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Biosorption of Toxic Metals from Solid Sewage Sludge by Marine Green Algae

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Abstract: Biosorption of toxic metals from solid sewage sludge by marine green (*Codium iyengrii)* algae were observed. The process of removal of toxic metals by certain plants species are called biosorption. For this purpose the contaminated sewage sludge from sewage farm of SITE area Karachi from three different places were collected. In these places vegetables like onion (*Allium cepa*), spinach (*Spinacia oleracea*) and turnip (*Brassica rapa*) were cultivated which is commonly supplied to Karachi city. Heavy metals analysis of sewage sludge and plants were performed by atomic absorption spectrophotometry before and after treatment of sludge at laboratory scale. The effect of removal of metal was observed on plant growth. The results were also compared by garden soil. Results showed that concentration of metals in soil decreases by bio-sorption of toxic metal on seaweed. This helps in improving the soil quality by adsorption of metals on seaweeds. Significant improvement in size of leaves of bean plants in treated sewage sludge, were observed due to which chlorophyll contents founds to be increased as compare to non-treated plant. Reduction in metals uptake by plants from sludge as compared with non-treated sludge were recorded due to which nutritional level of plants were enhanced.

Key words: Bio-sorption, toxic metals, solid sewage sludge, sewage farm, nutritional level

INTRODUCTION

Investigations about new technologies (Chen and Cutright, 2001) involving the removal of toxic metals from wastewater has direct attention to biosorption, based on metal binding capacities of various biological materials. Biosorption can be defined as the ability of biological material to accumulate heavy metals from wastewater through metabolically mediated or physico-chemical pathway of up takes (Stirk and Staden, 2002). It can be use to detoxify sites containing metals; pesticides, solvent, explosives, crude oil, hydrocarbons and land fill leachates. This is often referred to as bioremediation, botanical-bio remediation and green remediation (Bizily *et al*., 1999).

As a matter of fact that larger parts of agricultural soil are contaminated with toxic metals including Pb, Cd, Hg and As (Strik *et al*., 2002). Therefore approaches to develop techniques to reduced heavy metal from soil are important. Many techniques have been developed to control the toxicity of heavy metals in the environment. However, to reduce heavy metal in food chain, that transfer less heavy metals to the shoot is required. The seaweed (Azmat *et al*., 2006a) is an organic non pollutant renewable resource which helps in increasing the water holding capacity of soil and conditioning the soil by absorbing (Same *et al*., 2002) toxic metal on its surface. Biosorption of heavy metals from soil by seaweeds offers a low cost method to reduced the toxic metals from the sludge and some extracted metals may be recycled for value. Pb (Blaylock *et al*., 1997) and Cr^{+6} may be inactivated in the soil by binding the metal (Azmat *et al*., 2006b) with chelating compound found in seaweeds (Strik and Staden, 2000)

Sludge originates from the process of treatment of wastewater. Due to physical-chemical processes involved in the treatment, the sludge (Joohyjun *et al*., 2003) tends to concentrate heavy metals and poorly biodegradable, trace organic compound as well as potentially pathogens (virus, bacteria etc.) present in wastewaters (Azmat *et al*., 2005). Sludge is however, rich in nutrients such as nitrogen and phosphorous and contains valuable organic matter that is useful when soils are depleted or subjected to erosion. Presence of metal in soil plays an integral role in the growth of plant. Some metals (e.g., Ca, Co, Cr, Fe, K, Mg, Na, Ni and Zn) are essential, serve as micronutrients and are used for redox process, to stabilize molecule through electrostatic interaction, while other metals (i.e., Ag, Al, Cd, Au, Pb and Hg) have no biological role and are toxic to physiological processes of plants.

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Toxicity of non-essential metals occurs through the displacement of essential metals from their native binding sites or through ligand interaction. In addition, at high level both essential and non-essential metals can damage cell membrane; disrupt cellular functions and damage the structure of DNA.

Keeping these above views in sight, this research has been design to conditioning the solid sewage sludge (used for the cultivation of vegetables) from SITE area Karachi by green seaweeds, as a biosorbent to detoxify the metals found in sludge. The effect of seaweeds on sludge will test on plant growth and results will discus in relation with growth, health, nutritional quality, essential and non-essential ions uptake by plants.

MATERIALS AND METHODS

Solid sewage sludge from SITE farm of Karachi were collected from three different sampling stations in the month of April 2005 where vegetables like onion (*Allium cepa*), spinach (*Spinacia oleracea*) and turnip (*Brassica rapa*) were cultivated and referred as sludge A, sludge B and sludge C, respectively in the laboratory.

The green seaweed *Codium iyengrii* were harvested in the morning from Bullijee coastal beach of Arabian Sea, Karachi, in the months of February and March 2005 when it is abundant at the beach. The seaweed were dried at room temperature and then finally grounded by rotary mill to dry seaweed powder. The bean plants (*Vigna radiata*) were chosen to study the effect of sludge on the physiological process of plants. Three pots of sludge A, B and C were prepared and seeds of bean plants were soaked in water and surface sterilized which later on introduced in to the pots of sludge. Similarly three pots of sludge A, B and C in which 1 g of dry seaweeds were added and one pot of garden soil were also prepared for comparative study. The pots were placed in natural environment.

Morphology of root, shoot and leaves were compared with plants grown into garden soil.

Analysis of essential and non essential metals: The ash method was use to prepared the solution for detection of metals ions in the sludge and plants. The solutions were subjected to flame photometry and atomic absorption spectrophotometry for the analysis of essential, non-essential mineral and toxic metals ions.

Physiological process of bean plant cultivated into the sludge and treated sludge were observed by visible spectrophotometer as designated by Tandon (1993). These values were compared with control plant, grown in to the garden soil.

RESULTS AND DISCUSSION

Analysis of sludge A, B and C before addition of seaweeds showed that it contain essential and non essential macro and micronutrients ions with toxic metals (Table 1). Solid sewage shows high percentages of macronutrients like Na, K, Ca and Mg which is useful for plant growth.

Results showed that sludge A contain high concentration of toxic metal, in this sludge onion was cultivated where as sludge b showed less concentration of toxic metals, spinach were grown in this area which is a leafy vegetable and leafy vegetable accumulate maximum (Azmat *et al.*, 2005) concentration of toxic metals in their broad leaf size while C shows moderate concentration of non-essential metals, turnip were cultivated in this place. Plants grown in to this sludge showed that it accumulate (Hammaini, 2003) maximum amount of essential and

Table 1: Analysis of macro nutrient, micronutrient and toxic metal (mg kg^{-1}) found in sewage sludge

Place of sludge	Na	K	Ca	Mg	Fe	Zn	Mn	Cd	As	Hg	Pb
Garden soil	155.0±12.0	125.0±13.3	150.0±17.6	45.0±9.60	2.1±0.01	2.5±0.2	2.9±0.3	-	0.03±0.02	-	-
Sludge A	150.0±15.0	87.50±15.0	112.5±15.5	70.0±6.50	1.4±0.10	3.5±0.1	2.9±0.3	0.75±0.001	0.62±0.02	0.20±0.03	0.53±0.01
Sludge B	172.5±16.2	102.5±16.3	112.5±13.0	77.5±12.6	1.6±0.20	2.2±0.3	1.5±0.2	0.50±0.020	0.65±0.05	0.30±0.01	0.35±0.01
Sludge C	187.5±16.2	115.0±14.2	135.0±13.3	97.4±12.5	1.5±0.20	4.6±0.1	2.8±0.3	0.45±0.100	0.72±0.06	0.35±0.02	0.48±0.02

Table 2: Accumulation trend of essential and non essential metals (mg kg^{-1}) from sewage sludge into bean plant

Place of sludge	Na	K	Ca	Mg	Fe	Zn	Mn	Cd	As	Hg	Pb
Root											
Garden soil	137.5±11.0	55.0±5.1	130.0±6.3	72.50±3.1	0.6±0.10	0.5±0.01	0.60±0.02	-	-	-	-
A	112.5±12..6	37.5±6.2	87.5±5.1	50.00±4.1	0.5±0.02	0.2±0.03	0.30±0.02	0.20±0.01	0.12±0.01	0.01± 0.01	0.24±0.01
B	117.5±14.4	45.0±9.3	97.5±4.1	62.50±3.1	0.7±0.03	0.3±0.01	0.50±0.01	0.15±0.01	0.25±0.01	0.20±0.01	0.50±0.02
C	127.5±15.3	52.5±9.1	112.5±3.1	67.50±3.2	0.3±0.01	0.4±.0.02	0.30±0.01	0.20±0.01	0.30±0.01	0.02±0.01	0.60±0.01
Shoot											
A	125.0±15.6	30.0±8.1	97.5±21.2	67.50±11.2	0.4±0.01	0.1±0.01	0.20±0.01	0.10±0.01	0.12±0.01	0.01±0.01	0.24±0.01
B	140.0±15.6	32.5±6.1	110.0±12.2	72.50±11.3	0.3±0.01	0.4±0.01	0.20±0.01	0.20±0.01	0.25±0.01	0.02±0.01	0.50±0.01
C	150.0±12.6	45.0±4.2	122.5±14.5	82.50±12.3	0.2±0.01	0.1±0.01	0.30±0.01	0.15±0.01	0.30±0.01	0.10±0.02	0.60±0.02
Garden soil	159.5±14.0	47.5±4.2	134.5±12.3	90.00±21.3	0.5±0.02	0.4±0.01	0.20±0.01	-	-	-	-

non-essential metals as compared to plant grown in garden soil (Table 2). Nutritive values like chlorophyll a and b, proteins and carbohydrate were found to be less as compared with control pot which contain garden soil while amino acids were absent both in roots and shoots (Table 3). Table 3 shows the growth rate of seedling in sewage sludge. Comparison of average growth rate of bean plant with the seedling grown into garden soil, indicated that length of root and shoot of bean plant were less as compared with the plant grown in garden soil.

Addition of seaweed powder to the sludge reduces the concentration of essential and non-essential metals available to plant (Table 4) while concentration of micronutrients were found to be increased and remarkable differences were recorded on the seed germination (El-Sheekh *et al*., 2000) and leaves size (Table 5). Seaweed also reduced the metal accumulation in the roots of plants results in the healthy growth of plant consequently change in the morphology of plants were observed with larger and broader size of leaves. It also helps in increasing the length of the root and makes it powerful due to which healthy growth of bean plants were observed (Table 5). This also represent the improvement in the nutritive value of edible plant.

It was also observed that toxic metals like Pb, Hg, Cd and As were not reported in shoot and root of plant (Table 6). This indicated that seaweeds correct the soil condition by providing the ligands interaction with heavy metals (Kamnev and lelie, 2000).

Mechanism of complexation: The biosorption of metals (Nortan, 2003) take place through both adsorption and formation of coordination bonds between metals and amino and carboxyl groups of cell wall polysaccharides of seaweeds. The metal removal from sewage sludge may also take place by complex formation on the cell surface after the interaction between the metal and the active groups of proteins and amino acids found in green algae. Complexation was found to be only mechanism responsible for calcium, magnesium, cadmium, zinc, copper and mercury accumulation by marine algae.

Investigation showed that application of dry seaweed powder to the sludge provides multiple levels of potential benefits. These potential benefits have been identified during seaweed spray including nutritional level, physiological process, morphology, mineral and metal ion (Schiewer and Wong, 2000) uptake by plants (Table 6). The physico-chemical interaction occurs

Table 3: Average growth rate and nutritive values in bean plant before treatment

Place of sludge	Ave. growth rate of	Ave. growth rate of	% of chlorophyll a	% of chlorophyll b	% of Carbohydr-ate in root	% of carbohydr-ate in	% of protein in root	% of protein in shoot	% of amino acid in root	% of amino acid in shoot
Garden soil	2.0±0.01	14.7±3.5	0.289±0.02	0.058±0.02	0.340±0.01	0.50±0.01	1.20±0.01	3.00±0.02	2.0 ±0.01	2.1±0.05
A	1.4±0.10	10.0±2.1	0.014±0.02	0.075±0.3	0.126±0.01	0.378±0.01	1.08±0.01	0.40±0.01	-	-
B	1.8±0.20	12.7±0.03	0.189±0.02	0.081±0.02	0.229±0.01	0.22±0.01	0.79±0.01	0.36±0.01	-	-
C	1.3±0.30	9.0±0.02	0.239±0.01	0.015±0.02	0.301±0.1	0.27±0.01	0.75±0.01	2.55±0.01	-	-

Table 4: Analysis of macro, micronutrient and toxic metal (mg kg^{-1}) in sewage sludge after addition of seaweed

Place of Sludge	Na	K	Ca	Mg	Fe	Zn	Mn	Cd	As	Hg	Pb
A	145.0±12.9	80.0±9.8	125.0±13.0	50.0±9.8	1.5±0.02	2.1±0.2	2.1±0.02	0.05±0.01	0.07±0.02	0.1±0.01	0.13±.02
B	167.5±31.1	87.5±6.2	140.0±15.0	60.0±9.3	1.7±0.03	2.3±0.3	2.3±0.3	0.02±0.01	0.12±0.01	0.12±0.01	0.14±0.01
C	182.5±23.1	100.0±8.5	150.0±12.6	65.0±5.5	1.8±0.02	1.7±0.03	2.0±0.2	0.01±0.01	0.17±0.01	0.11±0.01	0.11±0.02

Table 5: Average growth rate and nutritive values in bean plant after treatment

Place of sludge	Ave. growth rate of root (cm)	Ave. growth rate of shoot (cm)	% of chlorophyll a	% of chlorophyll b	% of Carbohydrate in root	% of carbohydrate in shoot	% of protein in root	% of protein in shoot	Ave. % of Amino acid in root	Ave. % of amino acid in shoot
A	2.9±0.02	25.0±2.1	0.238±0.01	0.107±0.02	0.22±0.01	0.38±0.01	1.5±0.02	0.6±0.01	0.03±0.01	0.05±0.01
B	3.9±0.01	23.5±0.01	0.199±0.01	0.101±0.01	0.40±0.02	0.364±0.02	1.4±0.01	0.6±0.01	0.04±0.01	0.02±0.01
C	2.3±1.2	24.0±5.1	0.246±0.03	0.03±0.02	0.235±0.01	0.39±0.01	1.62±0.01	2.76±0.01	0.09±0.01	0.12±0.01

Table 6: Accumulation trend of essential and non essential metals (mg kg^{-1}) from sewage sludge into bean plant after treatment

Place of sludge	Na	K	Ca	Mg	Fe	Zn	Mn	Cd	As	Hg	Pb
Root											
A	112.5±17.2	77.5±12.10	87.5±11.3	97.5±10.2	0.60±.0.01	0.30±0.02	0.36±0.02	-	-	-	-
B	110.0±14.6	87.5±11.30	95.0±14.0	87.5±11.3	0.70±0.30	0.50±0.02	0.60±0.02	-	-	-	-
C	112.5±14.3	100.0±12.6	100.0±12.6	97.5±12.3	0.40±0.02	0.50±0.02	0.34±0.01	-	-	-	-
Shoot											
A	102.5±8.9	102.5±11.3	122.5±13.3	97.5±10.2	0.20±0.01	0.20±0.01	0.10±0.01	-	-	-	-
B	122.0±14.3	115.0±12.0	130.0±15.1	125.0±10.2	0.32±0.01	0.50±0.01	0.20±0.01	-	-	-	-
C	137.5±4.5	112.5±4.20	137.5±11.3	125.0±10.6	0.31±0.01	0.20±0.01	0.30±0.01	-	-	-	-

between the toxic metal and the surface polysaccharides of the biomass (algae), ion-exchange, complexation and adsorption takes place and the phenomena is not metabolism dependent. The surface of the seaweeds is constituted of polysaccharides and proteins that provide a wide range of ligands for heavy metal ions. These processes are rapid and reversible. Seaweed contains all known trace element and these elements can be made available to plant by chelating i.e., by combining the mineral ion with organic molecules, starches, sugars and carbohydrates in seaweed and seaweed products possess such chelating properties (Kapoor and Viraroghvan, 1998). As a result, these constituents are in natural combination with the iron, cobalt, copper, manganese, zinc and other trace elements found naturally in seaweed. That is why these trace elements in seaweed product do not settle out in alkaline soils, but remain available to plants, which need them. Table 5 shows that when seaweeds mixed with the sludge, biosorption of toxic metals takes place, which stimulate the growth rate and physiological processes.

CONCLUSION

Today's industrial world has contaminated our soil, sediments and aquatic resources with hazardous material. Metal water interaction is often result of industrial activities, such as mining, refining and electroplating. Hg, Pb, As, Cd and Cr are often prevalent at highly contaminated sites. Therefore it is our responsibility to check and develop the low cost techniques to remove the toxic metals by methylation, complexation or changes in valance state from the environments for humanity. This research suggests that marine green algae may be use for removal of heavy metals from contaminated soil as a very low cost method.

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