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Bioremoval of Gobalt Chloride by Sargassum Biomass

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ABSTRACT

Bioadsorption is an effective low cost tool for cleaning polluted environment. In the present study the seedlings of Vigna mungo (L) Hepper were treated with various concentrations of cobalt chloride and their impact on the growth, biochemical and enzyme characters were studied. After fourteen days treatment with different concentration of nickel chloride (2 mM, 4 mM, 6 mM, 8 mM and 10 mM) the growth parameter such as,

shoot and root lengths, leaf area, fresh weight and dry weight were decreased than the control. Biochemical parameters such as chlorophyll, carotenoids, soluble sugar, and protein content and nitrate reductase activity also decreased with the increase in the concentration of cobalt chloride. But the content of free amino acid, proline, phenol and leaf nitrate content were increased with the increase in the concentration of cobalt chloride. The activities of enzyme such as catalase, peroxidase and polyphenol oxidase were found to be increased with the increase in the concentration of nickel chloride. Application of seaweed (Sargassum wightii) in different concentrations (2 gm/L, 4 gm/L and 6 gm/L) on 6 mM nickel chloride treated plants has shown the stress relieving effect caused by cobalt chloride. It is good low cost bioadsorbant for cobalt chloride.

Keywords: Cobalt chloride; Bioremoval; Bioadsorbant; Seaweed

Introduction

Heavy metal pollution is one of the most serious environmental problems which have been a subject of extensive research in recent years. Heavy metals are dominantly found in almost all kinds of industrial and sewage wastes. The concentration of heavy metals in air, water and soil leads to many hazardous effects to living organisms. Excessive metal concentrations in contaminated soils can result in decreased soil microbial activity, soil fertility and yield losses [1].

Cobalt (Co) as a trace element can be a contaminant in soils due to agricultural additives or metal refineries [2]. Cobalt is a brittle, hard metal, resembling iron and nickel in appearance. Certain plant species have the ability to extract metals (such as Co) from soil, thus reducing their hazardous effects in the environment. Co is known to cause irreversible damage metabolic constituents, plant cell walls, and cell membranes. Co is an essential element for humans, animals, and prokaryotes. Co is the component of vitamin B12. Trace elements are necessary for normal metabolic functions of the plants, but at higher concentrations these metals are toxic and may severely interfere with physiological and biochemical functions. Cobalt and its salts are used in variety of processes to make super alloys which maintain their strength at high temperature, in paint as a drier, in porcelain enamel finishes as a drying agent, as an ingredient of coloured pigments and in formulating vitamin B12. Some radioactive isotopes of cobalt, such as cobalt-60 (60Co), are used in treating patients in nuclear medicine and in research [3].

Seaweeds are one of the most important resources in the world. They are large group of marine benthic algae. Seaweeds are the best material for biosorption because their macroscopic structures offer a convenient basis for the production of biosorbent particles for sorption process. Macro algae are the group of marine plants classified on the basis of pigmentation into green, brown and red algae and also major primary producers in the marine environment play an important role in energy transfer. Bioadsorbants are prepared from naturally abundant biomass. They offer several advantages for bioadsorption because of their large surface area. The use of seaweed as manure is very common in coastal areas throughout the world. Seaweed extracts have been marketed for several years as fertilizer additives and beneficial results from their use. Seaweed extracts contain macronutrients, trace elements, and plant growth regulators such as auxin, cytokinin and gibberellins (Williams *et al.*, 1981). Seaweeds used as a soil amendment increases soil N, K and Mg, which may be beneficial for crop production.

This study aimed to found out the impact of various concentration of cobalt chloride on *Vigna mungo*(L) Hepper and the effect of varying amount of dried natural biomass of Sargassum wightii with 6 mM cobalt chloride on the growth, pigment, biochemical and enzymatic characteristics of *Vigna mungo*(L.) Hepper.

Materials and Method

In the present work, seeds of *Vigna mungo* (L.) Hepper (Black gram) were selected, which were obtained from a certified seed centre, Srivilliputtur. The healthy and viable seeds of *Vigna mungo* (L.) Hepper were surface sterilized with 0.1% of mercuric chloride for

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one minute and washed with running tap water followed by distilled water. Both control and experimental seeds were allowed to grow in plastic trough containing uniform amount of sand soil. Seedlings were irrigated everyday with tap water for seven days. After seven days the seedlings were treated with different concentration of cobalt chloride (2 mM, 4 mM, 6 mM, 8 mM and 10 mM). Various concentration of the dried powdered biomass of Sargassum wightii 2 g, 4 g and 6 g were added to 1000 mL of the synthetic solution of 6 mM cobalt chloride separately. 6 mM cobalt chloride was found to be optimum by LSD analysis [4]. The solutions were kept in shaker for 24 hours. After that, all the biomass was filtered through filter paper. The filtrate was applied to the experimental pots individually on the seven days old seedlings. Three replicates along with suitable control were kept for each treatment. After the treatment, the plants were analyzed for the growth; biochemical, enzymatic characteristics and metal ion content were analyzed.

Fifteen days old plants of *Vigna mungo*(L.) Hepper were used for measuring the growth characters such as shoot length, root length, leaf area, fresh weight and dry weight were measured. The biochemical and enzymatic characters were analysed by the following methods, chlorophyll and carotenoid (Wellburn and Lichtenthalar, 1984), anthocyanin (Mancinelli *et al.*, 1973), Total soluble sugar (Jayaraman, 1981), protein content (Lowry*et al.*, 1951), amino acid content (Jayaraman,1981), leaf nitrate (Cataldo *et al.*, 1978), phenol (Bray and Thorpe, 1954) proline (Bates *et al.*, 1973) in vivo nitrate reductase activity (Jaworski, 1971), peroxidase and catalase activity (Kar and Mishra,1976) polyphenol oxidase activity (Addy and Goodman, 1972) [5-13].

Cobalt concentration in control and treated plants were analysed by atomic absorption spectrometry (AAS). Cobalt present in plants was analyzed using the method of Baker [14]. The plant sample (root, leaf and stem) were washed, dried in oven at 160°C for 40 minutes and digested in a mixture of nitric acid and perchloric acid (10:1). Then the solution was centrifuged at 5000 rpm for 5 minutes and double filtered with Whatmann filter paper No.4 and the collection was analysed for cobalt concentration by Atomic Absorption Spectrometry (Toshvin Company Model AA-6300 Shimadzu, Japan made).

Results

Effect of five different concentrations (2 mM, 4 mM, 6 mM, 8 mM, and 10 mM) of cobalt chloride on the growth, biochemical and enzyme activities are represented in Tables 1-4. 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 lcobalt chloride. 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, phenol and the antioxidant enzyme such as peroxidase, catalase and polyphenol oxidase activity increase with the increase in the metal concentration.

Table 1: Effect of various concentration of Cobalt chloride on the Growth parameters of *Vigna mungo* (L.) Hepper.

Growth Parameters	Control	2 mM	4 mM	6 mM	8 mM	10 mM
Root Length (cm)	13.9 ± 0.148 (100)	11.66 ± 0.789 84)*	9.73 ± 0.167 (70)*	8.72 ± 0.647 (63)*	6.30 ± 0.114 (45)*	4.19 ± 0.158 (30)*
Shoot Length (cm)	18.69 ± 0.152 (100)	14.74 ± 0.164 (79)*	12.55 ± 0.336 (67)*	10.23 ± 0.390 (55)*	8.03 ± 0.705 (43)*	6.03 ± 0.416 (32)*
Leaf Area (cm2)	4.6 ± 0.370 (100)	3.761 ± 0.378 (81)*	3.33 ± 0.354 (72)*	2.73 ± 0.365 (59)*	1.94 ± 0.249 (42)*	1.19 ± 0.476 (26)*
Fresh Weight (gm)	0.690 ± 0.021 (100)	0.575 ± 0.146 (79)*	0.475 ± 0.049 (69)*	0.351 ± 0.067 (51)*	0.268 ± 0.041 (39)*	0.183 ± 0.015 (27)*
Dry Weight (gm)	0.139 ± 0.070 (100)	0.086 ± 0.022 (61)*	0.065 ± 0.014 (47)*	0.043 ± 0.123 (31)*	0.033 ± 0.015 (24)*	0.025 ± 0.419 (18)*
Values in parenthesis indicate percent activity; value represents mean of 10 samples with their standard error; *Significantly different from the control at P<0.05						

Table 2: Effect of various concentration of Cobalt chloride on the Pigments of *Vigna mungo* (L.)Hepper.

Pigments	Control	2 mM	4 mM	6 mM	8 mM	10 mM
Total Chlorophyll (mg/gLFW)	2.710 ± 0.007 (100)	2.184 ± 0.032 (81)*	1.709 ± 0.023 (63)*	1.465 ± 0.048 (54)*	1.298 ± 0.035 (48)*	0.986 ± 0.017 (36)*
Carotenoids (mg/ gLFW)	1.513 ± 0.008 (100)	1.079 ± 0.016 (71)*	0.936 ± 0.018 (62)*	0.823 ± 0.011 (54)*	0.727 ± 0.011 (48)*	0.606 ± 0.013 (40)*
Anthocyanin (A. units/g LFW)	1.024 ± 0.011 (100)	1.438 ± 0.01 (140)*	1.673 ± 0.009 (163)*	1.824 ± 0.017 (178)*	2.085 ± 0.01 (203)*	2.210 ± 0.007 (216)*
Values in parenthesis indicate percent activity; value represents mean of 3 samples with their standard error; *Significantly different from the control at P<0.05						

Table 3: Effect of various concentration of Cobalt chloride on the Biochemical characteristics of *Vigna mungo* (L.) Hepper.

Parameters	Control	2 mM	4 mM	6 mM	8 mM	10 mM
Total Soluble Sugar (mg/g LFW)	69.06 ± 0.424 (100)	55.07 ± 0.112 (78)*	47.41 ± 0.291 (69)*	39.78 ± 0.208 (58)*	23.62 ± 0.275 (34)*	15.71 ± 0.103 (23)*
Total Soluble Protein(mg/g LFW)	9.16 ± 0.213 (100)	7.42 ± 0.239 (81)*	5.94 ± 0.073 (65)*	4.23 ± 0.087 (46)*	3.30 ± 0.171 (36)*	2.03 ± 0.034 (22)*
Amino acid (µg/g LFW)	23.07 ± 0.265 (100)	33.51 ± 0.318 (145)*	42.54 ± 0.664 (184)*	55.99 ± 0.167 (243)*	70.05 ± 0.268 (304)*	74.03 ± 0.256 (322)*
Proline (µg/g LFW)	2.417 ± 0.033 (100)	2.751 ± 0.021 (114)*	2.919 ± 0.010 (121)*	3.154 ± 0.041 (130)*	3.423 ± 0.007(142)*	3.671 ± 0.010 (152)*
Leaf Nitrate (µg/g LFW)	19.55 ± 0.306 (100)	27.17 ± 0.450 (139)*	35.05 ± 0.167 (179)*	47.49 ± 0.579 (243)*	51.11 ± 0.100 (261)*	65.54 ± 0.256 (335)*
Total Phenol	2.710 ± 0.007 (100)	2.710 ± 0.007 (100)	2.710 ± 0.007 (100)	2.710 ± 0.007 (100)	2.710 ± 0.007 (100)	2.710 ± 0.007 (100)
(µg/g LFW)	0.159 ± 0.0014 (100)	0.180 ± 0.0017 (113)*	0.198 ± 0.0003 (125)*	0.215 ± 0.0015 (135)*	0.253 ± 0.0026 (159)*	0.282 ± 0.002 (177)*
Values in parenthesis indicate percent activity; value represents mean of 3 samples with their standard error; *Significantly different from the control at P<0.05						

Table 4: Effect of various concentration of Cobalt chloride on the Enzymes activities of *Vigna mungo* (L.) Hepper.

Parameters	Control	2 mM	4 mM	6 mM	8 mM	10 mM
Nitrate Reductase activity (µ mole of NO_2 formed/ hour LFW)	15.83 ± 0.064 (100)	12.31 ± 0.118 (78)*	10.93 ± 0.073 (69)*	9.79 ± 0.040 (62)*	8.12 ± 0.026 (51)*	6.91 ± 0.098 (44)*
Catalase activity (µ mole of H_2O_2 formed/min. LFW)	1.73 ± 0.032 (100)	2.34 ± 0.037 (135)*	3.79 ± 0.051 (219)*	4.91 ± 0.023 (284)*	5.84 ± 0.056 (338)*	7.14 ± 0.116 (413)*
Peroxidase activity (µmole of H_2O_2 formed/ min. LFW)	0.017 ± 0.0005 (100)	0.024 ± 0.0003 (141)*	0.029 ± 0.0005 (171)*	0.032 ± 0.0003 (188)*	0.036 ± 0.0003 (212)*	0.040 ± 0.0003 (235)*
Polyphenol Oxidase activity (µ mole of H_2O_2 formed/ min. LFW)	0.986 ± 0.008 (100)	1.147 ± 0.009 (116)*	1.433 ± 0.084 (145)*	1.683 ± 0.009 (171)*	1.850 ± 0.006 (188)*	2.045 ± 0.077 (207)*
Values in parenthesis indicate percent activity; value represents mean of 3 samples with their standard error; *Significantly different from the control at P<0.05						

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 with 6 mM Cobalt chloride solution treated *Vigna mungo* plant (Table 5). The chlorophyll and carotenoid contents had been significantly increased after the application of seaweed treated metal solution in *Vigna mungo* seedlings. The anthocyanin content was decreased by the application of seaweed treated metal solution seedlings (Table 6). Total soluble sugar, soluble protein and in vivo nitrate reductase contents were significantly increased in the seedlings after the application of seaweed treated heavy metal solution. In contrary, total free amino acid, proline content and phenol content were reduced after the application of treated cobalt chloride (Table 7). The activities of enzymes such as catalase, peroxidase and polyphenol oxidase activity in the *Vigna mungo* seedlings had been reduced after the application of seaweed treated cobalt chloride solution, whereas the nitrate reductase activity was increased by the application of seaweed powdered (Table 8). AAS study on the concentration of Cobalt chloride in *Vigna mungo* (L.) Hepper treated with Cobalt chloride and also in the plants of Cobalt chloride+Sargassum wightii treatment is being tabulated in Tables 9 and 10.

Table 5: Effect of Cobalt and Sargassum wightii on the Growth parameters of *Vigna mungo* (L.) Hepper.

Growth Parameters	Control	Control (6 mM)	6 mM Cobalt		
			2 gm/L SWP	4 gm/L SWP	6 gm/L SWP
Root Length (cm)	13.9 ± 0.148 (100)	8.72 ± 0.647 (63)*	10.38 ± 0.114 (75)*	11.91 ± 0.529 (86)*	12.99 ± 0.180 (93)*
Shoot Length (cm)	18.69 ± 0.15 (100)	10.23 ± 0.390 (55)*	14.77 ± 0.236 (79)*	16.03 ± 0.332 (86)*	17.42 ± 0.271 (93)*
Leaf Area (cm2)	4.6 ± 0.370 (100)	2.73 ± 0.365 (59)*	3.56 ± 0.170 (77)*	3.88 ± 0.116 (84)*	4.35 ± 0.138 (95)*
Fresh Weight (gm)	0.690 ± 0.021 (100)	0.351 ± 0.067 (51)*	0.579 ± 0.446 (84)*	0.616 ± 0.284 (89)*	0.679 ± 0.183 (98)*
Dry Weight (gm)	0.139 ± 0.070 (100)	0.043 ± 0.123 (31)*	0.105 ± 0.067 (76)*	0.616 ± 0.284 (89)*	0.133 ± 0.056 (96)*
Values in parenthesis indicate percent activity; value represents mean of 10 samples with their standard error ;*Significantlydifferent from the control at P<0.05; SWP- Sea Weed Powder.					

Table 6: Effect of Cobalt and Sargassum wightii on the Pigments of *Vigna mungo* (L.) Hepper

Pigments	Control	Control (6 mM)	6 mM Cobalt		
			2 gm/L SWP	4 gm/L SWP	6 gm/L SWP
Total Chlorophyll (mg/gLFW)	2.710 ± 0.007(100)	1.465 ± 0.048(54)*	1.983 ± 0.008(73)*	2.245 ± 0.008(83)*	2.505 ± 0.010(93)*
Carotenoids	4.6 ± 0.370 (100)	2.73 ± 0.365 (59)*	3.56 ± 0.170 (77)*	3.88 ± 0.116 (84)*	4.35 ± 0.138 (95)*
(mg/gLFW)	1.513 ± 0.008(100)	0.823 ± 0.011(54)*	0.993 ± 0.007(66)*	1.183 ± 0.009(78)*	1.364 ± 0.0093(90)*
Anthocyanin (A. units/g LFW)	0.139 ± 0.070 (100)	0.043 ± 0.123 (31)*	0.105 ± 0.067 (76)*	0.616 ± 0.284 (89)*	0.133 ± 0.056 (96)*
Values in parenthesis indicate percent activity; value represents mean of 3 samples with their standard error ;*Significantlydifferent from the control at P<0.05; SWP- Sea Weed Powder.					

Table 7: Effect of Cobalt and Sargassum wightii on the Biochemical characteristics of *Vigna mungo* (L.) Hepper.

Parameters	Control	Control (6 mM)	6 mM Cobalt		
			2 gm\L SWP	4 gm/L SWP	6 gm/L SWP
Total Soluble Sugar (mg/g LFW)	69.06 ± 0.424 (100)	39.78 ± 0.208 (58) *	49.87 ± 0.511 (72)*	55.40 ± 0.313 (80)*	66.71 ± 0.383 (97)*
Total Soluble Protein(mg/g LFW)	9.16 ± 0.213 (100)	4.23 ± 0.087 (46) *	6.46 ± 0.215 (71)*	7.28 ± 0.157 (79)*	7.94 ± 0.057 (87)*
Amino acid (µg/g LFW)	23.07 ± 0.265 (100)	55.99 ± 0.167 (243) *	40.89 ± 0.261 (177)*	32.86 ± 0.102 (143)*	26.43 ± 0.240 (115)*
Proline (µg/g LFW)	2.417 ± 0.033 (100)	3.154 ± 0.041 (130) *	3.038 ± 0.013 (126)*	2.926 ± 0.014 (121)*	2.779 ± 0.026 (115)*
Leaf Nitrate (µg/g LFW)	19.55 ± 0.306(100)	47.49 ± 0.579 (243) *	32.57 ± 0.055 (167)*	24.87 ± 0.148 (127)*	21.12 ± 0.201 (108)*
Total Phenol (µg/g LFW)	0.159 ± 0.0014 (100)	0.215 ± 0.0015 (135) *	0.243 ± 0.0034 (153)*	0.202 ± 0.0034 (127)*	0.185 ± 0.0024 (116)*
Values in parenthesis indicate percent activity; value represents mean of 3 samples with their standard error ;*Significantly different from the control at P<0.05; SWP- Sea Weed Powder.					

Table 8: Effect of Cobalt and Sargassum wightii on the Enzymes activities of *Vigna mungo* (L.) Hepper.

Parameters	Control	Control (6 mM)	6 mM Cobalt		
			2 gm/L SWP	4 gm/L SWP	6 gm/L SWP
Nitrate Reductase activity (µ mole of NO_2 formed/hour LFW)	15.83 ± 0.064 (100)	9.79 ± 0.040 (62)*	11.90 ± 0.086 (75)*	13.27 ± 0.0145 (84)*	14.21 ± 0.155 (90)*
Catalase activity (µ mole of H_2O_2 formed/min. LFW)	1.73 ± 0.032 (100)	4.91 ± 0.023 (284)*	3.33 ± 0.043 (170)*	2.72 ± 0.076 (157)*	2.06 ± 0.015 (114)*
Peroxidase activity (µmole of H_2O_2 formed/ min. LFW)	0.017 ± 0.0005 (100)	0.032 ± 0.0003 (188)*	0.027 ± 0.0015 (159)*	0.020 ± 0.0016 (118)*	0.018 ± 0.0003 (105)*
Polyphenol Oxidase activity (µ mole of H_2O_2 formed/ min. LFW)	0.986 ± 0.008 (100)	1.683 ± 0.009 (171)*	1.1540 ± 0.0043 (122)*	1.089 ± 0.0040 (115)*	1.015 ± 0.0035 (107)*
Values in parenthesis indicate percent activity; value represents mean of 3 samples with their standard error ;*Significantly different from the control at P<0.05; SWP- Sea Weed Powder.	19.55 ± 0.306(100)	47.49 ± 0.579 (243) *	32.57 ± 0.055 (167)*	24.87 ± 0.148 (127)*	21.12 ± 0.201 (108)*
Values in parenthesis indicate percent activity; value represents mean of 3 samples with their standard error ;*Significantly different from the control at P<0.05; SWP- Sea Weed Powder.					

Table 9: AAS study on the concentration of Cobalt in *Vigna mungo* (L.) Hepper

Concentration	Leaf (ppm)	Root (ppm)	Stem (ppm)
Control	0.0592	0.0496	0.0324
2 mM	0.0916	0.0935	0.0649
4 mM	0.1260	0.1298	0.1622
6 mM	0.2805	0.2004	0.3015
8 mM	0.4198	0.3626	0.3168
10 mM	0.7366	0.4790	0.5210

Table 10: AAS study on the concentration of Cobalt after Sargassum wightii treatment in *Vigna mungo* (L.) Hepper

Concentration	Leaf	Root
Control	0.0592	0.0496
Control(6 mM cobalt chloride)	0.2805	0.0935
2 g/L SWP	0.0528	0.0459
4 g/L SWP	0.0389	0.0389
6 g/L SWP	0.0359	0.0319

Discussion

In the present study, the increase in the concentration of cobalt chloride caused considerable reduction on the root and shoot length of *Vigna mungo* (L.) Hepper. Similar results were also observed due to excess supply of cobalt in black gram [3]. Co at high levels may inhibit the root and shoot growth directly by inhibition of cell division or cell elongation or a combination of both resulting in the limited exploration of the soil volume for uptake and translocation of nutrients and water and induced mineral deficiency was reporte [15]. The pronounced inhibition of shoot and root growth are the main case for the decrease in freshand dry weight of seedlings, uptake of metals occurs primarily through the root [16].

The decrease in leaf area at higher concentrations of Co could be attributed to either, a reduction in the number of cell in the leaves of Phaseolus vulgaris, or to a reduction in cell size was reported. The decreased in leaf area under heavy metal stress was due to the reduced cell size and decreased intercellular spaces [17]. The decrease in fresh weight and dry matter content can be attributed to decrease in potassium, calcium and magnesium content of plants when they are supplied with high levels of metal ions. Hosono *et al.* (1979) showed that the heavy metal toxicity depends upon one or more nutrients concentration within the plants [18].

Excess supply of heavy metals such as cobalt sulphate, nickel sulphate, sodium molybdate and sodium dichromate with nutrient solution caused depressed germination, length of radicles, lowered the mobilization of reserved materials from the cotyledons to the developing embryo axis and adversely affected the number of respiratory enzymes in the case of green gram [19].

The photosynthetic process is related with the inhibition of biomass accumulation, which in turn relies upon the pigment level. The photosynthetic pigments such as chlorophyll "a", chlorophyll "b", total chlorophyll and carotenoid contents of black gram decreased with increasing concentration of cobalt chloride. In the present investigation, the excess cobalt treatment brought about a marked depression in photosynthetic pigments in plants. It might be due to excess supply of cobalt resulting in interference with the synthesis of chlorophyll. The formation of chlorophyll pigments depends on the adequate supply of iron [20]. It was suggested that protoporphyrin, the precursor for chlorophyll synthesis decreased after cobalt treatment. Cobalt prevents the incorporation of iron protoporphyrin molecule resulting in the reduction of chlorophyll pigment. This was strengthened by the fact that excessive amounts copper, cobalt (El-Sheekh *et al.*, 2003) induced chlorosis in plants [21].

The anthocyanin content was significantly increased with increasing concentration of cobalt. Anthocyanins are cationic polyphenol and exhibits antioxidant activity by inhibiting the lipogenase. The anthocyanin accumulated in the upper epidermal cells of the leaves, exposed to heavy metal could act as scavengers, before it reaches the sensitive targets such as chloroplast and other organelles [22]. Hence the increase in anthocyanin after metal treatment can be ascribed with its protective function. The synthesis of anthocyanin has been proposed as a regulator for the increased synthesis of phenol.

The reduction in sugar contents may be attributed to reduction in chlorophyll contents of the leaf and also a decline in protein. This change might have already affected the photosynthetic activity of the plant and hence the reduction in contents [23]. Kastori *et al.*, (1992) reported in *Helianthus annus* that content of soluble proteins decreased with high concentration of heavy metals [24]. Protein content under heavy metal influence may be affected due to the enhanced protein hydrolysis resulting in decreased concentration of soluble proteins.

Accumulation of free amino acid is considered as an adaptive mechanism employed by the plant cell to overcome post stress metabolism. In the present investigation the free amino acid content was increased with the increase in cobalt chloride treatment. It may be due to destruction of protein or to increase in the biosynthesis of amino acids from the nitrate source which were not utilized in the protein synthesis [25].

Accumulation of proline has been frequently used as biochemical marker for water stress inplants [26]. In stress condition the inhibition of growth of cells, leaves and the whole plant were accompanied by an accumulation of nitrate in plant tissue particularly in leaves [27]. The leaf nitrate content was found to be more in nickel treated plants paralleling with the reduction in nitrate reductase activity.

Peroxidase is an enzyme which utilizes hydrogen peroxide as a substrate and it also oxidizes a wide range of hydrogen donors such as phenolic substances cytochrome – c oxidase. The increased peroxidase activity caused a major impact on the chlorophyll degradation. Peroxidase could also act as IAA oxidase [28]. The peroxidase activity was reported to be increased with the increase in the concentration of the cobalt chloride, it cause a major impact on the chlorophyll degradation. Catalase is antioxidant and scavenging enzyme, it was found to be increased with the increasing concentration of cobalt chloride. Catalase is special type of peroxidase enzyme which catalase the degradation of H_2O_2, which is natural metabolite and also toxic to plants [28].

Polyphenoloxidase and peroxidase are the two major enzyme which are responsible for oxidation of phenolic compound such as polyphenols and ideal for radical scavenging activity. Polyphenoloxidase involved in the reduction of oxidative damage triggered by reactive oxygen species through stress experienced plant. These antioxidant enzymes and metabolites are reported to increase under various environmental stresses and with the application of fungicide and salt treatment in medicinal plants [29]. AAS study reported that the increasing concentration of nickel caused an accumulation in cobalt treated plants. Higher concentration was observed in 10 mM cobalt treated plant.

The dry seaweed biomass reduces the toxic effect of cobalt chloride and thereby promotes the growth of *Vigna mungo* the algae Sargassum wightii is a very good biosorbent of heavy metals. This finding clearly indicates that addition of dry seaweed biomass reduces the toxic effect of cobalt chloride and thereby promotes the growth of *Vigna mungo*. The increased in the growth may be due to the presence of phenyl acetic acid (PAA) in the seaweed extract [30]. The growth promoting activity of the plants may be attributed to the presence of hormone in the seaweed extracts. It has been suggested that hormones like cytokinin present in the seaweed extracts may be responsible for their growth promoting activity [31]. Marine algae contain a high amount of organic substances, such as carbohydrates, protein, lipids and pigments. Menon and Srivastava, (1984) reported an increase in the number and size of the chloroplast and better grana development after seaweed application [32]. The cell wall of brown algae generally contain components namely cellulose, alginic acid and salts of potassium, sodium, magnesium and calcium and sulfated polysaccharides [33].

An increase in sugar, protein and decrease in free amino acid,

proline and phenol accumulation, after the application of seaweed powder indicated the promotive nature of the seaweed. This positive response was observed even in low concentration of seaweed. It was also reported that, after seaweed application there was increase in the protein level and decrease in proline level [34]. The seaweed application have caused a reduction in proline and phenol accumulation than the stressed plants, indicating the plants response in overcoming stress effect by the seaweed application even at low concentration. This finding coincides with the findings of Jayakumar and Ramasubramanian (2009) who reported that the application of Sargassum wightii powder on the chromium treated plants showed improvement in plant growth [35].

Nandagobal and Subramanian (2004) who observed that the seaweed stimulates the nitrate reductase activity and protein synthesis [36]. Catalase, peroxidase and polyphenol oxidase activity are the enzymes responsible for scavenging the plant materials from the stressed impact. On application of seaweed, these enzyme activities decreased considerably than the control plants. Arunkumar *et al.*, (2002) reported that, seaweed is having potential to reduce the activity of scavenging enzymes [37].

The peroxidase activity was found to be decreased by seaweed treatment. This showed the remediation property of the seaweed application. The dried algal biomass used in the present study is available in large quantities which could be used to remove metals. Thus seaweed could be used as an effective, safe and economical alternative was also reported [35]. The finding of present study shows that the algae Sargassum wightii and dry powder can efficiently remove the cobalt chloride toxicity.

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 cobalt chloride. 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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