kg^{-1}) was recorded from (TSP 0.0). The application (TSP) significantly increased Zn uptake by wheat grains. Wheat grain uptake of Zn was (886.26, 557.94, 548.01 and 511.65 mg kg^{-1}) in the (TSP 160, 120, 80, 40) treatments higher compared to (TSP 0.0) treatment. The application of TSP fertilizer has a significant effect ($P \leq 0.05$) on Fe uptake in wheat grains. Application of TSP fertilizer significantly increased Fe uptake in wheat grains. The highest value of Fe in wheat seeds (627.42 mg kg^{-1}) was recorded from (TSP 160), while the lowest value (264.99 mg kg^{-1}) was recorded from (TSP 0.0) treatment. These results agree with (Ali *et al.*, 2014) which reported that the application of P caused an increase in concentration of cationic metals such as Fe and Mn due to reducing the soil pH. In the present study results indicated that increase of heavy metals concentration cause decrease plant growth this is agree with (Ahmed and Abd-Alhamid, 2019, and Ahmed and Khoshnaw, 2019).

3.2 Effect of different levels of (TSP) fertilizer on heavy metals concentration in bean grains *(Vicia faba-Somar)* plant.

The application of (TSP) has significant effect ($P \leq 0.05$) on the uptake of all studied heavy metals (Cd, Ni, Zn and Fe) by bean grains table (3) and figure (3). This may be due to increase in plant biomass this is agree with (Ahmed and Khoshnaw, 2019) reported that soil P application had significant influence on dry matter production of bean plants. This increase is attributed to adequate P supply which an essential nutrient element for plant growth due to its important role in plant biological processes like the creation and division of living cells in the transfer of genetic materials and protein synthesis, and ion transport across cell walls (Havlin, et al., 1999). But the application of (TSP) has no significant change on (Pb). The uptake of Pb by bean grain was (7.384, 6.99, 6.846 and 5.124 mg kg^{-1}) in the (TSP 80, 160, 40, 120) treatments and (6.487 mg kg^{-1}) in (TSP 0.0) treatment. The highest value of Pb in bean grains (7.384 mg kg^{-1}) was recorded in (TSP 80) treatment in wheat plant, while the lowest value (5.124 mg kg^{-1}) was obtained in (TSP 120) treatment, this results disagree with (Ahmed and Khoshnaw, 2019) lead accumulated and only small portion of this metal was transported to the bean tissue. The Pb balance in different ecosystem shows that the input of this metal highly exceeds its output (Kabata and Pendias, 2001). The grain uptake of Cd significantly increased by the effect of (TSP) fertilizer in bean grains compared to control sample. Cd uptake were (0.153, 0.115, 0.107 and 0.067 mg kg^{-1}) in the (TSP 160, 120, 80, 40) treatments higher compared to (TSP 0.0). The highest value of Cd in bean grains (0.153 mg kg^{-1}) was obtained in (TSP 160) treatment, while the lowest value (0.067 mg kg^{-1}) was recorded in (TSP 40) treatment.

The uptake of Ni in bean grains ranged between (52.95-95.87 mg kg^{-1}) the highest value (95.87 mg kg^{-1}) was recorded from the (TSP 160) treatment, while the lowest value (52.95 mg kg^{-1}) was obtained from (TSP 40) treatment, it means that the synergistic relations exist between levels of (TSP) fertilizer and Ni uptake in bean grains. The bean grain uptake of Ni significantly increased by the effect of the applied (TSP) compared to control sample. Generally, the concentration of Ni in the solution of heavy metal contaminated soils is higher depending on the soil total concentration, source of Ni, and age of pollution (Alloway, 2013).

The application (TSP) significantly increased Zn uptake by bean grains. Bean grain uptake of Zn was (1585.01, 1486.28, 1115.46 and 646.46 mg kg^{-1}) in the (TSP 120, 160, 80, 40) treatments higher compared to (TSP 0.0) treatment. The uptake of Zn in bean grain was higher (1585.01 mg kg^{-1}) at (TSP 120) treatment, while the lowest value (646.46 mg kg^{-1}) was recorded from (TSP 0.0) treatment. Cd and Zn are movable and have the potential to pollute the food chain due to higher bioaccumulation factor (Saha *et al.*, 2017). These results disagree with (Ahmed and Khoshnaw, 2019) reported that decrease the Zn content in plant body by increasing levels of P fertilizer.

Application of (TSP) fertilizers significantly increased Fe uptake in bean grains. The highest value of Fe in seeds (1580.45 mg kg^{-1}) was recorded from (TSP 160), while the lowest value (578.94 mg kg^{-1}) was recorded from (TSP 40), this result disagrees with those recorded by (Edmeades, 2003) stated that the application of high amount of P may lead to nutrient imbalance then decrease in iron concentration. The (TSP) supply generally increased (Pb, Cd, Ni, Zn, and Fe) concentrations in bean grains this may be due to decrease the soil pH by the effect of TSP fertilizer resulting increase the availability of heavy metals (Kabata-Pendias and Pendias, 2001).

Table 2: The effect of different levels of triple super phosphate (TSP) on heavy metals concentration (mg kg^{-1}) in grain and the heavy metals uptake (mg pot^{-1}) by wheat plant.

Part of plant	TSP mg kg^{-1}	Pb	Cd	Ni	Zn	Fe	Grains Dry Matter Weight (g)	Pb	Cd	Ni	Zn	Fe
		Conc. (mg kg^{-1})						Uptake (mg pot^{-1})				
Wheat Grains	0	1.215[a]	0.008[b]	1.026[c]	109.8[a]	61.27[a]	4.325[d]	5.254[c]	0.034[b]	4.437[c]	474.88[b]	264.99[c]
	40	1.123[a]	0.015[a]	1.175[b]	91.71[ab]	57.34[ab]	5.579[cd]	6.265[c]	0.084[b]	6.555[b]	511.65[b]	319.89[bc]
	80	1.115[a]	0.006[b]	1.159[b]	81.78[b]	53.17[b]	6.701[bc]	7.471[c]	0.040[b]	7.766[b]	548.01[b]	356.29[b]
	120	1.868[a]	0.007[b]	1.153[b]	80.64[b]	56.83[ab]	6.919[b]	12.93[ab]	0.048[b]	7.978[b]	557.94[b]	393.20[b]
	160	1.669[a]	0.017[a]	1.373[a]	79.06[b]	55.97[ab]	11.21[a]	18.71[a]	0.191[a]	15.391[a]	886.26[a]	627.42[a]

Table 3. The effect of different levels of triple super phosphate (TSP) on heavy metals concentration (mg kg^{-1}) in grain and the heavy metals uptake (mg pot^{-1}) by bean plant.

Part of plant	**TSP mg kg^{-1}**	**Pb**	**Cd**	**Ni**	**Zn**	**Fe**	**Seeds Dry Matter Weight (g)**	**Pb**	**Cd**	**Ni**	**Zn**	**Fe**
		Conc. (mg kg^{-1})						**Uptake (mg pot^{-1})**				
Bean Seeds	0	0.496[a]	0.006[a]	4.258[a]	55.58[a]	46.04[b]	13.08[d]	6.487[a]	0.078[d]	55.69[c]	726.98[c]	602.20[cd]
	40	0.506[a]	0.005[ab]	3.914[ab]	47.78[b]	42.79[b]	13.53[d]	6.846[a]	0.067[d]	52.95[c]	646.46[c]	578.94[d]
	80	0.343[ab]	0.005[bc]	3.555[bc]	51.81[ab]	39.51[b]	21.53[c]	7.384[a]	0.107[c]	76.53[b]	1115.46[b]	850.65[bc]
	120	0.177[b]	0.004[c]	3.134[c]	54.75[ab]	35.51[b]	28.95[a]	5.124[a]	0.115[b]	90.72[a]	1585.01[a]	1028.01[b]
	160	0.274[b]	0.006[a]	3.757[abc]	58.24[a]	61.93[a]	25.52[b]	6.99[a]	0.153[a]	95.87[a]	1486.28[a]	1580.45[a]

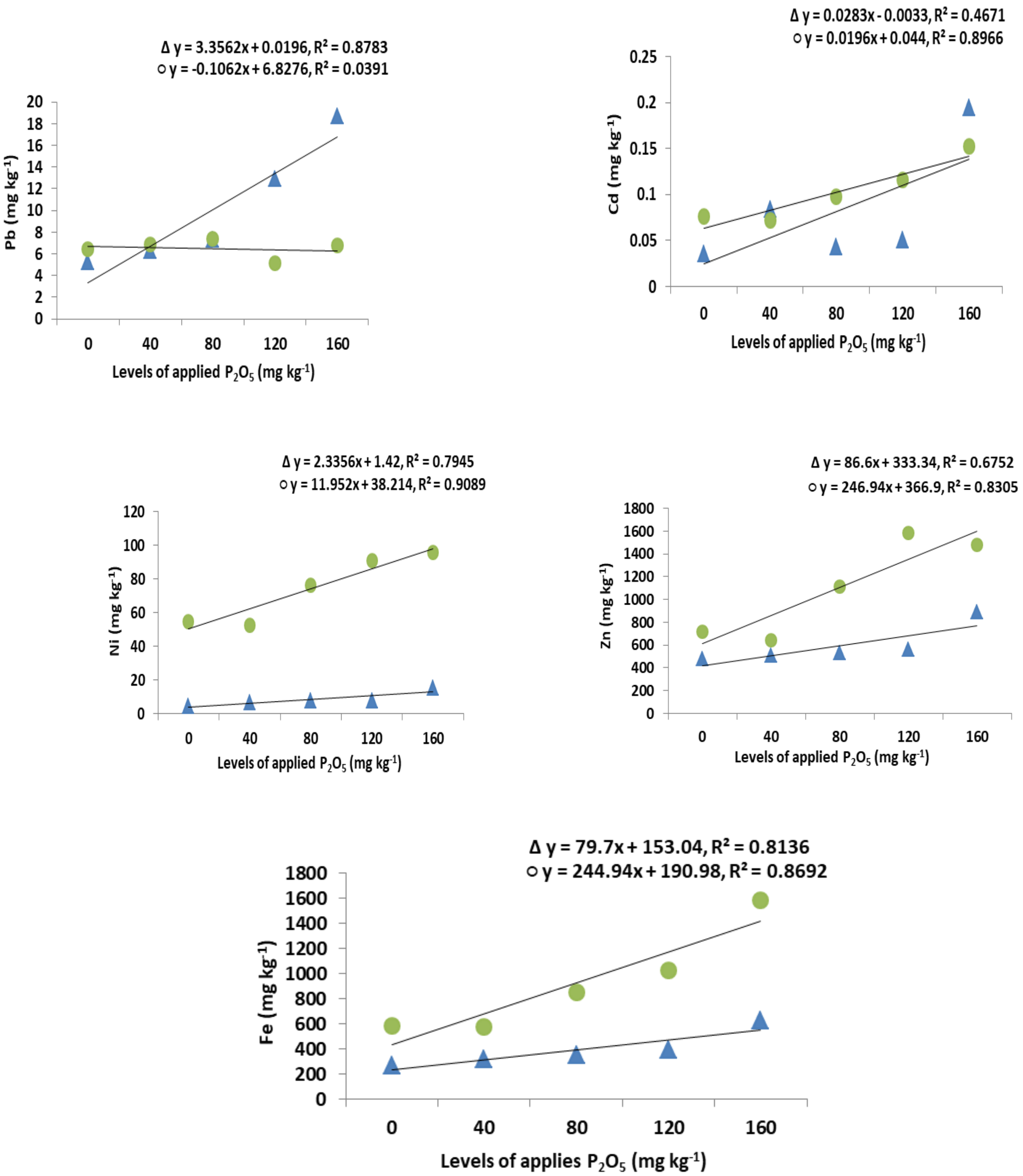

Fig. 3. The relationship between levels of applied phosphorus mg P_2O_5 kg^{-1} soil and heavy metals uptake mg kg^{-1} in grain.
Δ Wheat TSP
○ Bean TSP

3.3.The efficiency comparison of wheat and bean plants on phytoremediation of the polluted soil

Phytoavailability of heavy metals depends upon different physico-chemical properties of the soil, such as organic C and pH, organic C responsible for the release higher quantity of cationic heavy metals and consequently results into more holding of heavy metals in the soil with lower availability to the plant (Singh *et al.,* 2010). Variation in element concentrations between plant species indicated that the ability to accumulate trace elements differs between various types of plants and among various plants on the same species (Säumel *et al.,* 2012). In general the application of (TSP) fertilizer accumulated higher amount of heavy metals in bean tissues compared to wheat plant and increase the concentration of (TSP) increased heavy metals uptake by wheat and bean plants. The overall results regarded that application of the higher amount of phosphorus cause an increase in the heavy metal concentration in the wheat and bean plant parts, probably due to the positive interaction between cationic metals and applied phosphorus in the soil. Because increased applied phosphorus lowered the soil pH and increased the extractable (Pb, Cd, Ni, Zn and Fe), causing these metals to be highly soluble in the soil. Similar results were testified by (Shuman, 1988).

These results disagree with (Ahmed and Khoshnaw, 2019) reported that application of the higher levels of phosphorus caused a decrease in the heavy metals concentration in the wheat plant parts, and with (Haldar and Mandal, 1981) which declared that the addition of P caused a significant decline in concentration of Fe. These results evidence that phytoextraction of (Pb, Cd, Ni, Zn and Fe) by bean plant was much higher than that by wheat plant from municipally contaminated soil after adding phosphorus fertilizer to the soil.

4. CONCLUSIONS

The application of the higher amount of phosphorus cause an increase in the heavy metal uptake in the wheat and bean grains, probably due to the positive interaction between cationic metals and applied phosphorus in the soil. The phytoextraction of (Pb, Cd, Ni, Zn, and Fe) by bean plant was much higher than that by wheat from municipally contaminated soil after adding phosphorus fertilizer to the soil.

Acknowledgments

This study is a part of a thesis for a Master of Science degree at Salahaddin University-Erbil. The authors of this study would like to acknowledge the head of Soil and Water department of Salahaddin University-Erbil / College of Agricultuer for his helping.

We would like to acknowledge all of them who providing the required information for this study.

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