Comparative Study on Seed Germination of Vigna Radiata with the Effect of Tannery Effluent

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Abstract- Disposal of tannery effluent will result in heavy metal contamination of land and will lead to many important health and environmental hazards. Aim of the work is to determine the effects of untreated tannery effluent and treated tannery effluent on seed germination and to biodegrade the effluent using fungi. Chemical methods of remove the heavy metals in tannery effluent will have metal bearing solid waste. Hence degrading tannery effluent using Trichoderma harzianum cultured from tannery effluent is an economical and easy method. T.harzianum isolated from the effluent is used to degrade the tannery effluent. Effect of tannery effluent on germinating seeds and relative toxicity are measured at different concentrations of tannery effluent. The degradation level of chromium is also analysed in this present work.

I. INTRODUCTION

The leather industry is associated with the generation of huge amounts of solid waste and disposal of this waste become a serious problem (Amita *et al*., 2005). Chromium in the effluent is a major concern for tanning industry. Chemical precipitation methods are commonly employed for the removal of chromium but this leads to formation of chrome-bearing solid waste, plus it is uneconomical when the concentration of chromium in the effluent is low. Tanning is one of the major industries in India and the effluent which is discharged from this industry is highly complex and may cause serious pollution. The conventional physical and chemical methods used for removal of heavy metals from the effluent, such as precipitation with carbonates, sulphides and hydroxide, adsorption on activated carbon, use of ion-exchange resins and membrane-separation processes, are responsible for generation of pollution and are not cost-effective (Volesky and Holzen, 1995; Kratochvil *et al.,* 1998; Camargo *et al.,* 2003). An alternative to these methods is the removal of heavy metal contaminants by microorganisms. The metal removal ability of microorganisms, including bacteria (Cheung and Gu, 2005; Thacker *et al*., 2007), microalgae (Kratochvil *et al*.,1998; Matsunaga *et al*.,1999; Gupta *et al.,* 2001; Gupta and Rastogi, 2008) and fungi (Tobin and Roux, 1998; Srivastava and Thakur, 2006), has been studied extensively. Fungi, in general, are well-known for their ability to biosorb and bioaccumulate metals (Pillichshammer *et al*., 1995; Dursun et al., 2003; Nouri et al., 2005; Park et al., 2005) and have also been reported to be involved in reduction (biotransformation) of Cr (VI) to Cr (III) form (Pal, 1997; Gouda, 2000; Acevedo-Aguilar *et al*., 2006; Morales-Barrera and Cristiani-Urbina, 2008). The common Cr(VI) detoxification mechanisms reported in Cr-resistant microorganisms are periplasmic biosorption, intracellular bioaccumulation and biotransformation through direct enzymatic reaction (Lovley, 1993; Lee *et al*., 2000; Valls *et al*., 2000) or indirectly with metabolites (Camargo *et al*., 2003). In Cr(VI)-resistant filamentous fungi, such as *Aspergillus* (Gouda, 2000; Acevedo- Aguilar *et al*., 2006), Penicillium (Acevedo-Aguilar *et al.,* 2006), Trichoderma (Morales-Barrera and Cristiani-Urbina, 2008) and Phanerochaete (Pal, 1997), the Cr(VI) detoxification through transformation of Cr(VI) to Cr(III) form was observed due to cellular metabolism processes based on the reducing power of carbon sources. During the skin processing two types of effluents are discharged (Manivasagam, 1987); vegetable tannin which does not contain chromium; chrome tannin which contains chromium.

Higher level of chromium in tannery effluent adversely affects seed germination. Treatment of tannery effluent using ion exchange, adsorption on to activated carbon are excessively energy consuming. Selective fungi which are efficient for degradation of pollutants can be isolated from tannery effluent itself. Treating tannery effluent by using fungi is an efficient biodegradation method. Recently tannery effluent contributes one of the major industrial pollution problems. Chemical precipitation methods for effluent treatment are expensive and will produce solid waste. Biodegradation using effective microorganisms are economical and easy to use. Tannery effluent contains some harmful toxic dyes. Higher level of toxic components in the effluent including chromium, aluminum and dissolved salts are lethal to flora and fauna in the environment.

II. MATERIALS AND METHODS

The tannery (Pretreated) effluent was collected from tannery plant in Chennai. For isolation of *Trichoderma harzianum*, the effluent was inoculated in Potato Dextrose Agar (PDA) media for 48-96 hours at room temperature. The isolate was identified based on their morphological structures, such as colour, diameter of the mycelia and microscopic observation of spore formation provided by standard monograph.

Pure culture of the isolated fungal strain was grown in Potato Dextrose Broth (PDB) at 30ºC in a shaking incubator (100 rpm) for 72 h in dark condition. After incubation culture was centrifuged in a sterile centrifuge tube at 5000g for 10 min at room temperature to get the biomass in the form of a pellet. The pellet was washed thrice with sterile distilled water to make the biomass free from media components.

Throughout this study, all experiments were set up in a 250-ml Erlenmeyer flask containing different concentration (25%, 50%, 75% and 100%) of effluent. The inoculum size used

was 0.4 mg/ml and incubates 24hrs. After incubation period, pH (pH meter), Conductivity (Electrical Conductivity meter), Dissolved Oxygen and Biological Oxygen Demand (APHA, 1995) were measured.

Treated and untreated effluent sample was analysed in gas chromatography flame photometric detector. 0.5µl of untreated tannery effluent and 25% concentrations of tannery effluent was injected to gas chromatography flame photometric detector. The amount of chromium can be detected and measured from the chromatogram. The amount of analyte can be explained by single point extension method.

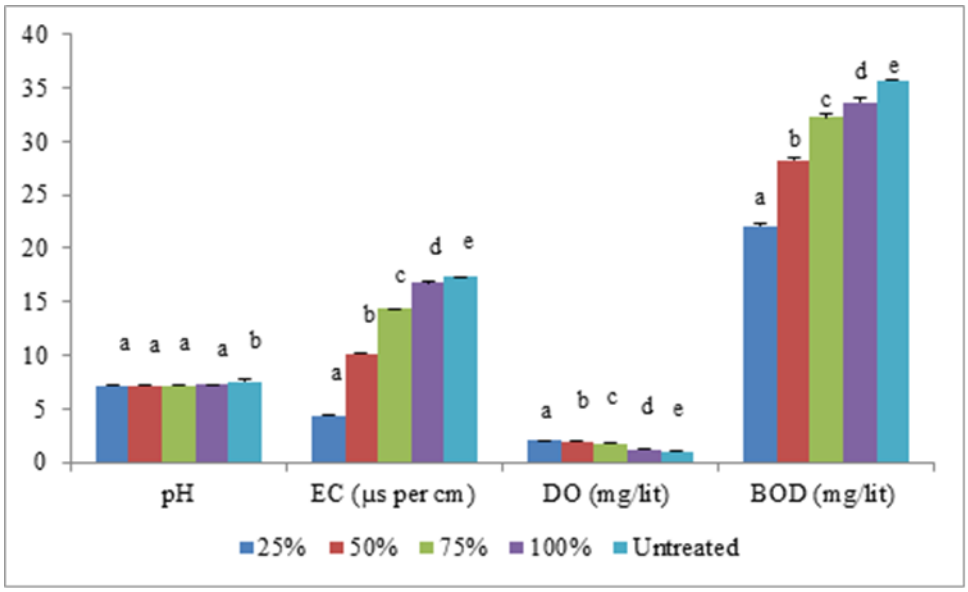
Response factor for standard run of chromium is calculated as,
Response factor = Peak area / sample amount
Amount of analyte is calculated by the following formula
Amount of analyte = Peak area / Response factor

Effects of treated and untreated tannery effluent on *Vigna radiata* shoot length were studied. Different concentration (25%, 50%, 75% and 100%) of treated and untreated tannery effluent was introduced in a culture tube and a tube maintained as control in triplicate. The shoot length was measured after 24hrs, 48hrs and 96hrs. Relative toxicity was assessed on germinating seeds using both effluents.

III. Results and Discussion

Physico-chemical parameters of untreated tannery and treated tannery effluent of pH, Electrical conductivity, Dissolved oxygen and Biological Oxygen Demand ranged was 7.15 to 7.58, 4.41-17.31 (µs per cm), 0.98-2.05 (mg/L) and 22.10-35.67(mg/L), respectively (Fig. 1). Karunyal *et al* (1994) reported that after treatment of vegetative effluent pH (6.0) and BOD (7678mg/L) was not permissible limit. In the present study, also physico-chemical parameters showed near to permissible limit as per WHO (1992) with related to potable water. It may use to gardening of plants. Anova for physico-chemical parameters showed significant different at 5% level. Anova followed by post-hoc test performed that untreated effluent significantly different when compare to other concentrations. Electrical conductivity, dissolved oxygen and biological oxygen demand showed that between concentrations significantly different at 5% level. Chromatogram of tannery effluent for chromium species showed in fig. 2. Degradation of chromium was significantly reduced of chromium species in 25% tannery effluent after treatment by *T. harzianum* (Fig. 3).

Effect of tannery effluent of seed germination on *V. radiate* stem height was high in 25% treated effluent after 24hrs (1.53±0.04cm) (Fig. 4) and 48hrs (2.50±0.02cm) (Fig. 5) than control and untreated effluent. In 96 hrs duration, it was 3.32±0.10cm at control than effluent samples (Fig. 6). Anova for effect of different concentration of treated tannery effluent and untreated effluent on seed germination showed significantly different ($P<0.05$ level) at 24, 48 and 96hrs (Table 2-4). Karunyal *et al* (1994) have reported that seed germination using tannery effluent showed that high biomass and more leaf surface area in 25% concentration tannery effluent. Anova followed by post-hoc test performed that in 25% concentration treated effluent stem length was significantly height when compare to control and untreated effluent at 24hrs duration (Fig. 4). In the case of 48 hrs duration in 25% concentration treated effluent shows that no difference in stem height when compare to control (Fig. 5). In 96 hrs duration, different concentrations of effluent between control, treated and untreated effluent stem length significantly different at $P<0.05$ level (Fig. 6).



Anova followed by Tukey's test performed
Different alphabets of same parameters shows significant different at 5% level
Figure 1. Physico-chemical characteristics of treated tannery effluent

Table 1. Anova for physico-chemical characteristics of tannery effluent between concentrations

Parameters	df	F value	P value
pH	4, 10	30.828	0.000*
Electrical conductivity	4, 10	35509.63	0.000*
Dissolved Oxygen	4, 10	2139.78	0.000*
Biological Oxygen Demand	4, 10	961.61	0.000*

*-significant different at P<0.05 level; NS-non significant different at P>0.05 level

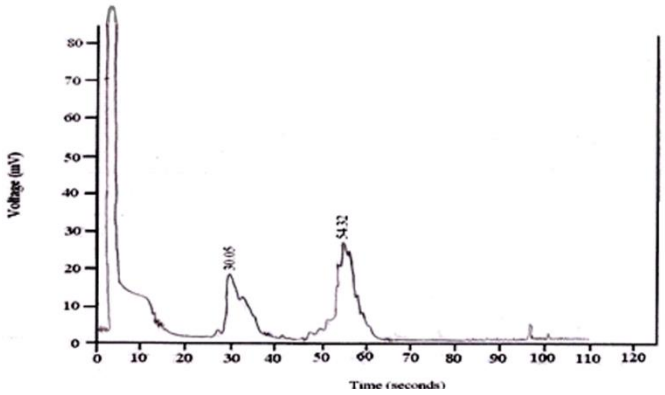


Figure 2. Chromatogram of untreated tannery effluent

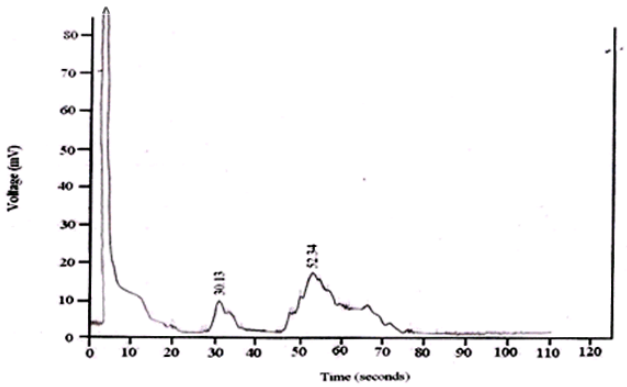
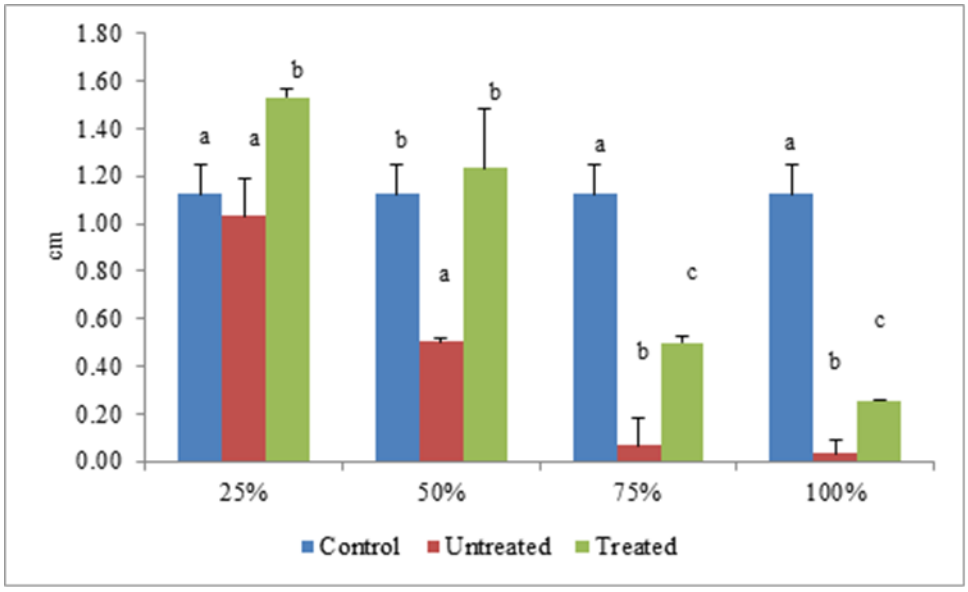


Figure 3. Chromatogram of 25% treated tannery effluent



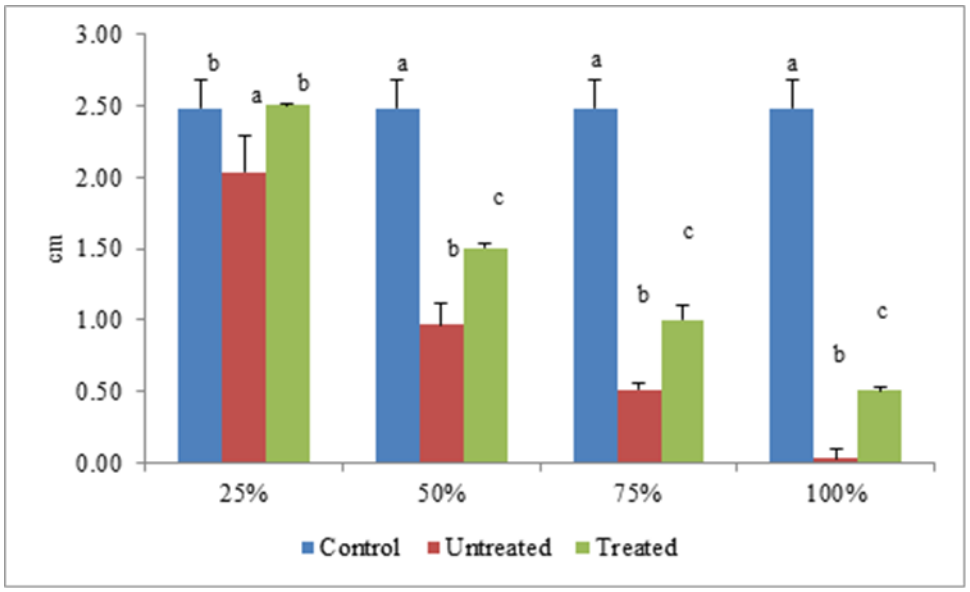
Anova followed by Tukey's test performed
Different alphabets of same parameters shows significant different at 5% level

Figure 4. Effect of tannery effluent on Seed germination after 24 hours

Table 2. Anova for effect of tannery effluent on Seed germination after 24 hours

Parameters	df	F value	P value
25%	2, 6	15.65	0.004*
50%	2, 6	17.60	0.003*
75%	2, 6	85.02	0.000*
100%	2, 6	157.33	0.000*

*-significant different at P<0.05 level; NS-non significant different at P>0.05 level



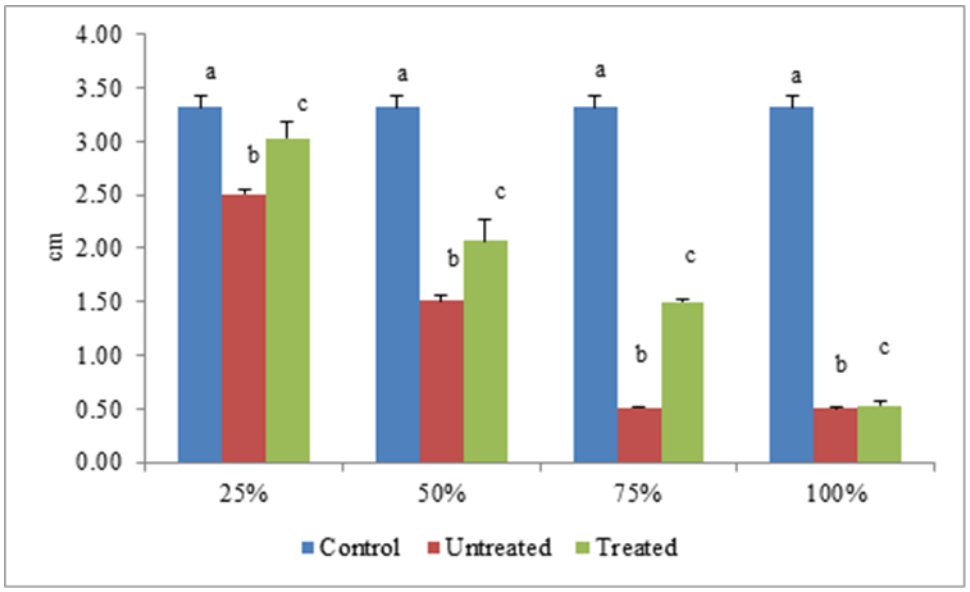
Anova followed by Tukey's test performed
Different alphabets of same parameters shows significant different at 5% level

Figure 5. Effect of tannery effluent on Seed germination after 48 hours

Table 3. Anova for effect of tannery effluent on Seed germination after 48 hours

Parameters	df	F value	P value
25%	2, 6	6.25	0.034*
50%	2, 6	85.10	0.000*
75%	2, 6	189.67	0.000*
100%	2, 6	360.56	0.000*

*-significant different at P<0.05 level; NS-non significant different at P>0.05 level



Anova followed by Tukey's test performed
Different alphabets of same parameters shows significant different at 5% level

Figure 6. Effect of tannery effluent on Seed germination after 96 hours

Table 4. Anova for effect of tannery effluent on Seed germination after 96 hours

Parameters	df	F value	P value
25%	2, 6	42.38	0.000*
50%	2, 6	137.41	0.000*
75%	2, 6	1632.40	0.000*
100%	2, 6	1857.04	0.000*

*-significant different at P<0.05 level; NS-non significant different at P>0.05 level

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