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Bioremediation Potential of *Brassica juncea* against Tannery Effluent

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Abstract

Heavy metal contamination caused by natural processes or by human activity is one of the serious ecotoxicological problems. Phytoremediation is an emerging plant based technology for the removal of toxic contaminants from the soil and water. It is relatively invasive and provides a low-cost remedial option suited too many sites. The present study is focused to remediate heavy metal contamination from tannery effluents using the plant *Brassica juncea*. The leather tanning effluent was found to have high pH indicating alkalinity of the sample with a large amount of suspended solids and dissolved salts of sodium, potassium, chromium, zinc, cadmium and copper. *Brassica juncea* was grown as a control plant using pure water used in the tanning industry and also using three different dilutions of the tannery effluent (25 %, 50 % and 100 % concentrations of the effluent). The biometric and biochemical observations of the plants were recorded on 30th and 60th days after sowing. Undiluted effluent did not favour the growth of the plants and the few germinated plants died soon. The plants treated with diluted effluents were found to have lower pigments, protein, DNA and RNA in the leaves as compared to the control plants. The 25 % concentration was found to be better for plants than 50% concentration of the effluent (**Keywords**: phytoremediation, *Brassica juncea*, bioremediation).

Introduction

Tanning industry is recognized as a serious environmental threat all over the world (Tariq *et al.*, 2005). It is one of the major consumers of water and most of it is discharged as waste water, containing high amounts of chromium along with high BOD. The presence of chromium in industrial effluents poses serious problem when discharged into water bodies or onto the waste land. Its constant deposition affects the soil surface and also ground water quality through seeping (Sinha *et al.*, 2002). Pollution of the water resources, both surface and underground, by indiscriminate discharge of spent wastes of chromium-based industries has become a serious global concern, for it has created an acute scarcity of safe drinking water in many countries (Chandra and Kulsheshtha, 2004).

Bioremediation is an environmental friendly and cost competitive alternative to chemical decomposition processes (Patil *et al.*, 2008). Phytoremediation is a new and novel strategy to remove toxic heavy metals from soil through hyper-accumulator plant species. This is a low-cost and eco-friendly means of reclaiming heavy metal contaminated soils, resulting from developmental activities such as discharge of industrial effluents and city wastes into drinking water (Panwar *et al.*, 2002; Wang *et al.*, 2002). The *Brassicaceae* family distinguishes with the ability to accumulate heavy metals in an extremely high degree (Broadley *et al.*, 2001). An attempt has been made in the present study to use the plant *Brassica juncea* for phytoremediation of tannery effluent.

Materials and Methods

Tannery Effluent

The effluent was collected from a selected leather processing industry situated at Dindigul in Tamil Nadu, India at weekly intervals for five weeks, pooled together and stored at 4 °C for analysis. The collected tannery effluent was analyzed for physicochemical properties like colour, odour, turbidity, pH, total suspended solids, total dissolved salts, chemical oxygen demand (COD), biochemical oxygen demand (BOD) (APHA, 1998), carbonate and bicarbonate, sodium and potassium (Natarajan *et al.*, 1988), chromium, copper, cadmium, nickel and zinc (APHA, 1998).

Soil

Red Soil of about five kilograms was filled in each pot of one foot diameter before sowing the seeds of the plant. Both the control soil and effluent treated soil were analyzed for certain parameters like pH, sodium and potassium (Natarajan *et al.*, 1988), chromium, copper, cadmium, nickel and zinc (APHA, 1998).

Plant

The seeds of the plant *Brassica juncea* selected for the study were collected from the Tamil Nadu Agricultural University, Coimbatore (India). The untreated soil was taken as control. *Brassica juncea* was grown in control soil and effluent treated soil in three different dilutions (25 % and 50 % concentrations and undiluted effluent) for a period of 60 days. On 30th and 60th days after sowing, the plants were analyzed for different biometric observations such as height of the plant, fresh and dry weight of the plant were taken. Biochemical analyses such as chlorophyll, carotenoids (Zakaria *et al.*, 1979), protein (Lowry *et al.*, 1951), vitamin A (Bayfield and Cole, 1994), DNA, RNA, Chromium, Copper, Cadmium, Nickel and Zinc (APHA, 1998) were also undertaken.

Statistical Aanalysis

All the observations were measured in triplicates and analyzed statistically by 'F' test wherever necessary.

Results and Discussion

Tannery Effluent

The collected leather tanning industrial effluent was assessed for its physicochemical properties and its toxic metal levels were also determined (Table 1). The presence of colour and odour in any water sample indicates the unpleasant nature of water. The leather tanning industrial effluent studied was found to be light brown-coloured, turbid and also had an offensive odour. According to BIS (2009), the normal pH range of water should be between 6.0 and 8.0. The effluent sample analyzed had a pH value of 10.5 which is high when compared to normal water indicating the alkaline nature of the effluent due to the presence of high concentrations of salts of sodium, potassium, chromium etc. (Voo and James, 2002).

Table 1. Physicochemical characteristics of the tannery effluent

Parameters	Effluent#	BIS limits*
Colour	Light brown	Absent
Odour	Offensive	Absent
Turbidity	Turbid	-
pH	10.5	6.0 - 8.0
Total suspended solids (mg L^{-1})	02300.0	100
Total dissolved salts (mg L^{-1})	12900.0	2100
Chemical oxygen demand	03180.0	250
Biochemical oxygen demand	01300.0	30
Carbonate (mg L^{-1})	07250.0	600
Bicarbonate (mg L^{-1})	10238.0	NM
Sodium (mg L^{-1})	02300.0	NM
Potassium (mg L^{-1})	00600.0	NM

* - Tolerance limits for textile effluent discharged into inland water source as per Bureau of Indian Standard (BIS), (2009)

\# - Mean value of duplicate samples; NM - Not mentioned

The total suspended solids (2300 mg L^{-1}) and total dissolved salts (12900 mg L^{-1}) in the effluent sample were found to be very high when compared to BIS standards. The presence of higher level of total suspended solids and total dissolved salts in the effluent might be due to the presence of insoluble organic matter from the animal skin and unused inorganic salts used for tanning (Nagarajan *et al.*, 2005). COD and BOD in the selected effluent sample were found to be 3180 mg L^{-1} and 1300 mg L^{-1} respectively. The record of high COD might be due to the presence of oxidizable organic matter removed from the animal skin (Umamaheshwari, 2004).

In the effluent sample analyzed, the presence of carbonate and bicarbonate were found to be very high (7250 mg L^{-1} and 10238 mg L^{-1} respectively). From the study by Balakrishnan and Karuppusamy (2005), it is clear that high carbonate and bicarbonate content contributes to the total alkalinity of the sample. The level of sodium and potassium in the tannery effluent was found to be 2300 mg L^{-1} and 600 mg L^{-1} respectively.

Chromium is the widely used heavy metal in tannery industries and was found to be 179 mg L^{-1} in the effluent. Other metals like cadmium, chromium, copper, nickel and zinc were present at levels of 4.81 mg L^{-1}, 179 mg L^{-1}, 64.321 mg L^{-1}, 132 mg L^{-1} and 171 mg L^{-1} respectively (Table 2).

Table 2. Metal levels in the selected tannery effluent

Parameters	Concentration# (mg L^{-1})	BIS limits*
Chromium	179.00	2.0
Copper	064.32	NM
Zinc	171.00	NM
Nickel	132.00	NM
Cadmium	004.81	NM

* - Tolerance limits for textile effluent discharged into inland water source as per Bureau of Indian Standard (BIS), (2009)

- Mean value of duplicate samples; NM - Not mentioned

Chromium and sulfide are among the most hazardous components of the tanneries effluent. The use of excessive amount of these chemicals in tanning process gives rise to their high concentrations in the effluent. Chromium VI is known to cause cancer. The recommended limit for maximum amount of chromium in the effluent samples is 1.0 mg L^{-1} (Bhalli and Quisor, 2003)

Soil

Both the control and effluent contaminated soil samples were analyzed for essential parameters as listed in table 3.

Table 3. Analysis of control and effluent contaminated soil

Parameters	Control soil	Effluent contaminated soil
pH	8.04	8.50
Metals (mg kg^{-1})		
Sodium	121.07	046.00
Potassium	513.05	650.00
Chromium	021.01	090.00
Zinc	047.92	082.10
Cadmium	003.21	006.81
Nickel	090.10	113.40
Copper	020.05	064.00

It is evident that the effluent contaminated soil markedly differs from the normal soil in the mineral and heavy metal content. The average levels of sodium, potassium, chromium, zinc, cadmium, nickel and copper were found to be more in the effluent contaminated soil collected from tannery effluent receiving sites. Krishna and Govil (2008) reported that the level of exchangeable cations (sodium and potassium) in the soil irrigated with tannery waste water varied differently from control sites. In general, the exchangeable sodium and potassium were found to be high. As a result of this the pH of the contaminated soil (8.5) was higher than that of the normal soil (8.04).

Plants -Biometric Observations

The biometric observations like height of the plant, fresh and dry weights of control plant and effluent treated plants were recorded on both 30th d and 60th d after sowing (Table 4).

Table 4. Biometric observations of control and tannery effluent treated *Brassica juncea* plants

Treatment	Plant Height (cm)		Fresh Weight (g)		Dry Weight (g)	
	30th d	60th d	30th d	60th d	30th d	60th d
Control	22	34	7.25	20.01	0.99	7.62
50 % concentration	15	22	3.63	16.23	0.48	4.32
25 % concentration	18	26	5.34	18.93	0.71	6.58
CD (P: 0.05)	5		3.71		0.37	

Values are mean of triplicates

In the plant treated with undiluted effluent there was no germination of the seeds and the few plants, that germinated, died within a week. Therefore biometric and biochemical observations were taken only in 25 % and 50 % concentrations of the effluent and compared with the control plants.

The plants receiving 25 % concentration showed better growth than the control plants and also the 50 % concentration treatment. Chandra and Kulsheshtha (2004) reported that when aquatic vascular plants were grown in the soil amended with different amounts of tannery sludge, collected from waste water treatment plant, the shoot length and root length increased with increase in sludge amendments. The same result was noticed in the present study wherein higher the dilution of the effluent, the plants had the ability to grow more or less similar to the control plants.

The fresh weights and dry weight of the plants were much higher in the control plants as compared to the effluent treated plants both on 30th d and 60th d. However the plants from 25 % concentration treatment were closer to the control and there was no significant differences between them. The fresh and dry weights of the plants of 50 % concentration were significantly lower than control. These results showed that the plants were able to tolerate 25 % concentration of the effluent.

Biochemical Analysis

The leaf chlorophyll content was comparatively less in the effluent treated plants than the control plants both on 30[th] d and 60[th] d of analysis. The decrease in chlorophyll content was more marked on 60[th] d than that on 30[th] d. The carotenoid concentration of control plants was higher (5.198 mg g^{-1} and 8.201 mg g^{-1} on 30[th] and 60[th] d respectively). Sinha *et al.* (2002) reported a similar decrease in chlorophyll content in *Vallisneria spiralis* plants grown in presence of heavy metals. The effluent treated plants showed a decrease in total chlorophyll, carotenoids and protein content in the leaves and roots of *Vallisneria* when compared to control plants. The protein and vitamin A content of the 50 % concentration treated plants showed a significant decrease in the quantities of protein than that of 25 % concentration. The increase in protein content at lower concentration of effluents could be due to adsorption of organic nitrogen present in the effluent by plants. Both DNA and RNA contents of the leaves of *Brassica juncea* plants also showed similar trend.

Both dilutions had the capacity to accumulate chromium (63.18 µg g^{-1} and 66.12 µg g^{-1} on 30[th] d and 60[th] d in 1:3 dilution and 44.08 µg g^{-1} and 62.01 µg g^{-1} on 30[th] and 60[th] d in 1:1 dilution) in their leaves (Table 5).

Treatment	Chromium		Copper		Zinc		Cadmium		Nickel	
	30[th] d	60[th] d	30[th] d	60[th] d	30[th] d	60[th] d	30[th] d	60[th] d	30[th] d	60[th] d
Control	0.513	0.716	40.13	84.89	21.06	23.05	30.12	36.19	0.513	0.716
50 % Concentration	63.18	66.12	42.39	79.08	39.09	58.06	43.23	62.53	0.198	0.245
25 % Concentration	44.08	62.01	53.19	89.70	56.03	69.18	60.81	75.31	0.280	0.315
CD (P: 0.05)	0.339		1.78		5.14		5.58		0.047	

The uptake ability of zinc, cadmium and nickel in *Brassica juncea* leaves under different dilutions of the effluent showed that zinc accumulation capacity in the 25 % concentration treated plant sample was found better (56.93µg g^{-1} and 69.18 µg g^{-1}) than the control and 50 % concentration. Similarly, cadmium and nickel accumulation was also noticed high in 25 % concentration compared to control sample on 30[th] and 60[th] d respectively. This might be due to the presence of high zinc and nickel content in the tannery effluent used for the study.

Cadmium accumulation in different plants increases with increasing concentration of cadmium and the exposure time. The gradient of accumulation of heavy metals was found to be highest in root followed by stem, branch, leaf and then in fruit (Aery and Rana, 2003). Nickel reduces photosynthetic activity of plants. Reduction of photosynthetic activity may result both from the disturbance of photochemical and biochemical photosynthetic reduction, and the damage of photosynthetic apparatus at all levels of organization as reported in *Brassica oleracea* (Molas, 2002).

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