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BAGASSE AS A LOW COST BIOADSORBENT ON THE GROWTH AND BIOCHEMICAL CHARACTERISTICS OF METAL TREATED *ELEUSINE CORACANA*, GAERTN

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ABSTRACT

In the bioadsorption treatment, various concentrations of Bagasse. as an effective and efficient adsorbent for the heavy metal treated *Eleusine coracana*, Gaertn. The seedlings of *Eleusine coracana*, Gaertn were treated with various concentrations of heavy metal ions and their impact on the growth; biochemical and enzyme characters were studied. After ten days treatment with different concentration of lead acetate (2mM, 4mM, 6mM, 8mM, 10mM) the growth parameter such as leaf area, fresh weight, dry weight, shoot and root lengths were decreased than the control. Biochemical parameters such as chlorophyll, carotenoids, soluble sugar, and protein content also decreased with the increase in the concentration of lead acetate. But the content of free amino acid, proline, phenol and leaf nitrate increased with the increase in the concentration of lead acetate. The activities of enzyme such as catalase and peroxidase were found to be increased with the increase in the concentration of lead acetate. Application of low cost bioadsorbent (Bagasse) in different concentrations (2gm/L, 4gm/L, 6gm/L) on 6mM lead acetate treated plants has shown the stress relieving effect caused by lead acetate. The results revealed that Bagasse is a good potential as an adsorbent for the removal of toxicity caused by lead.

Key Words: Heavy metal, biochemical, enzyme chlorophyll, carotenoids, *Datura metel*, L.

INTRODUCTION

Environmental pollution is one of the undesirable side effects of industrialization urbanization and population growth towards the environment. Though industrialization and development is necessary to meet the basic requirement of people, it is very much necessary to preserve the environment also. Heavy metals are metallic elements which have a high atomic weight and density much greater than water. Heavy metals are essential for the growth of plants in low concentration. But become toxic when the concentration limit exceeds. Many heavy metals are essential for the growth of the plants in low concentrations but they become toxic only when the concentration limit exceeded. 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 . All plants have the ability to accumulate “essential” metals such as Ca, Co, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, Se, V and Zn from the soil solution and plants need these elements in different concentrations for their growth and development. This ability also allows plant to accumulate other “non-essential” metals (Al, As, Au, Cd, Cr, Hg, Pb, Pd, Pt, Sb, Te, T and U) which have no known biological functions (Djingova and Kuleff, 2000). Moreover, metals cannot be broken down and when concentrations inside the plant cells accumulate above threshold or optimal levels, it can cause direct toxicity by damaging cell structure (due to oxidative stress caused by reactive oxygen species) and inhibit a number of cytoplasmic enzymes (Assche and Clijsters, 1990). In addition, it can cause indirect toxic effects by replacing essential nutrients at cation exchange sites in plants. Geological and anthropogenic activities are the sources of heavy metal contamination (Dembitsky, 2003). 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 agricultural waste material of bagasse (Sugarcane waste) with 6mM lead on the growth and biochemical characteristics of *Eleusine coracana*, Gaertn.

MATERIALS AND METHODS

Seedling of *Eleusine coracana* were treated with various concentration of lead acetate (2mM, 4mM, 6mM, 8mM, 10mM). After ten days, various morphometric and biochemical characters were analysed. The dried agricultural waste material of bagasse was collected from the road side. It was shade dried, finely powdered by milling and various concentrations (2, 4 and 6 gm/L) were prepared with 6mM solutions of lead. After 10 days all the growth and biochemical parameters were analysed. After ten days old plants of *Eleusine coracana* were used for measuring the growth parameters such as shoot length, root length, Leaf area, fresh weight, dry weight and for analyzing the Biochemical parameters such as Chlorophyll a, b, Total Chlorophyll and Carotenoid (Wellburn and Lichtenthaler, 1984). Anthocyanin content (Mancinell *et al.,* 1973). Total soluble sugar (Anthrone method) protein content (Lowry *et al.,* 1951), aminoacid content (Jayaraman, 1981) proline content (Bates *et al.,* 1973), phenol content (Bray and Thorpe, 1954) *in vivo* nitrate reductase activity (Jaworski, 1971) Catalase (Kar and Mishra, 1976) and Peroxidase activity (Addy and Goodman, 1978).

RESULTS AND DISCUSSION

Effect of five different concentrations (2mM, 4mM, 6mM, 8mM, and 10mM) 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.

Similarly Chlorophylls, Carotenoid, Total Soluble Sugar, protein and NR activity also shows a declining trend. In contrary the pigment Anthocyanin total free amino acid, proline, phenol and the antioxidant enzyme such as peroxidase and catalase increases with the increase in the metal concentration.

Table: 1

Effect of Various Concentrations of Lead acetate on the Growth of *Eleusine coracana*, Gaertn**.**

S.No	Parameters	Control	2 mM	4 mM	6 mM	8 mM	10 mM
1	Shoot length (cm)	6.4 +0.03 (100)	6.1 +0.02** (95)	5.9 +0.03** (92)	5.6 +0.01** (88)	5.4+0.02** (84)	5.03 +0.07** (79)
2	Root length (cm)	15.1 +0.09 (100)	9.6 +0.07** (64)	7.08 +0.02** (47)	6.3 +0.06** (42)	2.6 +0.08** (17)	1.3 +0.01** (9)
3	Fresh weight (g)	0.074 +0.002 (100)	0.04 +0.001** (57)	0.03+0.038** (52)	0.036+0.004** (49)	0.02 +0.001** (29)	0.01+0.007** (14)
4	Dry weight (g)	0.01+0.007 (100)	0.007 +0.01** (70)	0.005+0.002** (50)	0.0043+0.02** (43)	0.003 +0.04** (30)	0.001+0.002** (10)
5	Leaf Area (cm2)	1.07 +0.02 (100)	0.6 +0.009** (56)	0.5 + 0.04** (47)	0.4 +0.01** (37)	0.3 +0.007** (28)	0.1 +0.01** (18)

Values are an average of ten observations. Values in parentheses are percentage activity with respect to control. Mean (±) SE. ** Sigfinicance at $P < 0.01$ level.

Table: 2

Effect of Various Concentrations of Lead acetate on the Pigment Content of *Eleusine coracana*, Gaertn.

S.No	Parameters	Control	2 mM	4 mM	6 mM	8 mM	10 mM
1.	Chlorophyll *a* (mg/gLFW)	2.426 ±0.011 (100)	2.183±0.021** (90)	2.134±0.011** (88)	1.649 ±0.10** (68)	1.479±0.003** (61)	1.213 ±0.001** (50)
2.	Chlorophyll *b* (mg/gLFW)	1.944 ±0.003 (100)	1.594 ±0.071** (82)	1.438 ±0.012** (74)	1.30 ±0.014** (67)	1.127±0.013** (58)	0.758 +0.03** (39)
3.	Total Chlorophyll (mg/gLFW)	4.370 ±0.001 (100)	3.777 ±0.012** (87)	3.572 ±0.003** (84)	2.949±0.071** (67)	2.606±0.011** (59)	1.971 ±0.012** (46)
4.	Carotenoid (mg/gLFW)	2.149 ±0.002 (100)	2.02 ±0.004** (94)	1.805 ±0.002** (84)	1.72 ±0.001** (80)	1.611±0.012** (75)	1.375 ±0.001** (64)
5.	Anthocyanin (mg/gLFW)	1.023 ±0.01 (100)	1.125 ±0.001** (110)	1.166 ±0.024** (114)	1.411 ±0.01** (138)	1.493 ±0.03** (146)	1.565 +0.03** (153)

Values are an average of three observations. Values in parentheses are percentage activity with respect to control. Mean (±) SE. ** Sigfinicance at P < 0.01 level.

Table: 3

Effect of Various Concentrations of Lead acetate on the Biochemical Characteristics of *Eleusine coracana*, Gaertn.

S. No	Parameters	Control	2 mM	4 mM	6 mM	8 mM	*10 mM*
1.	Total soluble sugar (mg/g LFW)	13.41 ±0.102 (100)	11.93 ±0.021** (89)	9.52 ±0.015** (71)	7.51 ±0.11** (56)	6.43 ±0.12** (48)	*5.22 ±0.012** (39)*
2.	Protein (mg/g LFW)	13.53 ±0.02 (100)	10.9 ±0.074** (81)	10.41 ±0.14** (77)	9.06 ±0.10** (67)	7.98 ±0.016** (59)	*7.30 ±0.016** (54)*
3.	Amino acid (µ mole/g LFW)	3.245 ±0.001 (100)	3.89 ±0.01** (120)	4.41 ±0.013** (136)	4.890 ±0.012** (159)	5.32 ±0.001** (164)	*5.54 ±0.05** (171)*
4.	Nitrate (mg/g LFW)	256.5 ±0.015 (100)	282.1 ±0.013** (110)	318.0±0.011** (124)	325.5 ±0.032** (127)	348.8 ±0.02** (136)	*359.1 ±0.02** (140)*
5.	Proline (mg/g LFW)	1.153 ±0.003 (100)	1.279 ±0.005** (111)	1.33 ±0.024** (115)	1.418 ±0.016** (123)	1.54 ±0.021** (134)	*1.6 9 ±0.021** (147)*
6.	*Phenol (mg/g LFW)*	*3.26 ±0.012 (100)*	*3.78 ±0.042** (116)*	*4.17 ±0.013** (128)*	*4.423 ±0.012** (136)*	*4.66 ±0.001** (143)*	4.82 ±0.001** (148)

Values are an average of three observations. Values in parentheses are percentage activity with respect to control. Mean (±) SE. ** Sigfinicance at $P < 0.01$ level.

Table: 4

Effect of Various Concentrations of Lead acetate on the Enzyme activities of *Eleusine coracana*, Gaertn**.**

S. No	Parameters	Control	2 mM	4 mM	6 mM	8 mM	10 mM
1	Nitrate reductase (µ mole/g LFW)	581.4±0.011 (100)	505.8 ±0.013** (87)	459.3 ±0.021** (79)	406.9 ±0.001** (70)	366.2 ±0.01** (63)	337.2 ±0.016** (58)
2	Catalase activity (µ mole/g LFW)	2.834 ±0.002 (100)	3.174 ±0.10** (112)	4.052 ±0.01** (143)	4.61 ±0.012** (163)	4.846 ±0.14** (171)	4.987 ±0.012** (176)
2	Peroxidase activity (µ mole/g LFW)	0.389 ±0.016 (100)	0.420 ±0.01** (108)	0.501 ±0.013** (129)	0.552 ±0.001** (142)	0.587±0.021** (151)	0.602 ±0.021** (155)

Values are an average of three observations. Values in parentheses are percentage activity with respect to control. Mean (±) SE. ** Sigfinicance at $P < 0.01$ level.

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 agricultural waste material of bagasse with 6mM lead acetate solution treated Ragi plants (Table 5). The optimum recovery was observed even at 2mg/L of *bagasse* powder in 6mM metal solution compared to that of metal treated control.

The chlorophyll and carotenoid contents had been significantly increased after the application of treated metal solution in *Eleusine coracana* seedlings. The anthocyanin content was decreased on application of bioadsorbent treated metal solution seedlings (Table 6).

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

The activities of enzymes such as catalase and peroxidase in the *Eleusine coracana* seedlings had been reduced after the application of treated metal solution, where as the nitrate reductase activity was increased significantly (Table 8).

In the present investigation growth characteristics such as shoot length, root length, fresh and dry weight, and leaf area of the seedlings were increased in biosorbent treated *Eleusine coracana* than in the untreated metal solution applied plants. Peternele *et al.*, (1999) studied on adsorption of Cd(II) and Pb(II) on to the functionalized formic lignin from sugarcane bagasse. Mohan and Singh (2002) carried out research of single and multi component adsorption of cadmium and zinc using activated carbon derived from bagasse. They have reported the removal of Cd(II) and Zn(II) from aqueous solution.

The results of the study clearly indicates that addition bagasse biomass reduces the toxic effect of cand thereby promotes the growth of *Eleusine coracana*. The dry bagasse biomass in 6mM lead caused an increase in pigment content than the control lead treated plants.

Chand *et al*., (1994) studied the removal of hexavalent chromium from waste water by bagasse and coconut jute biosorbent. Quek *et al.*, (1998) reported that the use of sago waste for the sorption of lead and copper from solution. Activated carbon prepared from agricultural solid wastes such as silk cotton hulls, coconut tree saw dust, sago waste and maize and banana pith.

Table: 5

Effect of Lead acetate with Bagasse on the Growth of *Eluesine coracana*, Gaertn.

S. No.	Parameters	Control (water)	6 mM	2 g/L(w/v)	4 g/L(w/v)	6 g/L(w/v)
1.	Shoot length (cm)	6.4 ± 0.002 (100)	5.6 ± 0.015** (81)	6.9 ± 0.001** (108)	7.4 ± 0.011** (116)	5.2 ± 0.014** (88)
2.	Root length (cm)	15.1 ± 0.013 (100)	6.3 ± 0.013** (42)	17 ± 0.013** (113)	18 ±0.01** (119)	5.9 ± 0.01** (39)
3.	Fresh weight (g)	0.074 ± 0.02 (100)	0.036 ± 0.012** (49)	0.080 ± 0.01** (108)	0.089 ± 0.13** (120)	0.0226±0.001** (32)
4.	Dry weight (g)	0.0124 ± 0.012 (100)	0.0053±0.001** (43)	0.0113 ± 0.11** (113)	0.0109±0.012** (109)	0.0041 ± 0.14** (41)
5.	Leaf area (cm^2)	1.07 ± 0.011 (100)	0.14 ± 0.002** (37)	1.358 ±0.012** (127)	1.57 ± 0.014** (115)	0.3 ± 0.015** (28)

Values are an average of ten observations. Values in parentheses are percentage activity with respect to control. Mean (±) SE. ** Sigfinicance at P < 0.01 level.

Table: 6

Effect of Lead acetate with Bagasse on the Photosynthetic Pigment Content of *Eleusine coracana*, Gaertn.

S. No.	Parameters	Control (water)	6 mM	2 g/L(w/v)	4 g/L(w/v)	6 g/L(w/v)
1.	Chlorophyll *a* (mg/gLFW)	2.426 ±0.021 (100)	1.649 ±0.012** (68)	2.474 ±0.024** (102)	2.620 ±0.011** (108)	2.086 ±0.024** (86)
2.	Chlorophyll *b* (mg/gLFW)	1.944 ±0.016** (100)	1.3 ±0.01** (67)	1.866 ±0.016** (96)	2.041 ±0.1** (105)	1.632 ±0.001** (84)
3.	Total Chlorophyll (mg/gLFW)	4.370 ±0.1 (100)	2.949 ±0.002** (67)	4.340 ±0.004** (99)	4.661 ±0.16** (107)	3.718 ±0.002** (73)
4.	Carotenoid (mg/gLFW)	2.149 ±0.15 (100)	1.992 ±0.011** (80)	2.084 ±0.024** (97)	2.191 ±0.012** (102)	1.809 ±0.141** (87)
5.	Anthocyanin (mg/gLFW)	1.023 ±0.131 (100)	1.411 ±0.11** (138)	1.002 ±0.17** (98)	1.094 ±0.14** (107)	1.155 ±0.014** (113)

Values are an average of three observations. Values in parentheses are percentage activity with respect to control. Mean (±) SE. ** Sigfinicance at P < 0.01 level.

Table:7

Effect of Lead acetate with Bagasse on the Biochemical characteristics of *Eleusine coracana*, Gaertn**.**

S. No.	Parameters	Control (water)	6 mM	2 g/L(w/v)	4 g/L(w/v)	6 g/L(w/v)
1.	Total soluble sugar (mg/g LFW)	13.41 ±0.102 (100)	`7.51 ±0.121** (56)	12.33 ±0.001** (92)	13.81 ±0.013** (103)	9.65 ±0.12** (72)
2.	Protein (mg/g LFW)	13.53 ±0.002 (100)	9.06 ±0.031** (67)	12.98 ±0.13** (96)	14.61 ±0.014** (108)	10.68 ±0.14** (79)
3.	Amino acid (µ mole/g LFW)	3.245 ±0.001 (100)	4.89 ±0.01** (151)	3.175 ±0.048** (98)	3.725 ±0.13** (115)	4.535 ±0.045** (140)
4.	Nitrate (mg/g LFW)	256.5 ±0.015 (100)	325.5 ±0.012** (127)	243.65 ±0.14** (95)	264.15 ±0.021** (103)	292.5 ±0.003** (114)
5.	Proline (mg/g LFW)	1.15 ±0.003 (100)	1.42 ±0.015** (123)	1.210 ±0.004** (105)	1.245 ±0.10** (108)	1.325 ±0.012** (115)
6.	Phenol (mg/g LFW)	3.26 ±0.012 (100)	4.42 ±0.021** (136)	3.325 ±0.021** (102)	3.55 ±0.074** (109)	3.879 ±0.002** (119)

Values are an average of three observations. Values in parentheses are percentage activity with respect to control. Mean (±) SE. ** Sigfinicance at $P < 0.01$ level.

Table: 8

Effect of Lead acetate with Bagasse on the Enzyme Activities of *Eleusine coracana,Gaertn*.

S. No.	Parameter	Control (water)	6 mM	2 g/L(w/v)	4 g/L(w/v)	6 g/L(w/v)
1	Nitrate reductase (μ mole/g LFW)	581.427 ±0.011 (100)	406.9 ±0.015** (70)	523.2 ±0.15** (90)	546.5 ±0.021** (94)	500 ±0.10** (86)
2	Catalase activity (μ mole/g LFW)	2.834 ±0.002 (100)	4.61 ±0.012** (163)	3.54 ±0.17** (125)	2.94 ±0.028** (104)	3.74 ±0.016** (132)
3	Peroxidase activity (μ mole/g LFW)	0.389 ±0.018 (100)	0.552 ±0.011** (142)	0.470 ±0.13** (121)	0.462 ±0.014** (119)	0.497 ±0.01** (128)

Values are an average of three observations. Values in parentheses are percentage activity with respect to control. Mean (±) SE. ** Sigfinicance at $P < 0.01$ level.

Table 9: AAS Study on the Quantification of Lead on the in Heavy metal Treated Plants of *Eleusine coracana,Gaertn*

S.No	Treatment	Lead (in ppm)
1.	Control	0.0931
2.	2m M	0.4258
3.	4 mM	0.6789
4.	6 mM	1.0571
5.	8 mM	1.4732
6.	10 mM	2.658

Table 10: AAS Study on the Quantification of Lead on the post Bagasse treated *Eleusine coracana,Gaertn*

S.No	Treatment	Lead (in ppm)
1.	Control	0.0931
2.	6 mM	1.0571
3.	2 g/L	0.5431
4.	4 g/L	0.4201
5.	6 g/L	0.1235

An increase in protein content and decrease in free aminoacid and Proline after the application of dry bagasse biomass powder in 6mM lead observed in the present study indicates the active promotive nature of agricultural waste extracts on plant growth and metabolism.

Sugarcane bagasse ash is a good source of micronutrients like, Fe, Mn, Zn and Cu (Anguissola *et al.*, 1999). It can also be used as soil additive in agriculture due to its capacity of supplying the plants with small amounts of nutrients (Carlson and Adriano, 1993). Bagasse ash contains no N, but has high concentrations of K and P. Therefore, its use in agriculture for crop production will be proved more beneficial (Page *et al.*, 1979). Along with positive effect on soil nutrient contents, bagasse ash has also produced increased yield of wheat crop and sugar cane proved by Mlynkowiak *et al*., (2001)

Peroxidase and catalase are the enzymes responsible for scavenging the plant materials from the stressed impact. Upon the addition of dried biomass of bagasse in 6 mM lead treated seedlings of *Eleusine coracana*, 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`ddition of dried bagasse biomass.

Metal concentration in Plants

AAS study on the concentration of lead acetate in *Eleusine coracana* is tabulated in table 9.

The Atomic Adsorption Spectrometric (AAS) study revealed that metal concentration was more in metal treated plants. The Atomic Adsorption Spectrometric (AAS) study revealed that metal concentration was decreased in biosorbent treated plants table 10.

CONCLUSION

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 agricultural waste 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 used of dried agricultural waste biomass sugarcane bagasse can efficiently remove the toxicity of lead from the soil. Hence we strongly suggest that sugarcane bagasse can be used as a biosorbent of heavy metal in the metal polluted environment for sustainable agriculture.

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