

Original Research Article

Effect of some organic amendments on the availability and fractions of certain heavy metals in Abo Rawash soil and growing wheat plants

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 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 under irrigation with tap water, 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

Irrigation with wastewater in urban lands for raising crops has long been in practice both in developing and developed countries due to its high plant nutrient 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 (**Ahmad *et al.*, 2011**). 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 (Nanda and Abraham, 2013). 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 (Canellas *et al.*, 2015). 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 (-NH₂) 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 atom accepts pair of electrons from non-metal (Burlakovs *et al.*, 2013). 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 (Lado *et al.*, 2008). 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 (Islas-Valdez *et al.*, 2015). The organic amendments tend to increase organically bound metals compared to control treatment as showed by Chang *et al.* (2014). In addition, biochar, a carbon-rich material obtained from heating organic biomass under limited oxygen conditions appears to be more stable source of carbon (Sukartono *et al.*, 2011). 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 (Lehmann *et al.*, 2009). In that sense, Zeng *et al.* (2015) 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 (Xu *et al.*, 2014).

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 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 **Klute (1986); Page *et al.* (1982)**, 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.

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 ⁻¹	0.97	0.94	< 3.00 dS m ⁻¹
Elements concentration, ppm			
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 ⁻¹
Mg	136	134	5.00 me L ⁻¹
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 **Ayers and Westcot (1985)**.

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

Particle size distribution, %			Textural class	CaCO ₃	OM	BD	FC	WP	CEC
Sand	Silt	Clay		g kg ⁻¹		g cm ⁻³	%		cmol _c kg ⁻¹
62.6	20.5	16.9	Sandy loam	55.0	10.1	1.59	14.5	4.03	16.5
pH	EC _e	Soluble ions, mmol _c L ⁻¹							
(1:2.5)	dS m ⁻¹	Ca ²⁺	Mg ²⁺	Na ⁺	K ⁺	CO ₃ ²⁻	HCO ₃ ⁻	Cl ⁻	SO ₄ ²⁻
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 ⁻¹															
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 [#]	-	>1 [#]	150-300*	>1 [#]	50-140*	>1 [#]	50-300*	-	30-75*	-	-	-	1.0-3.0*	-
-	-	1500-3000**	-	300-400**	11.2 ^{##}	60-125**	7.7 ^{##}	100-400**	7.9 ^{##}	100-100**	2.6 ^{##}	25-50**	-	3.0-8.0**	3.6 ^{##}
				60-200***		20-100***		70-100***		15-70***				0.5-1.5***	

* Maximum total contents (mg kg⁻¹), European Union, **Alloway (1995)**.

** Maximum total contents (mg kg⁻¹), **Kabata-Pendias and Pendias (1992)**, levels considered as phytotoxic.

*** Maximum total contents (mg kg⁻¹), European Union, cited from **Hoalst-Pullen and Patterson (2010)**.

[#] Adequate levels extracted by DTPA (mg kg⁻¹), cited from **McKenzie (1992)**.

^{##} Maximum background levels extracted by DTPA (mg kg⁻¹), cited from **Logan and Miller (1982)**.

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 ⁻¹	23.8	10.9	2.65
OC, g kg ⁻¹	521	73.2	745
OM, g kg ⁻¹	898	126	1284
C/N ratio	11.2	3.47	41.8
Total content of macronutrients, %			
N	4.65	2.11	1.78
P	0.31	0.57	0.19
K	2.73	1.20	0.24
Total content of micronutrients and heavy metals, mg kg ⁻¹			
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

After one week from adding the amendments, columns were cultivated with wheat (*Triticum aestivum*, c.v Sakha 61) on 25th of November 2016. Tap water was used for irrigation 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⁻¹ 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⁻¹ 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 H₂SO₄/H₂O₂ mixture according to the method described by **Chapman and Pratt (1961)**, 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 H₂SO₄/H₂O₂ according to **Jackson (1958)**. Chemically available heavy metals were extracted with DTPA solution from soils according to **Lindsay and Norvell (1978)**. Soil samples were subjected to sequential extraction and the studied heavy metals were partitioned into five components according to **Tessier et al. (1979)**, as shown in Table 4.

Table 4. Determination of the studied heavy metals fractions in the investigated soil according to **Tessier et al. (1979)**.

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

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.

The experiment was designed in a completely randomized design and each treatment was replicated three times. Data were statistically analyzed using the analysis of variance adopting a SAS software package at P≤0.05 (**SAS, 2000**).

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 tap water. 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.* (1999)** 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 tap water. 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 **Ali and Mindari (2016)**. 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 **Adriano (2001)** 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 after harvesting wheat plants.

Amendment	Leachate mg L ⁻¹	Depth (cm)	Zn fractions, mg kg ⁻¹						
			EXC [#]	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
Mean			6.61	8.36	25.7	23.9	33.3	97.9	14.9
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
Mean			6.51	8.33	21.6	33.7	27.9	98.0	14.7
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
Mean			6.39	8.31	22.3	35.8	25.4	98.1	14.1
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
Mean			6.47	8.32	21.7	34.9	26.7	98.1	14.5
LSD_{0.05}	0.05		0.07	0.10	0.38	1.07	0.09		0.19

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. (1985)** 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 (**Lindsay, 1979**) 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 (**Papadopoulos *et al.*, 2007**). Copper ions however, form very stable complexes with organic substances over a wide range of pH (**Jones and Jarvis, 1981**). 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.

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

Amendment	Leachate mg L ⁻¹	Depth (cm)	Cu fractions, mg kg ⁻¹						
			EXC [#]	CAB	OXD	ORG	RES	Sum	DTPA-Cu
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
Mean			5.46	7.27	16.8	26.6	29.2	85.4	12.0
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
Mean			5.39	7.14	19.0	29.8	24.2	85.5	11.5
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
Mean			5.22	7.06	19.7	31.1	22.5	85.6	11.2
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
Mean			5.27	7.11	19.2	30.5	23.6	85.6	11.4
LSD_{0.05}	0.05		0.06	0.05	0.27	0.07	0.09		0.11

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

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. (2012); Zhang et al. (2013)** proposed various mechanisms for Pb^{2+} sorption by biochar that could include (1) the trace element (TE) exchange with Ca^{2+} , Mg^{2+} , 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^{2+} .

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

Amendment	Leachate mg L ⁻¹	Depth (cm)	Pb fractions, mg kg ⁻¹						
			EXC [#]	CAB	OXD	ORG	RES	Sum	DTPA-Pb
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
Mean			4.80	6.67	10.6	25.7	32.4	80.1	10.7
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
Mean			4.63	6.65	12.1	31.5	24.9	79.8	10.4
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
Mean			4.07	6.18	13.3	34.0	23.0	80.5	10.1
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

Mean		4.39	6.53	12.8	32.9	23.5	80.1	10.3
LSD _{0.05}	0.06	0.07	0.10	0.07	1.01	0.13		0.12

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

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 tap water. 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 (**Bradbury and Baeyens, 2005**). 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.

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, CaCO₃ and sand content. Similar results were obtained by **Abou El-Khir (2000)**; **Al-Azab (1997)**. 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 (**Gupta and Gupta, 1998**). In fact, **Berrow and Burridge (1979)** 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 (Fathi *et al.*, 2014). 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 (Kabbata-Pendias and Pendias, 1992). Soil carbonate content is related negatively to the studied heavy metals because of the higher affinity of these elements to react with carbonate fraction (Kabata-Pendias and Pendias, 1992). OM showed a positive linear relation with all the analyzed elements because of the high affinity of these metals to soil OM (Papadopoulos *et al.*, 2007). 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 (Kabbata-Pendias and Pendias, 1992). 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 (Abou El-Khir, 2000). 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 (Acosta *et al.*, 2011).

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

Amendment	Leachate mg L ⁻¹	Depth (cm)	Ni fractions, mg kg ⁻¹						
			EXC [#]	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
Mean			2.40	2.73	7.11	9.42	8.27	29.9	3.77
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
Mean			2.18	2.71	7.50	9.88	7.78	30.1	3.65
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
Mean			1.79	2.37	7.71	10.9	7.82	30.6	3.28
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
Mean			2.03	2.48	7.64	10.2	7.81	30.2	3.42

LSD _{0.05}	0.12	0.11	0.10	0.07	0.21	0.13	0.11
---------------------	------	------	------	------	------	------	------

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 ₃	-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

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 tap water. 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 **Canellas *et al.* (2015)** on the effect of humic acid on soil and plant, and **Lehmann *et al.* (2009)** 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 (**Bolan and Duraismy, 2003**). 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 **Karapanagiotis *et al.* (1991)** 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.* (2012)** 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 **Jones (1967)** and **Kabata-Pendias and Pendias (1992)**. 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 (**Mostafa *et al.*, 2012**).

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.

5. References

- Abd-Elrahman, Shaimaa H., Mostafa, M.A.M., Taha, T.A., Elsharawy, M.A.O. and Eid, M.A. 2012. Effect of different amendments on soil chemical characteristics, grain yield and elemental content of wheat plants grown on salt-affected soil irrigated with low quality water. *Annals Agric. Sci.*, 57: 175-182. <http://dx.doi.org/10.1016/j.aoas.2012.09.001>
- Abou El-Khir, AM. 2000. Some soil properties and trace elements as influenced by some biochemical activities in the Eastern Nile delta soils. *J. Agric. Sci., Mansoura Univ.*, 25:8421-8438.
- Acosta, JA; Faz, A; Martínez-martínez, S; Zornoza, R; Carmona, DM; Kabas, S. 2011. Multivariate statistical and GIS-based approach to evaluate heavy metals behavior in mine sites for future reclamation. *J. Geochemical Exploration*, 109:8-17. <http://doi.org/10.1016/j.gexplo.2011.01.004>

331 Adriano, D. C. 2001. Trace Elements in the Terrestrial Environments. Springer-Verlag, New York,
332 USA, p. 800.

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.

Amendment	Dry weight of whole plant (g/column)	Weight of 1000 grains, g	N	P	K	Fe	Mn	Zn	Cu	Pb	Ni	Co	Cd
				%					mg kg ⁻¹				
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
LSD_{0.05}	0.62	1.12											
		Root	0.07	0.07	0.05	10.2	12.3	5.07	2.28	0.59	0.12	0.18	0.10
		Shoot	0.05	0.04	0.04	9.35	5.93	1.53	1.58	0.17	0.04	0.06	0.02
		Grains	0.04	0.06	0.04	4.15	3.97	1.13	0.21	0.10	0.03	0.02	0.02
Normal range, mg kg⁻¹						50 - 250*	30 - 300**	27 - 150**	5 - 30**	5 - 10**	0.1 - 5**	0.02 - 1**	0.05 - 0.2**
Contaminated, mg kg⁻¹						-	400 - 1000**	100 - 400**	20 - 100**	30 - 300**	10 - 100**	15 - 50**	5 - 30**
According to						* Jones (1967)		** Kapata-Pendias and Pendias (1992)					

- 333 Ahmad H.R., M. Abbas Aziz, A. Ghafoor, M.Z. Rehman, M. Sabir and Saifullah. 2011. Wheat
334 assimilation of nickel and zinc added in irrigation water as affected by organic matter. J.
335 Plant Nutr., 34: 27-33.
- 336 Al-Azab, KM. 1997. Effect of different polluted irrigation waters on soil and plant. M.Sc.
337 Thesis, Fac. Agric., Kafr El-Sheikh, Tanta Univ., Egypt.
- 338 Ali, M. and Mindari, W. 2016. Effect of humic acid on soil chemical and physical
339 characteristics of embankment. MATEC Web of Conferences 58:1-6.
- 340 Alloway, B. J. 1995. Heavy metals in soils. 2nd Ed., John Wiley & Sons Press, Inc. New York,
341 USA.
- 342 Ayers, R. S. and D. W. Westcot. 1985. Water Quality for Agriculture. FAO, irrigation and
343 drainage. Paper 29, Rome, Italy.
- 344 Berrow M and Burridge JC. 1979. Sources and distribution of trace elements in soils and
345 related crops. In: Proceedings of the International Conference on Management and Control of
346 Heavy Metals in the Environment. CEP Consultants, Edinburgh UK, pp 304-311.
- 347 Bolan, N. S. and V. P. Duraisamy. 2003. Role of inorganic and organic soil amendments on
348 immobilization and phyto-availability of heavy metals: A review involving specific case
349 studies. Aust. J. Soil Res., 41: 533-555.
- 350 Bradbury, M. H. and B. Baeyens. 2005. Modelling the sorption of Mn(II), Co(II), Ni(II), Zn(II),
351 Cd(II), Eu(III), Am(III), Sn(IV), Th(IV), Np(V) and U(VI) on montmorillonite: Linear free
352 energy relationships and estimates of surface binding constants for some selected heavy
353 metals and actinides. Geochim. Cosmochim. Acta 69: 875-892.
- 354 Burlakovs, J., Klavins, M., Osinska, L., Purmalis, O. 2013. The impact of humic substances as
355 remediation agents to the speciation forms of metals in soil. APCBEE Procedia 5: 192-196.
356 <http://doi.org/10.1016/j.apcbee.2013.05.034>
- 357 Canellas, L.P.; Olivares, F.L.; Aguiar, N.O.; Jones, D.L.; Nebbioso, A.; Mazzei, P.; Piccolo, A.
358 2015. Humic and fulvic acids as biostimulants in horticulture. Sci. Hort., 196: 15-27.
359 <http://dx.doi.org/10.1016/j.scienta.2015.09.013>
- 360 Chang, H.Y., Ahmed, O.H., Majid, N.M.A. 2014. Improving phosphorous availability in an acid
361 soil using organic amendments produced from agro industrial wastes. The Scientific World
362 Journal 10: 1-6. <http://dx.doi.org/10.1155/2014/794756>
- 363 Chapman, H.D., and Pratt, P.F. 1961. Methods of analysis for soils, plants and waters. Division
364 of Agric. Sci., Berkeley Univ., California, USA, pp. 150-152.
- 365 Fathi, H., Aryanpour, H., Fathi, H., Moradi, H. 2014. Distribution of zinc and copper fractions
366 in acid and alkaline (highly calcareous) soils of Iran. Sky J. Soil Sci. Environ. Manag., 3: 6-
367 13.

- 368 Garcia Sanchez, A., Moyano, A. and Munez, C. 1999. Forms of cadmium, lead, and zinc in
369 polluted mining soils and uptake by plants (Soria province, Spain). *Communications in Soil*
370 *Science and Plant Analysis*, 30:1385-1402. <http://dx.doi.org/10.1080/00103629909370294>
- 371 Gupta UC, Gupta SK. 1998. Trace element toxicity relationships to crop production and
372 livestock and human health: implications for management. *Communications of Soil Science*
373 *and Plant Analysis* 29:1491-1522.
- 374 Hoalst-Pullen, N. and M.W. Patterson. 2010. Geospatial technologies in environmental
375 management. <https://books.google.com.eg/books?isbn=904819525X>
- 376 Islas-Valdez, S.; Lucho-Constantino, C.A.; Beltrán-Hernández, R.I.; Gómez-Mercado, R.;
377 Vázquez-Rodríguez, G.A.; Herrera, J.M.; Jiménez-González, A. 2015. Effectiveness of rabbit
378 manure biofertilizer in barley crop yield. *Environ. Sci. Pollut. Res.*, 1-10.
379 <http://dx.doi.org/10.1007/s11356-015-5665-2>
- 380 Jackson, M.L. 1958. *Soil chemical analysis*. Prentice-Hall, Inc., Englewood Cliffs, N.J., Library
381 of Congress, USA.
- 382 Jones, J.B. 1967. *Soil testing and plant analysis*. Prentice-Hall, Pvt., Ltd., New Delhi, India.
- 383 Jones, L.H.P. and Jarvis, S.C. 1981. The fate of heavy metals. In: D.J. Greenl and M.H.B. Hayes
384 (eds) *The Chemistry of Soil Processes*. John Wiley and Sons, Chichester, England, UK.
- 385 Kabata-Pendias, A. and Pendias, H. 1992. *Trace elements in soils and plants*. 2nd ed., CRC
386 Press, Inc. Boca Raton, Florida, USA.
- 387 Karapanagiotis, N. K.; R. M. Sterritt and J. N. Lester. 1991. Heavy metal complexation in
388 sludge amended soil: The role of organic matter in metal retention. *Environ. Technol.*, 12:
389 1107-1116.
- 390 Klute, A. 1986. *Methods of soil analysis, part I*, 2nd ed., Madison, Wisconsin, USA.
- 391 Lado LR, Hengl T, Reuter HI. 2008. Heavy metals in European soils: A geostatistical analysis
392 of the FOREGS Geochemical database. *Geoderma*, 148: 189-199.
- 393 Lehmann, J.; Czimczik, C.; Laird, D.; Sohi, S. 2009. Stability of biochar in soil. In: Lehmann,
394 J.; Joseph, S. (Eds.). *Biochar for environmental management*. London: Earthscan, pp.183-
395 205.
- 396 Lindsay W.L. 1979. *Chemical Equilibria in Soils*. John Wiley and Sons, New York, USA.
- 397 Lindsay, W.L. and Norvell, W.A. 1978. Development of DTPA soil test for zinc, iron,
398 manganese and copper. *Soil Sci. Soc. Am. J.*, 42: 421-426.
- 399 Logan, T. and R. H. Miller. 1982. Background levels of heavy metals in Ohio Farm soils.
400 Research Circular 275-283.
- 401 Lu H, Zhang W, Yang Y, Huang X, Wang S, Qiu R. 2012. Relative distribution of Pb²⁺ sorption
402 mechanisms by sludge-derived biochar. *Water Res.*, 46: 854-862.

- 403 Mahler, R. L., J. E. Hammel and R. W. Harder. 1985. The influence of crop rotation and tillage
404 methods on DTPA-extractable copper, iron, manganese, and zinc in Northern Idaho soils.
405 Soil Sci., 139: 279-286.
- 406 McKenzie, Ross H. 1992. Micronutrient requirements of crops. Source: Agdex 531-1.
- 407 Mostafa, M.A.M., Taha, T.A., and Abd-Elrahman, Shaimaa H. 2012. Distribution of heavy
408 metals in soil and elemental content of lettuce plants as affected by different amendments.
409 Egypt. J. Soil Sci., 52: 367-386.
- 410 Nanda, S., Abraham, J. 2013. Remediation of heavy metal contaminated soil. Afr. J.
411 Biotechnol., 12: 3099-3109. <http://doi.org/10.5897/AJB12.720>
- 412 Page, A. L., Miller, R.H., and Keeney, D.R. 1982. Methods of soil analysis, part II, 2nd ed.,
413 Wisconsin, USA.
- 414 Papadopoulos, A.; Prochaska, C.; Papadopoulos, F.; Gantidis, N.; Metaxa, E. 2007.
415 Determination and evaluation of cadmium, copper, nickel, and zinc in agricultural soils of
416 Western Macedonia, Greece. Environ Manage, 40:719–726. [http://doi.org/10.1007/s00267-](http://doi.org/10.1007/s00267-007-0073-0)
417 [007-0073-0](http://doi.org/10.1007/s00267-007-0073-0)
- 418 SAS. 2000. Statistical Analysis System, SAS User's Guide: Statistics, SAS Institute Inc., Cary,
419 USA.
- 420 Sukartono, Utomo W.H., Nugroho W.H., Kusuma, Z. 2011. Simple biochar production
421 generated from cattle dung and coconut. J. Basic Appl. Sci. Res., 1: 1680-1685.
- 422 Tessier, A.; P.G.C. Campbell and M. Bisson. 1979. Sequential extraction procedure for the
423 speciation of particulate trace metals. Anal. Chem., 51: 844-851.
- 424 Xu, X., Cao X., Zhao L., Zhao H., Luo Q. 2014. Interaction of organic and inorganic fractions
425 of biochar with Pb (II) ion: Further elucidation of mechanisms for Pb (II) removal by
426 biochar. RSC Adv., 4: 44930-44937. <http://doi.org/10.1039/C4RA07303G>
- 427 Zeng, G., Wu H., Liang J., Guo S., Huang L., Xu P., Liu Y., Yuan Y., He X., He, Y. 2015.
428 Efficiency of biochar and compost (or composting) combined amendments for reducing Cd,
429 Cu, Zn and Pb bioavailability, mobility and ecological risk in wetland soil. RSC Adv., 5:
430 34541-34548. <http://doi.org/10.1039/C5RA04834F>
- 431 Zhang X, Wang H, He L, Lu K, Sarmah A, Li J, Bolan N, Pei J, Huang H. 2013. Using biochar
432 for remediation of soils contaminated with heavy metals and organic pollutants. Environ Sci
433 Pollut Res., 20: 8472-8483.