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Biosorption of Chromium from Tannery Effluent Using Bacterial and Fungal Species

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Tannery effluent was collected from Common Effluent Treatment Plant, Dindigul, Tamil Nadu and used for analysis for physico-chemical, heavy metals and biological properties. Soil samples were collected from the field and *Aspergillus* sp. and *Pseudomonas* sp. were isolated and pure cultured. The pH of the tannery waste water was around 8.50 and electrical conductivity was 2.89 dS/m indicating the alkalinity of the effluent. Total dissolved solids(TSS) was found to be 1460 mg/L and BOD showed 2346 mg/l. The AAS reading of chromium in the effluent indicated 205 mg/L which means the effluent can not be released into the environment without proper treatment. The metal tolerance capacity of the organisms was tested at different concentrations of chromium salts dissolved in tannery effluent *viz*., 25 to 200 ppm with 25 ppm of increment for each of the treatment. *Pseudomonas* sp.tested tolerated upto 100 ppm,while the *Aspergillus* sp could resist upto 75 ppm of chromium concentration. It was also found that the *Pseudomonas* sp. had potentially removed chromium from aqueous spiked solution with effluent, hence it could be a good biosorbent for the removal of chromium from tannery effluent contaminated agricultural fields.

Key words: Tannery, Chromium biosorption, *Pseudomonas* sp. and *Aspergillus* sp.

Extensive use of chromium in industries such as leather tanning, stainless-steel production, electroplating and wood preservatives have resulted in chromium contaminated soil and ground water at production sites which pose a serious threat to human health. The list of heavy metals in which chromium is generally found to be contaminating the water and is carcinogenic when inhaled even at 0.05 ppm (maximum permissible concentration). Generally, chromium occurs in two oxidation states Cr^{3+} and predominantly Cr^{6+} in air, water and soil.

The tanning industry is one of the major sources of pollution in Tamil Nadu, India, as it releases large quantities of effluents and sludge rich in chromium and salts into the environment (Ramasamy *et al*., 2000). The chromium concentration in ground water was also markedly higher than the average back ground value reported in different parts of India. Cr^{6+} species are strong oxidants, which are carcinogenic, mutagenic and teratogenic in living systems (Daulton *et al*., 2007). It is hundred times poisonous and thousand times mutagenic than Cr^{3+} hence it has been listed as a priority pollutant and a human carcinogen by the USEPA (Srinath *et al*., 2002). At high levels, heavy metals like chromium damage cell membranes, alter enzyme specificity; disrupt cellular functions and damage structure of DNA. Hence, the need arises to remediate chromium before being discharged.

Conventional methods for treatment of toxic chromate include chemical reduction followed by precipitation, ion exchange and adsorption on activated coal, alum, kaolinite and ash, which require large amounts of chemicals therefore are unsuitable (Camargo *et al.,* 2003). Hence, an effective alternate approach is required to safeguard the environment and there comes the use of microfauna in the way of biosorption. Biosorption not only offers an innovative alternative to other remediation approaches, but also allows metals recovery (Kotrba, 2011).

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Material and Methods

Tannery effluent samples were collected from the Common Effluent Treatment Plan, Dindigul, Tamil Nadu as per the standard procedure mentioned below. The collected samples were preserved in plastic cans and analyzed for various heavy metals, physical and chemical properties.

Estimation of total chromium

Chromium stock solution preparation

Chromium stock solution was prepared by dissolving 1.414 g of AR grade $K_2Cr_2O_7$ in 1litre of double distilled water which gives a concentration of 1000 ppm. From this, different concentrations of chromium were prepared by using the following equation.

Preparation of standards

Concentration of stock solution = N1

Concentration (ppm) required = N2

Required volume of needed concentration = V2

According to the law of volumetric anlaysis,

V1*N1 = V2*N2

V1 = V2*N2/N1

Therefore, V1 ml of N1 solutionwas taken and made up to V2 ml to get N2 solution.

Accordingly,

Weight of sample taken for analysis = Xg

Volume of extract made up = 100 ml

Concentration of heavy metal read against AAS reading in the standard curve = A μgml^{-1}

Total Cr present in the sample = A * 100 $\mu g\ g^{-1}$

Isolation and pure culturing of native microorganisms

The isolation and purification of *Pseudomonas* sp. and *Aspergillus* sp. were carried out as per the standard procedure (Bachanan and Gibbons, 1974). These isolates were sub cultured with nutrient agar for *Pseudomonas* sp. and rose bengal agar for *Aspergillus* sp. The slants were prepared and stored for further subculturing. The organisms were tested with the different concentrations of chromium as T_1- 25 ppm of chromium, T_2- 50 ppm of chromium, T_3- 75 ppm of chromium, T_4- 100 ppm of chromium, T_5- 125 ppm of chromium, T_6- 150 ppm of chromium, T_7- 175 ppm of chromium, T_8- 200 ppm of chromium and T_9- control.

Table 1. Physico-chemical analysis of effluent

Character	Method	References
pH	pH meter	Falcon *et al.* (1987)
Electrical conductivity (EC)	Conductivity bridge	Falcon et al. (1987)
Biological Oxygen Demand (BOD)	Winkler method	Anon (1989)
Organic carbon	Chromic acid wet digestion	Piper (1966)
Total N	Semi-automatic Kjel dahl distillation	Jackson (1973)
Total P	Sodium bicarbonate method	Olsen et al. (1954)
Total K and Na	Flame photometer	Jackson (1973)
Total Ca and Mg	Versenate method	Jackson (1973)
Chromium	0.005 M DTPA extraction – AAS	Lindsay and Norvell (1978)

Results and Discussion

Heavy metal contamination due to natural and anthropogenic sources is a global environmental concern (Sardar *et al.*, 2013). Release of heavy metal without proper treatment poses a significant threat to public health because of its persistence, bio-magnification and accumulation in food chain. Non-biodegradability and sludge production are the two major constraints of metal treatment. Microbial metal bioremediation is an efficient strategy due to its low cost, high efficiency and eco-friendly nature. Recent advances have been made in understanding metal - microbe interaction and their applications for metal accumulation/detoxification(Rajendran *et al.,* 2003).

Table 2. Characterization of physical and chemical parameters of effluent sample

Parameters	Sample Details
Colour	Light yellowish brown
Odour	Foul
pH	8.50
EC (dS/m)	2.89
Total Dissolved Solids(mg/L)	1460
BOD (mg/L)	2346
Calcium(mg/L)	567
Magnesium (mg/L)	124
Potassium (mg/L)	54
Chromium (mg/L)	205

Characterization of tannery effluent

The treated effluent was light yellowish brown in colour. The foul odour was due to the presence of hydrogen sulphide, sulphur oxides, ammonia and amines, which was unbearable in nature. The pH of the tannery waste water was 8.50 and can be recommended for the discharge of wastewater into inland surface after treatment with lime. Electrical conductivity of the effluent was 2.89 dS/m, which could be highly injurious to the crop, when used for the irrigation. The EC of the chromium contaminated soil and groundwater due to tannery wastes disposals at Vellore district of Tamil Nadu was found to be in the range of 1.28 dS/m to 5.01 dS/m (Sunitha *et al.*, 2015). The TDS value of 1460 mg/L mainly comprises of carbonates, bicarbonates, chlorides, sulphates and phosphates, which holds well within the permissible limit for irrigation (*i.e.*, 2100 mg/L). Biological oxygen demand (BOD) is the amount of oxygen required by bacteria for stabilizing decomposable organic matter under aerobic conditions, where organic loading must be restricted to 50 mg/L, while the sample contained 346 mg/L. The result of study revealed that BOD level (820-1100 mg/L) had indicated high organic load surpassed the CPCB limit of 30 mg/L for effluent discharge into inland surface waters.

Hardness of the effluent contributed by the amount of calcium and magnesium was which accounted for 567 and 124 mg/L, respectively. This indicated that the effluent was very hard in nature, thus making it unsuitable for discharging into inland water sources or for irrigation. Kjeldahl nitrogen and phosphate content in the effluent were 0.5 and 0.19 mg/L, respectively and the permissible limit is 100 and 5 mg/L, respectively, hence, this parameter does not create any problem in discharging. Potassium content of the effluent was found to be 54 mg/L. Chromium salt concentration permissible for the discharge into the inland stream is 2.0 mg/L (CPHEEO, 2012). Here, the AAS reading of chromium reads about 205 mg/L, which cannot be released into environment without treatment. The effect of

chromium III showed 50% inhibition at a concentration of 85 mg/L, indicating that this metal was not causing process inhibition during performance operations.

Fig.1.*Pseudomonas* sp. with 100 ppm of chromium contained tannery effluent

Biosorption of chromium by Pseudomonas sp.

The present investigation aimed to study the effect of chromium on *Pseudomonas* sp.which was isolated from the soil. From this study, it is clear that the growth of bacterial cultures was observed upto 100 ppm of chromium concentration followed by decline in growth at concentration of 125, 150, 175 and 200 ppm of chromium. This may be due to the differential capacity

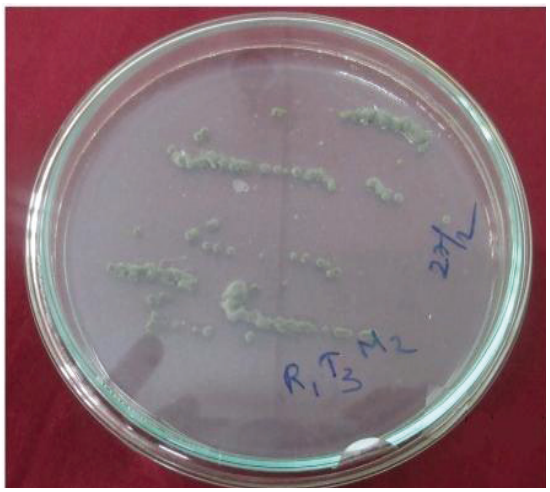

Fig.2.*Aspergillus* sp. with 75 ppm of chromium contained tannery effluent

of bacteria in metal degradation/transfer as they were inhibitors of electron from cytochrome b and c in mitochondria (Molina *et al.,* 2014). Deepali (2011) reported that three bacterial species i.e. *Bacillus* sp., *Pseudomonas aeruginosa* and *Pseudomonas putida* showed maximum tolerance even at 50.0 mg/L of Cr (VI). Bacterial populations resistant upto 500 mg/L Cr (VI) and fungal populations resistant upto 1000 mg/L Cr(VI) were directly isolated from soils (Bopp and Ehrlich, 1998).

Biosorption of chromium by Aspergillus sp.

Among the various concentrations of chromium, the growth of *Aspergillus* sp. was noticed up to 75 ppm in tannery effluent. The culture was found to grow well till the concentration of chromium was 50 ppm. No fungal growth was noticed atabove100 ppm level of chromium.This is in consonance with the findings of Mattar and Zain (2011), who reported that the three fungal species such as *Cunninghamella blakesleeana, C. homothallica* and *C. elegans* were able to grow and tolerate Cr(VI) till 1500 ppm.

Igwe and Abia (2006) found that the chromium was removed from tannery industry effluent by *Aspergillus oryzae.* This fungus was found to grow at different concentration of chromium (120-1080 mg/L).Adding this, the study of Ram Chandra Bajgai *et al.,* (2012) emphasized that 1.5 mM of Cr through $K_2Cr_2O_7$7 H_2O was lethal to the cultures of *Trichosporon cutaneum* R57.

Conclusion

The present laboratory study revealed that, among the tested Pseudomonas and Aspergillus strains, the bacterial strain could better remove chromium. The bacterial culture was also found to tolerate upto100 ppm level of chromium in tannery effluent. Confirmatory studies at CETP (Common Effluent Treatment Plant) level needs to be undertaken. The exact identity of the Pseudomonas at species level is also necessary to make further lead.

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