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The Inhibition of Bean Plant Metabolism by Cd Metal and Atrazine III: Effect of Seaweed *Codium iyengarii* on Metal, Herbicide Toxicity and Rhizosphere of the Soil

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Abstract: A seaweed-dried powder of *Codium iyengarii* was used to investigate the effect on bean plants. These cultivated in metal contaminated and atrazine herbicide environment. The use of seaweed increased the growth of seedlings under toxicity of metal and herbicide. Seaweed contains all trace elements and major and minor plant nutrients such as alganic acid, vitamins, auxins, gibberllins and antibiotics. Results were interpreted in relation with metabolic activity of plant, bioremediation of atrazine and soil rhizosphere. It was observed that seaweeds containing alganic acid act as a soil conditioner, which improved the water holding characteristics of soil and helped in the formation of crumb structure. The toxicity of heavy metal Cd in conjunction with atrazine herbicide was inhibited up to 100 ppm of metal concentration. Significant improvement in seed germination, morphology and physiological processes were observed. Seaweeds helps in improving the rhizosphere of the contaminated soil which results in the bioremediation of atrazine by soil bacteria. This can be attributed with the growth of the plant in heavy metal contaminated environment and also controls the toxicity of trizine herbicide. While an increase in the concentration of Cd metal shows that it retarded the growth of the bean plant in the presence of seaweeds too.

Key words: Seaweeds, alganic acid, metabolic activity, Cd, atrazine

INTRODUCTION

The discharge of heavy metal into aquatic ecosystem has become a matter of concern in the world over the last few decades. These pollutants were introduced into the aquatic system significantly as a result of various industrial operations. The pollutants of concern include Pb, Cr, Hg, U, Se, Zn, As, Cd, Au, Ag, Cu and Ni (Alya *et al*., 2004).

The commonly used procedures for removing metals ions from aqueous streams include chemical precipitation, lime coagulation, ion exchange, reverse osmosis and solvent extraction. Biosorption of heavy metals from aqueous solution can be considered as an alternative technative technology in industrial wastewater treatment. It is based on the ability of biological materials to accumulate heavy metal from waste-water. Natural resources hidden in the sea are becoming more and more important in human life. It is an establishing fact that seaweeds are full of innumerable wealth of bioactive properties (Stephenson, 1968).

Seaweeds have been used by plant growers for centuries but the reason for beneficial results has only recently bean attributed to the naturally occurring growth regulators and micro nutrients in the seaweed. The green alga, *Bryopsis corymbasa* was found to display an anti fungal activity. *Sargassum species* was used to remove Cu and Cr from aqueous solution (Cossich *et al*., 2002; Antunes *et al*., 2003). Seaweeds selectively, absorb from the seawater elements like Na, K, Ca, Mg, Cl, I and Br, which are accumulated in their thalli. Seaweeds are known as alkaline food since their inorganic components play a very important role in preventing blood acidosis (Veglio and Beilchini, 1997). The seaweeds can be used directly on the plant in the form of a spray and minerals in seaweed spray, are absorbed through the skin of the leaf through stomata into the sap with not only mineral but auxins too (Volesky and Holen, 1995). Seaweed sprays stimulate metabolic processes in the leaf and so helped the plant to exploit leaf locked nutrients and thus increase the rate of photosynthesis (Matheickal and Yu, 1999; Macaskie *et al*., 1987; Peng and Koon, 1993).

Although a lot of study on seaweed has been reported on their taxonomy, distribution, morpho-ecological studies, phytochemistry and antibacterial activity, but insufficient data are available in literature

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to control the toxicity of heavy metal contaminated water. Therefore this study was planned to control the toxicity of heavy metals and atrazine by green seaweed.

Codium iyengarii is abundant at the Karachi Bulljee coastal area. The present study considered the effect of seaweed on growth, morphology, physiology and rhizosphere of the soil.

MATERIALS AND METHODS

Macro Algae *Codium Iyengarii* has been collected from Bullijea coastal area of Karachi at low tide season in the morning at 9.00°clock during February and March 2004. Algae collected in this study was taken from free-floating tides at the beach side. Sample were cleaned from seawater and rinsed with deionised water. Samples was 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^{+2} were prepared like 20, 100 and 200 ppm from $CdCl_2$ for Hoagland solution. Dry seaweed was introduced from 0, 5, 10, 50 and 100 ppm with the fixed concentration of Cd and atrazine in separate pots. Plants were analyzed after fifteen days for different biochemical tests and mineral ions analysis.

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

Physiology of plant: Physiological processes of plants were checked by determining its nutritive value like carbohydrate, protein, amino acid and chlorophyll a and b by visible spectrophotometric technique (Chapman, 1976; Ahmed *et al.*, 1985; Tandom, 1933; Lowery *et al.*, 1951).

Analysis of mineral ions: The contents of sodium, potassium, manganese, magnesium, calcium and iron were estimated through dry ash method by using 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 was recorded at 660 nm by visible spectrophotometer (Chapman, 1976).

Analysis of roots for soil microorganism

Standard plate count: Samples of roots from different concentration of sea weed viz., 0, 5, 10, 50 and 100 ppm were taken for determining the effect of green algae on Colony Forming Units (CFU) per mL after 10 weeks of germination. Plant roots were soaked and incubated at 37°C in sterile saline for 24 h. Sample were diluted up to 10^{-5} and the last dilution was spread on nutrient agar plates and were incubated at 37°C for 24 h (Brown, 2005).

Identification of microorganisms: 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, Imvic, oxidase and catalase tests for the confirmation of organism.

RESULTS AND DISCUSSION

Seaweed extract treatment may be expected to increase the plant growth, even when the plant is under nutrient stress (Hasni and Sarwar, 1985). *Codium iyengarii* belongs to chlorophyta, which was used as protein and mineral enriched seaweed to control the toxicity of heavy metal contaminated water, which is now a days are commonly used for cultivation of crops in Asia. It was observed that seaweed containing alganic acid acts as a soil conditioner because alganic acid in the seaweed combined with the metalic radicals in the soil to form polymer with greatly increased molecular weight of the type known as cross-linked. The salts formed by alganic acid with soil metals swell when wet and retain moisture tenaciously, so helping the soil to form a crumb structure (Hasni and Sarwar, 1985; Kuyucak and Volesky, 1989). Healthy growth of the plants were observed with seaweeds (Fig. 1 and 2) and seaweed produces the retarded effect in accumulation of Cd metal with the roots of bean plant. Hydrolized seaweed carrying trace elements is alkaline having pH of 9 so alkaline solution would automatically precipitate trace elements. Results showed that it increases the seed germination, length of main root and shoots systems and of lateral roots. It also increased the protein contents in shoots. Seaweed also has chelating properties. The seaweed increases the water holding power by 11% which in turns leads to better aeration and capillary action and these stimulate the root system of plant to further growth and so stimulate the soil bacteria to greater activity. Table 1 shows that as the concentration of seaweed increases it controls the toxicity of Cd metal in conjunction with atrazine herbicide due to which concentration of contents of chlorophyll a and b, carbohydrate and proteins were found to be increased. At higher concentration of Cd metal used, Cd decreases the nutritive value of bean plant (Azmat *et al.*, 2005). The seaweed indeed any undecomposed organic matter,

Table 1: Effect of *Codium iyengarii* on growth and nutritive value of bean plant

Conc. of Seaweeds (ppm)	Average length of plant (cm) Roots	Average length of plant (cm) Shoots	Chlorophyll (a) mg g^{-1}	Chlorophyll (b) mg g^{-1}	% of protein Roots	% of protein Shoots	% of Carbohydrate 10^2 Roots	% of Carbohydrate 10^2 Shoots	% Amino acids 10^4 Roots	% Amino acids 10^4 Shoots
Atrazine 50 ppm					Cd 20 ppm					
0	40.2±0.2	21±0.2	0.128±0.01	0.047±0.02	1.166±.002	0.83±0.1	2.65±0.01	1.56±0.01	1.475	3.94±0.01
5	10.1±0.1	21±0.02	0.136±0.02	0.056±0.03	1.52±0.003	0.58±0.1	0.917±0.03	1.791±0.02	1.2±0.02	0.84±0.01
10	1.5±0.2	23±0.10	0.124±0.03	0.048±0.04	1.375±0.02	0.631±10.5	0.983±0.04	2.008±0.03	1.21±0.01	2.68±0.02
50	1.5±0.3	24±0.3	0.112±0.04	0.043±0.03	1.291±0.02	0.726±0.21	1.037±0.01	2.119±0.03	1.31±0.01	3.52±0.01
100	1.9±1.0	24±0.20	0.116±0.10	0.49±0.04	1.466±0.03	0.83±0.03	1.11±0.01	2.404±0.03	1.32±0.01	3.91±0.01
Atrazine 50 ppm					Cd 100 ppm					
0	3.0±0.1	16.0±0.1	0.095±0.02	0.033±0.01	1.166±0.001	0.716±0.001	2.47±0.01	1.42±0.03	-	-
5	2.5±0.1	16.0±0.1	0.083±0.001	0.088±0.02	1.708±0.002	0.466±0.001	3.22±0.17	2.87±0.02	-	-
10	3.5±0.4	17.0±0.2	0.119±0.003	0.097±0.01	1.416±0.004	0.50±.005	3.66±0.18	2.66±0.12	-	-
50	4.5±0.5	18±0.4	0.149±0.004	0.15±0.04	1.166±0.006	0.5166±0.17	3.69±0.12	2.63±0.17	-	-
100	4.5±0.1	19±0.02	0.326±0.006	0.323±0.05	1.041±0.001	0.6±.005	3.97±0.13	1.35±0.18	-	-
Atrazine 50 ppm					Cd 200 ppm					
0	2.0±0.1	11±0.2	0.0512±0.001	0.016±0.001	1.145±0.01	0.55±001	1.32±0.01	0.91±0.1	-	-
5	2.5±0.2	11±0.2	0.055±.001	0.032±0.001	1.45±001	0.443±0.02	2.051±0.04	2.462±0.1	-	-
10	2.0±0.3	15±0.3	0.054±0.04	0.0104±0.001	1.40±0.02	0.51±0.03	2.001±0.17	2.437±0.1	-	-
50	1.5±0.1	16±0.4	0.051±0.04	0.0189±0.002	1.33±0.04	0.095±0.03	2.001±0.02	2.124±0.02	-	-
100	1.0±0.01	16±0.5	0.224±0.03	0.19±0.003	1.33±0.05	0.0783±0.01	2.0±0.01	2.31±0.3	-	-

Table 2: Effect of seaweeds on K^+ ions content in roots and shoots of bean plant

	g % of K^+					
	(20 ppm Cd)		(100 ppm Cd)		(200 ppm Cd)	
Conc. of seaweeds (ppm)	Root	Shoot	Root	Shoot	Root	Shoot
0	0.569±0.002	0.587±0.001	0.587±0.003	0.588±0.003	0.321±0.001	0.254±0.001
5	0.542±0.001	0.547±0.003	0.574±0.001	0.542±0.001	0.323±0.001	0.323±0.003
10	0.421±0.002	0.321±0.001	0.321±0.003	0.421±0.004	0.324±0.003	0.235±0.004
50	0.423±0.003	0.423±0.003	0.321±0.001	0.321±0.001	0.321±0.003	0.321±0.001
100	0.423±0.001	0.423±0.005	0.212±0.001	0.212±0.001	0.21±0.001	0.21±0.003

Fig. 1: Growth of bean plant before treatment with seaweed

attacked by bacteria breaks into simpler units. Thus the bacteria need nitrogen and this take from the soil therefore the amount of nitrogen available to plants were reduced which may be explained by the less concentration of amino acid from the roots of plant at all concentration of seaweed used while amino acids were absent at higher concentration of Cd due to the toxicity of metal. It was observed that the lower concentrations of Cd metal were torelable to plants in presence of *Codium* sp. (Matheickal and Yu, 1999). The essential minerals ions like Na, K, Mg, Mn, Ca and Fe were effected by the toxicity of Cd metal at 200 ppm of Cd. Table 2-5 shows that mineral ions

Table 3: Effect of seaweed on Mn^{+2} ions content in roots and shoots of bean plant

Conc. of seaweeds (ppm)	g % of Mn (20 ppm) Root (10^3)	Shoot (10^3)	(100 ppm) Root (10^3)	Shoot (10^3)	(200 ppm) Root (10^3)	Shoot (10^3)
0	2.76±0.0010	2.51±0.0001	2.76±0.0001	2.41±0.001	2.15±0.0001	0.84±0.0001
5	1.71±0.00010	2.52±0.0001	1.98±0.0001	2.58±0.003	2.51±0.001	7.33±0.0001
10	1.08±0.00020	2.15±0.003	1.08±0.0001	1.05±0.001	2.51±0.001	2.51±0.0002
50	9.68±0.0001	7.33±0.0004	7.33±0.0001	1.32±0.001	1.04±0.0001	2.51±0.0003
100	7.97±0.0001	7.97±0.001	7.33±0.0001	2.06±0.001	1.04±0.001	1.04±0.0001

Table 4: Effect of seaweed on PO^{-}_4 ions content in roots and shoots of bean plant

Conc. of seaweed (ppm)	g % of PO_4 (20 ppm Cd) Root (10^3)	Shoot (10^3)	(100 ppm Cd) Root (10^3)	Shoot (10^3)	(200 ppm Cd) Root (10^3)	Shoot (10^3)
0	1.09±0.002	24.1±0.001	1.05±0.002	0.84±0.001	2.15±0.003	3.38±0.001
5	1.05±0.001	2.58±0.003	1.18±0.001	2.41±0.003	2.51±0.001	4.04±0.001
10	1.09±0.003	2.76±0.001	1.12±0.003	7.33±0.004	1.04±0.001	7.33±0.001
50	2.41±0.004	2.52±0.001	2.41±0.001	9.22±0.001	1.04±0.001	2.06±0.001
100	1.05±0.001	2.06±0.001	1.07±0.001	1.04±0.003	1.06±0.001	1.71±0.001

Table 5: Effect of seaweed on Fe ions content in bean plants in roots and shoots of bean plant

Conc. of seaweed (ppm)	g % of Fe (20 ppm Cd) Root (10^3)	Shoot (10^3)	(100 ppm Cd) Root (10^3)	Shoot (10^3)	(200 ppm Cd) Root (10^3)	Shoot (10^3)
0	2.76±0.003	2.52±0.0001	2.76±0.0003	2.15±0.001	2.15±0.001	2.52±0.003
5	1.71±0.0001	2.52±0.0001	1.98±0.0001	1.09±0.001	2.51±0.001	2.51±0.0005
10	1.08±0.0001	2.15±0.0001	1.08±0.0001	1.18±0.001	2.51±0.001	2.51±0.0001
50	9.68±0.0001	1.18±0.0001	7.33±0.0001	2.15±0.001	1.04±0.001	1.08±0.0001
100	1.97±0.0001	1.08±0.003	7.33±0.0001	2.15±0.001	1.04±0.001	1.09±0.0003

Fig. 2: Growth of bean plant after treatment with seaweed

concentration increases with the increase in concentration of seaweed but at higher concentration of heavy metal it become reduced.

Degradation of herbicide: Heavy bacterial populations with seaweeds were observed in presence of Cd metal in conjunction with atrazine herbicide. Bacterial activity in the presence of seaweed has two results, first the secretion of substance which further helps to conditioning in the soil and second, an effect on nitrogen contents of the soil. The substances secreted by soil bacteria in presence of seaweed include organic chemicals known as polyuronides, similar to the soil conditioner alganic acid whose direct effect on the growth of the plant explains the control of toxicities of heavy metal in contaminated water and soil.

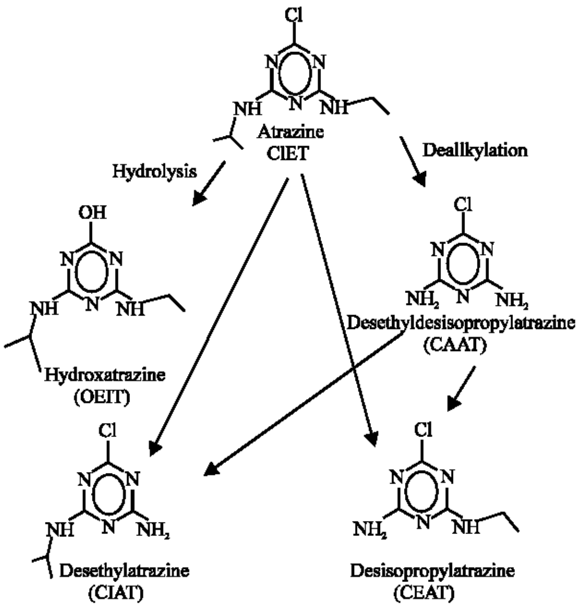

Fig. 3: Structure of Atrazine and its Metabolites

The addition of nutrients of Codium sp. causes an increase of microbiol population, there by increasing the number of indigenous microorganisms capable of degrading the contaminants, which results the metabolites of atrazine as shown in Fig. 3 (Geoffrey, 1990; Daniel and Ewing, 1994). The metabolites (CIAT) and (CEAT) finally degraded to biuret and then to urea. The urea is then broken down into carbon dioxide and ammonia which is ultimately converted in to nitrate for the use of plants (Azmat and Uddin, 2005).

CONCLUSIONS

The present investigation suggests that plants treated with seaweed powder develop a resistance to pests diseases and toxicity of metal and herbicide. Such a possibility may seen noval which is attributed to the spray and wet used of green seaweed which can be used to improve the quantity and quality of crops. It also improves the growth and nutritive value of crops and helps in improving the rhizosphere of the soil.

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