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Bioremediation of tannery effluent by using *Pseudomonas fluorescens* and *Eichhornia crassipes* and its effect on Wheat seed germination and plant growth

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ABSTRACT

Tannery industries discharge a large quantity of toxic substances like chromium, sodium sulphide, sodium carbonate, ammonium sulphate and chlorides in their effluent, which manifold soil pollution and affect on seed germination and plant growth. In this study, two investigational systems are attempted: i) tannery effluent was treated by aerobic bacteria *Pseudomonas fluorescens* and aquatic macrophyte *Eichhornia crassipes* and ii) the impact of treated and untreated effluent and soil on seed germination and plant growth were studied. The physicochemical properties such as color, pH, COD, BOD, total solids, suspended solids, dissolved solids, and chromium concentration were found decreased in effluent that treated with bacterial strain for 72 h and Water hyacinth for 20 days. These treated effluent also significantly enhanced chlorophyll content, and biomass production over other of wheat plant. The results revealed that effluent treated by microbes and plant has no negative impact on the seed germination and plant growth. Thus, it can be effectively used for irrigation.

1) INTRODUCTION

The tanning industry is one of the oldest industries in India. About 30 tanneries and other industries are discharging their untreated and semi treated effluents in to the loamy drain, a storm natural drain at Unnao, Uttar Pradesh, India. This contaminated drain water has been widely using for irrigation of cereals, vegetables and other economically important plants [1]. The wastewater, discharged from these industries, contains various pollutants including high amount of chromium (1.07 to 7.80 mg l^{-1} Cr). Cr (VI) is a toxic, powerful epithelial irritant and established as human carcinogen by International Agency for Research on Cancer and World Health Organization (WHO) [2]. Irrigation by this contaminated water may cause various effects in plants, as it interferes with several metabolic processes causing phytotoxicity like reduced growth, chlorosis, ultrastructural effects on organelles, chromatin condensation, swelling of mitochondria etc. and finally leading to plant death [3].

Conventional methods used for removal of Cr (VI) are very expensive and lack specificity. Biological approaches by utilizing microorganisms offer an alternative potential method for selective removal of toxic metals. They may couple with considerable operational flexibility hence, they can be applied for both *in situ* or *ex situ* in a range of bioreactor configurations as discussed by Fatima Benazir [4].

Chromium remediation studies have been carried out by employing microorganisms [5, 6] have been reported including *Pseudomonas* [7], *Bacillus* [8], aquatic weeds [9, 10, 11, 12] and plants [13,14]. The present investigation deals with physicochemical analysis of the tannery effluent and study of the effect of irrigation of treated effluent on seed germination, seedling growth, photosynthetic efficiency and yield of wheat plant.

2) MATERIALS AND METHODS

2.1 Sample collection- The tannery effluent was collected from the outlet of the tannery industry, which is located in Unnao District, Uttar Pradesh in clean glass containers.

2.2 Analysis of physicochemical parameters- The physicochemical parameters such as color, pH, EC, COD,

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BOD, total solids, suspended solids, dissolved solids, chromium were estimated following the standard methods as given by APHA [15].

2.3 Isolation of bacteria- The chromate resistant bacteria were isolated and incorporated on nutrient agar media with different concentration of Cr^{6+} as $K_2Cr_2O_7$ following standard pour plate technique [15, 16]. Plates were incubated at 37^0c for 24 hrs and the total numbers of bacteria were determined as CFU ml^{-1}. Isolates were selected randomly and microscopic and biochemical tests were applied to identify these isolates according to bergey's manual of systematic bacteriology. The identify isolates were stored on the nutrient agar medium at 4^oc for further studies.

2.4 Effluent Treatment- The effluent was treated as following

(a) By *Eichhornia crassipes* for 10 and 20 days.

(b) By *Pseudomonas fluorescens* for 48 and 72 h.

2.5 Effluent treatment with aquatic plant- Young plants of water hyacinth were collected from local ponds located in Pantnagar, Uttarakhand. The plants were thoroughly washed with distilled water and placed in plastic tub diameter 60 cm and depth 40 cm containing 20 L of tannery waste water effluent. Each tub was containing the same weight (300gm) of plants. Plants were cultured in triplets for 10 days and 20 days.

2.6 Effluent treatment by bacterial inoculation- A loopful culture of *Pseudomonas fluorescens* was inoculated in sterilized 100 ml nutrient broth. The flask was placed in a shaker at 120 rpm for 16-18 h at 30°c. The culture broth was centrifuged at 10,000 rpm for 20 min. Cell suspension was prepared using sterile distill water and adjusted to 0.5 OD using UV-Visible spectrophotometer (Electronic Corporation of India, ECIL). 1% (10^5 CFU/ml) of the above suspensions was inoculated in sterilized 500 ml of tannery effluent in 1 lit bottle having a side arm outlet. Sterile dry air was passed continuously using an aerator for proper aeration. After 72 hrs, the sample was filtered under aseptic condition. Then the bacterially treated effluent was collected in a sterile conical flask and used in a pot culture study. Physicochemical parameters were estimated before and after treatment.

2.7 Germination of wheat seeds **-** To know the effect of treated effluent on seed germination, wheat seeds were placed on set were replicated 3 times involving 18 plates. These petridish as moist chamber. Seeds germination was tested in presence of treated effluent by *Pseudomonas* for 24 and 48 h (2) and by *Eichhornia* for 10 and 20 day (2), untreated effluent (1) and distilled water as control (1). All these 6 experimental petriplates were converted into moisture chamber in which 10 wheat seeds per plate were spread after shocking with respective treated effluent for 12 h. All these petridishes were incubated at 25 ± 2° C in an incubator and the seed germination and time taken for complete germination was recorded after 48 hrs for 10 days. The treatment were designated as control (without effluent), untreated , bacterial treated for 48 hrs and 72 hrs as 42 HB and 72 HB, *Eichhornia* treated for 10 days and 20 days as 10 DE and 20 DE respectively.

2.8 Plant growth study- The experiment, involving wheat plant growth, was performed in plastic pots of 10-inch diameter carrying 4 kg of soil. There were 6 treatments having three replicates each. Wheat seeds allowed to germinate in the pot soil and after seedling establishment the plant were thinned to four seedlings per pot. The seedlings were irrigated with the respective treated effluent as and when required. All the pots were kept in polyhouse for further growth. The total chlorophyll, chlorophyll a and chlorophyll b of the wheat plant leaves growing in the different effluent treatment was monitored dissolving in acetone by spectrophotometric method after 9 weeks of plant growth. The final fresh weight and dry weight of the plant were estimated after harvesting the plants after 9 weeks of growth.

3) RESULTS AND DISCUSSION

3.1 Effluent Treatment- Physico-chemical analysis of untreated polluted effluent and the 48 hr and 72 hr bacterial treated and the 10 days and 20 days *Eichhornia crassipes* treated effluent are shown in **Table 1**. It showed a substantial decrease in pollution load of the tannery industry effluent. These observations were supported by the study done by Mythili and Karthikeyan [17]. In general the bacterial treatment for 72 h and the *Eichhornia crassipes* growth for 20 days reduced the pollution load maximum. Indira and mycin [18] also suggested that higher amount of TDS was decreased after 72 h of bacterial treatment. Bioremediation often involves the manipulation of environmental parameters to allow microbial growth and degradation at a faster rate as given by Fulekar [19]. Vasanthy [20] reported the metal removal efficiency of *Bacillus* and *Pseudomonas* species isolated from tannery effluent. Hossain [21] studied the biosorption of chromium on *Bacillus subtilis*.

Table 1. Effect of bacterial treatment and phytoremediation on some physico-chemical parameters of tannery industry effluent

	Untreated	48HB	72 HB	10DE	20DE
Color	Greyish black	Light Greyish black	Light Greyish black	Pale	Pale
pH	8.7	7.9	7.5	7.3	7.2
BOD (mg/L)	1230	115	80	120	95
COD (mg/L)	2350	360	340	498	302
TS (mg/L)	4560	560	420	510	290
TDS (mg/L)	2310	220	320	360	245
TSS (mg/L)	365	95	105	110	86
Cr µg/L	7.6	1.02	0.06	0.08	0.04

3.2 Seed germination- The irrigation of wheat seeds were done by the untreated, bacterial treated and phytoremediated tannery effluent and the germination percentage of wheat seed were recorded, that are shown in **Table 2**. Relatively better germination percentage of wheat seed was recorded in the effluent that was phytoremediated by the *Eichhornia crassipes.*

Pseudomonas fluorescens may reduced the pollution load substantially. The irrigation of wheat seeds with this treated effluent increased the germination percentage and has beneficial effect on the chlorophyll content and plant growth. It can be proposed that chromium contaminated effluent can be used for irrigation purposes after treatment with this microbe and aquatic weed.

Table 2. Effect of Bioremediated effluent of tannery industry effluent on wheat seed germination

Treatment	Cumulative germination %	Days taken for germination
Control(0%effluent)	75.0	5
Untreated effluent	25.0	10
48HB	75.0	4
72HB	72.0	5
10DE	82.0	6
20DE	85.4	5

Table 3. Effect of bioremediated effluent on leaf chlorophyll content in nine week old wheat plant (mg/g dry weight)

Treatment	Chlorophyll a	Chlorophyll b	Total chlorophyll
Control(0%effluent)	0.32	0.24	0.59
Untreated effluent	ND	ND	ND
48HB	0.54	0.39	0.93
72HB	0.38	0.32	0.68
10DE	0.45	0.33	0.74
20DE	0.42	0.25	0.61

Table 4. Fresh weight and dry matter production (g/shoot) of wheat grown for nine weeks in different effluent treatment

Treatment	Fresh weight	Dry weight
Control(0%effluent)	19.6	3.01
Untreated effluent	ND	ND
48HB	21.2	3.4
72HB	24.2	3.2
10DE	20.1	3.1
20DE	17.4	3.0

Indira and mycin [19]clearly reported the toxicity of tannery effluent on the growth, physiological and biochemical contents of blackgram. The time taken for the wheat seed germination was also reduced in the phytoremediated and bacterial treated effluent so that there is increase in the rate of wheat seed germination. Whereas the untreated effluent irrigated seed took longer time to germinate and with the lowest germination percentage.

3.3 Plant Growth- The effect of *E. crassipes* and bacterial treated effluent was studied on wheat plant growth with the particular reference to chlorophyll content and dry matter production. A significant increase in chlorophyll a, b and total content was recorded especially in response to 48 h bacterial treated and 10 days *Eichhornia* treated effluent irrigation **(Table 3)**. The fresh weight and dry matter production of wheat grown for nine weeks in different effluent treatement are shown in **Table 4**. There was an increase in the fresh weight and dry matter production of wheat plant in the bacterial treated and phytoremediated effluent.

4) CONCLUSION

Results from the present study clearly depicted that chromium rich effluent treatment by *Eichhornia crassipes* and

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