Ecological Risk Assessment of Heavy Metals along Three Main Drains in Nile Delta, and Potential Phytoremediation by Macrophyte Plants

Yasser A. El-Amier[1], Giuliano Bonanomi[2], Saud L. Al-Rowaily[3] and Ahmed M. Abd-ElGawad[1,3]*

[1] Department of Botany, Faculty of Science, Mansoura University, Mansoura 35516, Egypt; yasran@mans.edu.eg, dgawa84@mans.edu.eg
[2] Department of Agriculture, University of Naples Federico II, 80055, Portici, Naples, Italy; giuliano.bonanomi@unina.it
[3] Plant Production Department, College of Food & Agriculture Sciences, King Saud University, P.O. Box 2460 Riyadh 11451, Saudi Arabia; aibrahim2@ksu.edu.sa, srowaily@ksu.edu.sa
* Correspondence: aibrahim2@ksu.edu.sa, Tel.: +966562680864 (A.M.A-E.)

Supplementary Materials

Table S1. Pearson correlation matrix various sediment parameters and heavy metals from the three studied drains in Nile Delta.

Variables	Fe	Mn	Pb	Cr	Zn	Cu	Ni	Co	Cd	pH	EC	OM	$CaCO_3$	Sand	Silt
Mn	0.22														
Pb	0.65	-0.32													
Cr	-0.01	0.18	0.12												
Zn	-0.62	**-0.69**	-0.06	0.01											
Cu	-0.49	-0.34	-0.14	**-0.71**	0.36										
Ni	0.43	-0.42	0.32	**-0.71**	0.06	0.35									
Co	-0.26	-0.09	0.31	-0.05	0.44	0.43	-0.17								
Cd	-0.05	-0.05	-0.03	**0.89**	0.13	**-0.74**	-0.48	-0.38							
pH	-0.42	-0.21	0.00	0.49	0.54	-0.14	-0.51	0.42	0.42						
EC	-0.07	-0.11	-0.24	**-0.95**	0.05	0.63	0.56	0.11	**-0.85**	-0.39					
OM	0.43	-0.14	0.33	**-0.85**	-0.09	0.46	**0.81**	0.21	**-0.84**	-0.44	**0.78**				
$CaCO_3$	0.28	0.35	-0.41	-0.20	-0.20	-0.41	0.24	-0.48	0.02	-0.32	0.32	0.20			
Sand	-0.29	-0.32	-0.37	**-0.93**	0.24	**0.74**	0.61	-0.03	**-0.71**	-0.31	**0.90**	0.64	0.22		
Silt	0.24	0.40	0.15	**0.94**	-0.31	**-0.83**	-0.63	-0.22	**0.82**	0.32	**-0.91**	**-0.74**	-0.06	**-0.95**	
Clay	0.30	0.22	0.54	**0.83**	-0.16	-0.60	-0.54	0.25	0.55	0.28	**-0.81**	-0.49	-0.34	**-0.96**	**0.81**

Values in bold are significance at $p \leq 0.05$. OM: soil organic matter, EC: electric conductivity.

Table S2. Microelement concentrations (mg kg^{-1}) in roots and shoots of three studied emergent hydrophytes naturally growing along studied drains.

Plant species	Plant part	Drain	Metals								
			Fe	Mn	Zn	Cu	Cr	Co	Cd	Ni	Pb
Echinochloa stagnina	Root	(Drain 11)	2585.87	1564.19	50.28	33.17	11.76	10.48	8.66	63.13	16.65
		(Drain 9)	2761.67	1739.99	78.10	48.98	18.72	20.94	20.46	78.94	32.45
		(Drain 7)	2755.20	1733.52	71.63	42.50	12.25	14.47	13.99	72.47	25.98
		Mean	2700.91	1679.23	66.67	41.55	14.24	15.30	14.37	71.51	25.03
		±SE	57.55	57.55	8.41	4.59	2.24	3.05	3.41	4.59	4.59
	Shoot	(Drain 11)	326.40	1252.56	55.46	16.30	1.93	4.21	9.68	13.18	25.92
		(Drain 9)	275.09	1098.84	34.44	14.66	2.74	3.76	4.58	16.07	11.76
		(Drain 7)	331.29	1372.08	60.36	21.19	6.82	9.10	14.58	18.07	30.81
		Mean	310.93	1241.16	50.09	17.38	3.83	5.69	9.61	15.77	22.83
		SE	17.97	79.08	7.95	1.96	1.51	1.71	2.89	1.42	5.71
Phragmites australis	Root	(Drain 11)	2778.39	1756.71	94.81	65.69	35.44	37.66	37.18	95.65	21.16
		(Drain 9)	2813.09	1791.41	129.52	100.39	70.14	72.36	71.88	130.36	55.87
		(Drain 7)	2783.70	1762.02	100.12	71.00	40.75	42.97	42.49	100.96	26.47
		Mean	2791.73	1770.05	108.15	79.03	48.78	51.00	50.52	108.99	34.50
		±SE	10.79	10.79	10.79	10.79	10.79	10.79	10.79	10.79	10.79
	Shoot	(Drain 11)	2457.55	1435.87	47.98	5.51	1.16	2.17	6.50	3.30	2.33
		(Drain 9)	350.76	430.08	96.46	13.87	3.01	8.27	22.01	9.61	35.74
		(Drain 7)	2459.35	1437.67	49.78	7.31	1.38	3.97	8.30	5.10	4.13
		Mean	1755.89	1101.21	64.74	8.90	1.85	4.80	12.27	6.00	14.07
		±SE	702.56	335.56	15.87	2.54	0.58	1.81	4.90	1.88	10.85
Typha domingensis	Root	(Drain 11)	641.01	841.58	69.38	29.15	8.55	2.65	11.86	86.75	39.43
		(Drain 9)	664.64	865.22	93.02	52.79	32.19	26.29	35.50	110.38	63.07
		(Drain 7)	650.13	850.70	78.50	38.27	17.67	11.77	20.98	95.87	48.55
		Mean	651.93	852.50	80.30	40.07	19.47	13.57	22.78	97.67	50.35
		±SE	6.88	6.88	6.88	6.88	6.88	6.88	6.88	6.88	6.88
	Shoot	(Drain 11)	555.08	728.77	60.08	25.25	7.40	2.30	10.27	75.12	31.43
		(Drain 9)	473.71	303.43	26.45	21.59	16.55	16.92	16.84	26.59	18.84
		(Drain 7)	614.52	831.84	64.80	29.96	12.12	2.40	14.99	79.84	36.14
		Mean	547.77	621.35	50.44	25.60	12.02	7.21	14.03	60.52	28.80
		±SE	40.81	161.72	12.07	2.42	2.64	4.86	1.96	17.02	5.16

Table S3. Results of analysis of variance of various studied heavy metals in either roots or shoots of *Echinochloa stagnina*, *Phragmites australis*, and *Typha domingensis* collected from the three studied drains in Nile Delta.

Element	Effect	SS	df	MS	*F*	*P*
Fe	Species	8425504.60	2	4212752.3	16.88	**0.0003*** **
	Organ	6230391.17	1	6230391.2	24.97	**0.0003*** **
	Species × Organ	3963382.91	2	1981691.5	7.94	**0.0064** **
Mn	Species	2023828.70	2	1011914.3	13.62	**0.0008*** **
	Organ	895211.20	1	895211.2	12.06	**0.0046** **
	Species × Organ	143819.38	2	71909.69	0.97	0.4074
Zn	Species	2561.46	2	1280.73	3.68	0.0566^{ns}
	Organ	4036.51	1	4036.51	11.61	**0.0052** **
	Species × Organ	539.77	2	269.89	0.78	0.4819^{ns}
Cu	Species	690.51	2	345.25	3.43	0.0662^{ns}
	Organ	5915.09	1	5915.09	58.84	**<0.0001*** **
	Species × Organ	2652.34	2	1326.17	13.19	**0.0009*** **
Cr	Species	802.95	2	401.48	4.50	**0.0348***
	Organ	2098.66	1	2098.66	23.52	**0.0004*** **
	Species × Organ	1450.35	2	725.17	8.13	**0.0059** **
Co	Species	1219.32	2	609.66	6.01	**0.0156***
	Organ	1932.14	1	1932.14	19.04	**0.0009*** **
	Species × Organ	1467.77	2	733.88	7.23	**0.0087** **
Cd	Species	1172.46	2	586.23	5.54	**0.0197***
	Organ	1339.03	1	1339.03	12.66	**0.0039** **
	Species × Organ	1003.88	2	501.94	4.74	**0.0303***
Ni	Species	3829.69	2	1914.84	7.98	**0.0063** **
	Organ	19183.83	1	19183.83	79.92	**<0.0001*** **
	Species × Organ	3456.15	2	1728.07	7.20	**0.0088** **
Pb	Species	957.76	2	478.88	2.65	0.1117^{ns}
	Organ	975.79	1	975.79	5.39	**0.0386***
	Species × Organ	354.12	2	177.06	0.98	0.4040^{ns}

Notes: Tested effects included plant species and plant organs. For each tested effect, sum of squares (SS), degrees of freedom (df) mean squares (MS) and Duncan's test results (*F* and associated *s* value [significant values in boldface type]) are shown. Significance level fixed at *p*-values < 0.05. ***: significant at $p \leq 0.001$, **: significant at $p \leq 0.01$, *: significant at $p \leq 0.05$, ^{ns}: non-significant.

Table S4. Comparison among the three plant species (*Echinochloa stagnina*, *Phragmites australis*, and *Typha domingensis*) based on the bioaccumulation factors of root and shoot as well as the translocation factor.

Factor		Fe	Mn	Zn	Cu	Cr	Co	Cd	Ni	Pb
Root bioaccumulation factor (BAF)										
Echinochloa stagnina	Mean	0.058 ^{A}	2.130 ^{A}	0.093 ^{A}	0.169 ^{B}	0.493 ^{A}	1.558 ^{A}	2.860 ^{B}	2.517 ^{A}	1.199 ^{A}
	±SE	0.005	0.038	0.006	0.009	0.136	0.266	0.338	0.301	0.180
Phragmites australis	Mean	0.060 ^{A}	2.245 ^{A}	0.154 ^{A}	0.319 ^{A}	1.743 ^{A}	5.236 ^{A}	10.109 ^{A}	3.880 ^{A}	1.609 ^{A}
	±SE	0.005	0.004	0.014	0.011	0.536	0.955	1.067	0.517	0.292
Typha domingensis	Mean	0.014 ^{B}	1.081 ^{B}	0.115 ^{A}	0.161 ^{B}	0.728 ^{A}	1.443 ^{A}	4.514 ^{B}	3.456 ^{A}	2.373 ^{A}
	±SE	0.001	0.002	0.010	0.011	0.267	0.494	0.710	0.432	0.290
F-value		12.164	275.197	2.701	25.232	1.171	3.796	8.215	0.894	1.759
P-value		0.0077**	<0.0001***	0.1457 ^{ns}	0.0012 **	0.3721 ^{ns}	0.0860 ^{ns}	0.0191 *	0.4573 ^{ns}	0.2505 ^{ns}
Root bioaccumulation factor (BAF)										
Echinochloa stagnina	Mean	0.007 ^{A}	1.576 ^{A}	0.078 ^{A}	0.073 ^{AB}	0.103 ^{A}	0.533 ^{A}	1.957 ^{A}	0.541 ^{A}	1.097 ^{A}
	±SE	0.001	0.065	0.015	0.008	0.016	0.063	0.333	0.051	0.231
Phragmites australis	Mean	0.042 ^{A}	1.404 ^{A}	0.087 ^{A}	0.035 ^{B}	0.070 ^{A}	0.504 ^{A}	2.429 ^{A}	0.206 ^{A}	0.641 ^{A}
	±SE	0.011	0.251	0.006	0.004	0.025	0.138	0.520	0.041	0.276
Typha domingensis	Mean	0.012 ^{A}	0.792 ^{A}	0.081 ^{A}	0.107 ^{A}	0.418 ^{A}	0.806 ^{A}	2.812 ^{A}	2.153 ^{A}	1.374 ^{A}
	±SE	0.001	0.121	0.017	0.010	0.124	0.344	0.165	0.506	0.239
F-value		3.115	2.081	0.039	7.037	2.255	0.196	0.449	4.169	0.734
P-value		0.1181 ^{ns}	0.2058 ^{ns}	0.9620 ^{ns}	0.0267 *	0.1860 ^{ns}	0.8273 ^{ns}	0.6582 ^{ns}	0.0733 ^{ns}	0.5185 ^{ns}
Translocation factor (TF)										
Echinochloa stagnina	Mean	0.115 ^{B}	0.741 ^{A}	0.796 ^{A}	0.430 ^{AB}	0.289 ^{A}	0.403 ^{B}	0.795 ^{A}	0.221 ^{B}	1.035 ^{A}
	±SE	0.014	0.095	0.334	0.113	0.232	0.225	0.496	0.025	0.611
Phragmites australis	Mean	0.631 ^{AB}	0.624 ^{A}	0.583 ^{A}	0.108 ^{B}	0.037 ^{B}	0.088 ^{A}	0.225 ^{A}	0.053 ^{B}	0.302 ^{A}
	±SE	0.438	0.333	0.140	0.028	0.006	0.029	0.071	0.020	0.293
Typha domingensis	Mean	0.841 ^{A}	0.731 ^{A}	0.659 ^{A}	0.686 ^{A}	0.689 ^{A}	0.572 ^{A}	0.685 ^{A}	0.647 ^{A}	0.613 ^{A}
	±SE	0.118	0.334	0.325	0.244	0.176	0.338	0.197	0.352	0.274
F-value		6.0846	0.1630	0.4431	10.3529	11.4825	3.2809	2.8323	6.7596	2.2782
P-value		0.0360 *	0.8532 ^{ns}	0.6615 ^{ns}	0.0113 *	0.0089 **	0.1090 ^{ns}	0.1361 ^{ns}	0.0290 *	0.1836 ^{ns}

Different superscript letters within each element and factor mean values significance. Significance level fixed at *p*-values < 0.05. ***: significant at $p \leq 0.001$, **: significant at $p \leq 0.01$, *: significant at $p \leq 0.05$, ^{ns}: non-significant.

Table S5. Various pollution indices formulas used in the present study.

Index	Formula	References
Single indices of pollution		
Enrichment factor (E*f*)	$\mathbf{EF} = \left(\frac{\mathbf{C_{sample}}}{\mathbf{Fe_{sample}}}\right) / \left(\frac{\mathbf{C_{ref}}}{\mathbf{Fe_{ref}}}\right)$	Franco-Uria et al. (2009)
Contamination factor (C*f*)	$\mathbf{CF} = \boldsymbol{C_{sample} / C_{ref}}$	Hakanson (1980)
Geoaccumulation index (I*geo*)	$\boldsymbol{Igeo} = \mathbf{Log2}\left(\frac{\mathbf{C_{sample}}}{\mathbf{1.5Bn}}\right)$	Muller (1969); Lu and Bai (2010)
Ecological risk factor (E*r*)	$\mathbf{Er} = \mathbf{Ti} * \mathbf{Cf}$	Hakanson (1980)
Total complex indices (include integrated indices and indices of ecological risk)		
Degree of contamination (D*c*)	$\mathbf{Dc} = \sum_{\mathbf{i=1}}^{\mathbf{n}} \mathbf{CFi}$	Hakanson (1980); Caeiro et al. (2005)
Potential ecological risk index (PERI)	$\mathbf{PERI} = \sum_{\mathbf{i=1}}^{\mathbf{n}} \mathbf{ER}$	Kowalska et al. (2016)
Bioaccumulation factor (BAF)	$\mathbf{BAF_{shoot/root}} = \frac{\mathbf{Metal_{shoot/root}}}{\mathbf{Metal_{soil}}}$	Baker (1981)
Translocation factor (TF)	$\mathbf{TF} = \frac{\mathbf{Metal_{shoot}}}{\mathbf{Metal_{root}}}$	Baker (1981)

Abbreviation: C_{sample}: metal concentration in soil analyzed sample; Fe_{sample}: concentration of the reference metal in soil analyzed sample; C_{ref} : (background) metal concentration in the reference environment; Fe_{ref} (background), reference metal concentration in the reference environment; Bn: the geochemical background value in average shale of element *n*; 1.5: the background matrix correction due to terrigenous effects; Ti: the toxic-response factor for a given substance; Cf: the contamination factor.

Table S6. Classes of used indices for metals in the present study.

Index	Value	Soil quality	Ecological risk
	EF < 2 = natural, EF > 2 = anthropogenic		
	Ef < 1	Depletion or no enrichment	
	Ef < 2	Minor enrichment	
E*f*	Ef = 2-5	Moderate enrichment	
	Ef = 5-10	Moderately severe enrichment	
	Ef = 10-25	Severe enrichment	
	Ef = 25-50	Very severe enrichment	
	Ef > 50	Extremely severe enrichment	
	CF < 1	Low contamination factor	
C*f*	1 ≤ CF ≤ 3	Moderate contamination factor	
	3 ≤ CF ≤ 6	Considerable contamination factor	
	6 ≤ CF	Very high contamination factor	
	DC < 8	Low DC	
D*c*	8 ≤ Dc < 16	Moderate DC	
	16 ≤ Dc < 32	Considerable DC	
	Dc > 32	Very high	
	I*geo* ≤ 0	Uncontaminated	
	0 < I*geo* < 1	Uncontaminated to moderately contaminated	
	1 < I*geo* < 2	Moderately to heavily contaminated	
I*geo*	2 < I*geo* < 3	Moderately to strongly contaminated	
	3 < I*geo* < 4	Strongly contaminated	
	4 < I*geo* < 5	Strongly to extremely contaminated	
	I*geo* > 5	Extremely high contaminated	
	Er < 40		Low ecological risk
	40 ≤ Er < 80		Moderate ecological risk
E*r*	80 ≤ Er < 160		Considerable ecological risk
	160 ≤ Er < 320		High ecological risk
	Er ≥ 320		Very high ecological risk
	PERI < 150		Low risk
PERI	150 ≤ PERI < 300		Moderate
	300 ≤ PERI < 600		Considerable
	PERI ≥ 600		Very high.

Abbrivation: Enrichment factor (EF), Contamination factor (CF), Degree of contamination (Dc), Geoaccumulation index (I*geo*), Ecological risk factor (Er) and Potential ecological risk index (PERI)