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 alginic acid, vitamins, auxins, gibberellins and 
and antibiotics. Results were interpreted in relation with metabolic activity of plant, bioremediation of atrazine and 
soil rhizosphere. It was observed that seaweeds containing alginic acid act as a soil conditioner, which 
improves 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 help 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 trazine 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, alginic 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, Cu, 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 technicative 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 been attributed to the naturally occurring growth regulators and micro nutrients in the seaweed. The green 
algae, Bryopsis corymbosa was found to display an anti fungal activity. Sargassum species was used to remove 
Cu and Cr from aqueous solution (Cossioh 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 thallii. 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
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 Bulljee 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 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 Cd²⁺ were prepared like 20, 100 and 200 ppm from CdCl₂ 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 10⁻² 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, Invic, 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 metallic 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 a form a crumb structure (Hasni and Sarwar, 1985; Kinyucak 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. Hydrolyzed 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 Ccladium meneghinianum on growth and nutritive value of bean plant

<table>
<thead>
<tr>
<th>Cont. of</th>
<th>Average length</th>
<th>Chlorophyll</th>
<th>% of protein</th>
<th>% of Carbohydrate</th>
<th>% Amino acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seaweeds</td>
<td>of plant (cm)</td>
<td>(mg g⁻¹)</td>
<td>(mg g⁻¹)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Roots</td>
<td>Shoots</td>
<td>Roots</td>
<td>Shoots</td>
<td>Roots</td>
</tr>
<tr>
<td>0</td>
<td>40±0.2</td>
<td>21±0.2</td>
<td>0.12±0.01</td>
<td>0.04±0.02</td>
<td>1.16±0.02</td>
</tr>
<tr>
<td>5</td>
<td>10±0.2</td>
<td>21±0.2</td>
<td>0.16±0.02</td>
<td>0.06±0.03</td>
<td>1.52±0.02</td>
</tr>
<tr>
<td>10</td>
<td>1±0.2</td>
<td>22±0.2</td>
<td>0.14±0.02</td>
<td>0.08±0.04</td>
<td>1.55±0.02</td>
</tr>
<tr>
<td>20</td>
<td>1±0.2</td>
<td>22±0.2</td>
<td>0.14±0.02</td>
<td>0.08±0.04</td>
<td>1.55±0.02</td>
</tr>
<tr>
<td>50</td>
<td>1±0.2</td>
<td>22±0.2</td>
<td>0.14±0.02</td>
<td>0.08±0.04</td>
<td>1.55±0.02</td>
</tr>
<tr>
<td>100</td>
<td>1±0.2</td>
<td>22±0.2</td>
<td>0.14±0.02</td>
<td>0.08±0.04</td>
<td>1.55±0.02</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Cont. of seaweed (g)</th>
<th>% of K⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Roots</td>
</tr>
<tr>
<td>0</td>
<td>5.94±0.02</td>
</tr>
<tr>
<td>5</td>
<td>5.94±0.02</td>
</tr>
<tr>
<td>10</td>
<td>5.94±0.02</td>
</tr>
<tr>
<td>20</td>
<td>5.94±0.02</td>
</tr>
<tr>
<td>50</td>
<td>5.94±0.02</td>
</tr>
<tr>
<td>100</td>
<td>5.94±0.02</td>
</tr>
</tbody>
</table>

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

The concentration of Cd due to the toxicity of metal. It was observed that the lower concentrations of Cd metal were tolerable 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
\[
\text{\% of Mn} \quad \begin{array}{ccc}
\text{(20 ppm)} & \text{(100 ppm)} & \text{(200 ppm)} \\
\hline
\text{Conc. of seaweed (ppm)} & \text{Root (mg)} & \text{Shoot (mg)} & \text{Root (mg)} & \text{Shoot (mg)} & \text{Root (mg)} & \text{Shoot (mg)} \\
0 & 2.7640.0001 & 2.5140.0001 & 2.7640.0001 & 2.4140.0001 & 2.1540.0001 & 0.9440.0001 \\
5 & 1.7140.0001 & 1.9840.0001 & 1.9840.0001 & 1.8540.0001 & 1.8540.0001 & 1.7140.0001 \\
10 & 1.0840.0001 & 1.3540.0001 & 1.3540.0001 & 1.2240.0001 & 1.1840.0001 & 1.0440.0001 \\
50 & 9.6840.0001 & 7.3340.0001 & 7.3340.0001 & 6.1840.0001 & 5.1840.0001 & 4.0440.0001 \\
100 & 7.9740.0001 & 6.9740.0001 & 6.9740.0001 & 5.9740.0001 & 5.1840.0001 & 4.0440.0001 \\
\end{array}
\]

Table 4: Effect of seaweed on PO$_4^{3-}$ ions content in roots and shoots of bean plant
\[
\text{\% of PO$_4$} \quad \begin{array}{ccc}
\text{(20 ppm C4)} & \text{(100 ppm C4)} & \text{(200 ppm C4)} \\
\hline
\text{Conc. of seaweed (ppm)} & \text{Root (mg)} & \text{Shoot (mg)} & \text{Root (mg)} & \text{Shoot (mg)} & \text{Root (mg)} & \text{Shoot (mg)} \\
0 & 1.0440.0001 & 1.0940.0001 & 1.0940.0001 & 1.0440.0001 & 1.0440.0001 & 1.0440.0001 \\
5 & 1.0540.0001 & 1.1840.0001 & 1.1840.0001 & 1.0440.0001 & 1.0440.0001 & 1.0440.0001 \\
10 & 1.0940.0001 & 1.2240.0001 & 1.2240.0001 & 1.0440.0001 & 1.0440.0001 & 1.0440.0001 \\
50 & 2.4140.0001 & 2.4140.0001 & 2.4140.0001 & 1.0440.0001 & 1.0440.0001 & 1.0440.0001 \\
100 & 1.0540.0001 & 2.0540.0001 & 2.0540.0001 & 1.0440.0001 & 1.0440.0001 & 1.0440.0001 \\
\end{array}
\]

Table 5: Effect of seaweed on Fe ions content in bean plants in roots and shoots of bean plant
\[
\text{\% of Fe} \quad \begin{array}{ccc}
\text{(20 ppm C4)} & \text{(100 ppm C4)} & \text{(200 ppm C4)} \\
\hline
\text{Conc. of seaweed (ppm)} & \text{Root (mg)} & \text{Shoot (mg)} & \text{Root (mg)} & \text{Shoot (mg)} & \text{Root (mg)} & \text{Shoot (mg)} \\
0 & 2.7640.0001 & 2.7640.0001 & 2.7640.0001 & 2.7640.0001 & 2.7640.0001 & 2.7640.0001 \\
5 & 1.7140.0001 & 1.7140.0001 & 1.7140.0001 & 1.7140.0001 & 1.7140.0001 & 1.7140.0001 \\
10 & 1.0840.0001 & 1.0840.0001 & 1.0840.0001 & 1.0840.0001 & 1.0840.0001 & 1.0840.0001 \\
100 & 7.9740.0001 & 7.9740.0001 & 7.9740.0001 & 7.9740.0001 & 7.9740.0001 & 7.9740.0001 \\
\end{array}
\]

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 seaweed 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 alginic acid whose direct effect on the growth of the plant explains the control of toxicities of heavy metal in contaminated water and soil.
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 seem novel 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.

REFERENCES