Bioadsorbent effect of *Abutilon indicum*, L., on paper mill effluent and its impact on the Physico chemical characteristics of *Lycopersicum esculentum*, Mill.

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Abstract

Phytoremediation is the use of plant's natural ability to degrade, or remove toxic chemicals and pollutants from soil or water. In this regard, phytoremediation study was carried out by the biomass of commonly available weed plant *Abutilon indicum*, L. in different concentrations (0.5%, 1%, 1.5% and 2% (w/v)) with 30% (v/v) concentration of paper mill effluent on the growth and biochemical characteristics of *Lycopersicum esculentum*, Mill. After the phytotreatment of paper mill effluent with *Abutilon indicum*, L all the physicochemical parameters especially, total dissolved solids, biological oxygen demand, chemical oxygen demand, electrical conductivity and oil and grease were significantly reduced when compared to the untreated paper mill effluent. The beneficial effects of the phytotreated paper mill effluent were studied by treating the seedlings of *Lycopersicum esculentum*, Mill. from the 7th day of sowing and continued till 21st day; then the seedlings of *Lycopersicum esculentum* were analyzed for their growth and biochemical characteristics and compared with the control maintained with water and 30% effluent. The growth and biochemical characteristics were found to be improved significantly than with the application of untreated paper mill effluent. The photosynthetic pigment content such as chlorophyll a, chlorophyll b, total chlorophyll, carotenoid, soluble sugar and protein contents were increased after the application of phytotreated effluent. But as a contrast, anthocyanin, aminoacid, proline, leaf nitrate, total phenol and enzyme activities such as catalase and peroxidase were found to be reduced in the phytoremediated effluent treated seedlings. In the present study, it is concluded that, *Abutilon indicum*, L., is a suitable alternate for the remediation of paper mill effluent by reducing all the pollutants from the effluent and relieved the experimental plants from stress.

Introduction

The World's ever increasing population and her progressive adoption of industrial based life style and urbanization have yielded large quantities of sewage and effluent. Making wastewater a vital water resource for agriculture is one of the recently suggested remedies to tackle this environmental problem. Phytoremediation is an effective and inexpensive cleanup of hazardous waste sites. The use of plants to degrade, assimilate, metabolize or detoxify contaminants is cost effective and ecologically sound for the restoration and management of our natural water resource.

There is an urgent need to apply phytoremediation technology, using plants to decontaminate the polluted soils and water bodies in India. This will be effective in bringing new resources and technology to solve environmental problems in India, generated by industries.

Paper mill generate large volume of wastewater (252 m^3/t) for paper production (CPCB, 1991). It ranks third in terms of fresh water withdrawal in the world (Kallas and Munter, 1994). The production of paper and paperboard in India during the year 2002-2003 was 0.852 million tons per day (Ministry of Commerce and Industrial Policy, 2004). Hence, an investigation has been undertaken to study whether paper mill effluent, after phytoremediation, can be used as a water resource for agriculture, for which the effluent has been collected from a paper mill in

Sivakasi. This study intends to assess the characteristics of paper mill effluents, its effect on the growth and biochemical characteristics of crop plant the *Lycopersicum esculentum*, Mill. The plants used for this purpose must have both high potential capacity to absorb elements from soil or water and large biomass.

These plants not only had the BOD reducing capacity, but also reduced total suspended solids from sewage lagoons. Free floating plants such as *Spirodela*, *Lemna minor*, *Salvinia natum* and *Pistia* had a great significance in the treatment of sewage water (Reed *et al.*, 1988). The use of *Albizzia lebbeck* for the removal of alhyl benzene sulphonates from aquous solution was worked out (Ferrandez *et al.*, 1996). The *Populus* species removed the hazardous substances such as TNT, RDX from ammunition wastes (Thompson and Schnor, 1996). *Nicotiana tobaccum* volatilize mercury and absorb elemental mercury and methyl mercury from the soil and release volatile mercuric oxide from the leaves into the atmosphere (Heaton *et al.*, 1998; Rugh *et al.*, 1996).

In *Typha*, roots can hold heavy metals in the cell walls reducing the heavy metal combination with large weight molecules in plant cell plasma (Bringezu *et al.*, 1999). *Typha angustifolia* is able to tolerate heavy metals such as Ni, Cu, and Zn. The cell walls of the plant have the capacity to bind metal ions in negatively charged sites (Macfie and Welbourn, 2000). *Brassica juncea* is more effective in the removal of zinc from soil (Blaylock and Huang, 2000).

It was suggested that phytoremediation would be a suitable alternative to conventional remediation. This was proved experimentally with the help of *Pteris vittata* and *Brassica juncea* plants to remediate the arsenic and lead contaminated soil (Arthur *et al.*, 2003).

Planting Indian mallow (*Abutilon avicennae*) in (TNT) 2, 4, 6-trinitrotoluene contaminated soil enhanced TNT reduction both by stimulating microbial activity that enhances microbial TNT transformation, and by direct uptake and phytotransformation of TNT (Chang Yoon-Young *et al.*, 2004). After the application of seaweed fertilizer (*Gracilaria*) in match industry treated bhendi plant, the growth and biochemical characters increased over their effluent treated counter parts (Ramasubramanian *et al.*, 2004).

The present investigation attempts to use *Abutilon indicum*, L for the remediation of paper mill effluent on the growth and biochemical profile of *Lycopersicum esculentum*, Mill.

Materials and Methods:

Healthy and viable seeds of *Lycopersicum esculentum*, Mill. var. TMV 1 were surface sterilized with 0.1% mercuric chloride for one minute and washed with running tap water, followed by rinsing with distilled water. Various concentrations of paper mill effluent were prepared, such as 10%, 20% 30%, 40%, and 50% (v/v). The seeds were soaked in distilled water for two hours were allowed to grow in pots containing 9 kg of uniformly mixed red, black and sandy soil in 1:1:1 ratio. The experimental sets were kept in diffused light at room temperature. Seven days after sowing, the experimental plants were watered constantly every day with the respective concentration of the effluent (750 mL). Both experimental sets and control sets maintained with tap water were maintained in triplicates. On the twenty first day both the sets of plants were taken for analysis.

For the phytoremediation studies, the chosen plants *Abutilon indicum*, L were rinsed, dried in shade, cut into pieces and ground to powder. Exactly 0.5g, 1g, 1.5g and 2g of biomass were mixed separately with 30% (v/v) paper mill effluent. Phytoremediated paper mill effluent was obtained by keeping the different concentrations of plant biomass mixed effluent in the shaker for four hours. After that the physico chemical characteristics of the treated effluent was analysed and it was applied to seven days old seedling of *L. esculentum* which were grown in pots.

From the seventh day onwards the seedlings of *L. esculentum* in the respective labeled pots were treated with 750 mL/day of phytoremediated paper mill effluent in respective concentrations. On the 21st day, the seedlings were plucked out with out any damage and analyzed for the growth, pigment, biochemical and enzyme activities.

Biochemical characters:

For Biochemical characters such as chlorophyll and carotenoids, fresh leaf tissues were extracted with 100% acetone and were quantified using the formula Wellburn and Lichtenthaler (1984), Total sugar content (Jeyaraman,1981),Amino acid (Moore and stein,1948)Leaf soluble protein(Lowry, 1951), Proline(Bates et al.,1973), Total phenol (Bray and Thorpe, 1954), *in vivo* nitrate reductase activity (Jaworski, 1971), Peroxidase activity (Addy and Goodman, 1972), and Catalase (Kar and Mishra, 1976),were analysed both for control and experimental seedlings.

Physicochemical Characteristics of Phytoremediated Paper Mill Effluent

The major physicochemical parameters of phytoremediated paper mill effluent using 2% (W/V) concentrations of the biomass of *Abutilon indicum*, L., are shown in Table 1 and summarized as follows

The pH of the paper mill effluent was increased. The electrical conductivity and total dissolved solids of the treated paper mill effluent was highly reduced when compared with untreated one. Sukumaran (1997) stated, that macrophytes had the potentials of treating wastewater systems and reducing the suspended solids, nutrients, organic matter, BOD and COD. The *Lemna minor* is efficient in removing BOD, dissolved solids and nutrient from wastewater (Egbert Selwin Rose, 2000).

Likewise, the contents of potassium, magnesium, sulphate, sodium and chloride were reduced after phytoremediation. The biological and chemical oxygen demand had also been reduced in paper mill effluent after phytoremediation. Mueller *et al.*, (1995) studied the uptake and biotransformation of (TNT) 2, 4, 6-trinitrotoluene in cell suspension cultures and in whole plants of Jimson weed (*Datura innoxia*, Mill.) and tomato (*Lycopersicum peruvianum* (L.) Mill.)

Effect for Phytoremediated Paper Mill Effluent on the growth and Biochemical Characteristics of *Lycopersicum esculentum*, Mill. Seedling.

With the increase in the concentrations (0.5%, 1%, 1.5% and 2% (w/v)) of *Abutilon indicum*, L remediation the shoot length, root length, fresh weight, dry weight and leaf area of the seedlings enhanced in *L. esculentum.* The increase in growth was very significant when they were treated with 2% (w/v) concentration of *Abutilon indicum*, L remedidated paper mill effluent. The increase in the shoot length of *L. esculentum* was 78% than when they were treated with paper mill effluent alone.

A remarkable increase in the root length was noticed with 2% *Abutilon* remediated paper mill effluent treatment in *L. esculentum*, and the increase was 130%. A gradual increase was observed in fresh weight, dry weight and leaf area under 0.5%, 1%, 1.5% and 2% (w/v) concentrations of *Abutilon indicum*, remediated paper mill effluent treatment.

Photosynthetic Pigment Content

The chlorophyll and carotenoid contents had significantly increased after the application of phytoremediated paper mill effluent. The anthocyanin content was decreased to about 36%, 43%, 57% and 68%.

Biochemical Characteristics

The total soluble sugar increase was at the rate of 49% for 1% (w/v). Increase in the leaf protein was at the rate of *79%* for 2% (w/v) *Abutilon*. Similar positive trend was observed after the application of seaweed fertilizer (*Gracilaria*) in match industry effluent treated bhendi plant (Ramasubramanian *et al.*, 2004b).

But in contrast, amino acid, proline, leaf nitrate and total phenol contents were decreased. The reduction in their biochemical characteristics was high with 2% (w/v) *Abutilon*. It is to be pointed out that the application of 2%

(w/v) *Abutilon* remediated paper mill effluent caused pronounced reduction in proline and phenol content to about 69% and 84% respectively.

The SDS – PAGE profile of the total proteins was studied. In phytoremediation the degraded protein of 70 kDa and 30 kDa were recovered. In the case of *L. esculentum* the 30 and 20 kDa proteins responded better and increased than the plants treated with effluent.

Enzyme Activities

The effect of various concentrations of phytoremediated paper mill effluent on the activities of nitrate reductase enzyme in *L. esculentum* was found to be increased with the increase in the concentrations of phytoremediation. The enzyme nitrate reductase activity was increased to about 12%, 33%, 44% and 71% respectively with0.5%,1%,1.5% and *2*% (w/v) *Abutilon* than the plants treated with effluent alone.

Peroxidase and catalase are the enzymes responsible for scavenging the plant materials from the stressed impact. The enzyme catalase and the peroxidase activity followed a negative trend when treated with phytoremediated paper mill effluent. This is in accordance with the findings of Jeyaprakash, (2006).The enzyme peroxidase activity was reduced to about 28%, 51%, 60% and 77% respectively and the catalase activity was reduced to about 37%, 61%, 74% and 83% respectively with 0.5%,1%,1.5% and *2%* (w/v) of *Abutilon*. It is to be pointed out that among the four concentrations used for phytoremediation of the paper mill effluent, it was found that the effect was the highest for 2% (w/v) *Abutilon.*

Physico Chemical Characteristics of Phytoremediated Paper Mill Effluent

S. No.	**Parameters***	**Water**	**Control (Effluent)**	*Abutilon 2%* (w/v)
1.	Temperature	-	30^0C	29^0C
2.	pH	7 to 8.5	4.7	6.8
3.	Electrical Conductivity	400	10,500	4,300
4.	Total Solids	-	9,800	3,400
5.	Total Dissolved Solids	500	9,975	5,700
6.	Total Suspended Solids	-	9,175	6,130
7.	Total hardness	300	1,365	545
8.	Salinity	-	26.2	13.2
9.	Potassium	20	85.2	32.61
10.	Sodium	20	1,250	854
11.	Calcium	75	36.2	19.51
12.	Magnesium	30	15.7	6.54
13.	Sulphate	150	26.2	15.43

14.	Chloride	250	4,000	1,500
15.	Bicarbonate	-	7.6	5.43
16.	Phosphate	-	22.1	12.15
17.	Nitrogen	-	6.2	3.81
18.	Dissolved oxygen	-	4.5	2.86
19.	Dissolved carbon di oxide	-	41.2	25.43
20.	COD	-	850	416
21.	BOD	-	95.2	29.5
22.	Oil and grease	-	0.017	0.010
23.	Water Quality Index	-	50	73

- All the values are expressed in mg/L, except pH and Electrical Conductivity.

Table: 2 Effect of *Abutilon* with Paper Mill Effluent on the Growth of *Lycopersicum esculentum*, Mill.

S. No.	Parameters	Control (water)	Effluent 30% (v/v)	0.5% (w/v)	1% (w/v)	1.5% (w/v)	2% (w/v)
1	Shoot length (cm)	9.6 ± 0.001 (100)	6.66 ± 0.012** (69)	10.8 ± 0.013** (113)	12.3 ± 0.003** (128)	12.9 ± 0.012** (134)	14.1 ± 0.011** (147)
2	Root length (cm)	3.32 ± 0.011 (100)	0.6 ± 0.001** (18)	3.9 ± 0.011** (119)	4.2 ± 0.001** (128)	4.5 ± 0.001** (137)	4.9 ± 0.001** (148)
3	Fresh weight (g)	0.332 ± 0.01 (100)	0.281 ± 0.002** (36)	0.405 ± 0.001** (122)	0.448 ± 0.002** (135)	0.458 ± 0.015** (138)	0.52 ± 0.002** (157)
4	Dry weight (g)	0.0554 ± 0.003 (100)	0.0461±0.002** (32)	0.048 ± 0.013** (118)	0.052 ± 0.012** (128)	0.055 ± 0.001** (136)	0.061 ± 0.001** (148)
5	Leaf area (cm^2)	4.35 ± 0.001 (100)	1.45 ± 0.01** (33)	5.52 ± 0.001** (127)	5.8 ± 0.001** (134)	6.1 ± 0.011** (141)	6.8 ± 0.002** (156)

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.

Effect of Paper Mill Effluent with *Abutilon* on the Photosynthetic Pigment Content of *Lycopersicum esculentum*, Mill.

S. No.	Parameters	Control (water)	Effluent 30% (v/v)	0.5% (w/v)	1% (w/v)	1.5% (w/v)	2% (w/v)
1.	Chlorophyll *a* (mg/gLFW)	2.687 ±0.03 (100)	1.531 ±0.004** (57)	2.203 ±0.024** (82)	2.364 ±0.024** (88)	2.928 ±0.002** (109)	3.412 ±0.17** (127)
2.	Chlorophyll *b* (mg/gLFW)	1.822 ±0.01 (100)	0.843 ±0.02** (48)	1.430 ±0.007** (76)	1.750 ±0.006** (93)	2.051 ±0.10** (109)	2.239 ±0.16** (119)
3.	Total Chlorophyll (mg/gLFW)	4.569 ±0.05 (100)	2.165 ±0.15** (47)	3.633 ±0.021** (80)	4.114 ±0.017** (90)	4.979 ±0.011** (109)	5.651 ±0.018** (124)
4.	Carotenoid (mg/gLFW)	1.945 ±0.21 (100)	0.942 ±0.16** (48)	1.419 ±0.17** (73)	1.731 ±0.16** (89)	1.906 ±0.001** (98)	2.217 ±0.1** (114)
5.	Anthocyanin (mg/gLFW)	1.527 ±0.11 (100)	2.296 ±0.06** (150)	1.740 ±0.21** (114)	1.633 ±0.018** (107)	1.420 ±0.014** (93)	1.252 ±0.11** (82)

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.

Effect of Paper Mill Effluent with Abutilon on the Biochemical Characteristics of Lycopersicum esculentum, Mill.

S. No.	Parameters	Control (water)	Effluent 30% (v/v)	0.5% (w/v)	1% (w/v)	1.5% (w/v)	2% (w/v)
1.	Total soluble sugar (mg/g LFW)	11.6 ±0.001 (100)	7.07 ±0.02** (61)	10.9 ±0.001** (94)	12.8 ±0.014** (110)	13.3 ±0.01** (115)	15.1 ±0.04** (130)
2.	Protein (mg/g LFW)	10.73 ±0.074 (100)	7.71 ±0.013** (57)	9.76 ±0.013** (91)	13.09 ±0.021** (122)	13.73 ±0.001** (128)	14.5 ±0.05** (136)
3.	Amino acid (µ mole/g	2.84 ±0.01 (100)	4.068 ±0.002**	3.64 ±0.01** (128)	3.04 ±0.001** (107)	2.33 ±0.04** (82)	1.79 ±0.03**

	LFW)		(143)				(63)
4.	Nitrate (mg/g LFW)	185.5 ±0.013 (100)	228.1 ±0.01** (123)	207.5 ±0.002** (112)	193.0 ±0.03** (104)	161.5 ±0.02** (87)	133.5 ±0.001** (72)
5.	Proline (mg/g LFW)	1.012 ±0.002 (100)	1.356 ±0.12** (134)	1.163 ±0.01** (115)	0.920 ±0.002** (91)	0.759 ±0.02** (75)	0.718 ±0.02** (71)
6.	Phenol (mg/g LFW)	2.72 ±0.001 (100)	4.41 ±0.03** (149)	2.82 ±0.03** (104)	2.52 ±0.01** (93)	2.12 ±0.003** (78)	1.76 ±0.01** (65)

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.

Effect of Paper Mill Effluent with *Abutilon* on the Enzyme Activities of *Lycopersicum esculentum*, Mill.

S. No.	Parameters	Control (water)	Effluent 30% (v/v)	0.5% (w/v)	1% (w/v)	1.5% (w/v)	2% (w/v)
1	Nitrate reductase (µ mole/g LFW)	464.23 ±0.004 (100)	292.4 ±0.01** (63)	348.1 ±0.02** (75	445.6 ±0.001** (96)	496.7 ±0.004** (107)	622.0 ±0.015** (134)
2	Catalase activity (µ mole/g LFW)	3.315 ±0.013 (100)	5.038 ±0.025** (152)	3.812 ±0.012** (115)	3.016 ±0.02** (91)	2.585 ±0.11** (78)	2.2876 ±0.002** (69)
3	Peroxidase activity (µ mole/g LFW)	0.279 ±0.001 (100)	0.385 ±0.03** (138)	0.306 ±0.014** (110)	0.242 ±0.11** (87)	0.217 ±0.41** (78)	1.70 ±0.031** (61)

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.

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