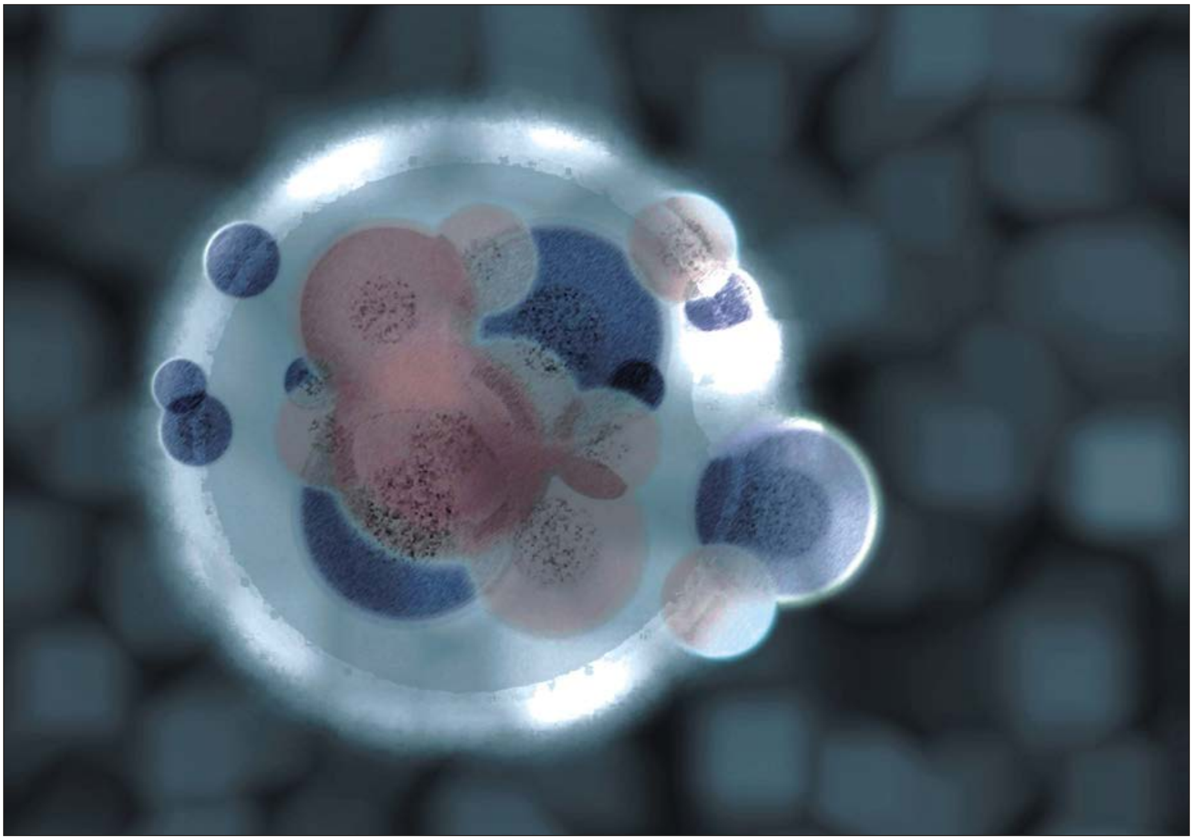

Journal of Biological Sciences

ISSN 1727-3048

science alert

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Journal of Biological Sciences 6 (1): 173-177, 2006
ISSN 1727-3048

Effect of Micronutrients of *Codium iyengarii* on Metal Toxicity in Bean Plants

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Abstract: Cd toxicity and problems with regards to tolerance, physiological processes and ecological significance with green seaweeds has been examined in this study. Seaweed *Codium iyengarii* belongs to chlorophyta which contains highest amount of essential mineral ions [Ca, Fe, K, Na and Mg] collected from Bullijee coastal area of Karachi coast, to control the toxicity of Cd metal in bean plant. Nutritive value and essential ions were determined by visible spectrophotometer, flame photometer and atomic absorption techniques. Microbial counts were determined by standard method. Results indicated that the biosorption of Cd by green seaweed *Codium iyengarii* was observed. Healthy growth of the plants were recorded up to 100 ppm of Cd concentration which improves in up taking of mineral ions from the soil into the roots and shoots of bean plant. It was observed that seaweed acts as a catalyst starter in soil irrigated with contaminated water. This water contains high percentages of heavy toxic metals that were toxic to the plant and harmful for its nutritive value. *Codium iyengarii* improves the morphology and physiological processes of plants also. Seaweeds help in the population of soil bacteria in heavy metal contaminated environment.

Key words: *Codium iyengarii*, Cd, biosorption, tolerance, soil bacteria

INTRODUCTION

From the agro industrial point of view, heavy metals can be presented as the chemical elements that are toxic to plant cultures, or chemical elements that decrease agricultural productivity, due its presence at high concentration in the soil. Seaweed are fascinating and diverse groups of organisms living in the earth oceans, attached to the rocks and floating on the oceans surface. Seaweeds are plants because they use the sun's energy to produce carbohydrates from carbon dioxide and water[1-3]. They are simpler than the land plants mainly because they absorb the nutrients and have no need of root. Seaweed extracts are used at the recommended times and rates. These extents will supply the amounts of iron, zinc, copper, molybedium, cobalt, boron, manganese and magnesium that most crops require[4].

Technologies, based on the use of biological material to concentrate toxic metals are currently under development, with the use of microbial species, as well as giant. Seaweed plants collected as waste material from the beaches in tropical countries[5]. The surface of the seaweed did constituted of polysaccharides and proteins that provide a wide range of ligands for heavy metal ions. Differently from metabolism dependent mechanisms, these processes are rapid and responsible. Seaweed serves as surface of food or polysaccharides to microorganism[6]. This outstanding natural occurrence stimulates the development of biotechnologies with the use of seaweeds for the up take of heavy metals from industrial effluents or decontaminates polluted areas through bioremediation[7]. It is also important to mention that seaweeds can be also used as biological indicators of contamination and based on their ability to concentrate heavy metals due to their chemical decomposition[8,9].

Studies about the technological aspects of the metal removal by algae are scare. In this sense, the objective of this research was to determine the potential of Cadmium uptake, a highly toxic metal present in several industrial effluents and have no known biological function, by the inactive biomass of the marine algae *Codium iyengarii* species abundant at the Karachi cost. The present investigation examined the removal of metal in relation with seed germination, morphology, physiology and rhizosphere.

MATERIALS AND METHODS

Green algae *Codium iyengarii* has been collected from Bullijea coastal area of Karachi at low tide season in the morning at 9.00 am during February and March 2004. Seaweed collected in this study was taken from

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free-floating tides at the beach side. Samples were cleaned from seawater and rinsed with deionised water. Weeds were dried at room temperature and ground to a homogenous mixture.

Pot experiments were conducted in growth chamber (30°C day and 25°C night) for 15 days in July 2004 in garden soil. Three concentration of Cd were prepared like 20, 100 and 200 ppm from $CdCl_2$ for garden soil. Dry seaweed was introduced from 5, 10, 50 and 100 ppm with the fixed concentration of Cd in separate pots. Plants were analyzed after fifteen days for different biochemical tests and mineral ions analysis.

Morphological changes in the plant were noted after passing 2 days. The length of shoot, root and size of leaves were recorded and compared with control plant in which no seaweeds were present.

Physiological processes of plants were checked by determined its nutritive value like carbohydrate, protein, amino acid and chlorophyll a and b by spectrophotometeric technique[10,11].

The essential mineral ions like sodium, potassium, manganese, magnesium, calcium and iron were estimated through dry ash method by flame photometer and atomic absorption spectrophotometer. The extract, for phosphate contents were prepared by ash method and shaken with ammonium molybdate and stannous chloride. A blue colored complex with phosphate ion was obtained whose absorbance of solution were recorded at 660 nm by visible spectrophotometer[10].

Analysis of roots for soil microorganism in presence of seaweeds: Samples of roots from different concentration of seaweed viz., 0,5, 10, 50, 100, 150 and 200 ppm were taken for determining the effect of green algae on colony forming units (cfu) per mL after 10 weeks of germination. Sample were diluted up to 10^{-5} and the last dilution were spread on nutrient agar plates and were incubated at 37°C for 24 h.

Growth obtained on nutrient agar was subjected for gram staining. Gram +ve bacteria were further streaked on mannitol salt agar and blood agar. While gram-ve bacteria on Eosin Methylene blue agar (EMB). Different biochemical tests were performed, e.g., TSI agar slant, urease, lmvic, oxidase and catalase tests for the confirmation of organism.

RESULTS AND DISCUSSION

Seaweed has been used by plant growers for centuries, but the reason for beneficial results has only recently been attributed to the naturally occurring growth regulators and micronutrients such as iron, copper, zinc,

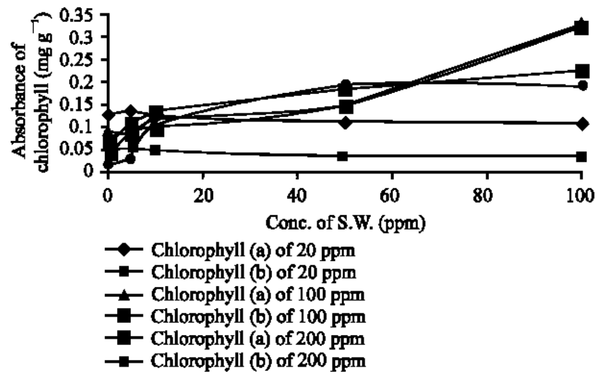

Fig. 1: Effect of seaweeds on chlorophyll a and b of bean plants

molybedenum, boron, manganese and cobalt in addition to highest amount of Na, K, Ca, Mg for healthy plant growth development[8].

Seaweed extract treatment may be expected to increase plant growth, even when the plant is under nutrient stress and facing toxicities of heavy metals due to pullulated water. It has been observed that seaweed extract increases the drought resistance of plant under stress of toxicity of metals. Seaweed spray evidently aid in changes the metabolic pathways thereby permitting the roots access of extremely low moisture levels, which during a drought are not normally available to plants[12,13]. The large numbers of micronutrients of seaweeds replace the soil nutrient deficiencies as well as increases the formation of humus and also helps to increase the colonies of soil bacteria, which were helpful in fixing the nitrogen in roots nodulates in leguminous plants.

When dry seaweeds powder were used to control the toxicity of Cd metal on bean plants, it was observed that plant become healthier under the metal stress which indicated that seaweeds adsorb the metal and it supply the amounts of iron, zinc, copper, molybdenum, cobalt, boron, manganese and magnesium that most crops require because micronutrients serve as catalyst starters. Activate enzymes, results in the growth of the plant and increase in the nutritive value of plant (Fig. 1-3). Table 1 and 2 shows effect of seaweed *Codium iyengarii* on the toxicity of Cd metal on bean plant and found that biosorption of Cd up to 100 ppm of Cd concentration takes place which increases the percentage composition of essential mineral ions like Na, k, Ca, Fe, Mn and Mg.

Biosorption from sludge can be considered as an alternative technology in industrial waste waters treatment[14]. It is based on the ability of biological materials to accumulate heavy metals from wastewater by either metabolically mediated or physiochemical pathways of uptake[15]. Investigation shows that seaweed supplies

Table 1: Effect of seaweed *Codium iyengarii* in conjunction with Cd metal on mineral ions contents of roots of bean plant

Seaweed ppm	K (%)	Na (%)	Ca (%)	Mg (%)	Mn (%)	Fe (%)	PO_4x10^{-2} (%)
[Cd] 20 ppm							
0	2.81±0.01	3.01±0.03	0.51±0.0	0.31±0.05	0.029±0.02	0.026±0.01	2.4 ±0.01
5	2.13±0.01	2.70±0.0	0.55±0.01	0.39±0.02	0.031±0.02	0.027±0.02	2.51±0.01
10	2.20±0.02	2.80±0.01	0.60±0.01	0.41±0.01	0.033±0.02	0.027±0.02	2.71±0.01
50	2.32±0.02	2.90±0.02	0.64±0.02	0.42±0.02	0.035±0.03	0.028±0.01	2.90±0.05
100	2.350±0.02	2.92±0.02	0.67±0.03	0.43±0.02	0.36±0.04	0.030±0.04	3.05±0.02
[Cd] 100 ppm							
0	1.90±0.01	1.5±0.05	0.35±0.04	0.022±0.01	0.024±0.20	0.021±0.03	1.5±0.01
5	2.00±0.03	1.6±0.01	0.38±0.01	0.026±0.03	0.027±0.02	0.023±0.02	1.7±0.02
10	2.19±0.02	1.60±0.03	0.39±0.02	0.028±0.03	0.029±0.01	0.025±0.02	1.9±0.01
50	2.20±0.02	1.70±0.02	0.41±0.02	0.030±0.01	0.031±.0.01	0.028±0.02	2.1±0.05
100	2.15±0.01	2.00±0.2.	0.45±0.02	0.032±0.02	0.032±0.01	0.030±0.01	2.3±0.03
[Cd] 200 ppm							
0	1.50±0.01	1.30±0.01	0.31±0.01	0.021.±0.01	0.020±0.01	0.017±0.01	1.5±0.02
5	1.30±0.02	1.11±0.021	0.30±0.01	0.020±0.01	0.020±0.01	0.17±0.01	1.4±0.02
10	1.10±0.01	1.01±0.02	0.31±0.02	0.018±0.01	0.017±0.02	0.17±0.01	1.4±0.02
50	1.20±0.02	1.21±0.02	0.30±0.02	0.017±0.02	0.018±0.01	0.18±0.02	1.5±0.00
100	1.5±0.02	1.3±0.01	0.34±0.02	0.19±0.01	0.022±0.02	0.21±0.05	1.6±0.02

Table 2: Effect of seaweed *Codium iyengarii* in conjunction with Cd metal on mineral ions contents of shoots of bean plant

Seaweed ppm	K (%)	Na (%)	Ca (%)	Mg (%)	Mn (%)	Fe (%)	PO_4 (%)
[Cd] 20 ppm							
0	3.23 ±0.01	3.6 ±0.01	0.45±0.02	0.25±0.01	0.045±0.01	0.0251±0.02	0.025±0.02
5	3.52 ±0.01	3.9±0.01	0.45±0.01	0.26±0.01	0.047±0.02	0.027±0.02	0.027±0.01
10	3.52 ±0.01	3.9±0.02	0.48±0.06	0.29±0.01	0.49±0.02	0.029±0.02	0.028±0.01
50	3.71 ±0.02	4.1±0.01	0.49±0.01	0.29±0.02	0.49±0.02	0.029±0.02	0.028±0.02
100	3.81±0.03	4.5±0.01	0.50±0.03	0.30±0.03	0.50±0.02	0.030±0.02	0.030±0.01
[Cd] 100 ppm							
0	3.01±0.01	3.21±0.01	0.37±0.02	0.24±0.01	0.032±0.01	0.023±0.01	0.020±0.01
5	3.25±0.01	3.5±0.01	0.38±0.01	0.29±0.01	0.035±0.01	0.024±0.01	0.020±0.01
10	3.42±0.01	3.6±0.02	0.34±0.02	0.29±0.01	0.036±0.01	0.025±0.01	0.022±0.01
50	3.41±0.02	3.6±0.01	0.34±0.01	0.30±0.02	0.036±0.01	0.025±0.01	0.22±0.05
100	3.65±0.02	3.7±0.02	0.35±0.01	0.31±0.02	0.039±0.01	0.026±0.01	0.024±0.01
[Cd] 200 ppm							
0	2.83±0.01	3.2±0.01	0.31±0.01	0.21±0.01	0.030±0.01	0.020±0.01	0.090±0.02
5	2.72±0.02	3.0±0.01	0.29±0.01	0.21±0.01	0.025±0.02	0.019±0.01	0.085±0.01
10	2.51±0.01	2.6±0.01	0.29±0.01	0.21±0.01	0.026±0.01	0.016±0.01	0.074±0.01
50	2.4±0.01	2.6±0.01	0.26±0.03	0.18±0.02	0.026±0.02	0.015±0.01	0.063±0.01
100	1.3±0.01	2.5±0.01	0.26±0.01	0.16±0.01	0.028±0.02	0.015±0.01	0.061±0.01

Table 3: Rhizosphere of bean plant in presence of Cd and seaweeds

Conc. of seaweed (ppm)	Dilution of sample	No. of colonies	No. of colonies X df = cfu	Average CFU (mL^{-1})
0	10^{-3}	Uncountable	-	
	10^{-4}	80	8x10^5	2.9x10^6
	10^{-5}	50	5x10^6	-
10	10^{-3}	Uncountable	-	-
	10^{-4}	"	-	-
	10^{-5}	"	-	-
50	10^{-3}	64	6x10^5	1.33x10^4
	10^{-4}	54	5.4x10^5	-
	10^{-5}	40	4x10^6	-
100	10^{-3}	240	2.4x10^4	3.42x10^6
	10^{-4}	200	2x10^6	-
	10^{-5}	140	1.4x10^6	-
150	10^{-3}	200	2x10^5	4.45x10^6
	10^{-4}	156	1.56x10^6	-
	10^{-5}	116	1.16x10^7	
200	10^{-3}	Uncountable	-	-
	10^{-4}	"	-	-
	10^{-5}	"	-	-

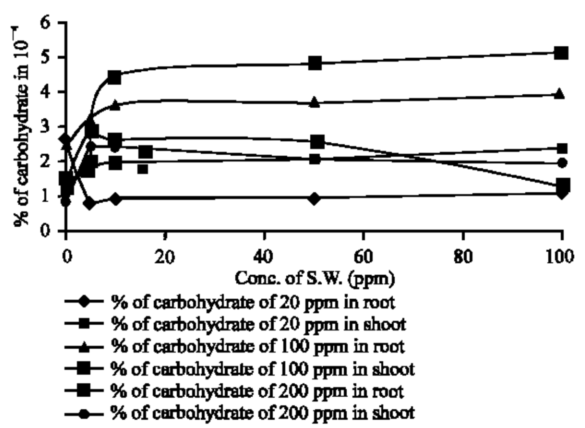

Fig. 2: Effect of seaweeds on carbohydrate of bean plants in presence of Cd metal

the plant growth regulator and micronutrients not available otherwise and speeding up seed germination of seed and control the harmful effect of heavy metals present in the aquatic resources. Seaweeds retarded the effect Cd at 20 to 100 ppm but at higher concentration of Cd, the toxicity of metal were dominant and decline in

Table 4: Identification of microorganism obtained in different concentration of seaweeds

Identified organism	Gram reaction	Growth on		TSI reaction										
		MSA	EMB agar	Slant	Butt	H_2S	Gas	Urease	Indol	Methyle red	Vogus proskure	Citrate	Oxidase	Catalase
E. coli	-ve	NF	LF	A	A	-	+	-	+	+	-	-	-	ND
Klebsiella pncumoniae	-ve	NF	LF	AG	AG	-	+	+	-	-	+	+	-	ND
Citrobacter	-	NF	Slow LF	AUK	AG	-	+	-	+	-	-	+	-	ND
S. aureus	-	MF	NF	ND	ND	ND	ND	+	-	+	+	-	-	+

MSA-Mannitol Salt agar, EMB-Eosin Methylene Blue agar, TSI-Triple sugar iron agar, NF-not found, LF-Lactose fermanter, A-Acidic, AG-acid with gas, ALK-alkaline, ND-not done

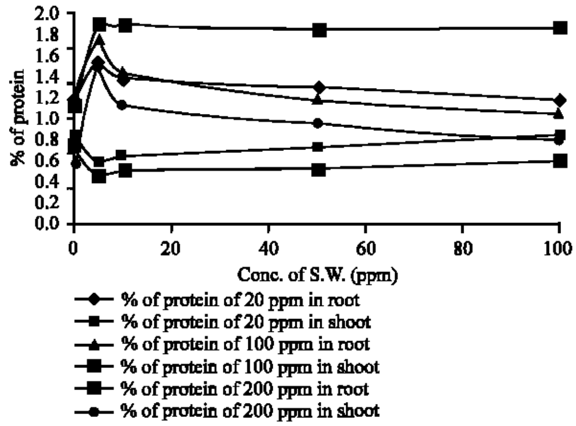

Fig. 3: Effect of seaweeds on protein of bean plants in presence of Cd

concentration of mineral ions was observed. Researches reveals that regular use of seaweed as soil feed encouraged development by fixing nitrogen in the roots and roots of plant accelerates the admission of nitrates, phosphorus, potassium and calcium into plant roots and shoots.

Mechanism of Biosorption: The mechanism by which microorganism remove metals from solution are: (i) extracellular accumulation/precipitation; (ii) cell-surface or complexation; (iii) intercellular accumulation. The mechanisms involved in the capture of heavy metal ions will depend on the biomaterial used, the charge of the metal ion[16], the genus of the seaweed and the chemical species of the element in aqueous solution.

Toxic metals in solution are not necessarily present as free metal ions. Some other ions, called ligands, are capable of interacting with metal ions, forming complexes. The knowledge of this interaction between free metal ions and ligands is the principle of the development of biosorption processes[17]. The same interaction is expected to happen between the free metal ions and the ligands present on the surface of the selected biomass for instance, polysaccharides from seaweeds.

There are several chemical groups in seaweeds that would attract and sequester the metals in biomass: acetamido groups of chitin, structural polysaccharides of fungi, amino and phosphate groups in nucleic acids, amido, amino, sulphohydryl and carboxyl groups in proteins, hydroxyls in polysaccharide and mainly carboxyls and sulphates in polysaccharides of marine algae that belong to the division Phaeophyta, Rhodophyyta and Chlorophyta[18,19]. However, it does not necessarily mean that the presence of some functional group guarantees biosorption, perhaps due to steric, conformational or others barriers[20].

Bacterial count in rhizosphere: The effect of seaweed on plant growth in presence of Cd metal on rhizosphere or bacterial population[13-15] was observed. Bacterial population increased with the increase in concentration. of seaweed (Table 3) which may be due to the polysaccharide available on the surface of seaweeds. The organism identified were both gram +ve and gram−ve bacteria; e.g., *Staphylococcus aureus* and among gram−ve bacteria were *E. coli, Klebsiella Pneumonia* and *Citrobacter* species (Table 4). Organism present in rhizosphere responsible for various metabolic activities, decomposition of compounds presents in soil and make it available to plant growth. Growth of microorganism were compared with control pot where it was found that growth were enhanced in presence of seaweed and produced effect on plant growth due to the favorable soil condition. Green seaweed helps to increase water-holding capacity of soil, which promote the growth of plants and rhizosphere population. Seaweeds stimulates beneficial soil microbial activity particularly in the pocket of soil around the feeder root resulting in a substantially larger root mass, where beneficial fungi and bacteria known as *mycorrhizae* make their home. This area of the soil is known as the rhizosphere. The rhizosphere activity improves the plant ability to form healthier, strong roots. The rhizosphere forms a nutrient food bank for the plants, it can draw on in time of stress. Having many actions it also enhance the plant own natural activity to ward off disease and pests. Another action seaweeds has on the roots in the rhizospere is due to the increased mass of depth of the roots the plants is able to draw more moisture from the soil increasing the drought tolerance level.

CONCLUSION

The present investigation reveals that biosorption of Cd metal takes place by using green seaweed *Codium iyengArii*. This results in the healthy growth of plants under metal toxicity. Seaweed also helps in developing the rhizosphere of soil.

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