International Journal of Research in Chemistry and Environment
Vol. 1 Issue 2 Oct. 2011(70-75)
ISSN 2248-9649

Research Paper

Effect of *Sargassum wightii* on the Growth and Biochemical Characteristics of Heavy Metal Treated *Vigna radiata* (L.) wilczek

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Available online at: **www.ijrce.org**

(Received 13th July 2011, Accepted 29th August 2011)

Abstract - The effects of lead acetate on the growth and biochemical characteristics of *Vigna radiata* (L.) Wilczek. Seedlings were studied with different concentrations of (5mM, 10mM, 15mM, 20mM, 25mM) lead acetate. The heavy metal treatment on green gram has caused a steep decline in its growth, pigment content, other biochemical characteristics and enzyme activities with increase in the concentration of the lead acetate. On the other hand, after bioremediation treatment with various concentration of seaweed (Sargassum wightii 2mg/L, 4mg/L, 6mg/L) on 15mM lead acetate treated plants a considerable increase on the growth and biochemical characteristics were noticed than in plants treated with heavy metal alone. Addition of seaweed has mitigated the toxic effect of the heavy metal to some extent and instigated the growth parameters, pigment content, biochemical characteristics and enzyme activities of *Vigna radiata* (L.) Wilczek. Seaweeds exhibited better performance in reducing all the pollutants from the heavy metal than its counterparts.

Keywords: Biochemical changes, Lead acetate, Enzyme activity, Growth, Heavy metal, Seaweed, etc.

Introduction

Environmental pollution, especially by chemicals, has received a great deal of attention in recent years. Among all chemical pollutants, heavy metals have specific ecological, biological and health significance. A particular concern has been generated over the possibility of toxic elements especially Pb and Zn which cause a more insidious problems for man and animals due to their accumulation in food plants [19]. Response of seed germination and plant growth to heavy metals has become the subject of great interest in recent years. Heavy metal toxicity causes multiple direct and indirect effects on plant growth and alter many physiological functions [30]. The toxicity of heavy metals is mainly attributed to their ability of binding to enzyme, resulting in the alteration of their catalytic functions and inactivation [25]. Heavy metals are metallic elements which have a high atomic weight and density much greater than water. Heavy metals influence and interfere with a variety of processes in higher plants such as protein and enzyme synthesis, disturbances in cytokinesis, lowering of DNA synthesis and stability. Lead does not evaporate, but it can be present in air as particles. Lead does not degrade not it can be destroyed. The major use of lead today is in the production of some type of batteries; also used in the ammunition, metal products (sheet metal, solder and pipes), medical equipment and high precision glass for lasers and other optical equipment.

Lead as an environmental Contaminant

Metal toxicity in plants has been reported by many workers [6]. Nicholls and Mal (2003) studied the effect of lead on growth of an invasive weed *Lythrum salicaria*,L. and explained that application of lead caused complete withering and death of the above ground parts of all plants. Al-Yemeni (2001) studied the effect of lead on seed germination and early seedling growth of *Vigna ambacensis* L. He observed that the apical and shoot length of *Vigna ambacensis* L. seedlings were significantly inhibited by lead.

Remediation of Lead using biosorbents

Biosorption or bioremediations consists of a group of applications which involve the detoxification of hazardous substances instead of transferring them from one medium to another by means of plants and microbes. This process is characterized as less disruptive and can be often carried out on site, eliminating the need to transport the toxic materials to treatment sites [9]. Biosorbents are prepared from naturally abundant and /or waste biomass. Due to the high uptake capacity and very cost effective source of the raw material, biosorption is a progression towards a perspective method. Seaweed are among the important marine resources with tremendous commercial application. They offer several advantages for biosorption because of their larger surface area. This feature offers a convenient basis for the production of biosorbent particles suitable for biosorption process. Seaweeds used as a soil amendment increases soil N, K and Mg, which may be beneficial for crop production. Soil acidity can be reduced by adding calcareous seaweeds *Lithothanmnion, Phymatolithon.* Seaweed manure is rich in potassium. Application of seaweeds in plants cause the resistances to bacterial, viral and fungal activities.

In the present study, it was aimed to find out the impact of various concentration of Lead acetate and the effect of varying amount of dried seaweed powered of *Sargassum wightii* with optimum concentration of 15mM lead acetate on the growth and biochemical characteristics of *Vigna radiata* (L). Wilczek.

Material and Methods

Healthy and Viable seeds of *Vigna radiata* (L). Wilczek were germinated in plastic troughs of uniform size containing acid washed riverbed sand. Seeds of equal number were sown in each pot. The three day old seedlings were irrigated everyday with 50ml of different concentration (5mM, 10mM, 15Mm, 20mM, 25mM) of lead acetate. Plants irrigated with half-strength of Hoagland's Nutrient Solution [11] served as control. Various concentration of *Sargassum wightii* *(*2g/L, 4g/L and 6g/L) was mixed with 15Mm lead acetateand used for further study.

After ten days of the treatment the *Vigna radiata* were used for measuring the growth parameters such as shoot length, root length, leaf area, fresh weight, dry weight. Biochemical parameters such us Chlorophyll *a*, *b*, Total Chlorophyll and Carotenoid [27]. Anthocyanin content [16]. Total soluble sugar (Anthrone method) protein content [15], aminoacid content [13] proline content [5], *invivo* nitrate reductase activity [12] Catalase [14] and Peroxidase activity [2] also analyse.

Results

Effect of five different concentrations (5mM, 10mM, 15mM, 20mM, and 25mM) of lead acetate on the growth, biochemical and enzyme activities are represented in Table 1 to 4. The results shows that growth parameters such as root length, shoot length, Leaf area, fresh weight and dry weight decreased with the increase in the concentration of lead acetate.

Similarly Chlorophylls, Carotenoid, Total Soluble Sugar, protein and NR activity were also in a declining trend. In contrary the pigment Anthocyanin, total free amino acid, proline and the antioxidant enzyme such as peroxidase and catalase increased with the increase in the metal concentration. Remediation studies shows that the growth parameters such as root length, shoot length, Leaf area, fresh and dry weight of the plant were increased by increasing the amount of dried seaweed powdered with15mM lead cetate solution treated *Vigna radiata* plants (Table 5).

The chlorophyll and carotenoid contents had been significantly increased after the application of seaweed treated metal solution in *Vigna radiata* seedlings. The anthocyanin content was decreased by the application of seaweed treated metal solution seedlings (Table 6).

Total soluble sugar and soluble protein contents were significantly increased in the seedlings after the application of seaweed treated heavy metal solution. In contrary, total free amino acid and proline contents were reduced after the application of treated lead acetate (Table 7).

The activities of enzymes such as catalase and peroxidase in the *Vigna radiata* seedlings had been reduced after the application of seaweed treated lead acetate solution, where as the nitrate reductase activity was increased by the application of seaweed powdered. (Table 8).

Discussion

The removal of heavy metals from our environment is now shifting from the use of conventional adsorbents to the use of seaweed biomass. Biosorbents are prepared from naturally abundant biomass. Seaweeds which originates from the ocean garden, is one of the best materials for an earth garden. Seaweeds helps in stimulating soil bacteria. This inturn increase fertility of the soil by humus formation (which feeds on bacteria), aeration and moisture retention. They contain many polyfunctional metal binding sites for both cationic and anionic metal complexes [7]. The algae *Sargassum wightii* is a very good biosorbent of heavy metals [20].

This finding clearly indicates that addition of dry seaweed biomass reduces the toxic effect of lead acetate and thereby promotes the growth of *Vigna radiata*. The increasing effect of seaweed biomass may be due to the presence of Phenyl Acetic Acid (PAA) and some growth promoting substances [24]. The dry seaweed biomass were mixed with 15mM lead acetate caused an increase in pigment content than the control ie lead acetate treated plants and also. Increase in the number and size of the chloroplast and better grana development [17]. An increase in protein content and decrease in free aminoacid and Proline after the application of *Sargassum wightii* biomass in 15mM lead acetate observed in the present study indicates the seaweeds contain many growth promoting hormones like auxin, gibberlins, and trace elements vitamins and mineral nutrient. In present investigation the *in vivo* nitrate reductase activity increases with the increase in the application of seaweed biomass. This may be due to the increase in the uptake of nitrate by the plants. Peroxidase and catalase are the enzymes responsible for scavenging the plant materials from the stressed impact. Upon the addition of dried biomass of *Sargassum wightii* in 15mM lead acetate treated seedlings of *Vigna radiata*, these enzyme activities decrease considerably than in plants treated only with the said metal. The present study shows that, the toxic effects of lead on plants can be almost removed by the addition of dried seaweed biomass.

Conclusion

In the present study, it has been experimentally proved that the use of seaweeds is the best biosorbent. We have reviewed the sources and toxicology of heavy metals as well as the reason why they need to be removed from our environment. Conventional methods of removal are expensive and hence the uses of low cost abundant environment friendly biosorbents have been tested. The present investigation on the use of dried algal biomass available in large quantities for removal of heavy metals has tremendous potential as an economic effective safe alternative. Innovative, economically feasible and novel biomass regeneration and conversion of the recovered metal into usable form are the best options to attract more usage of biosorbents.

The result of the present investigation clearly shows that the use of *Sargassum wightii* can efficiently remediate the toxicity of lead acetate. Hence we strongly suggest that *Sargassum wightii* can be used as a biosorbent of heavy metal in the metal polluted environment for sustainable agriculture.

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Table 1: Effect of Various concentration of Lead acetate on the growth of *Vigna radiata* (L.) wilczek

Parameters	Control	5mM	10mM	15mM	20mM	25mM
Shoot Length (cm)	32.50 ± 0.057 (100)	24.1 ± 0.057 (74)	21.0 ± 0.577 (64)	19.0 ± 0.577 (58)	18.2 ± 0.233 (56)	16.6 ± 0.333 (51)
Root Length (cm)	16.2 ± 0.057 (100)	13.4 ± 0.088 (82)	9.5 ± 0.251 (58)	9.2 ± 0.048 (57)	8.2 ± 0.088 (50)	7.2 ± 0.145 (44)
Fresh Weight (mg)	0.653 ± 0.008 (100)	0.490 ± 0.006 (75)	0.380 ± 0.005 (58)	0.283 ± 0.003 (43)	0.260 ± 0.005 (39)	0.186 ± 0.008 (28)
Dry Weight (mg)	0.146 ± 0.026 (100)	0.076 ± 0.0005 (51)	0.063 ± 0.0008 (43)	0.046 ± 0.0003 (31)	0.040 ± 0.0008 (27)	0.038 ± 0.0003 (25)
Leaf area (cm^2)	3.33 ± 0.166 (100)	2.86 ± 0.033 (86)	2.63 ± 0.033 (79)	2.23 ± 0.016 (67)	2.11 ± 0.016 (63)	1.95 ± 0.028 (58)

Values are an average of ten observations.

Values in parenthesis are percentage activity with respective control Mean ± Sε

Table 2 Effect of Various concentration of Lead acetate on the Photosynthetic pigment of *Vigna radiata (L).* Wilczek

Parameters	Control	5mM	10mM	15mM		25mM
Chlorophyll *a* (mg/gLFW)	0.123 ± 0.0005 (100)	0.106 ± .0008 (86)	0.098 ± 0.0005 (79)	0.095 ± 0.0005 (77)	0.085 ± 0.0005 (69)	0.013 ± 0.001 (24)
Chlorophyll *b* (mg/gLFW)	0.056 ± 0.001 (100)	0.043 ± 0.001 (77)	0.034 ± 0.0008 (61)	0.026 ± 0.0005 (46)	0.021 ± 0.0008 (37)	0.095 ± 0.001 (52)
Total Chrolophyll (mg/gLFW)	0.179 ± 0.0006 (100)	0.154 ± 0.001 (85)	0.135 ± 0.0005 (75)	0.116 ± 0.001 (65)	0.105 ± 0.001 (58)	0.095± 0.001 (52)
Carotenoids (mg/gLFW)	1.124 ± 0.001 (100)	0.894 ± .0008 (79)	0.765 ± 0.002 (68)	0.615 ± 0.001 (54)	0.576 ± 0.001 (51)	0.525 ± 0.001 (46)
Anthocyanin (mg/gLFW)	4.230 ± 0.011 (100)	5.546 ± 0.017 (131)	5.750 ± 0.011 (135)	6.673 ± 0.012 (157)	6.753 ± 0.017 (159)	7.346 ± 0.017 (173)

Values are an average of ten observations.

Values in parenthesis are percentage activity with respective control Mean ± Sε

Table 3: Effect of Various concentration of Lead acetate on the Biochemical Characteristics of *Vigna radiata (L.)* wilczek

Parameters	Control	5mM	10mM	15mM	20mM	25mM
Glucose (mg/gLFW)	64.23±0.145 (100)	29.60±0.115 (46)	25.43±0.145 (39)	22.70±0.115 (35)	17.60±0.115 (27)	14.70±0.152 (22)
Protein (mg/gLFW)	4.63 ± 0.088 (100)	3.25 ± 0.011 (70)	2.64 ± 0.012 (57)	2.50 ± 0.023 (53)	2.06 ± 0.017 (44)	1.46 ± 0.011 (31)
Starch (mg/gLFW)	2.05 ± 0.006 (100)	0.86 ± 0.014 (42)	0.64 ± 0.011 (31)	0.54 ± 0.014 (26)	0.44 ± 0.015 (21)	0.34 ± 0.012 (16)
Amino acid (µmole/gLFW)	26.66 ± 0.088 (100)	43.43 ± 0.233 (162)	47.39 ± 0.049 (177)	53.24 ± 0.023 (199)	56.06 ± 0.021 (210)	60.30 ± 0.050 (226)
Proline (mg/gLFW)	1.246 ± 0.008 (100)	1.763 ± 0.014 (141)	1.84 ± 0.011 (147)	1.953 ± 0.020 (156)	2.066 ± 0.017 (165)	2.150 ± 0.023 (172)
Leaf nitrate (mg/gLFW)	15.16 ± 0.088 (100)	20.64 ± 0.023 (136)	25.63 ± 0.027 (169)	28.45 ± 0.017 (187)	33.46 ± 0.015 (220)	36.69 ± 0.015 (241)

Values are an average of ten observations.
Values in parenthesis are percentage activity with respective control Mean ± Sε

Table 4: Effect of Various concentration of Lead acetate on Enzyme activities of *Vigna radiata* (L.) Wilczek

Parameters	Control	5mM	10mM	15mM	20mM	25mM
Nitrate reductase activity (µmole/gLFW)	7.65 ± 0.005 (100)	4.15± 0.017 (54)	3.16± 0.021 (41)	2.14± 0.008 (27)	1.63± 0.027 (21)	0.94± 0.023 (12)
Catalase activity (µmole/gLFW)	2.1± 0.057 (100)	3.36 ± 0.015 (160)	3.73± 0.020 (177)	4.65±0.018 (221)	6.71±0.142 (319)	8.27± 0.011 (393)
Peroxidase activity (µmole/gLFW)	0.0077± 0.0003 (100)	0.016 ± 0.001 (212)	0.017±0.0003 (216)	0.019±0.0003 (242)	0.026 ± 0.0008 (341)	0.035 ± 0.00 (458)

Values are an average of ten observations.
Values in parenthesis are percentage activity with respective control Mean ± Sε

Table 5: Effect of lead acetate and *Sargassum wightii* on the growth parameters of *Vigna radiata* (L.) Wilczek.

Growth	Control (Water)	Control (lead acetate 15Mm)	2gm/L	4gm/L	6gm/L
Root length (cm)	16.2± 0.057 (100)	9.5± 0.251 (58)	12.43± 0.233 (76)	13.83± 0.166 (85)	15.53± 0.290 (95)
Shoot length (cm)	32.5± 0.057 (100)	19 ± 0.577 (58)	21.73± 0.371 (66)	24.86± 0.133 (76)	28.63 ± 0.317 (88)
Leaf area (cm^2)	3.33 ± 0.166 (100)	2.23 ± 0.016 (67)	2.30 ± 0.003 (69)	2.66± 0.025 (79)	3.06 ± 0.033 (92)
Fresh weight (gm)	0.653 ± 0.008 (100)	0.283 ± 0.003 (43)	0.436± 0.008 (66)	0.566 ± 0.008 (86)	0610 ± 0.005 (93)
Dry weight (gm)	0.146 ± 0.026 (100)	0.046 ± 0.0003 (31)	0.097 ± 0.001 (66)	0.109± 0.010 (74)	0.13± 0.005 (88)

Values are an average of ten observations.
Values in parenthesis are percentage activity with respective control Mean ± Sε

Table 6: Effect of lead acetate and *Sargassum wightii* on the pigment content of *Vigna radiata*(L.) Wilczek.

Pigments	**Control (Water)**	**Control (lead acetate 15mM))**	**2 gm/L SWP**	**4 gm/L SWP**	**6gm/L SWP**
Chl. A (mg/g LFW)	0.123 ± 0.0005 (100%)	0.0950 ± 0.0006 (77)	0.0987± 0.0003 (80)	0.107± 0.0012 (87)	01133± 0.0008 (92)
Chl. b. (mg/g LFW)	0.0563 ± 0.0012 (100%)	0.0260± 0.0005 (46)	0.0290± 0.0006 (51)	0.0427± 0.0017 (75)	0.0473± 0.0012 (84)
Tot. Chlorophyll (mg/g LFW)	0.179± 0.0006 (100%)	0.116± 0.0012 (65)	0.136 ± 0.0015 (75)	01427± 0.0015 (79)	0.158± 0.001 (88)
Carotenoids (mg/g LFW)	1.1247± 0.0012 (100%)	0.615 ± 0.0017 (54)	0.812 ± 0.0008 (72)	0.911± 0.0045 (80)	1.020± 0.003 (91)
Anthocyanin (mg/g LFW)	4.23± 0.011 (100%)	6.673 ± 0.012 (157)	6.333 ± 0.008 (149)	5.843± 0.093 (138)	5.180± 0.041 (122)

Values in parenthesis indicate percent activity; value represents mean of 10 samples with their standard error (±)

Table 7: Effect of lead acetate and *Sargassum wightii* on the biochemical characteristics of *Vigna radiate* (L.) Wilczek.

Biochemical Parameters	**Control (Water)**	**Control (lead acetat 15mM))**	**2 gm/L SWP**	**4 gm/L SWP**	**6m/L SWP**
Total Soluble Sugar (mg/g LFW)	64.23± 0.145 (100%)	22.7± 0.115 (35)	28.6± 0.057 (44)	35.1± 0.321 (54)	41.13± 0.272 (64)
Total Soluble Protein (mg/g LFW)	4.63± 0.088 (100%)	2.50± 0.023 (53)	3.22± 0.066 (69)	3.75± 0.018 (81)	4.26± 0.027 (92)
Amino acid (µ mole/g LFW)	26.66 ± 0.088 (100%)	53.24± 0.023 (199)	48.51± 0.364 (181)	32.91± 0.303 (123)	30.33± 0.158 (113)
Proline (mg/g LFW)	1.246 ± 0.008 (100%)	1.953 ± 0.020 (156)	1.630 ± 0.015 (130)	1.443± 0.012 (115)	1.290± 0.005 (103)

Values are an average of ten observations.
Values in parenthesis are percentage activity with respective control Mean ± Sε

Table 8: Effect of lead acetate and *Sargassum wightii* on the enzyme activities of *Vigna radiate* (L.) Wilczek

Enzymes	**Control (Water)**	**Control (lead acetate 15mM))**	**2 gm/L SWP**	**4 gm/L SWP**	**6 gm/L SWP**
Nitrate reductase activity (□ mole/g LFW)	7.65± 0.005 (100%)	2.143± 0.008 (28)	3.850± 0.070 (50)	4.870± 0.090 (63)	6.460± 0.211 (84)
Catalase activity (□ mole/g LFW)	2.10± 0.057 (100%)	4.653± 0.018 (221)	4.10± 0.057 (195)	3.40± 0.058 (161)	2.66± 0.088 (126)
Peroxidase activity (□ mole/g LFW)	4.230.± 0.012 (100%)	6.673± 0.012 (157)	6333± 0.008 (149)	5.843± 0.093 (138)	5.180± 0.041 (122)

Values in parenthesis indicate percent activity; value represents mean of 5 samples with their standard error (±)