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Evaluation of Algae Dry Biomass as a Biochemical Soil Remediation for Polluted Soil

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ABSTRACT

A pot experiment was carried out to evaluate the effect of two seaweeds(*Ulva sp.*(green algae) and *Gelidium sp.*(red algae)) on remediation of a contaminated soil with heavy metals (Fe, Zn, Mn and Pb ions) and to assess their effects on growth of lettuce *(Lactuca sativa*) roots and shoots contents of heavy metals. Surface soil samples (0-30cm) were collected from El-Gable El-Asfer farm located 25km Northeast Cairo, Egypt. The experiment involved 21 pots comprised 7 treatments with three replicates in a completely randomized design. Two types of algae dry biomass was chopped to obtain two particle of size (0.125 and 0.08mm) were mixture with soil at different rates of 0.2, 0.4, and 0.6 ton hectare^{-1}., before harvest. Extractable amounts of heavy metals were decreased with increasing rates of applied algae dry biomass compared with the control. The lower values of extractable heavy metals were obtained when application of mixture algae dry biomass at rat of 0.6 ton hectare^{-1} with particles of size 0.08 mm after harvesting than that particles of size 0.125 mm compared with the control. The results suggested that the important role of application of seaweeds dry biomass at lower particles of size 0.08mm for remediation soil contaminated with heavy metals on the contrary lower uptake of heavy metals by lettuce shoots in contaminated soils than in roots with the control., The study recommends using a mixture of green and red algae dry biomass at particles of size 0.08 mm and rat of 0.6 ton hectare^{-1}, with the need to avoid plants that are eaten leaves or roots growing in the soil contaminated with heavy metals.

Key words: soil remediation, seaweeds, algae dry biomass, heavy metals, lettuce (*Lactuca sativa*).

Introduction

Heavy metals contamination has become a global issue of great concern due to their higher toxicities, higher bioaccumulation in human body and food chain, nature of non-biodegradability, and most likely carcinogenicities to humans. Iron, zinc, manganese, Lead, mercury, chromium, arsenic, cadmium, copper and nickel are the most common contaminants found in contaminated surface water and soil as well as industrial wastewater, (Sheng *et al.*, 2008). Adsorption is the one of the important procedure for the removal of the traces heavy metals from the environment. The main properties of the adsorbents for heavy metal removal are strong affinity and high loading capacity. Natural adsorbents have generally these properties, (Barbier *et al.,* 2000).

Biosorption is a sorption process, where biomaterial or biopolymer is engaged as sorbent, was found to be concentrated by several algae,(Sheng *et al.,* 2007).The basic biochemical constitution of the marine algae is responsible for their adsorption performance (Davis *et. al.,*2003 and Ahmady *et al.,*2008).

The mechanisms for biosorption are presented. Biosorption is a complicated process involving ion-exchange, complexation and coordination. Among various biosorbents reported in the literature, marine algal biomass is identified as a promising biosorbent, in view of their high uptake capacities, low cost, renewability as well as the ready abundance of the biomass in many parts of the world's oceans. The global harvest of seaweeds for food and algal products (e.g., agar, alginate, and carrageenan) is over 3 million tons annually, with potential harvests estimated at 2.6 million tons for red algae and 16 million tons for brown algae (Chen 2012 and Ibrhim 2011).

Many studies have shown the inactive, non-living(dead) biomass may be even more effective than active (living) one in removal of heavy metals and is based on metal sorption due to the high affinities between the metal ions and the biomass,(Wan Maznah *et al.,*2012 and Zakhama *et al.,*2011).

The results suggest that these algae may be employed in a metal removal/recovery process at low cost, (Deng *et al.,*2009).The biosorption capacity of these algae for heavy metals resides mainly in a group of linear polysaccharides known as alginates that occur as a gel in the algal thallus, (Davis and Mucci 2003).

Alginic acid and some sulfated polysaccharides such as fucoidan are important.These compounds contain functional groups such as amino, carboxyl, sulfate, and hydroxyl which play important roles in the biosorption. Red algae contain cellulose in the cell wall, but their biosorption capacities are attributed mainly to the presence of sulfated polysaccharides made of galactans, (Lim *et al*., 2008 and Chen 2012).

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The marine algae have high metal binding capacities due to the presence of polysaccharides, proteins and lipids in the cell wall structure which containing functional groups such as; amino, carboxyl, hydroxyl and sulphate, that act as binding sites for heavy metals, (JinSong and Chen 2014).

More specifically, it is the properties of cell wall constituents, such as alginate and fucoidan, which are mainly responsible for heavy metal sequestration. Typically, the algal cell walls of brown algae, red algae and many green algae are comprised of a fibrillar skeleton and an amorphous embedding matrix .Furthermore, the coordination or complexation formation is also observed in binding of heavy metals by alginate and sulfated polysaccharides (fucoidan) (Davis *et. al.,* 2003). It was reported that the affinity of metal ions to alginate or fucoidan was related to the stereochemical effects.Sulfated polysaccharides (galactanes) in the red algae were also found to be mainly responsible for the complexation formation of metal ions, (Romera *et al.,*2007, Kooh *et al.,*2013. and Prosenjit *et al.,*2015).

The present investigation was carried out to evaluate the efficiency of two seaweeds dry biomass on remediation of a contaminated soil with heavy metals ions and to assess their effects on growth of lettuce (roots and shoots) contents of heavy metals.

Materials and Methods

A pot experiment was carried out at the experimental farm of Faculty of Agriculture, Al-Azhar University, and Cairo, Egypt during the winter season of 2014.

Soil samples:

Surface soil samples 0-30cm were collected from El-Gable El-Asfer farm located at 25km Northeast Cairo, Egypt, this soil is irrigated continuously with sewage effluent for about 81 years. Soil sample was air dried and then ground to pass through a 2mm sieve. Some physical and chemical analysis was carried out according to the standard methods undertaken by (Klute 1986) and (Page *et. al.,* 1982). Also, extractable Fe, Zn, Mn and Pb ions were determined in the soil using ammonium bicarbonate-DTPA extractable according to (Soltanpour and Schwab, 1977), and their contents in the obtained extract were measured by atomic absorption spectrophotometer.

Some physical and chemical, properties of the studied soil are presented in Tables 1-3.

Table 1: Mechanical analysis of soil sample.

Particle size distribution (%)				
Coarse sand	Fine sand	Silt	Clay	Textural class
51.41	21.31	12.72	14.56	Sandy loam

Table 2: Chemical analysis of soil sample before harvest.

OM %	pH	EC dsm^{-1}	Cation ($mmol_c L^{-1}$)				Anion($mmol_c L^{-1}$)			
			Ca^{2+}	Mg^{2+}	Na^{1+}	K^{1+}	CO_3^{2-}	HCO_3^{1-}	SO_4^{2-}	Cl^{1-}
2.44	7.78	1.35	4.50	3.59	3.85	1.92	ND	3.82	6.06	3.98

Table 3: Concentration of heavy metals in none polluted and studied polluted soil.

Plant available heavy metals ions (mg kg^{-1})		
Elements	Critical limits of heavy metals in soil *	Studied polluted soil
Fe^{2+}	>10	88.74
Zn^{2+}	>0.6	92.13
Mn^{2+}	>0.6	21.85
Pb^{2+}	>1	27.18

** Lindsay and Norvell, 1978, U.S. Department of Agriculture, 2003.*

Seaweeds treatments:

Two types of (*Ulva sp.*(green algae)and Gelidium sp. (red algae)) were collected from Abo-Quir Bay, Alexandria, Egypt and washed with seawater, tap water, and then distilled water several time, to remove extraneous and salt. They were then dried in an oven at 60^0C for 24 hr. The dried algae biomass was chopped, to obtain two particle size of0.125 and 0.08 mm was used for biosorption experiment. The moisture percentage, the ash and nitrogen content; were determined for according to (AOAC. 2006).A factor of 6.25 was used to convert N to protein; fat; carbohydrate by (AOAC 2006); also, CEC, pH, heavy metals (Fe, Zn, Mn and Pb) and (Ca and P) contents were recorded according to (Page *et. al.,* 1982), as shown in Tables 4 and 5.

Table 4: Chemical analysis of seaweeds dry biomass.

Types	pH	Ash %	CEC $Cmol_c\ kg^{-1}$dry $biomass^{-1}$	Moisture %	Protein %	Fat %	Carbohydrates %
Ulvasp (Green algae)	6.58	29.9	25.8	89.3	18.41	2.3	53.11
Gelidium sp. (Red algae)	6.76	19.5	56.7	93.4	19.12	1.5	61.2

Table 5: Fe, Zn,Mn, Pb, P and Ca contents of seaweeds.

(mg kg^{-1}dry weight)		($mg\ kg^{-1}$ dry weight)			
Ca	P	Fe	Zn	Mn	Pb
1.8	6.6	0.21	0.39	0.46	0.08
1.9	6.7	0.12	1.13	0.64	0.16

Experimental treatments:

The experiment involved 21 pots comprised 7 treatments with three replicates in a completely randomized design. Two types of algae dry biomass was chopped to obtain two particle of size (0.125 and 0.08mm) were mixture with soil at different rates

0.2, 0.4, and 0.6 ton hectare^{-1}., before harvest. Then seeds of lettuce were sown, treatments of this study were as follows:

1- Contaminated soil (control)
2- Contaminated soil with mixture of algae dry biomass a particles size of (0.125 mm) at rate0.2 hectare fed^{-1}.
3- Contaminated soil with mixture of algae dry biomass a particles size of (0.125 mm) at rate0.4 hectare fed^{-1}.
4- Contaminated soil with mixture of algae dry biomass a particles size of (0.125 mm) at rate0.6 hectare fed^{-1}.
5- Contaminated soil with mixture of algae dry biomass a particles size of (0.08 mm) at rate0.2 hectare fed^{-1}.
6- Contaminated soil with mixture of algae dry biomass a particles size of (0.08 mm) at rate0.4 hectare fed^{-1}.
7- Contaminated soil with mixture of algae dry biomass a particles size of (0.08 mm) at rate0.6 hectare fed^{-1}.

The used soil was packed in polyethylene pots (30cm inside diameter and 40cm in height). Soil was mixed of seaweeds dry biomass. Plant lettuce seedlings in the nursery was forty days before planting ginseng and one in potted in mid-October 2014, Fertilization with N and K nutrients were according to the recommendations ofthe Agriculture Ministry.

Plant roots and shoots were harvested after 60 days from planting, at which time there was sufficient plant material for analysis. Plant organs were rinsed in distilled water and then dried at 60-70° for 24 hr, dry weights were recorded. The plant samples were ground and wet digested with acids mixture (HNO_3 and $HC1O_4$) according to (Jackson, 1973), Heavy metals (Fe, Zn, Mn and Pb ions) were determined according to (Page et al., 1982). Soil samples were collected from all pots after harvest, air dried and then sieve.

At the same time, DTPA extractable contents of the studied heavy metals were determined, as mentioned before, at harvest to evaluate the response of their potential mobility and biological uptake by grown plants.

Statistical analysis:

The collected data were subjected to the proper statistical analysis of complete randomized block design combined over locations according to (Steel and Torrie1980), Using LSD at 5 % level.

Results and Discussion

The effect of algae dry biomass on extractable and % of the residual from native of heavy metals in polluted soil after harvest.

Data in Table 6 represent the extractable and % of the residual in soil from native Fe, Zn, Mn and Pb ions as affected by different levels of seaweeds dry biomass after mixing with soil at different rates of 0.2, 0.4, and 0.6 ton hectare^{-1}with particles size of (0.125 and 0.08 mm)before harvest. The results showed that the extractable amounts of Fe, Zn, Mn and Pb ions were reduced with increasing rates of algae dry biomass compared with the control. However the data revealed that the lower values of extractable Fe, Zn, Mn and Pb ions were obtained when application of mixture algae dray biomass at rat of 0.6 ton hectare^{-1}with lower particles size of 0.08mm after harvesting than at particles size of 0.125 mm compared with the control., Lower particles size of 0.08 mm a relatively high surface area of structurally uniform and physiologically active cells of seaweeds. It's the important role both types of algae dry biomass to retain heavy metals in soils as unavailable form. This could be due to the increase adsorption surface dried of seaweeds. These results may be attributed to the formation of stable form compounds with a wide range of cationic contaminants or immobilization of heavy metals as oxide, hydroxide or phosphate, (Lim *et al*., 2008 and Chen 2012).

These results could be attributed to the important role both types of seaweeds dry biomass in the chemical behavior of heavy metals in soil and decomposition of organic matter is followed by formation of active groups which have the ability to retain the metal in the complex, chelated form and components.,

These compounds contain functional groups such as amino, carboxyl, sulfate, and hydroxyl which play important roles in the biosorption. Red algae contain cellulose in the cell wall, but their biosorption capacities are attributed mainly to the presence of sulfated polysaccharides made of gelatins, these findings could be enhanced with those obtained by (Lim *et. al.,* 2008 and Chen 2012).

Also (Romera*et. al.,* 2007; Kooh *et. al.,* 2013; and Prosenjit *et al.,* 2015), Indicated that Larger ions may better fit a binding site with two distant functional groups, such as the affinity sequence Pb^{2+}> Cu^{2+}> Cd^{2+}>

Zn^{2+}> Ni^{2+}> Ca^{2+}>Mg^{2+}. Sulfated polysaccharides (galactanes) in the red algae were also found to be mainly responsible for the complexation formation of metal ions. The results suggested that a particles size of 0.08 mm at 0.6 ton hectare^{-1} before harvest relatively high surface area of structurally uniform and physiologically active cells of seaweed dry biomass.

Table 6: The Effect of algae dry biomass treatments on extractable and % of the residual from native of heavy metals in polluted soil after harvest.

Particles of mm	Treatments No.	Extractable (mg kg^{-1})				% of the residual from native			
		Fe^{2+}	Zn^{2+}	Mn^{2+}	Pb^{2+}	Fe^{2+}	Zn^{2+}	Mn^{2+}	Pb^{2+}
Control	1	88.74	92.13	21.85	27.18	100	100	100	100
0.125 mm	2	77.46	83.62	19.20	25.61	87.24	90.76	87.87	94.22
	3	58.95	64.82	18.62	18.33	66.43	70.35	85.21	67.43
	4	56.72	58.94	13.72	14.42	63.91	63.97	62.69	53.05
0.08 mm	5	62.71	81.75	18.61	23.43	70.66	88.73	85.17	86.20
	6	42.81	51.71	12.67	15.61	48.24	56.12	57.98	57.43
	7	40.60	50.33	11.16	14.36	45.75	54.62	51.07	52.83
L.S.D. at 5%	A	2.45	1.32	0.39	0.37	2.25	1.50	1.41	1.92
	B	2.45	1.32	0.39	0.37	2.25	1.50	1.41	1.92
	AB	4.25	2.29	0.69	0.64	3.89	2.60	2.45	3.33

This could be due to the increased adsorption on surface of dry biomass. It's the important role both types of seaweed to retain heavy metals Fe, Zn, Mn and Pb ions in soils as unavailable form., And results indicated that the both types of seaweeds had directly greater potential to immobilize Fe, Zn, Mn and Pb ions in the studied contaminated soil at 0.6 ton hectare^{-1}before harvest.

The ability of both types of seaweeds dry biomass to immobilize heavy metals under consideration in a contaminated soil could be attributed to the high metal binding capacities due to the presence of polysaccharides, proteins such as amino, hydroxyl, carboxyl and sulphate, which can act as binding sites for metals, (Jinsong and Chen, 2014).

The effect of algae dry biomass on lettuce yield and uptake of heavy metals after harvest.

Data in Table 7 and 8 shows that the dry weight yields of roots and shoots of lettuce after harvesting in the contaminated soil samples were extremely higher than the control. The results showed that the relative dry weight of roots of lettuce were in treatment No. 2, 3 and 4 at particles of size 0.125 mm (102.8%),(106.0%) and (109.3%) then increased gradually until reached (102.5%), (117.0%) and (118.3%) with treatments No. 5, 6 and 7at particles of size 0.08mm respectively (Table 7)., The same remark was found regarding the shoots of lettuce were in treatments No. 2, 3 and 4 at particles of size 0.125 mm (100.6%), (103.3%) and (105.4%) then increased gradually until reached (111.1%), (118.1%) and (118.9%) with treatments No. 5, 6 and 7 at particles of size 0.08 mm respectively (Table 8).,This emphasized that the yield of lettuce increase with increasing the rate of seaweeds.

These results confirm again the important role of application of seaweeds dry biomass for remediation soil contaminated with heavy metals on the contrary lower uptake of heavy metals by lettuce roots and shoots after harvesting in contaminated soils than in roots and shoots of the control. This remark insured the important role of application amount to reduce solubility and concentration of heavy metals, which could led to low plant uptake of heavy metals in contaminated soils. Similar results were obtained by (Jinsong and Chen 2014). Data represent in Table 7 and 8 shows a positive effect of different materials in increasing the content and its uptake by lettuce plant. Concerning the effect of adopted treatments on Fe, Zn, Mn and Pb content and uptake, data in Table 7 showed that Fe, Zn, Mn and Pb contents and uptake are affected by application of seaweeds dry biomass to contaminated soil compared with the other treatments and the control. The dry weight of the plants was increased with the increases in the concentration and uptake of Fe, Zn, Mn and Pb ions. The dry weight reflected the high content and its uptake of heavy metals found at 0.2 ton hectare^{-1} followed by treatment 0.4 and 0.6 ton hectare^{-1}at particles of size 0.08 mm lower than particles of size 0.125 mm compared with the control.

This remark insured the important role particles of size 0.08mm from seaweeds dry biomass especially non living (dead) a relatively high surface area of structurally uniform and physiologically active cells of seaweeds dry biomass. This could be due to the increase adsorption on surface dried of seaweeds. It's the important role both types of seaweeds dry biomass to retain heavy metals in soils as unavailable form. Similar results were obtained by (Wan Maznah *et al*., 2012; Zakhama *et al*., 2011) they indicated that have shown the inactive, non-living(dead) biomass may be even more effective than active (living) one in removal of heavy metals and is based on metal sorption due to the high affinities between the metal ions and the biomass.

Table 7: The effect of algae dry biomass on lettuce yield and uptake of heavy metals in roots after harvest.

Particles of mm	Treatments No.	D.W. gm pot^{-1}	The relative yield%	Concentration (mg kg^{-1})				Uptake µg pot^{-1}			
				Fe^{2+}	Zn^{2+}	Mn^{2+}	Pb^{2+}	Fe^{2+}	Zn^{2+}	Mn^{2+}	Pb^{2+}
Control	1	6.97	100	40.43	41.16	35.61	1.19	281.7	286.8	248.2	8.29
0.125 mm	2	7.17	102.8	40.52	41.20	35.72	1.37	290.5	295.4	256.1	9.82
	3	7.39	106.0	40.53	41.31	35.74	1.40	299.5	305.2	264.1	10.43
	4	7.62	109.3	41.11	41.32	35.89	1.42	313.2	314.8	273.4	10.82
0.08 mm	5	8.11	102.5	42.18	41.30	36.61	1.41	342.0	334.9	296.9	11.43
	6	8.16	117.0	42.21	41.60	36.71	1.46	346.0	339.4	299.5	11.91
	7	8.25	118.3	42.23	41.65	36.77	1.49	348.3	343.6	303.3	12.29
L.S.D. at 5%	A	0.13	1.24	0.36	0.08	0.03	0.11	2.75	2.05	3.24	0.38
	B	0.13	1.24	N.S	0.08	0.03	N.S	2.75	2.05	3.24	0.38
	AB	N.S	2.14	N.S	0.14	0.06	N.S	4.77	3.55	5.62	N.S

The total uptake of Fe, Zn, Mn and Pb ionsby roots were increased gradually at 0.2 ton $hectare^{-1}$followed by, 0.4 particularly, and 0.6ton $hectare^{-1}$. On the contrary, total uptake of Fe,Zn, Mn and Pbions of shoots were decreased with the different treatments. These results confirm again the important role of application of seaweeds for improving soil contaminated with heavy metals on the contrary lower uptake of heavy metals by lettuce shoots after harvesting in contaminated soils than in roots of the control.

This remark insured the important role of the key functional groups sufficiently present in the red and green algae, such as carboxyl, hydroxyl, sulfate, phosphate, and amine groups, which play important roles in the biosorption. Red algae contain cellulose in the cell wall, but their biosorption capacities are attributed mainly to the presence of sulfated polysaccharides made of galactans. These results could be supported those obtained by (Lim *et al*., 2008; Chen, 2012)., (Gupta, Rastogi, 2008 and Johnson *et al*., 2007).

Table 8: The effect of algae dry biomass on lettuce yield and uptake of heavy metals in shoots after harvest.

Particles of mm	Treatments No.	D.W. gm pot^{-1}	The relative yield%	Concentration (mg kg^{-1})				Uptake µg pot^{-1}			
				Fe^{2+}	Zn^{2+}	Mn^{2+}	Pb^{2+}	Fe^{2+}	Zn^{2+}	Mn^{2+}	Pb^{2+}
Control	1	14.38	100	30.41	28.21	22.13	0.91	437.2	405.6	318.2	13.0
0.125 mm	2	14.48	100.6	23.15	21.72	16.31	0.85	335.2	314.5	236.1	12.3
	3	14.86	103.3	19.31	20.45	15.91	0.82	286.9	303.8	236.4	12.1
	4	15.16	105.4	18.11	19.31	14.49	0.73	274.5	292.8	222.7	11.0
0.08 mm	5	15.98	111.1	18.41	19.62	14.31	0.65	294.1	313.5	228.6	10.3
	6	16.99	118.1	16.50	17.86	12.35	0.61	280.3	303.4	209.8	10.3
	7	17.1	118.9	16.11	17.51	11.16	0.51	280.2	283.1	190.3	8.72
L.S.D. at 5%	A	0.19	1.32	0.38	0.26	0.04	0.10	2.75	2.31	3.18	0.32
	B	0.19	1.32	0.38	0.26	0.04	N.S	2.75	2.31	3.18	0.32
	AB	0.33	2.29	0.66	0.45	0.07	N.S	4.77	3.99	5.51	0.56

The results suggested that the important role of application of seaweeds dry biomass at particles of size 0.08mm for remediation soil contaminated with heavy metals on the contrary lower uptake of heavy metals by lettuce shoots in contaminated soils than in roots with the control.

Conclusion

The results of this study indicated that the extractable amounts of heavy metals were decreased with increasing rates of applied green algae or red algae compared with the control. The lower values of extractable heavy metals were obtained when application of mixture algae dry biomass at rat of 0.6 ton $hectare^{-1}$withparticles of size 0.08 mm after harvesting than that particles of size 0.125 mm compared with the control, it's the important role of seaweeds dry biomass to retain heavy metals in soils as unavailable form.

The results suggested that the important role of application of seaweeds dry biomass at lower particles of size 0.08mm for remediation soil contaminated with heavy metals on the contrary lower uptake of heavy metals by lettuce shoots in contaminated soils than in roots with the control., The study recommends using a mixture of green and red algae dry biomass at particles of size 0.08mm and rat of 0.6 ton $hectare^{-1}$, with the need to avoid plants that are eaten leaves or roots growing in the soil contaminated with heavy metals.

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