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Rubbia Maritime (L.) as a Phytoremediator for Uptaking and Translocating Toxic Heavy Metals from Alasfar Contaminated Wastewater at Al-Ahsa, Saudi Arabia

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***Abstract* – *Rubbia maritime* (L.) a halophyte submersed plant of the family *Potamogetonaceae*, was evaluated as a phytoremediator for removal of the heavy metals from Lake Alasfar (Saudi Arabia) contaminated wastewater. Lake Alasfar wastewater, eastern Saudi Arabia is contaminated with heavy metals. The plant accumulated in its lower parts (roots) heavy metals of Cr, Mn, Zn, Cd, Pb, and in its stem Cr, Zn, Cd at high levels above that suggested by the Food and Agriculture Organization and the World Health Organization (FAO/WHO, 2007), and also accumulated P and K at very high levels. The transfer factor (TF) of heavy metals and elements from soil to the plant parts was high as regards P, K, Cr, Mn, Fe, Zn, Cd and Pb. Also the transfer ratio (TR) between the different plant parts was high, it is high for Na, P, K, Zn from root to the stem. There was correlation between the different plant parts and water and soil. There is a positive correlation between water and the lower parts of the plant for concentration of Cu, and between soil and stem in Na, and between soil and lower plant parts in, Na and Cd,, and a correlation between lower plant parts and stem in concentrations of Na, Cr, Fe, Cd, Pb. Rubbia maritime is thus capable of accumulating the heavy metals Cr, Mn, Zn, Cd, Pb, at high levels, and is recommended as phytoremediator for cleaning Lake Alasfar wastewater from the contaminating heavy metals, and the species contains high concentrations of heavy metals so animals should be kept away from grazing on it..**

***Keywords* – Phytoremediation, Rubbia Maritime, Heavy Metals.**

I. Introduction

Phytoremediation is considered an effective, low cost, preferred cleanup option for moderately contaminated areas. Phytoremediation is an emerging technology that employs the use of higher plants for the cleanup of contaminated environments. Fundamental and applied research have unequivocally demonstrated that selected plant species possess the genetic potential to remove, degrade, metabolize, or immobilize a wide range of contaminants. Plants through several natural biophysical and biochemical processes, such as adsorption, transport and translocation, hyperaccumulation or transformation and mineralization, can remediate pollutants (Meagher, 2000).

The immersed and floating water plants can absorb heavy metals from bottom sediments via their root systems, if they have one, or via their whole surface directly from water. It was confirmed that concentrations of the analyses accumulated in water plants are correlated with their concentrations in water and/or bottom sediments (Guilizzoni, 1991). It is assumed that the process of sorption of metals via the surface of water plants by ions exchange (Schneider et al., 2001; André et al., 1999).

The presence of heavy metals in water and soil even in trace amounts can cause serious problems to all organisms, and heavy metal bioaccumulation, especially in the food chain, can be highly dangerous to human health (Islam et al., 2007). Concentration of Pb, Zn, Cd, Ni, Cr and Cu in shoots and roots of plants grown in contaminated soil were significantly higher as compared to the plants grown in uncontaminated soil (Singh and Agrawal, 2007). Plant roots take up metal contaminants and/or excess nutrients from growth substrates through rhizofiltration (root) process, the adsorption, or, precipitation onto plant roots or absorption into the roots of contaminants that are in solution surrounding the root zone. This process is for metals, excess nutrients, and radionuclide contaminants in groundwater, surface water, and wastewater medium (Kähkönen and Manninen, 1998).

In general, the uptake mechanism is selective, plants preferentially acquiring some ions over others, ion uptake selectivity depends upon the structure and properties of membrane transporters, these characteristics allow transporters to recognize, bind and mediate the trans-membrane transport of specific ions (Lasat, 2000). Phytoextraction involves the absorption of contaminants by roots followed by translocation and accumulation in the aerial parts, and mainly applied to metals (Cd, Ni, Cu, Zn, Pb) and organic compounds (Van der Ent et al., 2013). Phytofiltration uses plants to absorb, concentrate and/or precipitate contaminants, particularly heavy metals, from an aqueous medium through their root system or other submerged organs (Frers, 2009).

The aim of present study is to assess treatability potential of submersed macrophyte *Rubbia maritime* (L.), family (*Potamogetonaceae*) as a phytoremediator for heavy metals in the wastewater of Lake Alasfar wetland in the eastern part of Saudi Arabia.

II. Materials and Methods

Alasfar Lake Region is in Al-Ahsa Province in the southern eastern corner of the eastern region of Saudi Arabia, 13 km east of Al-Ahsa and extends between Latitudes 25^0 05’ and 25^0 40’ north and between Longitudes 49^0 10’ and 49^0 55’ east , and rises about 109 m above sea level (Al-Taher, 1999). It is one of the shallow lakes with moistened soils and of most importance in the eastern region of Saudi Arabia. Its water is polluted

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by heavy metals, and a number of water plant species dominate its water, of which is *Rubbia maritime* (L.).

Analysis of heavy metals in plant, water and Soil

Four plant samples were collected from each of the three sites chosen, and were separated into the roots, the stems and the leaves. Washed thoroughly with distilled water, and dried and ground, and plant extracts were made out of it. Then the concentration of heavy metals was determined from each of these three plant parts (roots, stems and leaves) using the absorption spectroscopy and was estimated as ppm. Soil samples were collected from three pits in each plant site, dried, digested and heavy metals were determined. Samples of water were collected from the same plant sites chosen, and heavy metal concentrations were determined.

The heavy metals and nutrients determined are Fe, Cu, Pb, Mn, Cd, Cr, Na, Ca, K, Mg and P using the absorption spectroscopy as ppm, and P was determined using An Inductively Compelled plasma-atomic emission spectrophotometer IL-Plasma200 according to method of (Allen et al., 1974).

Estimation of the transfer factor (TF) and transfer ratio (TR) for heavy metals

Heavy metals transferred from the soil to the different plant parts were estimated according to (Chamberlin, 1983) equation.

$$TF = \frac{\text{Concentration of an element in the plant body (ppm)}}{\text{concentration of the same element in the soil at the same site}}$$

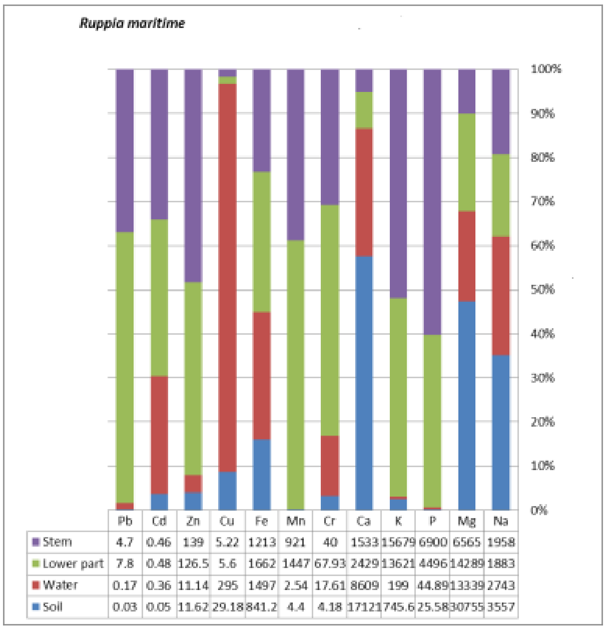
The TR is estimated to determine whether the plant is capable in transferring nutrients and heavy metals from the root to the shoot according to (Kim et al., 2003).

$$TR = \frac{\text{Concentration of an element in the shoot (ppm)}}{\text{concentration of the same element in the root (p}}$$

III. Results

Heavy metals and nutrient concentration in the different parts of rubbia maritime and in soil and alasfar lake water

The results in table (1) and figure (1) shows significant differences of all heavy metals and elements between the different plant parts and soil and water. The soil is characteristically high in contents of Na, Mg and Ca reaching 3557.20, 30755.49 and 17121.07 ppm respectively, and with low values in P, Cr, Fe, Cd and Pb reaching 25.58, 4.18, 841.24, 0.05 and 0.03 ppm respectively. In lake water Cu is high (294.99 ppm) and low values of K, Mn and Zn giving 199.02, 2.54 and 11.14 ppm respectively, and the lower parts (root) values are high in each of Cr, Mn, Fe, Cd and Pb giving 0.13, 67.93, 1446.89, 1662.35, 0.48 and 7.80 ppm respectively, but Na value is low 1882.83 ppm. Stem accumulations are high in P, K and Zn with 6900.01, 15678.90 and 139.02 ppm, respectively, and low in Mg, Ca and Cu with 6565.29, 1533.36 and 5.22 ppm, respectively.



	Pb	Cd	Zn	Cu	Fe	Mn	Cr	Ca	K	P	Mg	Na
Stem	4.7	0.46	139	5.22	1213	921	40	1533	15679	6900	6565	1958
Lower part	7.8	0.48	126.5	5.6	1662	1447	67.93	2429	13621	4496	14289	1883
Water	0.17	0.36	11.14	295	1497	2.54	17.61	8609	199	44.89	13339	2743
Soil	0.03	0.05	11.62	29.18	841.2	4.4	4.18	17121	745.6	25.58	30755	3557

Fig. 1. values of heavy metals and nutrient elements in plant parts of R. maritime and in water and soil samples.

Transfer factor (TF) and transfer ratio (TR) in R. maritime

The results of (Table 2) showed that metals of P, K, Cr, Mn, Fe, Zn, Cd, and Pb gave TF (absorption) more than one between all plant parts and the soil, and as is known TF is the division of the concentration of the element in the plant part by its concentration in the soil. As for the TR which is the division of the metal concentration in the stem by that in the root, Na, P, K, and Zn gave high values in their transference from root to stem i.e., K, Mn and Zn accumulate more in the stem than in the root.

Correlation between contents of heavy metals and elements in stem of r. Maritime and water

The results in Table (3) of Pearson simple linear correlation coefficient (r) between heavy metals and elements in water and stem illustrated a negative significant correlation for Fe (Fe=-0.879*), Zn (Zn=-0.813*) and Pb (Pb=-0.854*).

Table (1): Means of concentrations of heavy metals and nutrients of water and soil samples and R. maritime plant parts (first line) and standard error ± (second line) and F-Value based on ANOVA.

Element (ppm)	Soil	Water	Lower part	Stem	F-Value
Na	3557.20	2743.10	1882.83	1957.57	6.549**
	±457.74	±1409.98	±220.03	±100.20	
Mg	30755.48	13338.66	14288.98	6565.29	202.985***
	±2355.70	±329.83	±2221.84	±1371.16	
P	25.58	44.89	4496.44	6900.01	230.955***
	±2.08	±26.91	±510.57	±974.42	
K	745.59	199.02	13621.43	15678.90	105.710***
	±126.36	±63.48	±2639.11	±2655.59	
Ca	17121.07	8608.69	2429.23	1533.36	322.952***
	±816.57	±1722.09	±287.22	±356.06	
Cr	4.18	17.61	67.93	40.00	273.418***
	±0.14	±3.68	±5.89	±4.48	
Mn	4.40	2.54	1446.89	921.02	174.433***
	±0.49	±1.38	±239.16	±114.26	
Fe	841.23	1496.64	1662.35	1213.05	8.472***
	±116.29	±354.43	±431.81	±201.79	
Cu	29.18	294.99	5.60	5.22	37.253***
	±2.49	±113.34	±1.69	±1.84	
Zn	11.62	11.14	126.47	139.02	140.009***
	±0.84	±5.83	±15.32	±24.02	
Cd	0.05	0.36	0.48	0.46	8.324***
	±0.04	±0.20	±0.22	±0.14	
Pb	0.03	0.17	7.80	4.70	26.317***
	±0.01	±0.06	±3.05	±1.92	

Correlation between contents of heavy metals and elements in lower parts (root) and water

The results in Table (4) of Pearson simple linear correlation coefficient (r) showed negative significant correlation between heavy metals and elements in water and plant lower parts, for Fe (Fe=-0.898*) and a positive correlation for (Zn=0.904*).

Correlation between contents of heavy metals and elements in plant stem and soil

The results in Table (5) of Pearson simple linear correlation coefficient (r) between heavy metals and elements in plant stem and soil illustrated a positive significant correlation for Na (Na=0.904*).

Correlation between contents of heavy metals and elements in plant parts below soil surface and soil

The results in Table (6) of Pearson simple linear correlation coefficient (r) between heavy metals and elements in plant parts below soil surface and soil illustrated a positive significant correlation for Na (Na=0.915*) and Cd (Cd=0.825*).

Correlation between contents of heavy metals and elements in plant stem and root

The results in Table (7) of Pearson simple linear correlation coefficient (r) between heavy metals and elements in plant stem and plant root (parts below soil surface) illustrated a positive significant correlation for Na (Na=0.999**), Cr (Cr=0.814*), P (P=0.826**), Fe (Fe=0.0.955**), Cd (Cd=0.960**), and Pb (Pb=0.984**).

IV. Discussion

Rubbia maritime (L.) is a halophyte submersed plant of the family *Potamogetonaceae*, the results carried out on this species table (9) showed that *R. maritime* plant accumulated in its lower parts (roots) heavy metals of (Cr=67.93 mg/kg), (Mn=1447 mg/kg), (Zn=126.5 mg/kg), (Cd=0.48 mg/kg) and (Pb=7.8 mg/kg), and in its stem (Cr=40 mg/kg), (Zn=139 mg/kg), and (Cd=0.48 mg/kg), and all these heavy metal concentrations are above the permissible levels suggested by the Food and Agriculture Organization and the World Health Organization (WHO /FAO, 2007) table (9).

Table (2): Values of TF and TR in *R. maritime*

ppm	TF		TR
	Stem	**Lower part**	**Stem**
Na	0.55	0.53	1.04
Mg	0.21	0.46	0.46
P	269.74	175.78	1.53
K	21.03	18.27	1.15
Ca	0.09	0.14	0.63
Cr	9.57	16.25	0.59
Mn	209.32	328.84	0.64
Fe	1.44	1.98	0.73
Cu	0.18	0.19	0.93
Zn	11.96	10.88	1.10
Cd	9.20	9.60	0.96
Pb	156.67	260.00	0.60

Table (3): Pearson correlation coefficient (r) between heavy metals and elements in stem *R. maritime* and water. Significant correlation p ≤ 0.05* p ≤ 0.01** p ≤ 0.001*** .

Ruppia maritima		**Lower part (ppm)**									
		Na	**P**	**K**	**Ca**	**Cr**	**Mn**	**Fe**	**Cu**	**Cd**	**Pb**
water (ppm)	**Na**	.480	-.839*	.844*	.672	-.828*	.061	.350	-.610	-.213	-.179
	Mg	-.892*	.585	-.517	.387	-.019	.175	.346	-.080	-.835*	.547
	P	.345	-.377	.493	-.358	.349	-.850*	-.550	-.843*	-.155	-.628
	K	.826*	-.508	.587	-.544	.274	-.772	-.494	-.522	.433	-.699
	Mn	.608	-.912*	.872*	.223	-.316	-.286	-.356	-.479	.253	-.712
	Fe	.413	-.032	.029	-.900*	.820*	-.650	-.898*	.086	.710	-.688
	Cu	-.187	.342	-.454	.136	-.134	.608	.262	.904*	.461	.356
	Zn	.743	-.820*	.777	-.213	.040	-.452	-.619	-.394	.484	-.838*
	Cd	.637	-.908*	.903*	.449	-.685	-.027	.196	-.644	-.096	-.296
	Pb	.706	-.908*	.841*	.135	-.266	-.276	-.425	-.289	.509	-.777

Table (5): Pearson correlation coefficient (r) between heavy metals and elements in *R. maritime* stem and soil. Significant correlation p ≤ 0.05* p 0.01** p ≤ 0.001***

Ruppia maritima		**stem (ppm)**			
		Na	**Mg**	**Cd**	**Pb**
Soil (ppm)	**Na**	.904*	.795	.802	-.660
	Mg	.905*	.794	.803	-.662
	K	.900*	.790	.810	-.660
	Ca	-.897*	-.532	-.819*	.832*
	Mn	-.887*	-.765	-.817*	.622
	Fe	-.907*	-.794	-.801	.666
	Cu	.845*	.729	.823*	-.525
	Zn	-0.958	-.843*	-.469	.676
	Cd	.903*	.804	.802	-.670
	Pb	-.893*	-.809	-.806	.655

Table (6): Pearson correlation coefficient (r) between heavy metals and elements in *R. maritime* lower parts and soil. Significant correlation p ≤ 0.05* p ≤ 0.01** p ≤ 0.001***

Ruppia maritima		**Lower part (ppm)**			
		Na	**P**	**K**	**Cd**
Soil (ppm)	**Na**	.915*	-.666	.613	.822*
	Mg	.916*	-.666	.614	.822*
	K	.912*	-.658	.604	.829*
	Ca	-.915*	.625	-.637	-.740
	Cr	.815*	-.492	.431	.739
	Mn	-.901*	.617	-.567	-.830*
	Fe	-.918**	.670	-.618	-.820*
	Cu	.859*	-.532	.474	.834*
	Zn	-.950**	.912*	-.878*	-.504
	Cd	.914*	-.675	.620	.825*
	Pb	-.904*	.662	-.602	-.834*

Table (8): Pearson correlation coefficient (r) between heavy metals and elements in *R. maritime* stem and lower parts. Significant correlation p ≤ 0.05* p ≤ 0.01** p ≤ 0.001***

Ruppia maritima		**Lower part (ppm)**									
		Na	**P**	**K**	**Ca**	**Cr**	**Mn**	**Fe**	**Zn**	**Cd**	**Pb**
Stem (ppm)	**Na**	.999**	-.833*	.825*	-.202	-.171	-.372	-.269	-.483	.555	-.644
	Cr	-.391	.728	-.722	-.761	.814*	-.053	-.287	.107	-.031	.246
	Mn	-.211	-.061	.036	.885*	-.902*	.721	.923**	.677	-.433	.640
	Fe	-.406	.230	-.278	.699	-.713	.896*	.955**	.903*	-.474	.869*
	Zn	-.415	.510	-.492	.289	-.352	.659	.879*	.767	-.528	.937**
	Cd	.625	-.218	.163	-.670	.470	-.374	-.665	-.459	.960**	-.627
	Pb	-.749	.751	-.754	.289	-.178	.714	.753	.832*	-.573	.984**

IJAIR

International Journal of Agriculture Innovations and Research
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Table (9): Rate of concentration of nutrient elements and heavy metals in *R. maritime* in (mg/kg) and the standards permissible by (WHO/FAO, 2007), and the toxic limits for water (in mg/L) suggested by (FAO, 1985) and soil (in mg/kg) and toxic limits by (EU, 2002).

Elment	Water ASafer lake	FAO standard Cytotoxi Range (Mg/l)**	Soil ASafer lake	Eu Standards Cytotoxi Rang (Mg/Kg)***	Stem	Lower part	FAO standard Cytotoxi Rang (Mg/kg)*
Na	2743	-	3557	-	1958	1883	-
Mg	13339	-	30755	-	6565	14289	-
P	44.89	-	25.58	-	6900	4496	2000
K	199	-	745.6	-	15679	13621	10000
Ca	8609	-	17121	-	1533	2429	-
Cr	17.61	0.1	4.18	150	40	67.93	5
Mn	2.54	0.2	4.4	-	921	1447	1000
Fe	1497	5	841.2	-	1213	1662	-
Cu	295	0.2	29.18	140	5.22	5.6	40
Zn	11.14	2	11.62	300	139	126.5	50
Cd	0.36	0.01	0.05	3	0.46	0.48	0.2
Pb	0.17	0.5	0.03	300	4.7	7.8	5

Table (10): Pearson correlation coefficient (r) between heavy metals and elements in soil and water and different parts of *R. maritime*. Significant correlation $p \leq 0.05$* $p \leq 0.01$** $p \leq 0.001$***

	Stem (ppm)	Lower part (ppm)
Water (ppm)	Pb, Zn, Fe	Fe, Cu
Soil (ppm)	Na	Cd, Na
Stem (ppm)	-	Fe, Cr, Pb, Na, Cd

These metals are considered harmful according to the Agriculture and Food Organization, and the main source of these heavy metals is lake Alasfar wastewater with concentrations of Cr=17.61 mg/kg, Mn=2.54 mg/kg, Fe=1497 mg/kg, Zn=11.14 mg/kg, Cd=0.36 mg/kg and Cu=295 mg/kg and all these concentrations are above the permissible levels put forward by (FAO, 1985), so Lake Alasfar wastewater is polluted with heavy metals. It is observed that the plant accumulated P and K in its different parts at rates above the permissible level which is (2000 mg/kg) for P and (10000 mg/kg) for K, and this is mostly because these elements are movable inside the plant and so their concentrations increase inside plant tissues (Al-Wihaibi,2007). Also it is observed that the transfer factor (TF) of elements from soil to the different plant parts was high as regards P, K, Cr, Mn, Fe, Zn, Cd and Pb, and this is because the plant is good in selection and absorption of elements, particularly heavy metals, so can be considered a hyperaccumulator. Also the transfer ratio (TR) was high between the different plant parts, it is high for Na, P, K and Zn from the root to the stem and this is due to variation of elements movement inside the plant.

From table (10) can be seen that there is correlation between water, soil and the different plant parts in concentration of heavy metals and elements. There is a positive correlation between water- root (parts under soil level) in Cu concentration, soil - stem in Na, and soil–root in Na and Cd concentration, and between stem – parts under soil level (root) in concentration of Na, Cr, Fe, Cd and Pb. So there is correlation between the different plan parts themselves in the following metals (Cu=0.904 ppm), (Na=0.999 ppm), (Cd=0.960 ppm), (Pb=0.984 ppm) (Cr=0.814 ppm), and this can be attributed to the presence of this plant under water level.

The results of the analysis of the metals and elements in this study showed variations in their accumulation rates and this correlated with the magnitude with which that metal or element was absorbed from soil and water, and the plant has the ability to select specific metals and elements and reject others with high efficiency (Lasat, 2000). Phosphorus (P) was accumulated in high concentration in the upper parts of the plant and also K in the stem.

V. Conclusion

Lake Alasfar wastewater is contaminated with the heavy metals of Cr, Mn, Fe, Cu, Zn and Cd and the concentration of all these heavy metals are far above the standards put forward by (FAO, 1985). *Rubbia maritime* was found to accumulate in its lower parts (roots) heavy metals of Cr, Mn, Zn, Cd, Pb, and in its stem Cr, Zn, Cd and all these heavy metal concentrations are above the permissible levels suggested by the Food and Agriculture Organization and the World Health Organization (FAO/WHO, 2007) .The plant also accumulated P and K in its parts at rates above the permissible level. The transfer factor (TF) or absorption of heavy metals and elements from soil to the different plant parts was high as regards P, K, Cr, Mn, Fe, Zn, Cd and Pb. Also the transfer ratio (TR) between the different plant parts was high, it is high for Na, P, K, Zn from root to the stem. There was correlation between the different plant parts and water and soil. There is a positive correlation between water and the lower parts of the plant for concentration of Cu, and between soil and stem in Na, and between soil and lower plant parts in, Na and Cd, and a correlation between lower plant parts and stem in concentrations of Na, Cr, Fe, Cd, Pb. Generally *Rubbia maritime* accumulated the heavy metals Cr, Mn, Fe, Cu, Zn and Cd at very high levels and is thus recommended a good bio-remediator for cleaning wastewater of Lake Alasfar from toxic heavy metals, and animals are not allowed to graze on it.

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