Agricultural Waste Material as Potential Adsorbent for Heavy Metal Treated V*igna radiata (*L.)Wilczek Sequestering Heavy Metal Ions from Aqueous Solutions

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Abstract: ***The effect of lead acetate on the growth and biochemical characteristics of Vigna radiata, (L.)Wilczek. Seedling was studied with different concentration (5mM, 10mM, 15mM, 20mM, 25mM) of lead acetate. The heavy metal treatment on Vigna radiata has caused a steep decline in its growth, pigment content, other biochemical characteristics and enzyme activities with increasing concentration of lead acetate. On the other hand , a bioadsorption study carried out with the low cost biosorbent sugarcane bagasse in different concentration on lead acetate treated Vigna radiata, (L.)Wilczek on the growth, pigment content and biochemical characteristics was found improving to reduced parameters significantly than in plants subjected to untreated metal solution. The photosynthetic pigment content such chlorophyll a, chlorophyll b and total chlorophyll carotenoids and biochemical parameters such as glucose, protein content were increased after the application of bioadsorbed metal solution. But in contrast, anthocyanin, aminoacid, proline, leaf nitrate and enzyme activities such as catalase and peroxidase were found to be reduced in the bioadsorbed metal treated seedlings. From the present study, it is concluded that sugarcane bagasse is a suitable alternate for the remediation to reduce the toxic effect of the metal and to relieve Vigna radiata, (L.)Wilczek plants from metal stress.***

Keywords: sugarcane, bagasse, bioadsorbent, lead

1. Introduction

Excessive release of heavy metals into the environment due to industrialization and urbanization has posed a great problem world wide. Unlike organic pollutants, the majority of which are susceptible to biological degradation, heavy metal ions do not degrade into harmless end products. The presence of heavy metal ions is a major concern due to their toxicity to many life forms. Heavy metal contamination exists in aqueous wastes of many industries such as metal plating, mining operation, tanneries etc. Heavy metal toxicity causes multiple direct and indirect effects on plant growth and alter many physiological functions (1). The toxicity of heavy metals is mainly attributed to their ability of binding enzyme resulting in the alteration of their catalytic functions and inactivation (2). 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 process in higher plants such as protection and enzyme synthesis, disturbances in cytokinesis, lowering DNA synthesis and stability, explosives and petroleum product. Metal toxicity in plants has been reported by many workers (3). (4) 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. (5) 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. The lead treated sunflower (*Helianthus annuus)* plants at highter concentration showed stunted growth and reduced leaf expansion (6) . Hence, an investigation has been undertaken to study the effect of lead acetate on the growth and biochemical characteristics of bioadsorbed *Vigna radiata.*

Bioadsorption is one of the new approaches that offers more ecological benefits and a cost efficient alternative. Although it is cheaper method, but requires technical strategy, expert project designers with field experience that choose the proper species and cultivars for particular metals and regions.

The bagasse used in the bioadsorption technique must have a considerable capacity of metal absorption, its accumulation and strength to decrease the treatment time. Bagasse is a waste product from sugar industry. Bagasse pith is composed largely of cellulose, pentose and lignin (7)

Bioadsorption of Lead by bagasse

(8) reported that the use of bagasse for the elimination of hexavalent chromium from the waste water. (9) reported that increase in plant height, spike length and biomass of three native species of wheat treated with bagasse in wheat plant.

(10) reported that sugarcane bagasse used to remove the cadmium and nickel from the water. The use of lignin obtained from sugarcane bagasse was considered in the removal of heavy metals from water. The present investigation attempts to study sugarcane bagasse for the bioadsorption of lead on the growth and biochemical profile of *Vigna radiata* (L) wilczek.

2. Materials and Methods

Healthy and viable uniform size seeds of *Vigna radiata* (L). Wilczek were germinated in plastic troughs of uniform size containing acid washed riverbed sand. Seeds of equal number was 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 containing half- strength of Hoagland's Nutrient Solution(11). Plants irrigated with half- strength of Hoagland's Nutrient Solution served as control. Various concentration of dried powdered biomass of sugarcane bagasse (2g/L, 4g/L and 6g/L) was mixed with 15mM lead acetate and used for further study. 15Mm lead acetate was found to be optimum by LSD analysis (12)

After ten days of the treatment the seedlings of *Vigna radiata* were used for measuring the growth parameters such as shoot length, root length, leaf area, fresh weight, dry weight, pigment content such us chlorophyll *a*, *b*, total chlorophyll and carotenoid (13). Anthocyanin content (14). total soluble sugar (15) protein content (16), starch , aminoacid content (17) proline content (18), *invivo* nitrate reductase activity (19) catalase (20) and peroxidase activity (21).

3. Results and Discussion

Effects 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 result shows that the 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, starch content and NR activity also follow 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. Bioadsorption 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 powdered biomass of sugarcane bagasse with15mM lead acetate solution treated *Vigna radiata* plants (Table5) The chlorophyll and carotenoid contents had been significantly increased after the application in *Vigna radiata* seedlings. The anthocyanin content decreased with the application of biomass treated metal solution (Table 6).

Total soluble sugar and soluble protein contents were significantly increased in the seedlings after the application of sugarcane bagasse treated heavy metal solution. In contrary, total free amino acid and proline contents got reduced after the application of treated lead acetate(Table 7). The dried powdered biomass of sugarcane bagasse mixed with 15mM lead acetate caused increase in the pigment content than the untreated lead acetate treated plants and also. An increase in protein content and decrease in free aminoacid and proline after the application of bioadsorbed lead acetate observed in the present study indicates the use of plants natural ability to degrade and remove toxic effect from soil. In present investigation the *in vivo* nitrate reductase activity increases with the increase in the application of dried powder sugarcane bagasse. This may be due to the increase in the uptake of nitrate by the plants.

The activities of enzymes such as catalase and peroxidase in the *Vigna radiata* seedlings had been reduced after the application of sugarcane bagasse treated lead acetate solution, where as the nitrate reductase activity was increased by the application bioadsorbed metal solution.(Table 8). Peroxidase and catalase are the enzymes responsible for scavenging the plant materials from the stressed impact. Upon the addition of dried powdered biomass of sugarcane bagasse 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 powdered biomass of sugarcane bagasse.

AAS study also revealed that the accumulation of lead got reduced after the bioadsorption treatment (table9&10) and was in accordance with findings of (22). The result of present investigation clearly showed the sugarcane bagasse could used to remove the toxicity of lead in the pollution 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 ±.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)	9.6 ±0.305** (59)	9.5 ±0.251** (58)	8.2 ±0.088** (50)	7.2 ±0.145** (44)
Fresh Weight (mg)	0.653 ±.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 ±.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 five observations values in parenthesis are percentage activity with respective controlMean ± S .** Significance at P<0.05 level

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

Parameters	Control	5mM	10mM	15mM	20mM	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)	0.082±0.001** (66)
Chlorophyll b (mg/gLFW)	0.056 ±0.001** (100)	0.043 ±.001** (77)	0.034 ±0.0008** (61)	0.026±0.0005** (46)	0.021±0.0008** (37)	0.013±0.001** (24)
TotalChlorohyll (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±0.0008** (79)	0.765 ±0.002** (68)	0.615 ±0.001** (54)	0.576 ±.001** (51)	0.525±0.001** (46)
Anthocyanin (mg/gLFW)	4.230 ±0.011** (100)	5.546 ±0.017** (131)	5.750 ±.011** (135)	6.673 ±0.012** (157)	6.753 ±0.017** (159)	7.346±0.017** (173)

Values are an average of five observations values in parenthesis are percentage activity with respective control Mean ± Sε.** Significance at P<0.05 level

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 five observations values in parenthesis are percentage activity with respective control Mean ± Sε.** Significance at P<0.05 level

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 (mmole/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 (mmole/gLFW)	2.1±5.7E -102	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 (mmole/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

Parameter	**Control**	**15mM**	**2g/L(w/v)**	**4g/L(w/v)**	**6g/L(w/v)**
Shoot Length (cm)	32.50 ± 0.057** (100)	19.00 ± 0.577** (58)	18.80± 0.251** (57)	20.66 ± 0.348** (63)	24.33 ± 0.145** (74)
Root Length (cm)	16.2 ± 0.057** (100)	9.5 ± 0.251** (58)	10.40± 0.115** (64)	11.36 ± 0.409** (70)	12.53 ± 0.405** (77)
Fresh Weight (mg)	0.653 ± 0.008** (100)	0.283 ± 0.003** (43)	0.310 ± 0.015** (47)	0.383 ± 0.017** (58)	0.453 ± 0.020** (69)

Values are an average of five observations values in parenthesis are percentage activity with respective control Mean ± Sε.** Significance at P<0.05 level

Table 5: Effect of Lead acetate (15 mM) and Sugar cane bagasse on the growth parameter of *Vigna radiata* (L.) Wilczek

Dry Weight (mg)	0.146 ± 0.026** (100)	0.046 ± 0.0003** (31)	0.056± 0.002** (38)	0.064 ± 0.001** (43)	0.074 ± 0.001 ** (50)
Leaf Area (cm^2)	3.33 ± 0.166** (100)	2.23 ± 0.016 ** s(67)	2.41 ± 0.083** (72)	2.59 ± 0.041** (77)	2.75 ± 0.040 ** (82)

Values are an average of five observations values in parenthesis are percentage activity with respective control

Parameter	Control	15mM	2g/L(w/v)	4g/L(w/v)	6g/L(w/v)
Chlorophyll *a* (mg/g/LFW)	0.123 ± 0.0005** (100)	0.095 ± 0.0006** (77)	0.099 ± 0.002** (80)	0.105 ± 0.003** (85)	0.111 ± 0.010 ** (90)
Chlorophyll *b* (mg/g/LFW)	0.056 ± 0.001** (100)	0.026 ± 0.0005** (46)	0.028 ± 0.001** (50)	0.035 ± 0.002** (62)	0.044 ± 0.001 ** (78)
Total chlorophyll (mg/g/LFW)	0.179 ± 0.0006**	0.116 ± 0.001 ** (65)	0.127 ± 0.002** (71)	0.140 ± 0.005** (78)	0.155 ± 0.011 ** (86)

	(100)				
Carotenoids (mg/g/LFW)	1.124 ± 0.001** (100)	0.615 ± 0.001 ** (54)	0.665 ± 0.008** (59)	0.735 ± 0.055** (65)	0.870 ± 0.012 ** (77)

Mean ± Sε.** Significance at P<0.05 level

Table 6: Effect of Lead acetate (15 mM) and Sugar cane bagasseon the pigment content of *Vigna radiata* (L.) Wilczek

Anthocyanin (mg/g/LFW)	4.23 ± 0.011** (100)	6.67 ± 0.012 ** (157)	6.28 ± 0.170** (148)	5.43 ± 0.256** (128)	4.76 ± 0.088 ** (112)

Values are an average of five observations values in parenthesis are percentage activity with respective control

Mean ± Sε.** Significance at P<0.05 level

Table 7: Effect of Lead acetate (15 mM) and Sugar cane bagasse on the Biochemical parameter of *Vigna radiata* (L.) Wilczek

Parameter	**Control**	**15mM**	**2g/L(w/v)**	**4g/L(w/v)**	**6g/L(w/v)**
Glucose (mg/gLFW)	64.23 ± 0.145** (100)	22.70 ± 0.115 ** (35)	25.70 ± 0.208 ** (40)	29.66 ± 0.120** (46)	38.43 ± 0.120 ** (59)
Protein (mg/gLFW)	4.63 ± 0.088** (100)	2.50 ± 0.023 ** (53)	2.80 ± 0.080** (60)	2.98 ± 0.064** (64)	3.42± 0.157 ** (73)
Starch (mg/gLFW)	2.05 ± 0.006** (100)	0.54 ± 0.014 ** (26)	0.73 ± 0.029** (35)	0.92 ± 0.023** (44)	1.08 ± 0.082 ** (52)
Amino Acid (µmole/gLFW)	26.66 ± 0.088** (100)	53.24 ± 0.023 ** (199)	45.56 ± 0.145** (170)	42.80± 0.200** (160)	41.13 ± 0.735 ** (154)
Proline (mg/gLFW)	1.24 ± 0.008** (100)	1.95 ± 0.020 ** (156)	1.65 ± 0.061** (132)	1.53± 0.058** (122)	1.30 ± 0.029 ** (104)

Leaf Nitrate (mg/g/LFW)	15.16 ± 0.088** (100)	28.45 ± 0.017 ** (187)	26.66 ± 0.317** (175)	24.90 ± 0.305** (164)	22.10 ± 0.866 ** (145)

Values are an average of five observations values in parenthesis are percentage activity with respective controlMean ± Sε.** Significance at P<0.05 level

Table 8: Effect of Lead acetate (15 mM) and Sugar cane bagasse on the enzyme content of *Vigna radiata* (L.) Wilczek

Parameter	**Control**	**15mM**	**2g/L(w/v)**	**4g/L(w/v)**	**6g/L(w/v)**
NRA (µmole/gLFW)	7.65 ± 0.005** (100)	2.14 ± 0.008 ** (28)	2.79± 0.112** (36)	3.30 ± 0.121** (43)	3.84 ± 0.058 ** (50)
Catalase (µmole/gLFW)	2.10 ± 0.057** (100)	4.65 ± 0.018 ** (221)	4.48 ± 0.061** (213)	4.24 ± 0.012** (202)	3.74 ± 0.130 ** (178)
Peroxidase (µmole/gLFW)	0.007 ± 0.0003** (100)	0.018 ± 0.0003** (242)	0.017 ± 0.0005 ** (220)	0.014 ± 0.0006 ** (181)	0.012 ± 0.0015** (155)

Values are an average of five observations values in parenthesis are percentage activity with respective control

Mean ± Sε.** Significance at P<0.05 level

Table 9: AAS study on the concentration of lead in heavy metal treated *Vigna radiata* (L.)Wilczek

S.NO	**Treatment**	**Lead in ppm**
1	Control	0.0782
2	5mM	0.4313
3	10mM	0.8136
4	15mM	1.3273
5	20mM	1.9042
6	25mM	2.7410

Table 10: AAS study on the concentration of lead in Sugar cane bagasse treated *Vigna radiata* (L.)Wilczek

S.NO	Treatment	Lead in ppm
1	Control	0.0782
2	15mM	1.3273
3	2g/L	0.6412
4	4g/L	0.4315
5	6g/L	0.1135