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Accumulation of Potentially Toxic Metals in Egyptian Alluvial Soils, Berseem Clover (*Trifolium alexandrinum* L.), and Groundwater after Long-Term Wastewater Irrigation

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Abstract: The reduced availability of water resources in Egypt has imposed the need to intensify the use of wastewater for crop irrigation in the alluvial soils. Relevant effects can derive from contents of potentially toxic metals (PTMs) in supply resources soils, crops, and groundwater in these areas. For this reason the PTM content has to be monitored to evaluate and minimize health hazards. Therefore, in this context, two areas of the SE Nile Delta subjected to 25 year of wastewater irrigation, using agricultural drainage water (ADW) and mixed wastewater (MWW) were chosen and compared with a nearby site irrigated with Nile freshwater (NFW). At each of the three sites, ten samples of irrigation water, topsoil, berseem clover (*Trifolium alexandrinum* L.) plants, and seven groundwater samples were collected and analyzed for Cr, Co, Cu, Pb, Ni, and Zn. Results indicate that the total contents of Co, Cu, Ni, and Zn in soils collected from the three sampling sites and Pb in the MWW-irrigated soils were higher than their average natural contents in the earth's crust, indicating potential risks. The DTPA-extractable contents of Cu in the three sites, in addition to Pb and Zn in the MWW-irrigated soils, exceeded the safe limits. The MWW-irrigated soils showed a considerable degree of metal contamination, while the NFW- and ADW-irrigated soils showed moderate and low levels of contamination, respectively. The contents of the six PTMs in the three sites showed low individual ecological risks, except for Pb in the MWW-irrigated soils that showed a moderate risk; however, the overall ecological risk remained low in all samples. The values of Co, Cu, and Ni in berseem shoot in addition to Pb from the MWW-irrigated soils were over the maximum permissible levels for animal feeding. Values of root-to-shoot translocation factor were lower than 1.0 for Cr, Co and Ni but higher than 1.0 for Cu, Pb, and Zn. Berssem plant is a good candidate for phytofiltration of Cr, Co and Ni, while for extracting Cu, Pb and Zn from polluted soils. The groundwater samples collected from the three sampling sites showed lower metal concentrations than the safe limits for drinking standards. Further remediation studies should be taken into account to alleviate potential environmental and health-related risks when using supply resources different from freshwater.

Keywords: wastewater irrigation; alluvial soils; Nile Delta; berseem clover; groundwater; potentially toxic metals

1. Introduction

Chemical pollution is one of the most concerning environmental issues worldwide and plays a great role in the transformations of the biosphere [1]. Potentially toxic metals (PTMs) are ubiquitous inorganic pollutants that can pose serious environmental threats [2]. Zn and Cu are important elements for the metabolism in living organisms; however, their excessive levels can cause health problems. On the other hand, Cd, Cr, Pb, and Ni have no essential biochemical functions and are involved in developing cancer risk [3]. The PTMs are released during chemical weathering of parent rocks or enter into the environment by a wide range of anthropic activities [4].

The reduced availability of water in Mediterranean countries requires the efficient use of supply sources and alternative freshwater resources for irrigation. The use of wastewater raises, however, sanitary problems and risk both for farmers and crops and problems of agronomic nature due to the presence of biotic and abiotic toxic substances [5]. Wastewater irrigation is one of the principal contributing pathways for environmental metal pollution, especially in arid regions [6], where this practice has become necessary to compensate for freshwater scarcity coupled with limited rainfall [7]. In Egypt, recycling of treated sewage effluents in irrigation has been initiated since 1911 in a small farm (1250 ha) in the eastern desert [8], and extended to large-scale pilot projects occupying about 70,000 ha in several desert lands for irrigating trees beside some field crops [9]. In the Nile Delta alluvial soils, using agricultural drainage in irrigation has been adopted as an official policy since the early 1970s depending on collecting agricultural drainage water from main and branch canals in large-scale pumping stations to be mixed with the Nile freshwater in main and branch canals [10]. Uncontrolled use of wastewater in the Nile Delta has grown increasingly through unofficial reuse of drainage water by applying the drained water without blending directly to the fields [11] and direct use of raw (untreated) or/and partially treated effluents [12]. These waters usually contain considerable concentrations of PTMs, which accumulate in the food chain, causing potential environmental, and human health risks [4,13].

Soil is a major natural sink for metals discharged through historical wastewater irrigation, in particular for alluvial soils with high clay content which can retain excessive levels of PTMs due to their high sorption capacity [14]. Some studies have indicated that soil metal contamination has reached warning levels in the Nile Delta zones affected by wastewater irrigation [12,15–17]. Excessive metal concentrations in soils have adverse effects on ecosystems and human health through agricultural products or contaminated drinking water [1]. Unlike organic substances, the severity of PTMs results from their resistance to chemical or biological degradation in soils, and thus their total content persists. Their bioavailable fractions in turn may accumulate in different parts of the growing plants or reach shallow groundwater aquifer [18].

Berseem, or Egyptian clover (*Trifolium alexandrinum* L.), is one of the most common leguminous forage crops in the Mediterranean region and the Middle-East. It is considered as fresh forage, hay, silage, pasture, and green fertilizer. This crop has gained much attention because it improves soil properties, increases fertility, and contains more protein and minerals compared with grasses [19]. In Egypt, berseem is the major winter forage crop, occupying half of the cultivated area during winter, where it can be cultivated as early as October, and it can be harvested in May [20]. Studies indicated that berseem plants can uptake and accumulate high metal levels in different parts, which are transferred to animals and humans, resulting in potentially serious health risks [21–23].

Groundwater is the ultimate receptor of metal contaminants of anthropogenic origin; however, the occurrence of these toxicants in groundwater is of great human concern [1]. This is because excessive metal concentrations render the groundwater unfit for human consumption [24]. In the rural Nile Delta communities of Egypt, the shallow groundwater aquifer is the main source for drinking water, where the local residents usually make shallow wells operated hand pumps at a depth ranging from 15 to 20 m to extract the

groundwater [25]. Consequently, prolonged irrigation using wastewater may increase metal concentrations in the groundwater and cause human health risks.

Effective environmental management strategies impose comprehensive monitoring of metal contaminants in soil, crops, and groundwater from wastewater irrigated areas.

These considerations have motivated the current study that was conducted in an area of the Nile Delta to verify the implications of long-term wastewater irrigation on metal concentration in alluvial soils, different parts (root and shoots) of berseem plants, and groundwater. The study, therefore, provides a well-established database, helping policymakers in pollution control and alleviating potential human health risks.

2. Materials and Methods

2.1. Site Description

The studied area is located in Al-Qalubiya Governorate, south-eastern of the Nile Delta, Egypt (30°13'39'' to 30°17'27'' N and 31°17'22'' to 31° 20'22'' E) (Figure 1). It is characterized by hot arid summers and short rainy winter. The mean annual temperature (22 °C) and total annual precipitation (67 mm) indicate a *Thermic* temperature regime and *Torric* moisture regime, respectively [26]. The area is dominated by young alluvial plain landscapes, including terraces, flats and gently undulating surfaces with elevation ranging from 3 m below sea level in the south to 40 m above sea level in the north [27]. The geological map of Egypt indicates that the area is underlined by Quaternary sediments of the Nile silt deposits [28]. The soils are classified as *Typic Torriorthents* [29,30].
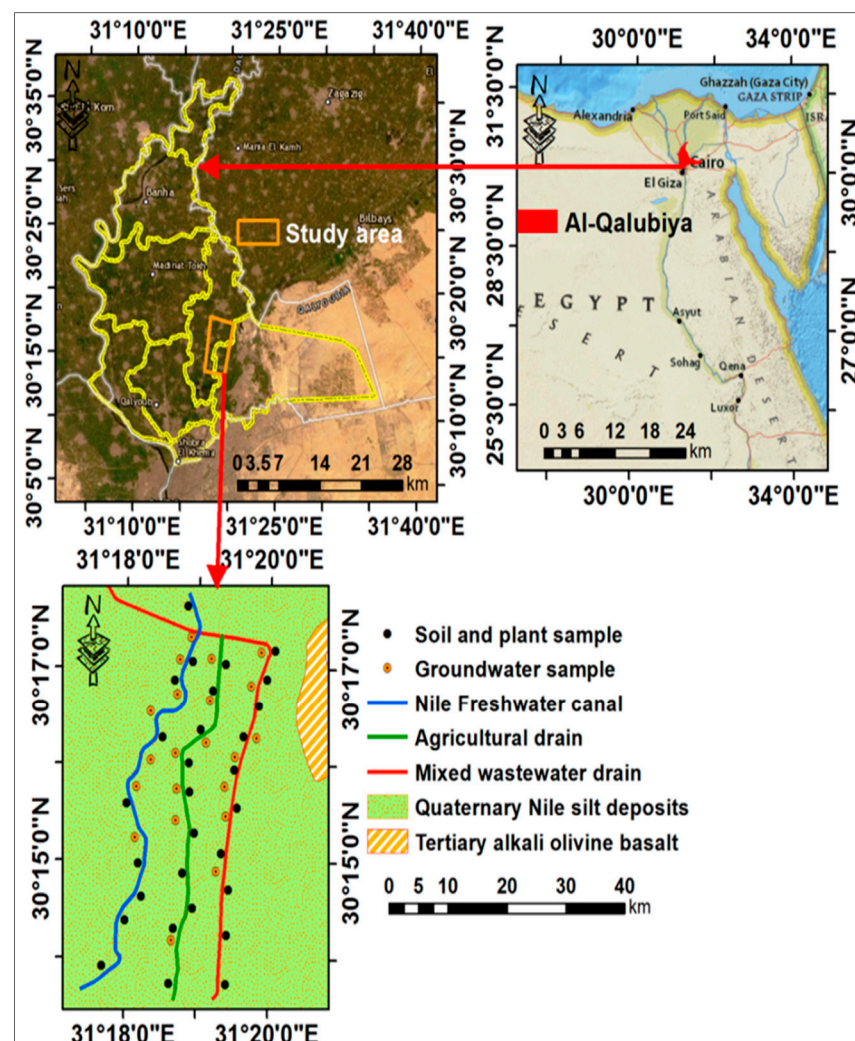

Figure 1. Maps of sampling location sites.

Field crops (wheat and clover in winter and maize in summer) are the predominant crop pattern. Irrigation is performed using traditional methods, using three surface water sources:

Nile freshwater (NFW) from the Al-Sharqua canal;
Agricultural drainage water (ADW) from the Sendiwah drain;
Mixed wastewater (MWW) from the Shebin Al-Kanater drain.

NFW was previously the primary source for irrigation; however, due to freshwater scarcity, farmers have used ADW and MWW (mixes of partially treated and untreated effluents of domestic, industrial, and agricultural activities) in irrigating their fields for over 25 years.

2.2. Collection of Samples

Irrigation waters, soils, berseem plants, and groundwater were collected during the winter of 2020. Irrigation water (30 samples) was collected from the three water sources (10 samples from each source) in the area shown in Figure 1. For groundwater samples, a total number of 21 samples were collected from hand pumps in the three different irrigated sites (7 samples from each site). At each site, composite water samples (with three sub-samples) were collected in 500 mL polypropylene bottles, previously washed with 50% HNO_3, deionized water, and acidified with 5 mL HNO_3, at 50 cm below the water surface for irrigation water samples, and after operating the hand pumps for 10 min. The collected samples were then transported to the laboratory and kept at 4 °C until being analyzed.

Similar to irrigation water, 30 top-soil samples (0–30) were collected from the three irrigated sites (10 samples from each site) adjacent to the point of irrigation water sampling in each area. At each site, composite soil samples with five sub-samples were collected, kept in plastic bags, and transported to the laboratory. At the same location of each soil sample, the whole berseem (*Trifolium alexandrinum* L.) plants (30 samples) were collected from the three sites (10 samples from each site) in paper bags and transported to the laboratory.

2.3. Laboratory Analyses

All analyses were performed in an ISO 17025: 2017 certified laboratory of the Central Laboratory for Environmental Quality Monitoring (CLEQM), National Water Research Center (NWRC), Egypt. All types of water sample (freshwater, wastewater, and groundwater) were digested in nitric acid according to method 3030 E [31]. Soil samples were air-dried, grounded, and passed through a 2 mm mesh. Soil physicochemical analysis, pH (1:2.5 soil: water suspension), electrical conductivity (EC) (in soil paste extract), organic matter (OM) content, exchangeable sodium percentage (ESP), and particle size distribution were performed using standard methods [32], whose results are shown in Table 1.

The total contents of PTMs were extracted according to method 3052 [33]: microwave-assisted acid digestion using concentrated HNO_3, HF, and HCl. On the other hand, the available contents were obtained with diethylenetriaminepentaacetic acid (DTPA) [34]. The whole berseem plants were cleaned and washed using tap water then deionized water. Thereafter, they were divided into roots and shoots and oven-dried at 70 °C for 48 h. After drying, samples were subjected to the traditional wet digestion method using a mixture of concentrated H_2SO_4, $HClO_4$, and HNO_3 (1:1:5) solutions.

All digested solutions (waters, soils, and plants) were filtered using Whatman 42 filter papers (pore size 2.5 μm), and brought to 50 mL volume by deionized water. These solutions in addition to the DTPA-extractable solutions were analyzed for PTMs using an Inductively Coupled Plasma Optical Emission (ICP-OES–Perkin Elmer Optima 5300, USA).

Table 1. Soil physicochemical characteristics.

Property	Unit	NFW-Irrigated Soils	ADW-Irrigated Soils	MWW-Irrigated Soils
pH	—	7.08 ± 0.03 b	7.22 ± 0.01 a	7.09 ± 0.03 b
EC	dS m^{-1}	1.26 ± 0.31 a	2.45 ± 0.55 a	1.41 ± 0.23 a
ESP	—	5.44 ± 0.49 b	10.98 ± 0.82 a	9.72 ± 0.55 a
OM	g kg^{-1}	15.77 ± 1.11 a	16.84 ± 2.61 a	17.95 ± 2.21 a
Sand	%	62.98 ± 3.08 a	42.95 ± 2.77 b	55.03 ± 2.88 a
Silt	%	19.09 ± 2.44 c	40.74 ± 2.63 a	29.12 ± 2.11 b
Clay	%	17.93 ± 3.52 a	16.31 ± 1.75 a	15.85 ± 1.58 a
Al		45.32 ± 0.94 a	39.89 ± 2.96 ab	37.83 ± 1.55 b
Fe	g kg^{-1}	41.95 ± 1.41 a	36.68 ± 2.04 b	33.68 ± 1.09 b
Mn		0.81 ± 0.02 a	0.74 ± 0.05 a	0.72 ± 0.03 a

Values are means $\pm$ standard error (SE) of ten soil samples; means with different letters indicate significant difference at 0.05 probability level; NFW, Nile freshwater; ADW, agricultural drainage water; MWW, mixed wastewater; EC, electrical conductivity; ESP, exchangeable sodium percentage; OM, organic matter.

2.4. Assessment of Soil Contamination by PTMs

2.4.1. Contamination Factor (C_f)

The C_f determines which metal poses the highest threat to the soil environment [35]. It was calculated considering the total metal content in soil (C_s) and the corresponding reference value (C_r) as follows:

$$C_f = \frac{C_s}{C_r}$$

Due to a lack of reference values for the Egyptian soils, geochemical values of the earth's crust were used as references [3]. The contamination levels are considered low, moderate, considerable, and high when the C_f is <1, 1–3, 3–6, and >6, respectively.

2.4.2. Contamination Degree (C_d)

The C_d gives a comprehensive picture of the contamination status [35]. This index was calculated based on the C_f of the number (n) of analyzed metals as follows:

$$C_d = \sum_{i=1}^{n} C_f$$

Contamination levels are low ($C_d < 8$), moderate ($8 \leq C_d < 16$), considerable ($16 \leq C_d < 32$), and very high ($C_d > 32$).

2.4.3. Potential Ecological Risk Index (PERI)

The PERI index was adopted to assess the degree of ecological risk caused by PTMs [36]. The PERI was calculated as follows:

$$PERI = \sum_{i=1}^{n} E_r$$

where: n is the number of metals and E_r is the single index of the ecological risk factor, which was calculated based on the equation:

$$E_r = T_t \times C_f$$

where: T_r is the toxic response factor, which is estimated by 2 for Cr, 5 for Co, Cu, Pb, and Ni, and 1 for Zn [36]. According to Kowalska et al. [35], the potential risk for the individual metal could be classified as low ($E_r < 40$), moderate ($40 \leq E_r < 80$), considerable ($80 \leq E_r < 160$), high ($160 \leq E_r < 320$), and very high ($E_r > 320$), while the overall risk as low (PERI < 150), moderate ($150 \leq$ PERI < 300), considerable ($300 \leq$ PERI < 600), and very high (PERI > 600).

2.4.4. Availability Ratio (AR)

The AR determines the soil metal supply to the growing plants in current and potential conditions [37]. The AR was calculated as follows:

$$AR = \left(\frac{DTPA - \text{extractable content}}{\text{Total content}} \right) \times 100$$

2.5. Accumulation and Translocation of PTMsin Berseem Plants

The soil-to-plant bioaccumulation factor (BAF) was determined to evaluate metal transfer from the soil to different parts of berseem plants (roots and shoots). It was calculated as a ratio of the metal content in plant tissue to its total content in the soil as follows [21]:

$$BAF = \frac{\text{Metal content in plant tissue } (mg\ kg^{-1})}{\text{Total metal content in soil } (mg\ kg^{-1})}$$

The translocation factor (TF) was determined to assess metal migration from roots to the aerial parts, i.e., shoots. The TF was calculated as a ratio of the metal content in roots to its content in shoots as follows [21]:

$$TF = \frac{\text{Metal content in shoots } (mg\ kg^{-1})}{\text{Metal content in roots } (mg\ kg^{-1})}$$

2.6. Data Analysis

All statistical analyses were performed using SPSS 19.0 software (SPSS Inc., Chicago, IL, USA) and MS Excel, using one-way ANOVA followed by Tukey's significance difference (HSD) test at 5% probability level ($p < 0.05$) to compare means values of PTMs in waters, soils, and berseem tissues. Pearson's correlation coefficient was calculated to analyze the relationship between metals in irrigation waters and soils.

2.7. Quality Control

All measurements were performed in triplicate using chemicals at analytical grade, and the results were presented as a mean value. The metal analysis was carried out along with blanks to reduce error. Blanks, triplicate measurements for each metal, and analyses of certified reference materials for each metal (Merck) were routinely included for quality control according to ISO/IEC 17025 for laboratory accreditation. A test of recovery for PTMs was performed at seven different concentration levels (1000, 500, 100, 50, 25, 12.5, and 6.25 $\mu g\ L^{-1}$). The average relative standard deviation was lower than 5%.

3. Results

3.1. Concentrations of PTMs in Irrigation Water

Results in Table 2 show that concentrations of the investigated metals in irrigation water were below the permissible levels for irrigation suggested by FAO 29 guidelines [38]. On average, the abundance of PTMs was Cu > Ni > Zn > Co > Pb > Cr in the NFW; Zn > Cu > Pb > Ni > Cr > Co in the ADW, and Cu > Zn > Ni > Pb > Cr > Co in the MWW. In general, the concentrations of Cr, Co, and Cu in the MWW were slightly higher than the corresponding values in the ADW and NFW. Moreover, the concentration of Zn in the ADW was slightly higher than that in the MWW and NFW. On the other hand, the MWW showed a higher Pb concentration ($p < 0.05$) than the NFW with a slight difference between MWW and ADW. Moreover, the MWW showed a higher Ni concentration ($p < 0.05$) compared with the ADW with a slight difference between MWW and NFW.

Table 2. Concentrations of PTMs (mg L^{-1}) in irrigation water.

PTM	Nile Freshwater		Agricultural Drainage Water		Mixed Wastewater		MAC
	Range	Mean $\pm$ SE	Range	Mean $\pm$ SE	Range	Mean $\pm$ SE	
Cr	Bdl–0.006	0.001 $\pm$ 0.001 a	bdl–0.008	0.003 $\pm$ 0.001 a	bdl–0.008	0.004 $\pm$ 0.001 a	0.100
Co	bdl–0.006	0.002 $\pm$ 0.001 a	bdl–0.008	0.002 $\pm$ 0.001 a	bdl–0.008	0.003 $\pm$ 0.001 a	0.050
Cu	bdl–0.073	0.021 $\pm$ 0.007 a	0.032–0.068	0.049 $\pm$ 0.005 a	0.029–0.413	0.087 $\pm$ 0.037 a	0.200
Pb	bdl–0.007	0.001$\pm$ 0.001 b	bdl–0.016	0.004 $\pm$ 0.002 ab	0.005–0.014	0.008 $\pm$ 0.001 a	5.000
Ni	bdl–0.017	0.007 $\pm$ 0.002 ab	bdl–0.008	0.004 $\pm$ 0.001 b	0.004–0.034	0.022 $\pm$ 0.003 a	0.200
Zn	bdl–0.020	0.003 $\pm$ 0.002 a	0.010–0.394	0.063 $\pm$ 0.047a	0.012–0.088	0.029 $\pm$ 0.008 a	5.000

Values are means $\pm$ standard error (SE) of ten soil samples; means with different letters indicate significant difference at 0.05 probability level; SE, standard error; MAC, maximum allowable concentration (FAO 29 guidelines).

3.2. Soil Contamination by PTMs

Descriptive statistics of the total metal content in the studied soils are shown in Table 3.

Table 3. Concentrations of PTMs in soils (mg kg^{-1}).

PTM	NFW-Irrigated Soils		ADW-Irrigated Soils		MWW-Irrigated Soils		Earth's Crust	MAC
	Range	Mean $\pm$ SE	Range	Mean $\pm$ SE	Range	Mean $\pm$ SE		
				Total content				
Cr	61.11–75.32	71.65 $\pm$ 1.61 a	56.51–85.52	69.19 $\pm$ 74.56 a	67.13–89.51	74.65 $\pm$ 2.85 a	100	50–200
Co	32.51–41.55	36.88 $\pm$ 0.97 a	24.53–40.61	30.63 $\pm$ 1.64 b	27.41–36.01	30.88 $\pm$ 1.12 b	10	20–50
Cu	84.11–141.21	106.25 $\pm$ 8.44 a	71.11–95.52	84.56 $\pm$ 2.81 a	76.56–206.12	110.63 $\pm$ 4.57 a	55	60–150
Pb	5.11–12.51	6.13 $\pm$ 0.61 b	0.67–4.11	1.81 $\pm$ 0.13 b	55.63–193.42	136.63 $\pm$ 9.14 a	15	20–300
Ni	30.35–39.12	34.06 $\pm$ 0.86 a	20.15–35.61	26.81 $\pm$ 1.77 b	25.61–40.12	32.25 $\pm$ 1.89 ab	20	20–60
Zn	61.51–89.12	74.88 $\pm$ 3.29 a	54.53–288.21	86.13 $\pm$ 6.62 a	67.56–155.67	94.97 $\pm$ 4.93 a	70	100–300
				DTPA-extractable content				
Cr		<0.002			0.05–0.26	0.06 $\pm$ 0.02		0.50
Co			<0.003					0.50
Cu	0.11–0.45	0.35 $\pm$ 0.04 b	0.67–1.54	0.98 $\pm$ 0.11 a	0.36–2.74	1.42 $\pm$ 0.22 a	—	0.20
Pb	0.05–0.46	0.22 $\pm$ 0.05 b	0.31–43	0.35 $\pm$ 0.01 b	1.58–36.71	12.21 $\pm$ 2.65 a	—	10.00
Ni	0.02–0.03	0.03 $\pm$ 0.01 b		<0.004	0.07–0.24	0.15 $\pm$ 0.01 a	—	1.00
Zn	0.02–0.13	0.05 $\pm$ 0.01 b	0.17–0.44	0.27 $\pm$ 0.03 b	0.31–2.72	1.01 $\pm$ 0.25 a	—	0.50

Values are means $\pm$ standard error (SE) of ten soil samples; means with different letters indicate significant difference at 0.05 probability level; NFW, Nile freshwater; ADW, agricultural drainage water; MWW, mixed wastewater.bdl, below detection limit; SE, standard error; MAC, maximum allowable concentration (FAO 29 guidelines).

On average, the concentrations of Cr in the three sampling sites in addition to Pb in the NFW- and ADW-irrigated soils did not exceed the average natural content (ANC) in the earth's crust, i.e., 55 mg kg^{-1} for Cr and 15 mg kg^{-1} for Pb [3]. On the other hand, potential risks were associated with Co, Cu, Ni, and Zn in all sampling sites and Pb in MWW-irrigated soils, since their concentrations were higher than their ANC of 10, 55, 20, 70, and 15 mg kg^{-1}, respectively [3]. The element abundance followed the order of Cu > Zn > Cr > Co > Ni > Pb in NFW-irrigated soils, Zn > Cu > Cr > Co > Ni > Pb in ADW-irrigated soils, and Pb > Cu > Zn > Cr > Ni > Co in MWW-irrigated soils. The statistical analysis indicated that the contents of Cr, Cu, and Zn in MWW-irrigated soils were slightly higher than the corresponding ones in NFW- and ADW-irrigated soils. On the other hand, significant differences ($p < 0.05$) were observed for Co, Pb, and Ni among the three sampling sites. The NFW-irrigated soils showed higher Co content with a slight difference between MWW- and ADW-irrigated soils. Moreover, Ni content in the NFW-irrigated soils was slightly higher than that in MWW-irrigated soils but significantly higher than that in

ADW-irrigated soils. The MWW-irrigated soils showed higher Pb concentrations with a slight difference between NFW- and MWW-irrigated soils.

Table 3 show that the concentrations of Co in the three sampling sites and Cr in the NFW- and ADW-irrigated sites were below the detection limit of 0.003 mg kg^{-1}. Although Cr occurred in the MWW-irrigated soils, its content stood below the permissible limit of 0.5 mg kg^{-1} [39]. The average Cu concentrations in the three sampling sites exceeded the safe limit of 0.20 mg kg^{-1} [39]. On average, Cu concentrations were 1.75, 4.9, and 7.1 fold higher than the safe limit in the NFW-, ADW- and MWW-irrigated soils, respectively. The Pb content in the NFW- and ADW-irrigated soils remained below the critical limit of 10.0 mg kg^{-1} [39], while it was 1.22-fold higher than that limit in the MWW-irrigated soils. The Ni content in the ADW-irrigated soils was below the detection limit (0.004 mg kg^{-1}), while it occurred in the NFW- and MWW-irrigated soils a lower than the safe level of 1.0 mg kg^{-1} [39]. The Zn content in NFW- and ADW-irrigated soils was below the safe limit (0.5 mg kg^{-1}) [39], while it was almost double that limit in the MWW-irrigated soils. The metal abundance was in the order Cu > Pb > Zn > Ni in NFW-irrigated soils; Cu > Pb > Zn in ADW-irrigated soils, and Pb > Cu > Zn > Ni > Cr in MWW-irrigated soils. In general, the MWW-irrigated soils showed higher significant ($p < 0.05$) concentrations of Pb and Zn compared with the NFW- and ADW-irrigated soils with slight differences between NFW- and ADW-irrigated soils. Furthermore, Cu content in MWW-irrigated soils was significantly ($p < 0.05$) higher than that in NFW-irrigated soils, while slightly higher than that in ADW-irrigated soils.

Pearson's correlation coefficients and statistical p-values between concentrations of PTMs in irrigation water and soil (Total and DTPA-extractable fractions) are reported in Table 4. Total Cr content in ADW- and MWW-irrigated soils showed significant positive correlations ($p < 0.05$) with irrigation water. Likely, the total Cu content in MWW-irrigated soils and the total Pb in ADW-irrigated soils correlated significantly with irrigation water. On the other hand, total Pb content in MWW-irrigated soils showed a significant negative correlation with irrigation water. Regarding the bioavailable soil metal content, Pb and Ni in NFW-irrigated soils showed highly significant correlations ($p < 0.01$) with irrigation water. Likewise, Cu content in MWW-irrigated soils correlated significantly with irrigation water. On the other hand, Ni content in MWW-irrigated soils showed a significant negative correlation with irrigation water.

Table 4. Pearson's correlation coefficient (r) and probability (*p*) value between metal concentrations in irrigation water and soils.

PTM	Content	NFW-Irrigated Sites		ADW-Irrigated Sites		MWW-Irrigated Sites	
		r	*p*-Value	r	*p*-Value	r	*p*-Value
Cr	Total	−0.005	ns	0.521	*	0.580	*
	Available	—	—	—	—	−0.318	ns
Co	Total	−0.018	ns	0.069	ns	−0.376	ns
	Available	—	—	—	—	—	—
Cu	Total	−0.171	ns	0.080	ns	0.546	*
	Available	0.176	ns	−0.401	ns	0.537	*
Pb	Total	0.176	ns	0.515	*	−0.507	*
	Available	0.771	**	−0.092	ns	−0.293	ns
Ni	Total	−0.463	ns	0.171	ns	0.064	ns
	Available	0.998	**	—	—	−0.686	*
Zn	Total	0.167	ns	−0.149	ns	−0.041	ns
	Available	−0.073	ns	−0.390	ns	−0.291	ns

ns, not significant; * significant at $p < 0.05$; ** significant at $p < 0.01$.

As shown in Figure 2, in the three sampling sites, values of C_f for Cr did not exceed 1.0 which indicated a low contamination level, moderate contamination levels were associated

to Cu, Ni and Zn (C_f values ranging from 1 to 3), while a considerable contamination level was related to Co with C_f values higher than 3.0 [35]. On the other hand, Pb showed different contamination levels among the three sampling sites. The C_f for Pb in NFW- and MWW-irrigated soils was less than 1.0 (low), while the corresponding value in the MWW-irrigated soils surpassed 6.0 indicating a high contamination level [35]. On average, the order of soil contamination was Co > Cu > Ni > Zn > Cr > Pbin NFW- and MWW-irrigated soils, and Pb > Co > Cu > Ni > Zn > Cr in MWW-irrigated soils. Regarding the overall contamination, the mean values of the Cd in NFW- and ADW-irrigated soils were 9.33 and 7.89, respectively, indicating moderate and low contamination levels [35]. On the other hand, the corresponding value in MWW-irrigated soils was 18.50, showing a considerable contamination level [35].
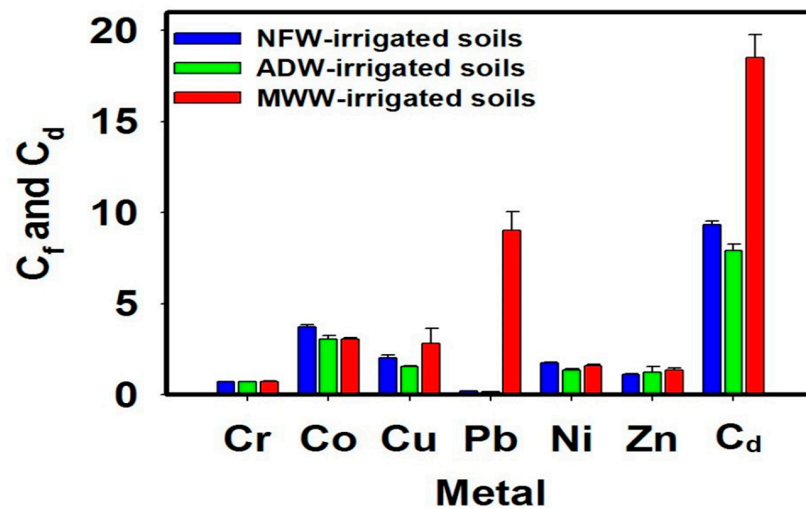

Figure 2. Contamination factor (C_f) and contamination degree (C_d).

With exception of Pb in MWW-irrigated soils, low ecological risks were associated with all metals in the three sampling sites (Figure 3) as their Er values did not exceed 40 [35]. On the other hand, the mean Er value for Pb in MWW-irrigated soils was 45.10, indicating moderate risks [35]. On average, the potential ecological risk factor was found in the order of Co > Cu > Ni > Cr > Zn > Pb in NFW- and ADW-irrigated soils, and Pb >Co > Cu > Ni > Cr > Zn in MWW-irrigated soils. Regarding the overall risk, values of PERI in the three sampling sites were below 150, indicating low potential risk [35].
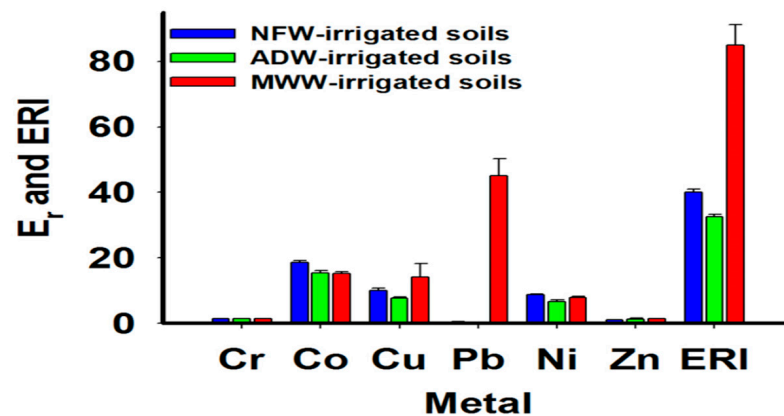

Figure 3. Single ecological risk (E_r) and poetical ecological risk index (ERI).

3.3. Availability Ratio (AR)

Figure 4 shows that the mean values of AR for Cu, Ni, and Zn in NFW-irrigated soils did not exceed 1.0, while Pb's mean value reached 7.94. For ADW-irrigated soils, the mean

values of AR were 0.37 for Ni, 1.16 for Cu, and 9.05 for Pb. In MWW-irrigated soils, the mean values of AR were 0.02 for Cr, 1.34 for Cu, 8.35 for Pb, 0.34 for Ni, and 1.14 for Zn.
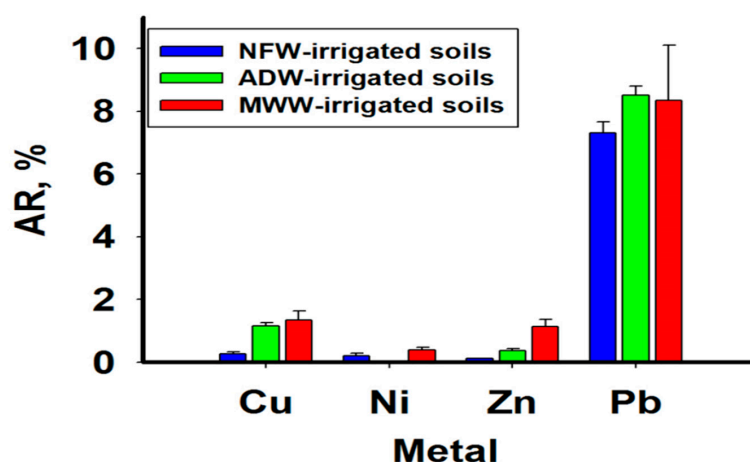

Figure 4. Metal availability ratio (AR).

In NFW-irrigated soils, a highly significant positive correlation ($p < 0.01$) occurred between AR of Pb and clay content (Table 5). A significant positive correlation ($p < 0.05$) occurred also between AR of Zn and clay content. The AR of Pb showed a significant positive correlation with the OM content. In ADW-irrigated soils as well as MWW-irrigated soils (except Ni), the ARs of all studied metals showed no significant correlations with soil properties. On the other hand, AR of Ni showed a significant negative correlation (r = −0.692*) with soil pH in the MWW-irrigated soils.

Table 5. Pearson's correlation between metal availability ratio (AR) and soil.

Soil Property	Cr	Cu	Pb	Ni	Zn
			Nile freshwater-irrigated soils		
pH	—	−0.193	−0.107	−0.132	−0.272
Organic matter	—	0.328	0.633 *	0.024	0.278
Clay	—	0.530	0.831 **	0.586	0.753 *
Al	—	0.095	0.030	0.053	0.124
Fe	—	−0.148	0.019	−0.158	0.063
Mn	—	−0.318	−0.050	0.041	0.119
			Agricultural drainage water-irrigated soils		
pH	—	−0.479	−0.054	—	−0.481
Organic matter	—	0.159	−0.232	—	0.500
Clay	—	0.108	−0.167	—	0.503
Al	—	−0.156	−0.451	—	−0.240
Fe	—	−0.190	−0.434	—	−0.282
Mn	—	−0.117	−0.193	—	−0.204
			Mixed wastewater-irrigated soils		
pH	−0.025	−0.581	−0.224	−0.692 *	−0.439
Organic matter	−0.060	0.368	−0.031	0.424	0.206
Clay	−0.160	0.082	0.171	−0.032	−0.159
Al	0.057	−0.210	−0.377	0.015	−0.250
Fe	0.106	−0.154	−0.296	0.055	−0.096
Mn	−0.317	0.155	0.322	0.014	0.470

* Significant at $p < 0.05$; ** Significant at $p < 0.01$.

3.4. Berssem Plant Contamination by PTMs

Results reported in Table 6 show that the order of metal contents observed in roots was Zn > Cu > Cr > Co > Ni > Pb in NFW- and MWW-irrigated soils, and Zn > Cu > Co > Cr > Ni > Pb in ADW-irrigated soils. Generally, with the exception of Zn, the magnitude of metal accumulation in berseem roots in the three sampling sites was similar to the metal content in soils. The lowest root Zn content was observed in plants grown on the ADW-irrigated soils despite the high total and bioavailable metal content of these soils.

Table 6. Concentrations of PTMs (mg kg^{-1}) in berseem (*Trifolium alexandrinum* L.) tissues.

PTM	NFW-Irrigated Soils		ADW-Irrigated Soils		MWW-Irrigated Soils		MAC
	Range	Mean $\pm$ SE	Range	Mean $\pm$ SE	Range	Mean $\pm$ SE	
				Roots			
Cr	17.11–21.62	18.38 $\pm$ 1.09 ab	10.51–13.62	12.75 $\pm$ 0.14 b	21.62–25.65	23.05 $\pm$ 1.30 a	—
Co	16.12–19.25	16.51 $\pm$ 0.29 a	12.65–14.63	13.44 $\pm$ 0.29 b	15.32–17.98	16.22 $\pm$ 0.45 a	—
Cu	17.43–22.51	19.33 $\pm$ 1.59 b	26.51–28.11	26.54 $\pm$ 0.29 a	24.62–33.31	28.76 $\pm$ 1.88 a	—
Pb	1.11–2.96	1.61 $\pm$ 0.27 b	1.33–2.96	1.93 $\pm$ 0.34 b	3.87–7.51	5.96 $\pm$ 0.87 a	—
Ni	13.34–19.11	16.96 $\pm$ 1.51 a	6.36–9.84	8.23 $\pm$ 0.29 b	7.32–12.69	9.62 $\pm$ 1.44 b	—
Zn	39.62–48.32	43.16 $\pm$ 1.91 a	24.94–31.23	29.31 $\pm$ 1.15 b	46.74–55.61	51.25 $\pm$ 1.88 a	—
				Shoots			
Cr	11.51–19.75	14.33 $\pm$ 1.71 a	8.44–10.93	9.26 $\pm$ 0.43 a	8.76–10.29	9.11 $\pm$ 0.14 a	15
Co	12.66–14.27	13.67 $\pm$ 0.46 a	11.33–12.66	12.54 $\pm$ 0.05 a	11.75–13.73	12.75 $\pm$ 0.58 a	0.50
Cu	36.52–54.25	45.46 $\pm$ 3.12 ab	25.63–35.71	30.38 $\pm$ 2.96 b	44.73–53.81	49.38 $\pm$ 1.96 a	15
Pb	0.95–3.78	2.17 $\pm$ 0.84 b	1.52–4.11	2.81 $\pm$ 0.75 b	5.11–8.78	6.95 $\pm$ 0.77 a	5
Ni	7.33–13.47	10.67 $\pm$ 1.74 a	3.62–7.01	5.91 $\pm$ 0.43 b	6.91–9.72	8.13 $\pm$ 0.51 ab	3
Zn	45.62–54.31	48.29 $\pm$ 1.39 a	17.51–60.19	38.75 $\pm$ 2.91 a	48.75–65.14	61.38 $\pm$ 1.44 a	100

Values are means $\pm$ standard error (SE) of ten soil samples; means with different letters indicate significant difference at 0.05 probability level; NFW, Nile freshwater; ADW, agricultural drainage water; MWW, mixed wastewater.bdl, below detection limit; SE, standard error; MAC, maximum allowable concentration (FAO 29 guidelines).

As shown in Table 6, the shoot concentrations of Cr and Zn in the three sampling sites and Pb in plants grown on the NFW- and ADW-irrigated soils were found below the safe limits of 15, 100, and 5 mg kg^{-1}, respectively [40–42]. On the other hand, concentrations of Co, Cu, and Ni in the three sampling sites and Pb in plants grown on the MWW-irrigated soils exceeded the safe limits of 0.5, 15, 5, and 3 mg kg^{-1}, respectively [3,41,43]. The order of metal content in shoots was Zn > Cu > Cr > Co > Ni > Pb in the NFW-irrigated soils, and Zn > Cu > Co > Cr > Ni > Pb in ADW- and MWW-irrigated soils. Out of six PTMs, plants grown on MWW-irrigated soils showed higher significant content of Pb ($p < 0.05$) in shoots compared with the two other sites. On average, the Pb increases represented 3.20 and 2.47 times the corresponding values in plants grown on the NFW- and MWW-irrigated soils, respectively.

As shown in Figure 5, the mean values of BAF of all observed PTMs did not exceed 1.0 in roots and shoots of plants grown on the three sites. Regarding metals' root-to-shoot transfer, mean values of TF for Cr, Co, and Ni were lesser than 1.0 in the three sampling sites (Figure 6). On the other hand, the mean values of TF for Cu, Pb, and Zn were found to be above 1.0.
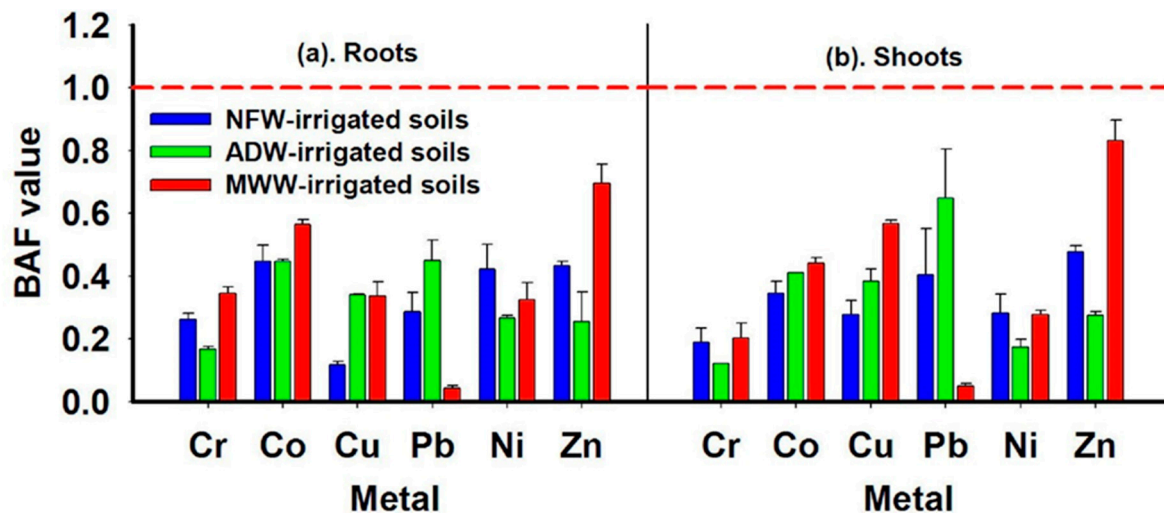
Figure 5. HM Bioaccumulation factor (BAC) of PTMs in berseem roots (**a**) and shoots (**b**).
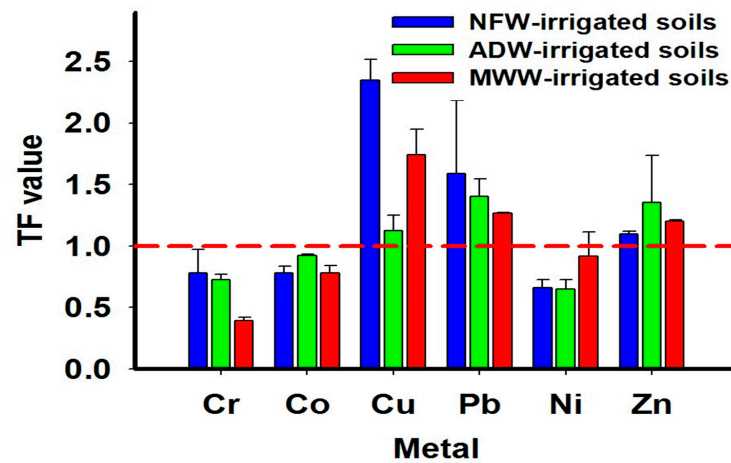

Figure 6. Metal translocation factor (TF) of berseem shoots.

3.5. Concentrations of PTMs in Groundwater

As shown in Figure 7, the concentrations of Cr and Co in the groundwater collected from the three sites were below the detection limits. In addition, the concentrations of Cu, Pb, Ni, and Zn were below the safe limits for drinking purposes, i.e., 0.20, 0.01, 0.07, and 3.0 mg L^{-1}, respectively [24]. The statistical analysis indicated no significant differences ($p < 0.05$) in metal concentration among the three sites.

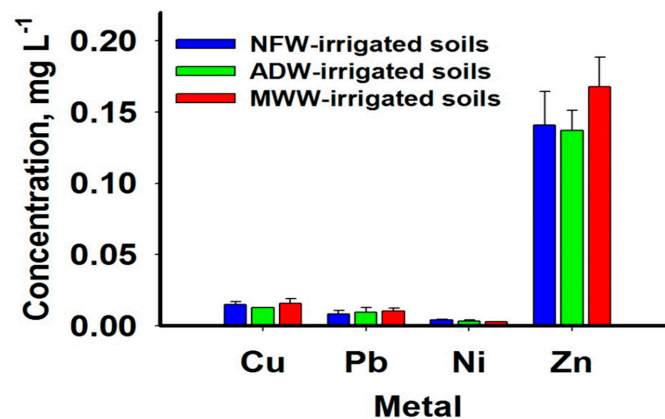**Figure 7.** Metal concentrations in groundwater.

4. Discussion

4.1. Concentrations of PTMs in Irrigation Water

Metal content in irrigation water plays an important role in determining water suitability for irrigation. Excessive quantities cause potential accumulation in irrigated soils and growing plants [38]. In the studied area, metal concentrations in the three water supplies (Table 2) indicated that they were suitable for irrigation. The concentrations of Cr, Co, and Cu in the three water supplies reflect the lower local anthropogenic inputs of these metals in agricultural and/or sewage drains. The higher concentration of Zn in the ADW compared with the NFW and MWW is probably due to leaching of agrochemicals (fertilizers and pesticides) from arable fields [6]. The raised concentrations of Pb and Ni in the three water supplies, in general, indicate that surface water bodies in the studied area undergo metal contamination due to the discharge of industrial and agricultural effluents [12].

4.2. Soil Contamination by PTMs

Soils can receive metals form various natural and/or anthropic sources [3]. In the studied soils, it is clear that soil parent rocks played a distinct role in the accumulation of Cr in the three sampling sites and Pb in the NFW-irrigated soils since their total concentrations did not exceed the ANC in the earth's crust (Table 3) [3]. On the other hand, man-made sources are likely to be the main driver for Pb in MWW-irrigated soils and Co, Cu, Ni, and Zn in the three sampling sites as their concentrations were above the ANC [4]. The abundance of PTMs in the studied soils was differing from those in irrigation waters, and this confirms that soils can sink contaminants from multiple sources [6]. Furthermore, metal accumulation in soils can be a sink for contaminants controlled mainly by key soil properties such as pH, clay, organic matter, and Fe-Mn oxides [44–46]. This hypothesis is confirmed by the higher total Co and Ni content in the NFW-irrigated soils. The NFW-irrigated soils contain higher Fe content compared with the ADW- and MWW-irrigated ones. Both Co and Ni have various oxidization states; thereby, Co (III) and Ni (III) often occur in isomorphous substitution of Fe (III) in the crystal structure of Fe oxides (goethite and hematite) [45,46]. The higher total Pb content in the MWW-irrigated soils is probably due to higher content in the MWW.

The DTPA-extractable fractions of Co (in the three sampling soils) and Cr (in the NFW- and ADW-irrigated soils) below the detection limit (Table 3) demonstrate that these metals are strongly bounded to less mobile fraction. In arid soils, Fe and Mn oxides can bind metals through inner-sphere complexes to unbounded oxygen atoms [47]. Generally, the orders of abundance of the DTPA-extractable fractions of the studied metals were far away from those for the total content. This in turn illustrates that soil properties and farming practices (chemical and organic fertilizers and pesticides) play key role in metal availability [39]. The MWW-irrigated soils showed higher concentrations of the DTPA-extractable contents of the studied metals, and thus plants grown on these soils may show higher metal content that those grown on the other two sites. This is because metal content in plants is closely related to the bioavailable fraction [48].

Results of Pearson's correlation coefficient (Table 4) indicate that ADW contributed mainly to enriching the soils with Cr and Pb, while the MWW contributed to Cr and Cu in the irrigated soils. On the other hand, the significant negative correlation between total Pb content in MWW-irrigated soils and MWW illustrates that other anthropic factors rather than irrigation water contributed mainly to Pb in soils [47]. Regarding the bioavailable metal content, the significant positive correlations between Ni and Pb in the NFW and irrigated soils indicate that NFW governed the availability of these metals in soils. In the same manner, the MWW controlled the Cu availability in irrigated soils [4]. On the other hand, the significant negative correlation between Ni content in MWW-irrigated soils and irrigation water demonstrate that Ni availability is regulated mainly by soil properties or farming practices [47].

Out of the six metals, only Cr in the three sampling sites showed a low contamination level, with values of C_f lower than 1.0. This affirms that the presence of Cr in the studied

soils resulted mainly from weathering processes and biochemical reactions in soils rather than wastewater irrigation [47]. The moderate contamination levels related to Cu, Ni, and Zn in the wastewater irrigation sampling sites indicate that soil contamination is associated mainly with human activities such as MWW irrigation (for Cu) and agrochemicals (for Ni and Zn). Values of C_f for Co exceeding 3.0 in the three sampling sites denote a considerable contamination level. In arid and semi-arid regions, Co contamination is mainly due to chemical weathering of ferromagnesian minerals, where Co (II) can substitute for Fe^{2+} and Mg^{2+} [47]. Pb, unlike the remaining metals, showed two different contamination levels, i.e., low contamination in the NFW- and ADW-irrigated soils but high contamination in the MWW-irrigated soils. This demonstrates that wastewater irrigation enriched the soils with Pb. The contamination degree in the MWW-irrigated soils was 1.98- and 2.34-fold higher than those of the NFW- and ADW-irrigated soils, respectively. This in turn reflects the great effect of irrigation water on metal accumulation in soils. Therefore, attention should be paid when growing food or feed crops on these contaminated soils. Values of Er showed that only Pb in the MWW-irrigated soils would pose potential risks. Thus, a suitable remediation strategy is necessary to alleviate Pb accumulation in crops and the food chain [48].

4.3. Availability Ratio (AR)

The AR is an index used for measuring the magnitude of metal supply in soils since it can determine metal transformations in soil environment [37]. In the studied soils, although the total contents of Cu, Ni and Zn in the three sampling sites surpassed the ANC, they showed low values of AR (Figure 4). These results suggest metal transformations from a loosely bound exchangeable fraction to strongly bound fractions, including hydrous oxides and organic matter-bound fractions [37]. Though the total Pb content in NFW- and ADW-irrigated soils are not perceived to be particularly high, the AR in these two sampling sites was higher than other metals. This may refer to recent soil contamination that has not yet allowed sequestering Pb strongly by the soil colloids [49]. On the other hand, higher AR of Pb in MWW-irrigated soils goes in line with the higher total Pb content than ANC, which indicates prolonged Pb enrichment in this site [50]. The significant positive correlations between the AR of Pb and Zn with clay in the NFW-irrigated soils demonstrate that clay minerals governed the transformations of these metals in soils. Pb and Zn are most reversibly adsorbed on 2:1 alumino-silicate clays due to their tendency to form outer-sphere linkages with the clay minerals, and thus they can be easily displaced by other cations [47]. Moreover, the significant positive correlation between AR of Pb with OM in the NFW-irrigated soils show that that the OM enhances Pb availability in the soil by supplying organic molecules to the soil solution as synthetic chelates [51]. The non-significant correlations between AR of all metals in the ADW- and MWW-irrigated soils (except Ni in the MWW-irrigated soils) reflect the recent uneven metal deposition in soils [37]. The significant positive correlation between AR of Ni and pH in the MWW-irrigated soils demonstrates that soil pH controlled Ni in soils since the mobility of Ni is low under neutral to alkaline soil conditions [52,53].

4.4. Berseem Plant Contamination by PTMs

Plants uptake their nutrients from soils; however, they are not selective enough to opt essential elements only but also absorb nonessential or even toxic elements [21]. The incept abundances in berseem roots (Table 6) collected from the three sampling sites indicate that Zn and Cu were the predominant metals, while Pb was the least prevalent. This is in line with the elemental requirements for plants since Zn and Cu are essential micronutrients. They are involved in physiological processes, i.e., enzyme activation, and thus were in higher concentrations than Cr, Co, Ni, and Pb, which are non-essential and phytotoxic [21]. Although the DTPA-extractable contents of Co (in the three sampling sites) in addition to Cr (in NFW- and ADW-irrigated soils) and Ni (in ADW-irrigated soils) were below the detection limits, they were found in considerable concentrations in root tissues. This goes

beyond the fact that a drop in the rhizosphere pH occurs when biological nitrogen (N_2) fixation is the primary source of N such as legumes and otherN_2-fixingplants. Such a decrease in pH results from the exchange of NH_4^+ for H^+ or uptake of NH_3, leaving H^+ behind [54]. As a result, a sizeable excess of metal uptake occurs with the concomitant acidification of the rhizosphere [55]. The rather similar magnitudes of metal accumulation in roots and soils demonstrate that the berseem plants can survive at high concentrations of essential and/or toxic metals [21,56]. Such ability is attributed mainly to the high biomass of shallow taproot system, and on the other hand the efficiency of berseem plants to reduce metal toxicity through several detoxification mechanisms on the other hand [57,58]. The lowest root Zn content in plants grown on ADW-irrigated soils might be due to the higher pH of these soils compared with those of the two other sites. An increase in the pH values higher than 7.0 increases the affinity of Zn to form strong bonds with Mn-Fe oxides, decreasing Zn availability to plants [59].

The concentrations of Co, Cu, and Ni in berseem shoots in the three sampling sites in addition to Pb in the MWW-irrigated soils exceeded the safe limits for animal feeding. As results, animal health in the studied region would suffer potential health risks that ultimately affect human health though food chain [21,48]. Therefore, it is not recommended to use berseem plants in the studied region for animal feeding but instead in other profitable purposes, such as phytomining. Phytomining is a concept in which valuable metals are recovered from hyper-accumulator plants to be used as a fuel or energy [21]. The orders of metal content in shoots (Table 6) were similar to that shown by roots in NFW-irrigated soils, but slightly different in ADW- and MWW-irrigated soils. However, they were also consistent with elemental requirements for plants [21]. Plants grown on the MWW-irrigated soils showed higher Pb contents in shoots compared with the two other sites. On average, the Pb increases represented 3.20 and 2.47 times the corresponding values in plants grown on the NFW- and MWW-irrigated soils, respectively. This is due to higher total and bioavailable Pb content in MWW-irrigated soils than that in the NFW- and ADW-irrigated ones.

When analyzing results of the BAF, it is clear that the studied metals showed low accumulation in both berseem roots and shoots. However, Galal [22] observed higher BAF for Cr, Co, Pb, Ni, and Zn than unity in berseem roots grown on metal-polluted soil in Egypt. In addition, Bhatti et al. [21] reported values of BAF > 1.0 for Cu and Zn in roots and shoots of berseem plants grown on alkaline sandy soils of Punjab, India. These findings illustrate that metal build-up in the studied soils was not high enough to cause considerable accumulation in berseem roots, probably due to high Fe-Mn oxides in soils, which play a great role in reducing their availability. This hypothesis was confirmed in some alluvial soils of Egypt where Fe-Mn oxides fraction was the abundant pool for Cu, Ni, Pb, and Zn [60].

Values of root-to-shoot transfer lower than 1.0 recorded for Cr, Co and Ni in the three sampling sites (Figure 6) indicate that roots can store these metals. These values are similar to those reported for Cr [22], and for Ni and Co [21]. Generally, legumes have exclusion mechanisms (binding to root cell walls and/or chelation by root exudates) by which they can sequester PTMs in roots and hinder their transfer to the aerial parts [54,55]. Therefore, the berseem plant has been known as a good candidate for phytofiltration to prevent the leaching of toxic metals to groundwater [57,58]. On the other hand, higher TF for Cu, Pb, and Zn than the unity demonstrates that these metals could be translocated to the shoots through xylem vessels, where they are stored in vacuoles [21]. These findings are similar to those reported in earlier studies for Zn [22], and Cu and Pb [21]. Usually, the xylem loading process in the non-metal accumulator plants is mediated by membrane transport proteins, in which metals must move symplastically to across this barrier [53]. However, metal translocation to shoots in legumes is facilitated mainly by complexing the metal with low-molecular-weight chelators, i.e., organic acids, phytochelatins, and histidine [51,53]. Therefore, legumes, especially berseem plants, have been known to have high potential for metal extraction from polluted soils [21,22,55]. The berseem plant produces multiple

harvests in the same season, which enhances its capability for phytoremediation [21]. The biomass produced could be used in phytomining purposes.

4.5. Concentrations of PTMs in Groundwater

Groundwater aquifers in the Egyptian rural communities are more susceptible to human activities, especially wastewater irrigation. This is because metal contaminants can easily percolates from soil and reach shallow aquifers [4,59–61]. Metal concentration in shallow aquifers is closely related to their corresponding contents in soils in addition to soil properties [61,62]. In groundwater samples collected from the three irrigated sits, the concentrations of Cr and Co were below the detection limits due to the low concentrations of these free metal ions in the studied soils. The concentrations of Cu, Pb, Ni and Zn in all groundwater samples remained below the WHO safe limit. However, the bioavailable concentrations of Cu (in all sampling sites) and Pb and Zn (in the MWW-irrigated soils) exceeded the safe limits in soils. These results suggest that the majority of metals released were mainly retained by soil colloids (clay, organic matter, and Fe-Mn oxides) or removed by the growing plants [39,62,63]. Although the metal concentrations in soil samples showed significant differences, all groundwater samples showed similar metal contents. This might be a result of relatively similar properties of all investigated soils, which governed metal migrations to the shallow aquifer.

5. Conclusions

The accumulation of six PTMs (Cr, Co, Cu, Pb, Ni, and Zn) in alluvial soils, berseem plants, and groundwater was observed in an area south-eastern the Nile Delta (Egypt) subjected to long-term wastewater irrigation, i.e., agricultural drainage water, and mixed wastewater in comparison with Nile freshwater irrigation. Total soil contents of Co, Cu, Ni, and Zn in the three sampling sites in addition to Pb in MWW-irrigated soils exceeded their ANC in the earth's crust, consequently increasing potential human risks. Co resulted in the highest contamination level in the NFW- and ADW-irrigated soils, while Pb posed the highest threat in MWW-irrigated soils. The NFW- and ADW-irrigated soils showed moderate and low contamination levels, while a considerable level was found in MWW-irrigated soils. Out of the six PTMs, Pb in MWW-irrigated soils showed moderate ecological risk, while the remaining showed low risks; therefore, the overall potential ecological risk in the three sites results was low, while the values of DTPA-extractable Cu, Pb, and Zn in MWW-irrigated soils exceeded safe limits. The metal availability ratio suggested abiotic transformation (redox processes) or binding processes in soil of Cr, Co, Cu, Ni, and Zn to less mobile fractions, i.e., Fe-Mn oxides-bound fraction. Moreover, a recent Pb deposition occurred in the NFW- and ADW-irrigated soils, but a historic enrichment occurred in MWW-irrigated soils. The contents of Co, Cu, and Ni in berseem shoots collected from the three sampling sites in addition to Pb collected from MWW-irrigated soils have been shown to be higher than the safe levels for animal feeding. Soil-to-plant BAF in shoots and roots was lower than 1.0, indicating that metal concentrations in soils were not high enough to be highly accumulated in plant tissues. Values of roots-to-shoots TF were <1.0 for Cr, Co and Ni, while >1.0 for Cu, Pb and Zn. These values indicate that berseem plants could be used for phytofiltration of Cr, Co, and Ni in polluted soils and for phytoremediation of Cu, Pb, and Zn in polluted ones. The groundwater samples collected from the three sampling sites showed lower concentrations of PTMs than the WHO permissible limits for drinking standards. Periodic monitoring of PTMs in water supplies, soils, and crops is essential to evaluate potential ecological risks. Moreover, future remediation studies are recommended to mitigate the accumulation of toxicants in food chain.

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