Current Botany 2011, 2(8): 40-45
ISSN: 2220-4822
Available Online: http://currentbotany.org/

Bioremediation of tannery effluent and its impact on seed germination (blackgram and sunflower)

Mythili, K*. and Karthikeyan, B.

Department of Microbiology, Annamalai University, Annamalai nagar- 608 002 (T.N)

Abstract

In this study, we attempted two investigational systems: one is treatment of tannery effluent by aerobic bacterial consortium and the other is impact of treated and untreated effluent on seed germination. For the effluent treatment, heavy metal resistant bacteria were isolated from tannery effluent. Among them, *Bacillus, Pseudomonas* and *Micrococcus* were selected for the further studies. The selected isolates were tested for their efficiency on the bioremediation of tannery effluent. The physico-chemical properties such as colour, odour, pH, electrical conductivity, total solids, suspended solids, dissolved solids, BOD, COD, chromium, zinc, iron, nickel were found decreased in effluent after 72 hrs. The study of seed germination (Blackgram and Sunflower) was carried out at 0, 20, 40, 60, 80, 100% concentrations of treated and untreated effluent using soil sowing method. Shoot length, fresh weight, dry weight and chlorophyll content showed an increase when treated effluent was tested whereas a decrease of growth was noticed in untreated effluent tested seedlings. The results revealed that effluent treated by aerobic microorganisms has no negative impact on the seed germination and can be effectively used for irrigation.

Keywords: Bioremediation, Tannery effluent, Blackgram, Sunflower

INTRODUCTION

Water pollution is prime cause of unavailability of the suitable water for irrigation purpose. Since many industries discharge their effluent on to open lands because of high cost of dilution and inadequate treatment facilities effective and profitable utilization of the effluent of the industries needs greater attention. The rapid industrialization is accompanied by both direct and indirect adverse effect on environment. Industrial development results in the generation of industrial effluents and if untreated, results in water sediment and soil pollution. It has been observed that a wide majority of industries discharge untreated effluent into river and only 10 % industries surveyed had primary treatment plants ranging from oxidation tanks, sedimentation tanks in developing countries.

High level of pollutants mainly organic matter in river water causes an increase in biological oxygen demand, chemical oxygen demand, total dissolved solids, total suspended solids and faecal coliforms. They make water unsuitable for drinking, irrigation or any other use. When the effluent is released into the environment without proper treatment, it alters the characters of ecosystem. Farmers are using these raw effluents for irrigation and found that the growth, yield and soil health are reduced (Nandy and Kaul, 1994).

The tannery industry represents an important sector in the economy of many countries. On the other hand, depending on the leather process, it generates large quantities of wastewater with ammonium, sulfates, surfactants, acids, dyes, sulfonated oils and organic substances, including natural or synthetic tannins. These chemical substances are applied to transform the animal skin into products with great capacities for dyeing, as well as to increase the mechanical and hydrothermal resistance. Considering that the greater part of these organic compounds are resistant to conventional chemical and biological treatments, the wastes discharged into natural waters increase environmental pollution and the health risks. The treatment of this type of wastewater is very complex mainly because of the variety of chemical products added in different concentrations (Scherank *et al*., 2004, 2005, Kurt *et al*., 2007, Preethi *et al*., 2009).

Several methods have been described in the scientific literatures; however, these treatment methods do not solve the problem because of the transfer of contaminants from one phase to another. However, in biological treatment, the microorganisms degrade the organic pollutants using them as a carbon source to produce metabolic energy to survive. The effects of various industrial effluents, sludge materials and metal elements on seed germination, growth and yield of crop plants have captivated the attention of many workers (Rahman *et al*., 2002; Selivanorskaya and Latypova, 2006; Yu and Gu, 2007; Gannis *et al*., 2008; Sahu and Arora, 2008). However no detailed experiments have been performed on the germination and plant growth using treated and untreated tannery effluents. Hence an attempt has been made to study the effects of tannery effluent (both treated and untreated) on the germination of blackgram and sunflower crops.

MATERIALS AND METHODS

Sample collection and Bacterial isolation:

The tannery effluent was collected in pre-cleaned containers from the outlet of the tannery industry, which is located in the Vaniyambadi, Vellore district, Tamil Nadu. For the selected isolation of heavy metals resistant bacteria, heavy metals incorporated Nutrient agar media were used. The Nutrient agar incorporated with heavy metals like Cr, Fe, Mn, Ni was prepared. The concentration of each metal was maintained at 50 μg/ml of the medium. The tannery effluent sample directly streaked on Nutrient media and incubated at

*Corresponding Author
Email: mythilikannaiyan@gmail.com

37°C for 24 hours. After the incubation period the plates were observed for growth on the media (Virender singh *et al*., 2010). Three isolates were selected randomly for the further studies. Microscopic and biochemical tests were applied to this isolates and identified according to Bergey's manual of systemic bacteriology. The genus to which the isolates belong were determined. The selected isolates were stored on the Nutrient agar medium at 4°C for further studies.

Physico-chemical analysis of effluent sample

Physiochemical parameters of the tannery effluent such as color, pH, electrical conductivity(EC), total solids(TS), suspended solids(SS), dissolved solids (DS), dissolved oxygen (DO), BOD, COD and other chemical constituents were estimated using the standard methods (APHA, 1998).

Preparation of inoculum:

A loopful of culture (*Bacillus* spp., *Pseudomonas* spp., and *Micrococcus* spp.,) was inoculated individually pre-sterilized 100ml Nutrient broth. The flask was kept in a shaker at 120rpm for 16 to 18 hrs at 30°C. The culture broth was centrifuged at 10000rpm for 20 minutes. Cell suspension was prepared using sterile distilled water and adjusted to 0.5 OD using UV-visible spectrophotometer. 1% (10^5 CFU/ml) of the above suspension was used as inoculum for the bioremediation of tannery effluent.

Bioremediation of tannery effluent by bacterial consortium:

500 ml effluent was taken under aseptic condition in 1 L bottle having a side arm outlet and sterilized. To this, 1% inoculum containing 10^5 CFU/ml by bacterial consortium was inoculated and sterile dry air was passed continuously using an aerator. After 72 hours, the sample was filtered under aseptic condition. Then, the bacterially treated effluent was collected in a sterile conical flask and used for pot culture study. Physico-chemical parameters were estimated before and after treatment.

Germination of Blackgram and Sunflower seeds in pot culture:

The impact of bacterially treated and untreated effluent on the germination of the blackgram, ADT 3 (*Vigna mungo* L.) and sunflower, CO 3 (*Helianthus annuus* L.) was investigated using soil pots. Clay loamy soil was collected from an agricultural field which was unpolluted and fertile.

The soil was sieved (2mm mesh) and taken in separate pots (15x15 cm). Each pot contained 2 kg of soil. Six different concentrations (20, 40, 60, 80 and 100%) of treated and untreated effluent were prepared and 1 L of effluent was poured in each pot. Control (0%) was also maintained and irrigated with tap water. Then, five seeds (blackgram and sunflower) already sterilized with 0.1% mercuric chloride were planted in each pot, slightly pressed and allowed to germinate. The effluent (1L) was irrigated periodically at every 48 hours interval. The percentage of germination was assessed (Rahman *et al*., 2002). The shoot length of the plants was recorded at every 72 hours for 15 days.

Fresh and total dry mass of blackgram and sunflower were determined after 15 days of the roots was measured. The plants were uprooted and washed thoroughly with distilled water and length of the roots was measured. The plants were dried under natural conditions at the open roof top garden for 2 hours. The fresh weight was taken and the plants were then packed in paper envelopes and oven dried for 36 hours at 70°C. The dry weight of each plant was also recorded. The total chlorophyll content was analyzed for each experimental plant leaves on the final day (Sadasivam and Manickam, 1996). All the experiments were conducted in triplicate, the average values were tabulated.

RESULTS AND DISCUSSION

Heavy metals resistant bacterial isolates were identified as *Bacillus* spp, *Pseudomonas* spp, and *Micrococcus* spp, according to Bergey's manual systemic Bacteriology. The physico-chemical characteristics of tannery effluent before and after treatment are presented in Table 1. The raw effluent was brown in colour, deficit in dissolved oxygen, rich in total solids high amount of BOD and COD with considerable amounts of sodium, calcium, chloride, sulphate, fluoride, nitrate. After treatment by bacterial consortium, there was a decrease in pH from 8.7 to 7.2 pH is an essential factor in the formation of algal blooms as stated by Palharyal *et al*., (1993). Low pH or high pH makes the water unfit for irrigation and the soil over large are becomes alkaline resulting in poor crop, growth and yield. Dissolved oxygen also increased from 2.2 to 6.5 mg/l. Generally, dissolved oxygen is one of the important parameter in the irrigated water system. Cherif *et al*., (1997) studied effect of tomato, lipid peroxidation and receptivity of roots under hydroponic condition. They found that increase of shoot and root system irrigated with aerated nutrient solution. The TDS (406 mg/L), BOD (1260 mg/L), and COD (2035 mg/L) were very high in the effluent, were as in the treated effluent the TDS was (105 mg/L), BOD (28mg/L), COD (230 mg/L). This observation is in good agreement with the report by Indira and Ravi mycin (2010), suggests that higher amount of TDS were also decreased after 72 hours. However, the amounts of suspended solids (215 mg/L) were found to be higher in effluent before treatment were as after the treatment the amount of suspended solids (93 mg/L). This was mainly due to higher concentration of bacterial biomass in the effluent.

Table.1: Physico-chemical properties of tannery effluent before and after treatment mg/L

Parameters	Untreated effluent	Treated effluent
Colour	Brown	Light brown
Temperature	32°C	37°C
pH	8.6	7.2
Electrical conductivity	358	65
Total dissolved solids	406	105
Total suspended solids	215	93
Hardness	412	89
BOD	1260	28
COD	2035	230
Calcium (mg/L)	150	70
Magnesium (mg/L)	58	23
Sodium (mg/L)	86	17
Chloride (mg/L)	462	100
Sulphate (mg/L)	345	96

Fluoride (mg/L)	6.0	2.4
Nitrate (mg/L)	46.4	15.5
Chromium (mg/L)	9.8	0.04
Iron (mg/L)	3.4	0.02
Nickel (mg/L)	1.2	0.01
Manganese (mg/L)	2.6	0.05
Sodium adsorption ratio	4.2	0.04
Dissolved oxygen	2.2	6.5

Germination and physical properties of blackgram and sunflower in soil pots irrigated with treated and untreated tannery effluent:

The results of treated and untreated tannery effluent on the germination of blackgram and sunflower are given in Fig.1 to 4. The seed of blackgram and sunflower germinates 100% in the effluent at the lower concentrations. The higher concentrations (80 and 100%) of tannery effluent suppressed the germination of blackgram and sunflower. It may be due to the effect of higher amount of total solids and heavy metals stress on the seed germination process in untreated effluent. Whereas in the untreated 80% effluent, the germination was found that 88 and 84%, In 100% effluent, the germination percentage was 72 and 68% for blackgram and sunflower respectively. This might be due to osmotic pressure of higher concentration of effluent that do not support the germination. Rodger *et al*., (1957) reported that high osmotic pressures of the germination solution makes imbibitions more difficult and retard germination, while the ability of seeds to germinate under high osmotic pressure differs with variety as well as species.

The pots irrigated with treated effluent showed 100% germination except 100% dilution of treated tannery effluent for blackgram. The germination percentage was 94%. The shoot length of blackgram was severally influenced by various concentration of untreated tannery effluent. The maximum shoot length was observed in control followed by 20%, 40%, 60%, 80% and 100% concentration of effluent (Fig 7).

Murkumar and Chaun (1987) have been reported that the higher concentration of effluent decrease enzyme dehydrogenase activity that is considered as one of the biochemical change which may have disrupt germinate and seedling growth. Studies on heavy metal tolerance in plants indicate that root growth is particularly to heavy metals (Punz and Sieghardt, 1993). After treatment by bacterial consortium, the heavy metals decreased from 9.8 to 0.04(Cr), 3.4 to 0.02 (Fe), 1.2 to 0.01(Ni), 2.6 to 0.05 (Mn). *Bacillus* spp., reduced chromium VI under aerobic conditions. This may be due to the presence of chromium reductase. Similarly chromium VI reduction has been reported by *Bacillus coagulans* (James, 2002). Removal of heavy metals by *Pseudomonas* spp might be due to the presence of chromium reductase gene (Jin, 2000; Mellor *et al*., 1996). The study establishes the role and efficiencies of *Bacillus*, *Pseudomonas* and *Micrococcus* spp., in the bioremediation of tannery effluents.

Some essential micronutrients are needed in small quantities for plant growth. However, the excessive level could prove toxic to plant growth. Indra and Ravi mycin (2009) clearly reported the toxicity of tannery effluent on the growth, physiological and biochemical contents of blackgram. The salt content outside the seed is known to act as liming factor and causes less absoption of water by osmosis and inhibit the germination of seeds (Gomathi and Oblisami, 1992; Palanivel et al 2004; Malla and Mohanty 2005). According in our study, the plant growth was highly affected due to the pH, presence of higher dissolved solids and heavy metals in the effluent. The germination of seeds under higher concentrations of effluent treatment would get low amount of oxygen which might have restricted the energy supply and retarded the growth and development of seedlings (Roa and Kumar, 1983). Inhibition of root and shoot length at higher concentrations of the effluent may be due to the high level of total dissolved and suspended solids present in the effluent which interfered and inhibited the uptake of other elements (Thabaraj et al., 1964). The root which continuously remains in direct with the effluent hence the higher concentrations of the effluent could affect cell multiplication or the growth (Kannan et al., 2008). There was slight variation in the shoot length when the pots irrigated with treated effluent at all the tested concentrations after the 15th day (fig7 and 8). Duraisamy and Pugalendhi (1997) tested in the effects of sago factory effluent, treated with various oxidizing chemicals for the germination of crops like paddy, maize, sorghum, pearl millet, finger millet, blackgram and cowpea. They found that the effluent treated with oxidizing chemicals increased the germination and vigour indices of paddy, maize, sorghum and cowpea over that of untreated effluent.

Similar to blackgram, the germination study was conducted using treated and untreated tannery effluent against sunflower. The similar influence was observed in sunflower when the pots irrigated with untreated effluent at different concentrations. There was significant variation noted in the shoot length when the study performed with various concentrations of treated effluent after 15th day. Majority of bacterial inoculation increased the seedling length. According to reports growth stimulatory bacteria released some chemotaxis, which helps plant for better growth. Rhizospheric bacteria improved plant growth and nutrient uptake of maize, wheat and legumes (Hoflich and Mets, 1997).

However, the root length of the blackgram and sunflower was severally influenced by various concentrations of effluent (Fig 5 and 6). Maximum root length was observed in control for blackgram and sunflower. In treated effluent, the root length was almost more or less similar in both cases. Impact of effluent on the fresh and dry weight of the plants was also checked (Fig 9- 12). Chlorophyll content also exhibited a similar trend when compared to the shoot and root length responses (Fig 13 and 14). Plants treated with higher concentration of untreated effluent (>20 %) showed lower chlorophyll content. However, an enhancement of germination and chlorophyll content was recorded when the pots irrigated with bacterially treated effluent.

From the above results it could be concluded that by treating the tannery effluent aerobically with bacterial consortium, the physico-chemical parameters were found to be relatively high in untreated effluent and severally affect the plant health. The germination of blackgram and sunflower was more or less similar to that of control in bacterially treated effluent. Treated effluent showed better response towards other parameters such as root length, fresh weight, dry weight and chlorophyll content under laboratory condition. Hence, it is suggested that tannery effluent can be aerobically treated using a bacterial consortium and may be suitable for irrigation purpose.

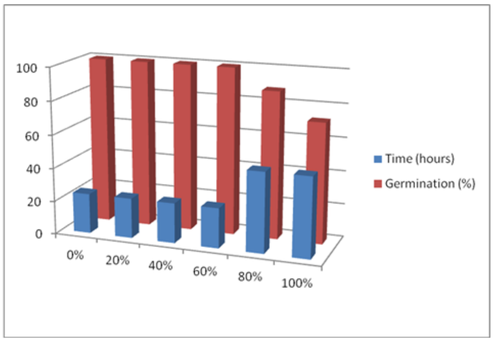

Fig1: Effect of untreated effluent on seed germination of Black gram

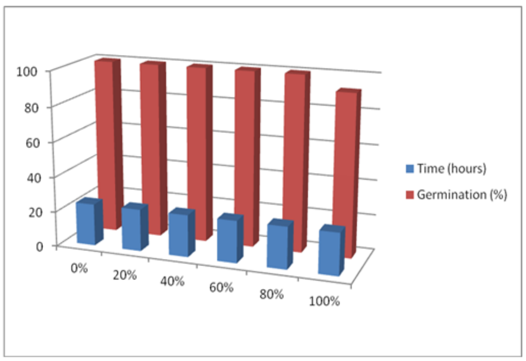

Fig-2: Effect of treated effluent on the seed germination of blackgram

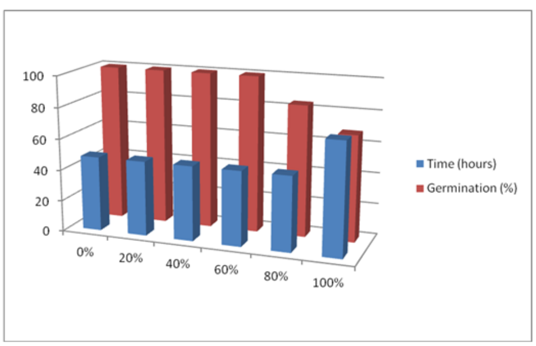

Fig.3: Effect of untreated tannery effluent on seed germination of sunflower

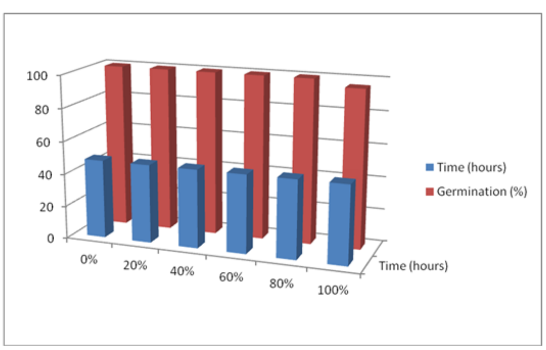

Fig.4: Effect of treated tannery effluent on seed germination (%) of sunflower

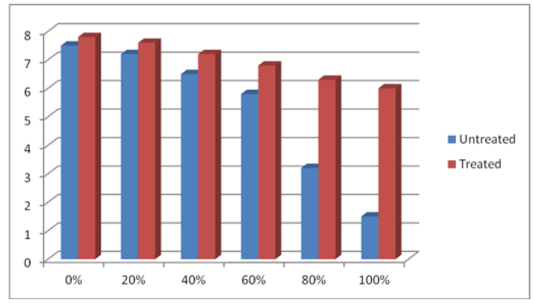

Fig.5: Effect of untreated and treated tannery effluent on root length(cm) of blackgram

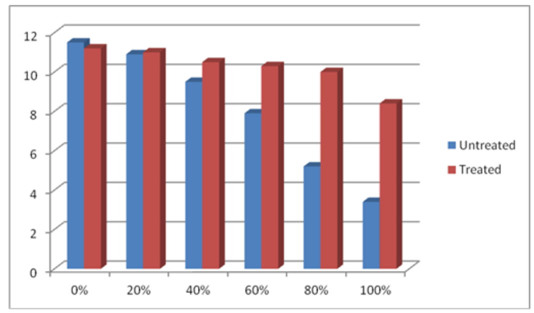

Fig.6: Effect of untreated and treated tannery effluent on root length(cm) of sunflower

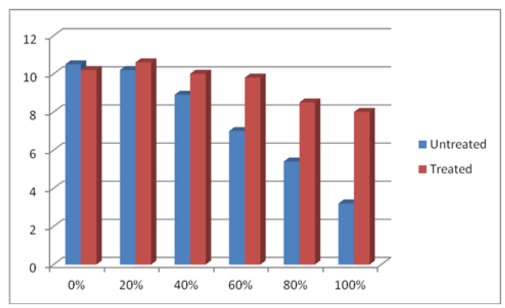

Fig.7: Effect of untreated and treated tannery effluent on shoot length(cm) of blackgram

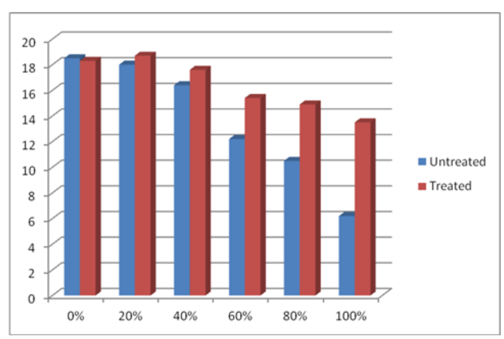

Fig.8: Effect of untreated and treated tannery effluent on shoot length(cm) of sunflower

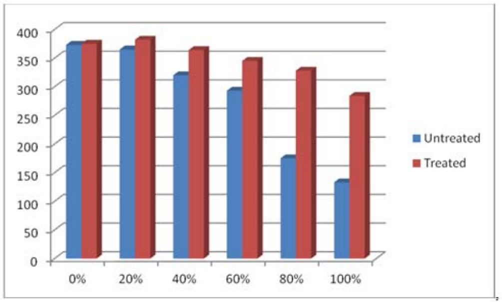

Fig.9: Effect of untreated and treated tannery effluent on fresh weight (mg) of blackgram

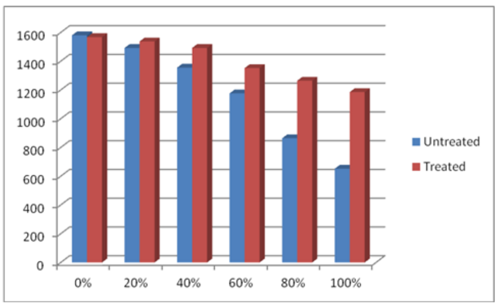

Fig.10: Effect of untreated and treated tannery effluent on fresh weight (mg) of sunflower

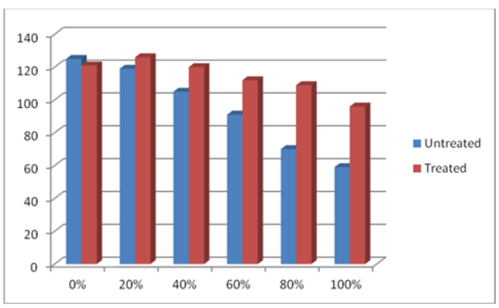

Fig.11: Effect of untreated and treated tannery effluent on dry weight (mg) of blackgram

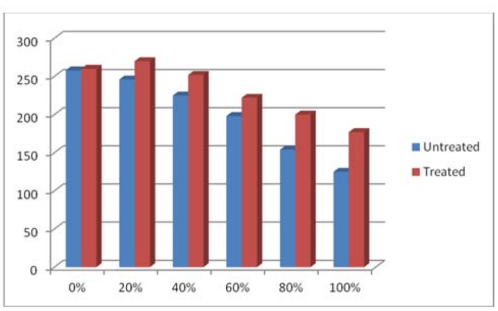

Fig.12: Effect of untreated and treated tannery effluent on dry weight (mg) of sunflower

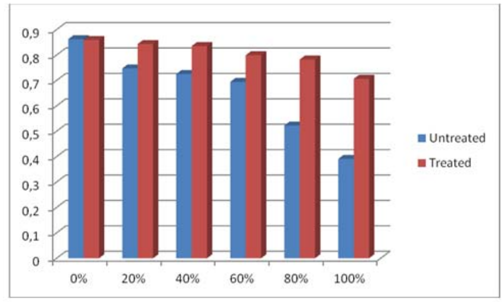

Fig.13: Effect of untreated and treated tannery effluent on chlorophyll content(mg/g) blackgram

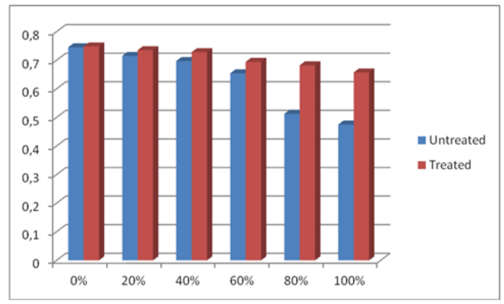

Fig.14: Effect of untreated and treated tannery effluent on chlorophyll content(mg/g) sunflower

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