ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Employment of tea waste as biosorbent to mitigate metal toxicity to plants

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Manuscript Info

Manuscript History:

Received: 11 January 2015
Final Accepted: 29 February 2015
Published Online: March 2015

Key words:

Dye effluent, heavy metal, antimony(III) chloride, morphometric, biochemical, enzymes.

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Abstract

Most of the environmental pollution studies were concerned mainly with water pollution. There are strong evidences of the progressive deterioration of water quality not only in India, but also all over the world. The polluted water contains various concentrations of heavy metal ions. That affected the living organisms. In the present study, the seedlings of *Cajanus cajan* (L.) Millspauk were treated with various concentration of textile dye effluent (20%, 40%, 50%, 60%, 80%) and antimony (III) chloride (5mM, 10mM, 15mM, 20mM, 25mM) individually. After 10 days of treatment the plants were analysed. The morphometric, pigmental, biochemical and enzymatic characteristics were decreased except proline, leaf nitrate and the activities of catalase and peroxidise with increasing the concentration of effluent and heavy metal. The declining trend was also noticed in the case of protein, soluble sugar, NR activity. But when the plants were applied with tea waste treated dye effluent and antimony the result was exactly opposite to what was noticed earlier, implying that biosorbent treatment caused the removal of pollutants, so, that the plants were recovered.

INTRODUCTION

Pollution is unpreventable owing to industrialization and causes considerable damage to the environment. Textile industry has been placed in the category of most polluting industries. The improper and indiscriminate disposal of textile effluents into natural waters and land is posing serious problems. The textile effluent contains organic and inorganic chemical compounds that have adverse effect on the growth of plants and animals, because it contains heavy metals, which accumulate in various parts of plants (Mishra and Dinesh, 1991). Presence of heavy metals even in traces is toxic and harmful to both flora and fauna. Most of the dyes are stable against photo degradation, bio-degradation and oxidizing agents. Some of the dyes used in the textile industries are found to be carcinogenic (Roshan Poi et al., 2000). Several methods are being used for the removal of heavy metals from waste water (chemical precipitation, ion-exchange, electro chemical treatment, membrane technologies). These methods have both merits and demerits and highly expensive. But biosorption is a suitable method of restoration and management of our natural water resources. There is an urgent need to apply bioadsorbtion technology, using agricultural waste and natural algal biomass to decontaminate the polluted water bodies in the world (Selvarathi and Ramasubramanian, 2010).

In the present study, aimed to investigate the effect of dye effluents and heavymetals on morphometric, biochemical and enzymatic characteristics of *Cajanus cajan* (L.) Millspauk also investigated biosorbent properties of the varying amount of tea waste on 50% effluent and 15mM heavy metal (antimony III chloride) to restore the reduced morphometric, biochemical and enzymatic characteristics of *Cajanus cajan* (L.) Millspauk.

Material and Methods

The dye industry effluents were collected from the cotton mill industry in Thalavaipuram, Rajapalayam Taluk. Seeds were procured from Agricultural University Coimbatore. The bioadsorbent tea waste collected from local tea shop in Thalavaipuram. Various concentration of dye effluent and heavy metal like (20%, 40%, 50%, 60% and 80%) and (5mM, 10mM, 15mM, 20mM and 25mM) were prepared. Both control and experimental plants were allowed to grow in soil mixture (red: black: garden soil) the ratio of 1:1:1. After ten days seedlings of *Cajanus cajan* were treated with dye effluent and heavy metal solution. Various morphometric, biochemical and enzymatic characteristics were analysed on the 7 day treated plants. The optimal concentration of dye (50%) and antimony (15mM) was mixed with various concentration of tea waste (2g/L, 4g/L and 6g/L w/v) and kept in shaker for 24 hours. The filtrate was used to treat plants. After 7 days of treatment, the same morphometric, biochemical and enzymatic characteristics were analysed as follows : chlorophyll (Wellburn and Lichtenthalar, 1984), anthocyanin (Swain and Hills, 1959), protein content (Lowry et al., 1951), total soluble sugar (Jayaraman, 1981), aminoacid content Jayaraman, (1981), proline (Bates et al., 1973), *in vivo* nitrate reductase (Joworski, 1971), peroxidise and catalase activity (Kar and Mishra, 1976).

Results and Discussion

Table – 1: Effect of various concentration of heavy metal on the morphometric characteristics of *Cajanus cajan* (L.) Millspauk.

S.No	Parameters	Control	5mM	10mM	15mM	20mM	25mM
1.	Shoot length (cm)	29.8 ± 0.173 (100)	23.00 ± 0.400 (77)	18.53 ±0.523 (62)	13.966 ±0.433 (46)	12.533±0.260 (42)	11.433±0.290 (38)
2.	Root length (cm)	17.6 ± 0.585 (100)	12.66 ± 0.578 (72)	8.7 ± 0.472 (49)	6.566 ± 0.348 (37)	5.5 ± 0.208 (31)	5.233± 0.145 (30)
3.	Fresh weight (mg)	1.041 ± 0.107 (100)	0.731 ± 0.072 (70)	0.50 ± 0.017 (49)	0.32 ± 0.030 (32)	0.30 ± 0.013 (29)	0.25 ± 0.005 (25)
4.	Dry weight (mg)	0.20 ± 0.021 (100)	0.15 ± 0.006 (176)	0.12 ± 0.008 (61)	0.09 ± 0.003 ± (46)	0.08 ± 0.006 (39)	0.04 ± 0.003 (23)

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

Table – 2: Effect of various concentrations of heavy metal on biochemical characteristics of *Cajanus cajan* (L.) Millspauk.

S.No	Parameters	Control	5mM	10mM	15mM	20mM	25mM
1.	Total chlorophyll (mg/gLFW)	5.459 ± 0.104 (100)	4.676 ±0.314 (85)	3.892 ±0.239 (71)	3.471±0.054 (64)	1.887±0.423 (34)	1.587 ±0.026 (29)
2.	Carotenoids (mg/gLFW)	0.417 ± 0.011 (100)	0.357 ±0.050 (86)	0.281 ±0.016 (67)	0.217±0.060 (52)	0.194±0.109 (47)	0.182± 0.010 (44)
3.	Total soluble sugar (mg/gLFW)	7.15 ± 0.050 (100)	6.51 ± 0.060 (91)	5.856 ±0.081 (81)	4.766 ±0.063 (66)	4.30 ±0.061 (60)	4.04 ± 0.004 (56)
4.	Total soluble Protein (mg/gLFW)	6.85 ± 0.031 (100)	5.40 ± 0.041 (79)	4.45 ±0.002 (64)	3.27 ±0.002 (47)	2.5 ± 0.031 (36)	1.125±0.0003 (16)
5.	Aminoacid (mg/gLFW)	5.24 ± 0.005 (100)	5.280 ±0.075 (101)	5.617 ±0.020 (107)	6.015±0.003 (115)	6.76 ±0.003 (129)	9.480 ± 0.008 (181)
6.	Proline (mg/gLFW)	0.617 ±0.112 (100)	0.768 ±0.084 (124)	0.973 ±0.025 (157)	1.146±0.052 (186)	1.628±0.005 (263)	2.322 ± 0.082 (376)
7.	Leaf Nitrate (mg/gLFW)	0.821± 0.008 (100)	1.483± 0.011 (180)	1.899± 0.005 (231)	2.129±0.006 (259)	2.422±0.003 (295)	2.716±0.003 (330)

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

Table – 3: Effect of various concentrations of heavy metal treatment on enzyme characteristics of *Cajanus cajan* (L.) Millspauk.

S.No	Parameters	Control	5mM	10mM	15mM	20mM	25mM
1.	NR activity (μMole/ gLFW)	29.52 ±0.976 (100)	26.07 ±0.596 (88)	22.407±0.369 (76)	17.499 ± 0.076 (59)	14.323 ± 0.087 (49)	8.999± 0.072 (30)
2.	Catalase activity (μMole/gLFW)	3.433±0.033 (100)	3.644 ±0.044 (106)	3.932 ±0.066 (114)	4.556± 0.044 (132)	4.77 ± 0.044 (139)	4.955±0.003 (144)
3.	Peroxidase activity (μMole/gLFW)	4.825 ±0.105 (100)	5.878 ±0.044 (122)	6.34 ± 0.030 (130)	6.615±0.044 (136)	6.906± 0.050 (144)	8.072± 0.086 (164)

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

Table – 4: Effect of various concentrations of dye effluent on biochemical characteristics of *Cajanus cajan* (L.) Millspauk.

S.No	Parameters	Control	20%	40%	50%	60%	80%
1.	Shoot length (cm)	29.8± 0.173 (100)	25.9 ± 0.433 (87)	22.8 ±0.448 (77)	19.4 ± 0.435 (65)	16.5 ± 0.643 (55)	14.2 ± 0.352 (48)
2.	Root length (cm)	17.6± 0.585 (100)	14.5 ± 0.480 (83)	11.6± 0.409 (66)	10.00 ±0.163 (57)	8.6 ± 0. 057 (49)	7.9 ± 0.260 (45)
3.	Fresh weight (mg)	1.04± 0.107 (100)	0.95 ± 0.029 (92)	0.83 ±0.027 (80)	0.66 ± 0.034 (64)	0.58 ± 0.003 (56)	0.42 ± 0.006 (41)
4.	Dry weight (mg)	0.20 ±0.021 (100)	0.14 ± 0.027 (72)	0.13 ±0.002 (63)	0.09 ±0.006 (47)	0.06 ± 0.003 (33)	0.04 ± 0.002 (22)

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

Table – 5: Effect of various concentrations of dye effluent treatment on biochemical characteristics of *Cajanus cajan* (L.) Millspauk.

S.No	Parameters	Control	20%	40%	50%	60%	80%
1.	Total chlorophyll (mg/gLFW)	5.459 ± 0.104 (100)	3.438 ±0.001 (71)	3.664±0.004 (64)	3.014 ±0.051 (58)	3.087 ±0.043 (57)	2.987± 0.026 (55)
2.	Carotenoids (mg/gLFW)	0.417± 0.073 (100)	0.351 ±0.010 (73)	0.296±0.050 (61)	0.237± 0.004 (49)	0.175± 0.032 (36)	0.151± 0.053 (31)
3.	Total soluble sugar (mg/gLFW)	7.15 ± 0.050 (100)	6.04 ± 0.055 (84)	5.756±0.034 (80)	4.69± 0.072 (66)	4.45± 0.045 (62)	4.176 ± 0.058 (58)
4.	Total soluble Protein (mg/gLFW)	6.85 ± 0.392 (100)	6.525±0.229 (95)	5.45± 0.139 (79)	4.325±0.264 (63)	3.125±0.304 (46)	0.95 ± 0.319 (14)
5.	Aminoacid (mg/gLFW)	5.245±0.053 (100)	5.508±0.157 (105)	5.792±0.588 (110)	6.299±0.273 (120)	6.54 ± 0.704 (125)	6.768±0.341 (129)
6.	Proline (mg/gLFW)	6.17 ± 0.031 (100)	9.28 ± 0.021 (150)	11.48±0.023 (186)	16.21±0.004 (263)	19.28±0.051 (312)	21.82±0.053 (353)
7.	Leaf Nitrate (mg/gLFW)	0.838±0.008 (100)	1.076±0.001 (128)	1.292±0.002 (154)	1.554±0.002 (185)	1.67± 0.001 (199)	1.971± 0.002 (235)

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

Table – 6: Effect of various concentrations of dye effluent treatment on enzyme characteristics of *Cajanus cajan* (L.) Millspauk.

S.No	Parameters	Control	20%	40%	50%	60%	80%
1.	NR activity (μMole/g LFW)	29.524 ± 0.975 (100)	27.231±0.094 (92)	25.851±0.200 (87)	20.57±0.176 (69)	17.736±0.017 (60)	16.38±0.158 (55)
2.	Catalase activity (μMole/gLFW)	3.4 ± 0.044 (100)	4.6 ± 0.001 (133)	4.9 ± 0.033 (143)	5.53 ± 0.023 (161)	5.8 ± 0.001 (168)	6.13± 0.042 (179)
3.	Peroxidase activity(μMole/g LFW)	4.825 ± 0.015 (100)	5.467 ± 0.037 (113)	6.129 ± 0.052 (127)	6.458±0.067 (133)	6.995 ± 0.038 (144)	7.378±0.068 (152)

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

Table – 7: Effect of Heavy metal and tea waste on the Morphometric characteristics of *Cajanus cajan* (L.) Millspauk.

S.No	Parameters	Control	15mM	2gm/L	4gm/L	6gm/L
1.	Shoot length (cm)	29.8 ± 0.173 (100)	13.96 ± 0.433 (47)	19.53 ± 0.700 (66)	22.16 ± 0.405 (74)	25.63 ± 0.592 (86)
2.	Root length (cm)	17.6 ± 0.585 (100)	7.56 ± 0.348 (37)	9.13 ± 0.202 (56)	10.36 ± 0.0.317 (63)	13.00 ± 0.305 (78)
3.	Fresh weight (mg)	1.04 ± 0.107 (100)	0.32 ± 0.030 (32)	0.63 ± 0.036 (61)	0.71 ± 0.030 (69)	0.83 ± 0.019 (80)
4.	Dry weight (mg)	0.20 ± 0.021 (100)	0.09 ± 0.003 (46)	0.11 ± 0.008 (64)	0.13 ± 0.004 (76)	0.14 ± 0.004 (78)

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

Table – 8: Effect of heavy metal and tea waste on biochemical characteristics of *Cajanus cajan* (L.) Millspauk.

S.No	Parameters	Control	15mM	2gm/L	4gm/L	6gm/L
1.	Total chlorophyll (mg/gLFW)	5.459 ± 0.104 (100)	3.471±0.054 (64)	3.666 ± 0.005 (67)	3.971 ± 0.043 (74)	4.479 ± 0.011 (82)
2.	Carotenoids (mg/gLFW)	0.417 ± 0.011 (100)	0.217 ± 0.060 (52)	0.307 ± 0.076 (74)	0.326 ± 0.007 (78)	0.351 ± 0.145 (84)
3.	Total soluble sugar (mg/gLFW)	7.15 ± 0.050 (100)	4.766 ± 0.063(66)	6.13 ± 0.046 (86)	6.82 ± 0.147 (95)	7.163 ± 0.162 (100)
4.	Total soluble Protein (mg/gLFW)	6.85±0.031 (100)	3.27±0.002 (47)	4.35 ± 0.012 (64)	6.15 ± 0.043 (90)	8.25 ± 0.058 (120)
5.	Aminoacid (mg/gLFW)	5.24± 0.005 (100)	6.015± 0.003 (115)	5.805 ± 0.040 (110)	5.617 ± 0.075 (107)	5.490 ± 0.023 (104)
6.	Proline (mg/gLFW)	0.617± 0.112 (100)	1.146±0.052 (186)	0.94 ± 0.036 (158)	0.873 ± 0.026 (141)	0.748 ± 0.029 (121)
7.	Leaf Nitrate (mg/gLFW)	0.821± 0.008 (100)	2.129± 0.006 (259)	1.560 ± 0.006 (186)	1.229 ± 0.007 (146)	1.037 ± 0.003 (123)

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

Table – 9: Effect of heavy metal and tea waste on enzyme characteristics of *Cajanus cajan* (L.) Millspauk.

S.No	Parameters	Control	15mM	2gm/L	4gm/L	6gm/L
1.	NR activity (µMole/gLFW)	29.527 ± 0.097 (100)	17.499 ± 0.761(59)	19.037 ±0.029 (64)	21.564 ± 0.045 (73)	27.379 ±0. 072 (92)
2.	Catalase activity (µMole/gLFW)	3.444 ± 0.044 (100)	4.55 ± 0.044 (132)	3.844 ± 0.040 (112)	3.110 ± 0.004 (90)	2.688 ± 0.088 (78)
3.	Peroxidase activity (µMole/ gLFW)	4.825± 0.105 (100)	6.582 ± 0.094 (136)	6.261 ± 0.118 (129)	5.624 ± 0.006 (116)	5.236 ± 0.014 (108)

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

Table – 10:Effect of dye effluent and tea waste on the Morphometric characteristics of *Cajanus cajan* (L.) Millspauk.

S.No	Parameters	Control	50%	2gm/L	4gm/L	6gm/L
1.	Shoot length (cm)	29.8 ± 0.173 (100)	25.9 ± 0.433 (87)	22.8 ± 0.448 (77)	19.4 ± 0.435 (65)	16.5 ± 0.064 (55)
2.	Root length (cm)	17.6 ± 0.585 (100)	14.5 ± 0.480 (83)	11.6 ± 0.409 (66)	10.0 ±0.163 (57)	8.6 ± 0.057 (49)
3.	Fresh weight (mg)	1.04 ± 0.107 (100)	0.66 ± 0.034 (64)	0.81 ±0.015 (78)	0.90 ± 0.020 (87)	0.10 ± 0.037 (99)
4.	Dry weight (mg)	0.20 ± 0.020 (100)	0.09 ± 0.006 (47)	0.13 ± 0.005 (64)	0.17 ± 0.005 (83)	0.23 ± 0.026 (115)

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

Table – 11: Effect of dye effluent and tea waste on biochemical characteristics of *Cajanus cajan* (L.) Millspauk.

S.No	Parameters	Control	50%	2gm/L	4gm/L	6gm/L
1.	Total chlorophyll (mg/gLFW)	5.459 ± 0.104 (100)	3.014 ±0.051 (58)	3.549 ± 0.003 (65)	3.852 ± 0.025 (71)	4.232 ± 0.167 (78)
2.	Carotenoids (mg/gLFW)	0.417 ± 0.011 (100)	0.237 ± 0.004 (49)	0.259 ± 0.521 (57)	0.273 ± 0.421 (61)	0.328± 0.005 (72)
3.	Total soluble sugar (mg/gLFW)	7.15 ± 0.050 (100)	4.69 ± 0.072 (66)	6.263 ± 0.042 (88)	6.86 ± 0.079 (95)	7.636 ± 0.043 (107)
4.	Total soluble Protein (mg/gLFW)	6.85 ± 0.392 (100)	4.325 ± 0.264 (63)	4.65 ± 0.008 (68)	5.75 ± 0.022 (84)	6.12 ± 0.013(107)
5.	Aminoacid (mg/gLFW)	5.245 ± 0.053 (100)	6.299 ± 0.273 (120)	5.617 ± 0.200 (107)	5.389 ± 0.702 (102)	5.123 ± 0.306 (98)
6.	Proline (mg/gLFW)	6.17 ± 0.031(100)	16.21 ± 0.004 (263)	14.08 ± 0.069 (228)	11.19 ± 0.038 (181)	8.04 ± 0.008 (130)
7.	Leaf nitrate (mg/LFW)	0.838 ± 0.008 (100)	1.554 ± 0.002 (185)	1.439 ± 0.006 (171)	1.310 ± 0.001 (156)	1.213 ± 0.004 (145)

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

Table – 12: Effect of dye effluent and tea waste on enzyme characteristics of *Cajanus cajan* (L.) Millspauk.

S.No	Parameters	Control	50%	2gm/L	4gm/L	6gm/L
1.	NR activity (µMole/gLFW)	29.524 ± 0.975 (100)	20.576 ±0.176 (69)	22.407 ± 0.369 (76)	22.870 ±0.168 (77)	24.657 ± 0.182 (83)
2.	Catalase activity (µMole/gLFW)	3.4 ± 0.044 (100)	5.53 ± 0.023 (161)	4.022 ± 0.022 (116)	3.44 ± 0.022 (100)	2.577 ± 0.044 (75)
3.	Peroxidase activity(µMole/g LFW)	4.825 ± 0.015 (100)	6.458 ± 0.067 (133)	5.488 ± 0.169 (113)	4.398 ± 0.138 (91)	3.524 ± 0.156 (73)

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

There was a decrease in shoot length, root length, fresh weight and dry weight with increasing the concentration of dye effluent and heavy metal (Table 1 & 4). The pronounced inhibition of shoot and root growth and leaf area were the main cause for the decrease in fresh and dry weight of seedlings. The inhibition of biomass accumulation is directly related to the photosynthetic process. At higher concentration, the effluent and heavy metal showed inhibitory effect on both photosynthetic pigments and total soluble sugar. The chlorophylls, carotenoids, total soluble sugar, protein (Table 2 & 5). In contrary, the leaf nitrate, free amino acids, proline (Table 2 & 5) and catalase and peroxidase activity was (Table 3 & 6) increased with increasing the concentration of dye effluent and antimony chloride. This result coincides with the results of (Panday and Pathak 2006). 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 (Swaminathan et al., 1998; Dowton, 1997). Nitrate reductase activity also showed declining trend.

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 plants. Accumulation of proline & free amino acid has been frequently used as biochemical marker for water stress in plants (Alia and Saradhi, 1991). In stress condition the inhibition of growth of cells and whole plant is accompanied by an accumulation of nitrate in plant tissue particularly in leaves (Sinha and Nicholas, 1981). The leaf nitrate content was found to be more in metal treated plants paralleling with the reduction in nitrate reductase activity. The peroxidase activity was reported to be increased with the increase in the concentration of Lead acetate, causing chlorophyll degradation. Catalase is special type of peroxidase enzyme which catalase the degradation of H_2O_2 which is natural metabolite, and also toxic to plants Balasinha (1982).

Biosorption studies showed increase in morphometric characteristics after the applications of tea waste powder. (Table 6-12). Similarly the chlorophyll content was increased. The total soluble protein content and nitrate activity also increased after the application of tea waste powder (Table 8 & 11). In contrary proline (Table 8 & 11) and the activity of enzymes such as catalase and peroxidise (Table 9 & 12) were dexcreased after the application of tea waste powder. Conventional methods of removal of toxins which are expensive in dye industry effluent and hence the use of low cost environment friendly biosorbent has been used. The tea waste powder used in the present study to remove the large quantity of heavy metals from dye industry effluent.

Result of the present study clearly shows that the tea waste can efficiently remove the toxins from effluent and heavy metal. Hence we strongly suggest that tea waste can be used as a bioadsorbant to remove the toxicity of effluent polluted environment for sustainable agriculture.

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