



## Effect of Some Organic Amendments on the Availability and Fractions of Certain Heavy Metals in Abo Rawash Soil and Growing Wheat Plants

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### *Author's contribution*

The sole authoress designed, analyzed and interpreted and prepared the manuscript.

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### ABSTRACT

Due to good water shortage, accumulation of heavy metals in soil under irrigation with wastewater can cause serious hygienic and environmental problems. However, the availability of metal ions in contaminated soils can be reduced by addition of organic and inorganic amendments. In this study, columns experiment was conducted during the winter season of 2016/2017 to evaluate the effect of applying some organic amendments such as humic acid, rabbit manure and biochar on the availability and forms of some heavy metals (i.e. Zn, Cu, Pb and Ni) in contaminated soil of Abo Rawash area irrigated with wastewater and their impact on growing wheat plants as a strategic crop. The forms of the concerned heavy metals in soil were determined by the sequential extraction method. Results showed that treating the soil with such amendments decreased heavy metals concentrations in soil. The amount of the studied heavy metals was distributed among the different soil fractions following the descending order: organically bound > residual fraction > Fe-Mn oxides bound > carbonate associated > exchangeable.

The dry weight of whole plant, weight of 1000 grains and NPK concentrations in wheat plants were significantly increased due to application of these amendments especially humic acid compared to the control. In addition, heavy metals concentrations were significantly decreased in wheat plants

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under irrigation with wastewater, with the highest effect for humic acid treatment. These results went hand by hand with the effect of humic acid on increasing heavy metals solubility and leachability in soil, and increasing heavy metals concentrations values in the collected drainage water from soil columns under investigation.

*Keywords: Contaminated soil; heavy metals; amendments; humic acid; rabbit manure; biochar; fractionation; wheat plants.*

## 1. INTRODUCTION

Irrigating the agricultural soils with wastewater for raising crops productivity has long been practiced both in developing and developed countries due to its high nutrients content as well as due to lack of infrastructure and facilities for the safe disposal of untreated effluent, and good water shortage. However, its use in crop production imposed limitations by the presence of heavy metals, concentrations of which are variable in both time and space [1]. Heavy metals are present in soil as free metal ions, soluble metal complexes, exchangeable metal ions, organically bound metals, precipitated or insoluble compounds like oxides, carbonates, and hydroxides, or a part of silicate materials [2]. The need to apply some treatments to reduce the hazardous effect of heavy metals by increasing their leachability out of soil profile or precipitation became necessary. Humic substances can help to reduce fertilizer application rates, enhance efficiency of nutrient use, replace synthetic plant regulators, enhance early growth and flowering, and improve fruit quality, increase water stress tolerance, decrease disease incidence, chelate micronutrients and heavy metals [3]. Humic acids (HA) can be considered as important remediation agent for the immobilization of heavy metals in soils. They contain functional groups such as carboxyl (-COOH), amine (-NH<sub>2</sub>) and hydroxyl (-OH) groups which are capable of binding metal ions through two types of mechanisms: 1) covalent binding, where each attached atom donates one of associated electrons and 2) coordinated binding, where each metal ion accepts pair of electrons from non-metal [4]. Humic-metal complex stability may be different for different metals. Metals such as Cu, Hg, Cd and Pb have higher stability, while Ca, Na, Mg and Zn have lower stability [5]. Also, rabbit manure is one of the few fertilizers that rich in macro and micronutrients, will not burn plants when added directly to the soil, can be safely used on food plants and play an important role in reducing heavy metals availability in polluted soils [6]. The organic amendments tend to increase organically bound metals compared to

control treatment as showed by [7]. In addition, biochar, a carbon-rich material obtained from heating organic biomass under limited oxygen conditions appears to be more stable source of carbon [8]. Four objectives may motivate biochar applications for environmental management: soil improvement (for improved productivity as well as reduced pollution); waste management; climate change mitigation; and energy production [9]. In that sense, Zeng et al. [10] found that by adding biochar to contaminated soil, it cause changes in the total organic carbon, water extractable organic carbon and pH. Also, it decreased the available Cd, Cu, Zn and Pb concentrations in the soil and reduced the ecological risk of heavy metals. Biochar has many favorable immobilization properties as heavy metal modifier, such as micro-porous structure, active functional groups, and high pH and cation exchange capacity. Moreover, it has been proved that biochar has a strong adsorptive power for heavy metals [11].

Thus, this study was planned to evaluate the effect of applying some organic materials such as humic acid, rabbit manure and biochar on the availability and fractions of some heavy metals i.e. Zn, Cu, Pb and Ni in contaminated soil of Abo Rawash area irrigated with wastewater and their impact on growing wheat plants as a strategic crop.

## 2. MATERIALS AND METHODS

A column experiment was carried out during winter season of 2016 under greenhouse conditions at Faculty of Agriculture, Ain Shams University, Qalubia governorate, Egypt. The experiment was left in the air temperature (21.7±3.2°C). Soil samples were collected from Abo Rawash area, Giza governorate, which was irrigated with wastewater (mixture of sewage and agricultural drainage water) for several years (Table 1), at a depth of 0-20 cm as the most polluted layer in soil profile. PVC columns of 60 cm length and 20 cm internal diameter were packed uniformly with the studied soil which was already air dried and ground to pass through a 2

mm sieve. Some physical and chemical properties of the studied soil were determined before cultivation according to the standard methods outlined by [12,13], and the obtained results are presented in Tables 2a and 2b. The columns were allowed to vibrate during packing and each soil sample was packed to a height of 55 cm. The following amendments were mixed well with the soil during packing; humic acid, rabbit manure and biochar at a rate of 1% OM. Some characteristics of the used amendments are shown in Table 3.

After one week from adding the amendments, columns were cultivated with wheat (*Triticum aestivum*, c.v Sakha 61) on 25<sup>th</sup> of November 2016. Wastewater of Abo Rawash area used for irrigating its soils was used for irrigation in this study to keep the moisture of the soil before and after plant cultivation at the field capacity till the end of the experimental work. The fertilization of N and P were applied in form of ammonium sulfate and ordinary superphosphate. The mineral fertilization were applied at doses of 180 kg N ha<sup>-1</sup> added in three batches after plant cultivation (after grains germination, at the vegetative growth stage and before the expulsion of spikes stage), and 7.86 kg P ha<sup>-1</sup> added before plant cultivation, according to the

extensions of the Egyptian Ministry of Agriculture for wheat cultivation. Plant samples were collected at harvest (132 days from sowing), separated into roots, shoots and grains, oven dried at 70°C for 48 hrs, digested by H<sub>2</sub>SO<sub>4</sub>/H<sub>2</sub>O<sub>2</sub> mixture according to the method described by [14], and kept for the elements determination (N, P, K, Fe, Mn, Zn, Cu, Pb, Ni, Co and Cd).

Soil samples were collected after plant harvest at depths of 0 to 15, 15 to 30 and 30 to 55 cm. The collected samples were air dried, crushed, sieved through a 2 mm sieve and stored until analyzed for their chemical analyses. Total heavy metals in the soil were determined by digestion with a mixture of H<sub>2</sub>SO<sub>4</sub>/H<sub>2</sub>O<sub>2</sub> according to [15]. Chemically available heavy metals were extracted with DTPA solution from soils according to [16]. Soil samples were subjected to sequential extraction and the studied heavy metals were partitioned into five components according to [17], as shown in Table 4.

Afterwards, soil columns were irrigated with excess water (120% of FC) for 15 minutes to allow draining of some water from each studied treatment. Drainage water were collected, filtered and stored at 4°C till analyzed.

**Table 1. Chemical composition of the collected water samples of irrigation and drainage water used in the studied area**

Property	Irrigation water	Drainage water	Recommended concentration*
pH	7.78	8.05	Normal range 6.50 - 8.40
EC, dS m <sup>-1</sup>	0.97	0.94	< 3.00 dS m <sup>-1</sup>
<b>Elements concentration, ppm</b>			
N	13.5	12.9	15.0 ppm
P	90.8	57.7	2.00 ppm
K	5.24	4.70	2.00 ppm
Ca	12.6	12.6	20.0 me L <sup>-1</sup>
Mg	136	134	5.00 me L <sup>-1</sup>
Na	39.7	43.7	SAR < 9.00
Fe	0.047	0.044	5.00 ppm
Mn	0.130	0.116	0.20 ppm
Zn	0.063	0.062	2.00 ppm
Cu	0.169	0.169	0.20 ppm
Pb	0.141	0.142	5.00 ppm
Ni	0.154	0.153	0.20 ppm
Co	0.311	0.310	0.05 ppm
Cd	0.153	0.153	0.01 ppm

\*Recommended maximum concentration according to [18]

Table 2a. Some physical and chemical properties of the studied soil (0-20 cm)

Particle size distribution, %			Textural class	CaCO <sub>3</sub>	OM	BD	FC	WP	CEC
Sand	Silt	Clay		g kg <sup>-1</sup>			g cm <sup>-3</sup>	%	cmol <sub>c</sub> kg <sup>-1</sup>
62.6	20.5	16.9	Sandy loam	55.0	10.1	1.59	14.5	4.03	16.5
pH (1:2.5)	EC <sub>e</sub> dS m <sup>-1</sup>	Soluble ions, mmol <sub>c</sub> L <sup>-1</sup>							
		Ca <sup>2+</sup>	Mg <sup>2+</sup>	Na <sup>+</sup>	K <sup>+</sup>	CO <sub>3</sub> <sup>2-</sup>	HCO <sub>3</sub> <sup>-</sup>	Cl <sup>-</sup>	SO <sub>4</sub> <sup>2-</sup>
6.96	0.34	2.28	1.90	0.97	0.34	n.d*	0.91	1.25	2.48

\*n.d means not detected.

Table 2b. Total and DTPA-extractable heavy metals of the studied soil irrigated with wastewater (0-20 cm)

Fe		Mn		Zn		Cu		Pb		Ni		Co		Cd	
Total	Ext.	Total	Ext.	Total	Ext.	Total	Ext.	Total	Ext.	Total	Ext.	Total	Ext.	Total	Ext.
mg kg <sup>-1</sup>															
973	15.7	235	21.3	97.8	14.7	85.1	11.9	79.7	10.1	29.5	3.73	11.2	1.97	18.3	2.31
-	>4.5 <sup>#</sup>	-	>1 <sup>#</sup>	150-300*	>1 <sup>#</sup>	50-140*	>1 <sup>#</sup>	50-300*	-	30-75*	-	-	-	1.0-3.0*	-
-	-	1500-3000**	-	300-400**	11.2 <sup>###</sup>	60-125**	7.7 <sup>###</sup>	100-400**	7.9 <sup>###</sup>	100**	2.6 <sup>###</sup>	25-50**	-	3.0-8.0**	3.6 <sup>###</sup>
				60-200***		20-100***		70-100***		15-70***				0.5-1.5***	

\* Maximum total contents (mg kg<sup>-1</sup>), European Union, [19]\*\* Maximum total contents (mg kg<sup>-1</sup>), levels considered as phytotoxic, [20]\*\*\* Maximum total contents (mg kg<sup>-1</sup>), European Union, cited from [21]<sup>#</sup> Adequate levels extracted by DTPA (mg kg<sup>-1</sup>), cited from [22]<sup>###</sup> Maximum background levels extracted by DTPA (mg kg<sup>-1</sup>), cited from [23]

**Table 3. Some characteristics of the studied amendments**

Character	Humic acid	Rabbit manure	Biochar
pH (1:5)	9.82	9.01	8.77
EC, dS m <sup>-1</sup>	23.8	10.9	2.65
OC, g kg <sup>-1</sup>	521	285	328
OM, g kg <sup>-1</sup>	898	491	565
C/N ratio	11.2	13.5	18.4
<b>Total content of macronutrients, %</b>			
N	4.65	2.11	1.78
P	0.31	0.57	0.19
K	2.73	1.20	0.24
<b>Total content of micronutrients and heavy metals, mg kg<sup>-1</sup></b>			
Fe	4913	6081	1870
Mn	112	127	315
Zn	27.1	35.4	32.1
Cu	8.95	11.3	13.7
Pb	3.59	5.93	4.25
Ni	0.31	0.54	0.52
Co	0.15	0.28	0.13
Cd	0.12	0.23	0.15

**Table 4. Determination of the studied heavy metals fractions in the investigated soil according to [17]**

Metal fractions	Extraction procedure
Exchangeable fraction (EXC)	1 M MgCl <sub>2</sub> (pH 7, 1 h, 25 °C)
Carbonate associated fraction (CAB)	1 M CH <sub>3</sub> COONa (pH 5, 5 h, 25 °C)
Fe-Mn oxides bound fraction (OXD)	0.04 M NH <sub>2</sub> OH.HCl in 25% acetic acid (5 h, 96 °C)
Organically bound fraction (ORG)	30% H <sub>2</sub> O <sub>2</sub> + 0.02 M HNO <sub>3</sub> (pH 2, 5 h, 85 °C), and after cooling 3.2 M NH <sub>4</sub> OAc in 20% HNO <sub>3</sub>
Residual fraction (RES)	HNO <sub>3</sub> conc. (2 h, 100 °C)

The experiment was designed in a completely randomized design and each treatment was replicated three times. The obtained data were then statistically analyzed using SAS software package [24]. Mean values were compared for each other using the least significant differences (LSD) at  $P \leq 0.05$ .

### 3. RESULTS AND DISCUSSION

#### 3.1 Status of Some Heavy Metals in the Studied Soil as Affected by Organic Amendments

##### 3.1.1 Zinc (Zn)

Data in Table 5 show the distribution of different forms of Zn, after harvesting wheat plants, throughout Abo Rawash soil columns treated with certain organic amendments under irrigation with wastewater. Generally, the control treatment contained Zn forms in the following

descending order: RES > OXD > ORG > CAB > EXC. While, in organically amended soil Zn was distributed among the soil fractions in the descending order: ORG > RES > OXD > CAB > EXC. The distribution of Zn among the different fractions in the untreated soil showed that the residual fraction is the prevalent form in soil in which it represented about 34.0% of the sum of all Zn fractions. Fe-Mn oxides bound fraction was the second dominant fraction; on average it represented 26.3% of the sum of Zn fractions in the soil. Garcia Sanchez et al. [25] mentioned that the greatest amounts of Zn were found in the residual and oxides fractions. On the other hand, the organically bound Zn fraction represented 24.4% of the sum followed by Zn associated with carbonates (8.54%). The exchangeable fraction of Zn gave the lowest values among the operationally defined ones. It amounted 6.75% of the sum of the extracted Zn fractions in the studied soil.

Regarding the studied treatments, the organically bound and Fe-Mn oxides bound Zn fractions were higher in rabbit manure treatment followed by biochar one. At the same time, the exchangeable, carbonate fraction, residual fraction and chemically available Zn values were higher in humic acid treatment, but still lower than the control treatment. Application of the different amendments reduced the EXC-Zn as compared to the control under irrigation with wastewater. Rabbit manure was the most effective treatment as it is amounted 3.20% reduction in EXC-Zn followed by biochar (2.12%). This may be due to the fact that these treatments have higher pH than the other treatment (humic acid) that when added to the soil cause lowering in the pH. This result about the effect of humic acid on soil pH is in agreement with that obtained by [26]. In addition, rabbit manure caused significant decreases in CAB-Zn, OXD-Zn and RES-Zn, recording 0.55, 13.4 and 23.7% less than the control treatment, respectively. This could be due to the action of organic manure on chelating Zn from other fractions and/ or making organo-metal complexes. This explained the highest amount of Zn which is bounded with the

organic fraction under the effect of the studied organic amendments. DTPA-extractable Zn was decreased by applying the organic treatments compared to the control. The highest value was obtained by applying the treatment of humic acid, followed by biochar treatment. The studied treatments increased the amount leached of Zn throughout soil columns, compared to the control treatment. It means that organic treatments increased solubility and leachability of Zn among soil columns through moving its form from RES or OXD to ORG-Zn and increasing downward movement in fractions of EXC and CAB-Zn.

### 3.1.2 Copper (Cu)

Data in Table 6 show the distribution of copper in soil as affected by different amendments. The untreated soil contained Cu forms in the following descending order: RES> ORG> OXD> CAB> EXC, but in treated soils with organic amendments they were ORG> RES> OXD> CAB> EXC. Similar results were obtained by [27] who reported that Cu was bound strongly to clay minerals and organic matter in soil by two mechanisms; complexation and adsorption.

**Table 5. Effect of different amendments on Zn fractions in Abo Rawash soil irrigated with wastewater after harvesting wheat plants**

Amendment	Leachate mg L <sup>-1</sup>	Depth (cm)	Zn fractions, mg kg <sup>-1</sup>						
			EXC <sup>#</sup>	CAB	OXD	ORG	RES	Sum	DTPA-Zn
Control	0.75	0-15	6.65	8.35	25.7	24.9	32.3	98.0	15.3
		15-30	6.61	8.38	25.9	23.5	33.5	97.9	14.9
		30-55	6.56	8.34	25.6	23.3	34.0	97.8	14.5
<b>Mean</b>			<b>6.61</b>	<b>8.36</b>	<b>25.7</b>	<b>23.9</b>	<b>33.3</b>	<b>97.9</b>	<b>14.9</b>
Humic acid	1.25	0-15	6.47	8.35	21.5	34.1	27.5	97.9	14.5
		15-30	6.51	8.34	21.6	33.8	27.9	98.2	14.7
		30-55	6.54	8.31	21.6	33.3	28.3	98.1	14.8
<b>Mean</b>			<b>6.51</b>	<b>8.33</b>	<b>21.6</b>	<b>33.7</b>	<b>27.9</b>	<b>98.0</b>	<b>14.7</b>
Rabbit manure	1.10	0-15	6.39	8.30	22.2	36.4	25.0	98.3	14.1
		15-30	6.38	8.35	22.6	35.7	25.4	98.4	13.9
		30-55	6.42	8.28	22.1	35.2	25.7	97.7	14.2
<b>Mean</b>			<b>6.39</b>	<b>8.31</b>	<b>22.3</b>	<b>35.8</b>	<b>25.4</b>	<b>98.1</b>	<b>14.1</b>
Biochar	1.18	0-15	6.45	8.32	21.8	35.3	26.1	98.0	14.3
		15-30	6.47	8.33	21.9	34.7	26.4	97.8	14.5
		30-55	6.48	8.31	21.5	34.6	27.5	98.4	14.6
<b>Mean</b>			<b>6.47</b>	<b>8.32</b>	<b>21.7</b>	<b>34.9</b>	<b>26.7</b>	<b>98.1</b>	<b>14.5</b>
<b>LSD<sub>0.05</sub></b>	<b>0.05</b>		<b>0.07</b>	<b>0.10</b>	<b>0.38</b>	<b>1.07</b>	<b>0.09</b>		<b>0.19</b>

# EXC, CAB, OXD, ORG and RES means exchangeable, carbonate, reducible bound, organically bound and residual fractions, respectively

Humic acid treatment showed significant decreases in Cu in the EXC fraction (1.34%), CAB fraction (1.74%), RES fraction (17.2%) and DTPA-extractable Cu fraction (4.44%) as compared to the control treatment. The rabbit manure amendment decreased EXC fraction by 4.46%, CAB fraction by 2.84%, RES fraction by 23.1% and chemically available Cu fractions by about 6.67% as compared to control. In the meantime, biochar treatment recorded significant decreases in EXC fraction by 3.48%, CAB fraction by 2.25%, RES fraction by 19.3% and DTPA-extractable Cu fraction by 5.28% as compared to the control treatment. Mahler et al. [28] stated that extractable levels of heavy metals in soil were known to be affected by soil organic matter content and other many factors. Thus, the chemistry of these metals in soils is often very complex. It was obvious that the organic treatments increased Cu in form of Fe-Mn oxides bound and organically bound rather than the control treatment, as a type of metal complexation. Complexation may be in the form of chelation, where the complex forming ligands from two or more co-ordination bonds with the metal ion [29], such as humic acid. Furthermore, humic substances in soils can serve as strong reducing agent and can influence the processes controlling mobilization of many toxic metals [30]. Copper ions however, form very stable complexes with organic substances over a wide range of pH [31]. It is known that organic substances accumulate at the soil surface, so the movement of ORG-Cu downward soil profile throughout soil columns is very slow or not recorded.

It was shown that the organic treatments increased the leached amount of Cu, compared to the control, with higher values for humic acid treatment followed by biochar.

### **3.1.3 Lead (Pb)**

Data in Table 7 show in general that the untreated soil contained Pb forms as follows: RES> ORG> OXD> CAB> EXC, whereas in soil treated with the organic amendments the distribution followed the order: ORG> RES> OXD> CAB> EXC. This trend may be attributed to the increase of the negative charges of the soil as a result of applying organic amendments; consequently Pb complexation increased.

Data revealed that sum of Pb fractions in the soil amended with rabbit manure was higher

than in the soils amended with the other amendments. This means that rabbit manure treatment was more effective in increasing the sum of Pb fractions than the other treatments (Table 3). On the other hand, leached and DTPA-extractable Pb in the soil treated with rabbit manure were lower than in the soils treated with the other amendments. Such decreases in solubility and leachability went hand by hand with increasing the sum of Pb fractions in the soil treated with rabbit manure, followed by biochar treatment. In addition, it is obvious that EXC, CAB, RES and DTPA-extractable Pb fractions significantly decreased (owing to the humic acid applied to soil) by about 3.41, 0.20, 23.0 and 2.43% relative to the control, respectively. At the same time, the rabbit manure treatment decreased EXC, CAB, RES and chemically available Pb fractions by about 15.1, 7.35, 28.9 and 5.11% relative to the control, respectively. In meantime, biochar recorded significant decreases in EXC fraction by 8.48%, CAB fraction by 2.05%, RES fraction by 27.5% and DTPA-extractable Pb fraction by 3.68% relative to the control. Contrarily, both OXD and ORG-Pb fractions increased due to the application of the organic amendments to the soil. Lu et al. [32]; Zhang et al. [33] proposed various mechanisms for Pb<sup>2+</sup> sorption by biochar that could include (1) the trace element (TE) exchange with Ca<sup>2+</sup>, Mg<sup>2+</sup>, and other cations associated with biochar, attributing to co-precipitation and inner sphere complexation with complexed humic matter and mineral oxides of biochar, (2) the surface complexation of TE with different functional groups and inner sphere complexation with the free hydroxyl of mineral oxides and other surface precipitation, and (3) the physical adsorption and surface precipitation that contribute to the stabilization of Pb<sup>2+</sup>.

### **3.1.4 Nickel (Ni)**

Data in Table 8 show the distribution of different forms of Ni, after harvesting wheat plants, throughout Abo Rawash soil treated with certain organic amendments under irrigation with wastewater. On the average, the presence of total Ni associated with different fractions in the untreated soil (the control) was in the following order: Organically bound fraction (31.5%)> residual fraction (27.6%)> Fe-Mn oxides bound fraction (23.8%)> carbonate associated fraction (9.12%)> exchangeable fraction (8.01%). The amended soil showed similar trend to that of the control treatment. This result may be attributed to the adsorption of Ni in soil which is dependent on

pH, temperature and type of sorbent (minerals or organic matters), as well as the concentration of aqueous complexing agents, competition with other adsorbing cations and the ionic strength in ground water [34]. In this respect, DTPA-extractable Ni values in the amended soil were lower than in the control. On contrary, leached Ni

in the treated soil with organic amendments was higher than that in the untreated soil, with the highest value for humic acid treatment. These results indicated that organic treatments increased the solubility of Ni and almost increased its movement throughout soil columns in most of its fractions in the studied soil.

**Table 6. Effect of different amendments on Cu fractions in Abo Rawash soil irrigated with wastewater after harvesting wheat plants**

Amendment	Leachate mg L <sup>-1</sup>	Depth (cm)	Cu fractions, mg kg <sup>-1</sup>					Sum	DTPA-Cu
			EXC <sup>#</sup>	CAB	OXD	ORG	RES		
Control	0.66	0-15	5.52	7.29	17.0	27.8	28.8	86.4	12.3
		15-30	5.46	7.28	16.8	26.2	29.3	85.0	11.9
		30-55	5.41	7.24	16.7	25.8	29.5	84.7	11.8
<b>Mean</b>			<b>5.46</b>	<b>7.27</b>	<b>16.8</b>	<b>26.6</b>	<b>29.2</b>	<b>85.4</b>	<b>12.0</b>
Humic acid	1.14	0-15	5.34	7.18	18.9	30.1	24.0	85.5	11.3
		15-30	5.39	7.14	19.2	29.8	24.2	85.7	11.5
		30-55	5.44	7.11	19.0	29.4	24.3	85.3	11.6
<b>Mean</b>			<b>5.39</b>	<b>7.14</b>	<b>19.0</b>	<b>29.8</b>	<b>24.2</b>	<b>85.5</b>	<b>11.5</b>
Rabbit manure	1.02	0-15	5.14	7.11	19.6	31.3	22.3	85.4	10.9
		15-30	5.25	7.06	19.8	31.1	22.4	85.6	11.3
		30-55	5.27	7.02	19.7	31.0	22.7	85.7	11.4
<b>Mean</b>			<b>5.22</b>	<b>7.06</b>	<b>19.7</b>	<b>31.1</b>	<b>22.5</b>	<b>85.6</b>	<b>11.2</b>
Biochar	1.11	0-15	5.25	7.15	19.1	31.0	22.9	85.4	11.1
		15-30	5.29	7.12	19.3	30.4	24.0	86.1	11.4
		30-55	5.28	7.05	19.2	30.0	23.8	85.3	11.6
<b>Mean</b>			<b>5.27</b>	<b>7.11</b>	<b>19.2</b>	<b>30.5</b>	<b>23.6</b>	<b>85.6</b>	<b>11.4</b>
<b>LSD<sub>0.05</sub></b>	<b>0.05</b>		<b>0.06</b>	<b>0.05</b>	<b>0.27</b>	<b>0.07</b>	<b>0.09</b>		<b>0.11</b>

# EXC, CAB, OXD, ORG and RES are denoted under Table 5.

**Table 7. Effect of different amendments on Pb fractions in Abo Rawash soil irrigated with wastewater after harvesting wheat plants**

Amendment	Leachate mg L <sup>-1</sup>	Depth (cm)	Pb fractions, mg kg <sup>-1</sup>					Sum	DTPA-Pb
			EXC <sup>#</sup>	CAB	OXD	ORG	RES		
Control	0.54	0-15	4.91	7.33	12.5	26.3	30.7	81.7	10.8
		15-30	4.75	6.46	9.98	25.8	32.8	79.8	10.6
		30-55	4.73	6.21	9.23	25.1	33.6	78.9	10.6
<b>Mean</b>			<b>4.80</b>	<b>6.67</b>	<b>10.6</b>	<b>25.7</b>	<b>32.4</b>	<b>80.1</b>	<b>10.7</b>
Humic acid	1.09	0-15	4.40	7.06	12.9	32.1	23.8	80.3	10.3
		15-30	4.70	6.52	12.2	31.6	24.1	79.1	10.4
		30-55	4.80	6.38	11.1	30.8	26.9	80.0	10.6
<b>Mean</b>			<b>4.63</b>	<b>6.65</b>	<b>12.1</b>	<b>31.5</b>	<b>24.9</b>	<b>79.8</b>	<b>10.4</b>
Rabbit manure	0.87	0-15	3.87	6.32	14.0	35.3	22.1	81.6	9.94
		15-30	4.09	6.14	13.8	33.7	23.1	80.8	10.2
		30-55	4.25	6.07	12.0	33.1	23.8	79.2	10.3
<b>Mean</b>			<b>4.07</b>	<b>6.18</b>	<b>13.3</b>	<b>34.0</b>	<b>23.0</b>	<b>80.5</b>	<b>10.1</b>
Biochar	0.94	0-15	3.95	6.87	13.4	33.6	22.4	80.2	10.2
		15-30	4.34	6.49	13.3	32.8	23.7	80.6	10.3
		30-55	4.88	6.23	11.8	32.3	24.3	79.5	10.4
<b>Mean</b>			<b>4.39</b>	<b>6.53</b>	<b>12.8</b>	<b>32.9</b>	<b>23.5</b>	<b>80.1</b>	<b>10.3</b>
<b>LSD<sub>0.05</sub></b>	<b>0.06</b>		<b>0.07</b>	<b>0.10</b>	<b>0.07</b>	<b>1.01</b>	<b>0.13</b>		<b>0.12</b>

# EXC, CAB, OXD, ORG and RES are denoted under Table 5.



Moreover, humic acid treatment significantly decreased EXC, CAB and RES-Ni by about 9.18, 0.73 and 5.52% less than the control, respectively. Also, application of rabbit manure decreased EXC, CAB and RES-Ni fractions by about 25.3, 13.1 and 5.44%, respectively. Similarly, biochar treatment decreased EXC, CAB and RES-Ni fractions in the studied soil by about 15.4, 9.04 and 5.64% relative to the control, respectively. While, humic acid, rabbit manure and biochar treatments significantly increased OXD and ORG-Ni by about (5.44 and 4.92%), (8.43 and 15.9%) and (7.45 and 8.46%) over the control, respectively. This may be due to the positive effect between organic substances and Ni in the studied soil.

### 3.2 Relationships between DTPA-Extractable Heavy Metals and Soil Properties

The DTPA-extractable heavy metals positively and significantly correlated with the EC, OM, clay and silt content (Table 9), however, negatively correlated with soil pH,  $\text{CaCO}_3$  and sand content. Similar results were obtained by [35,36]. Statistical analysis showed significant and negative correlation between the studied chemically extractable heavy metals and soil pH. Soil pH correlated more strongly with Ni and Cd because soil pH controls these elements concentration in soils [37]. In fact, [38] reported that an increase of soil pH from 4.5 to 6.5 decreased the Ni content of oats grains by a factor of approximately 8. This justifies the negative correlation of pH and Ni in the examined soil, also, with the same manner for all the studied heavy metals. The soil pH is generally acknowledged to be the main factor governing concentrations of soluble metals [39]. EC showed a linear positive relation with all the studied heavy metals. The substitution of Na in the exchange positions can produce desorption and higher mobility of these elements, especially with Pb. This result may suggest a higher bioavailability of heavy metals in some soils with salinity problems [20]. Soil carbonate content is related negatively to the studied heavy metals because of the higher affinity of these elements to react with carbonate fraction [20]. OM showed a positive linear relation with all the analyzed elements because of the high affinity of these metals to soil OM [30]. The formation of stable Cu complexes with OM and its humic substances content can explain why the lowest value of correlation (0.70) of

extractable Cu with OM content was obtained [20]. The clay content exhibited a positive relation with the studied chemically available heavy metals, especially with Zn because of the higher availability of Zn that is adsorbed by clay soils [35]. Similar trend was obvious with silt content. Negative correlation between sand fraction and DTPA-extractable heavy metals was found. Trace elements and heavy metals are generally more strongly retained in the fine fractions than in coarse ones [40].

### 3.3 Dry Weight and Elements Concentration of Wheat Plants

Data in Table 10 show the dry weight of whole plant, weight of 1000 grains, N, P and K concentrations in wheat plants cultivated in Abo Rawash soil as affected by different organic amendments, under irrigation with wastewater. It is clear that wheat plant and its grains dry weights as well as N, P and K concentrations were significantly higher due to application of such amendments to the studied soil. Probably due to the relatively improvement of soil physical and chemical properties, beside of decreasing bioavailability of heavy metals in soil, which in turn promoted plant growth, improved general plant vigour and encouraged plant yields. Also, application of the organic amendments to the soil improved biological properties, as well as they are themselves are sources of plant nutrients (Table 3). The highest effect on increasing yield was obtained from the treatment of humic acid followed by biochar. These results agree with those obtained by [3] on the effect of humic acid on soil and plant, and [9] on the effect of biochar.

Heavy metals concentrations of wheat plants as affected by the different organic amendments are shown in Table 10. The obtained results showed that heavy metals concentrations in wheat plants, in general, significantly decreased due to application of such amendments, with the highest effect due to humic acid treatment. These results went hand by hand with the effect of humic acid on increasing solubility and leachability of heavy metals in soil. In this concern, the decrease in heavy metals concentrations may be due to growth dilution, which occurred with an increase in biomass production and partially decreased heavy metals concentration in soil solution with all the amendments through formation of less soluble compounds [41]. Also, it can be noticed that organic substances significantly decreased

heavy metals concentrations and their translocations in roots, shoots and grains of wheat plant compared to the control treatment. Similar results were obtained by [42] who stated that metal-organic matter associations in both solution and solid phases by way of complexation and specific adsorption are the important mechanisms responsible for rendering the indigenous and applied metals less available for absorption by the plants. The results showed also that heavy metals concentration in wheat plants accumulated in roots > shoots > grains. Abd-Elrahman et al. [43] found that heavy metals tend to accumulate in roots of wheat plants rather than shoots and grains.

In view of the potential toxicity of these elements to plant, the current results indicate that the concentrations of Fe, Cu, Co and Cd in the roots, at least, of wheat plants, exceeded the normal ranges reported by [44,20]. Meanwhile, application of such organic amendments reduced the concentrations of these elements in different plant parts.

However, the long term use of wastewater for irrigating soils and plants is suspected to increase the content of heavy metals in soils. Plants grown on these soils will portray high concentrations of these elements in their tissues. Thus, these heavy metals might cause hazardous effects on human health [45].

**Table 8. Effect of different amendments on Ni fractions in Abo Rawash soil irrigated with wastewater after harvesting wheat plants**

Amendment	Leachate mg L <sup>-1</sup>	Depth (cm)	Ni fractions, mg kg <sup>-1</sup>						
			EXC <sup>#</sup>	CAB	OXD	ORG	RES	Sum	DTPA-Ni
Control	0.40	0-15	2.60	2.91	7.21	9.50	8.23	30.5	3.84
		15-30	2.41	2.73	7.09	9.41	8.28	29.9	3.78
		30-55	2.18	2.55	7.04	9.35	8.31	29.4	3.69
<b>Mean</b>			<b>2.40</b>	<b>2.73</b>	<b>7.11</b>	<b>9.42</b>	<b>8.27</b>	<b>29.9</b>	<b>3.77</b>
Humic acid	0.96	0-15	2.12	2.60	7.50	10.3	7.75	30.2	3.61
		15-30	2.18	2.70	7.54	9.85	7.78	30.1	3.64
		30-55	2.23	2.83	7.46	9.55	7.82	29.9	3.70
<b>Mean</b>			<b>2.18</b>	<b>2.71</b>	<b>7.50</b>	<b>9.88</b>	<b>7.78</b>	<b>30.1</b>	<b>3.65</b>
Rabbit manure	0.74	0-15	1.49	2.33	7.83	11.8	7.81	31.2	3.22
		15-30	1.87	2.39	7.68	10.9	7.82	30.6	3.29
		30-55	2.01	2.40	7.63	10.2	7.84	30.0	3.34
<b>Mean</b>			<b>1.79</b>	<b>2.37</b>	<b>7.71</b>	<b>10.9</b>	<b>7.82</b>	<b>30.6</b>	<b>3.28</b>
Biochar	0.87	0-15	1.96	2.40	7.84	10.7	7.79	30.6	3.35
		15-30	2.03	2.51	7.59	10.2	7.81	30.1	3.42
		30-55	2.09	2.54	7.50	9.85	7.82	29.8	3.49
<b>Mean</b>			<b>2.03</b>	<b>2.48</b>	<b>7.64</b>	<b>10.2</b>	<b>7.81</b>	<b>30.2</b>	<b>3.42</b>
<b>LSD<sub>0.05</sub></b>	<b>0.12</b>		<b>0.11</b>	<b>0.10</b>	<b>0.07</b>	<b>0.21</b>	<b>0.13</b>		<b>0.11</b>

# EXC, CAB, OXD, ORG and RES are denoted under Table 5.

**Table 9. Correlation coefficients (r) between some soil properties and constituents with DTPA-extractable heavy metals of the studied soil**

Soil properties and its constituents	Fe	Mn	Zn	Cu	Pb	Ni	Co	Cd
pH	-0.96	-0.90	-0.82	-0.80	-0.98	-0.99	-0.98	-0.99
EC	0.86	0.74	0.65	0.61	0.96	0.92	0.91	0.95
CaCO <sub>3</sub>	-0.89	-0.90	-0.83	-0.86	-0.75	-0.84	-0.88	-0.83
OM	0.91	0.82	0.86	0.70	0.80	0.79	0.94	0.93
Clay	0.90	0.94	0.98	0.76	0.81	0.87	0.86	0.82
Silt	0.90	0.80	0.71	0.68	0.98	0.95	0.94	0.97
Sand	-0.94	-0.96	-0.98	-0.91	-0.86	-0.92	-0.90	-0.87

**Table 10. Dry weight, weight of 1000 grains, NPK and heavy metals concentrations of wheat plants cultivated in Abo Rawash soil as affected by different amendments, under irrigation with wastewater**

Amendment	Dry weight of whole plant (g/column)	Weight of 1000 grains, g	N	P %	K	Fe	Mn	Zn	Cu	Pb	Ni	Co	Cd
Control	12.8	11.6											
Root			0.45	0.28	0.43	535	217	59.3	28.6	5.13	2.32	1.41	0.89
Shoot			0.51	0.33	0.49	365	73.0	28.5	13.9	2.25	0.74	0.53	0.16
Grains			1.31	0.45	1.09	224	26.1	11.8	4.55	0.93	0.22	0.14	0.07
Humic acid	16.6	19.9											
Root			0.78	0.71	0.73	568	173	32.0	15.3	2.64	1.71	1.27	0.25
Shoot			0.98	0.78	1.06	429	45.0	19.4	4.24	1.16	0.35	0.27	0.03
Grains			2.95	0.79	1.43	237	13.5	6.33	1.83	0.23	0.11	0.05	0.01
Rabbit manure	14.2	16.5											
Root			0.66	0.35	0.59	581	191	52.1	19.1	4.19	2.01	1.33	0.60
Shoot			0.86	0.49	0.91	447	57.3	24.0	6.35	1.73	0.51	0.42	0.09
Grains			2.42	0.56	1.17	245	18.9	9.07	2.11	0.52	0.17	0.09	0.03
Biochar	15.9	18.7											
Root			0.73	0.48	0.64	547	231	47.4	21.7	3.67	1.89	1.28	0.36
Shoot			0.94	0.61	0.96	387	79.6	22.7	8.15	1.54	0.46	0.39	0.05
Grains			2.74	0.66	1.21	229	30.4	7.48	2.33	0.44	0.15	0.06	0.01
<b>LSD<sub>0.05</sub></b>	<b>0.62</b>	<b>1.12</b>											
		Root	<b>0.07</b>	<b>0.07</b>	<b>0.05</b>	<b>10.2</b>	<b>12.3</b>	<b>5.07</b>	<b>2.28</b>	<b>0.59</b>	<b>0.12</b>	<b>0.18</b>	<b>0.10</b>
		Shoot	<b>0.05</b>	<b>0.04</b>	<b>0.04</b>	<b>9.35</b>	<b>5.93</b>	<b>1.53</b>	<b>1.58</b>	<b>0.17</b>	<b>0.04</b>	<b>0.06</b>	<b>0.02</b>
		Grains	<b>0.04</b>	<b>0.06</b>	<b>0.04</b>	<b>4.15</b>	<b>3.97</b>	<b>1.13</b>	<b>0.21</b>	<b>0.10</b>	<b>0.03</b>	<b>0.02</b>	<b>0.02</b>
Normal range, mg kg <sup>-1</sup>						50 - 250*	30 - 300**	27 - 150**	5 - 30**	5 - 10**	0.1 - 5**	0.02 - 1**	0.05 - 0.2**
Contaminated, mg kg <sup>-1</sup>						-	400 - 1000**	100 - 400**	20 - 100**	30 - 300**	10 - 100**	15 - 50**	5 - 30**
According to *[44]; **[20]													

#### 4. CONCLUSION

Application of organic amendments to the soil contaminated with heavy metals decreased contents of these heavy metals in soil and plants grown thereon. The amounts of the studied heavy metals were distributed among the soil fractions following the order: organically bound> residual> Fe-Mn oxides bound> carbonate associated> exchangeable. Metals associations with organic and oxides or in residual fractions are not readily available for plant uptake, due to complexation and adsorption or precipitation. In addition, applying organic amendments to the studied soil enhanced its physical, chemical and biological properties which in turn reflected positively on the grown wheat plants.

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#### COMPETING INTERESTS

Authoress has declared that no competing interests exist.

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