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EFFECT OF DINDIGUL TANNERY EFFLUENT ON THE GROWTH OF EGG PLANT *SOLANUM MELONGENA* (L). USING BIOREMEDIATION PROCESS

M. NAZEEMA[1] AND T. NIRMALA[2]

[1]*PG & Research Centre of Zoology, Jayaraj Annapackiam College for Women (Autonomous), Affiliated to Mother Teresa Women's University, Periyakulam, Tamil Nadu, India*
[2] *Jayaraj Annapackiam College for Women, Periyakulam.*
Annai Scholastica Arts and Science College Tharuvai Thoppu, Thangachimadam Ramanathapuram

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ABSTRACT

Leather industry is vital for the economic status of the country. Leather industry is the major source of revenue for the millions of people in India. Similarly agriculture is also important and the irrigation with treated effluent water is the need of the hour to meet the people's demand. The effluent water should be treated to remove the hazardous material and heavy metals including cadmium, lead and nickel before irrigation. Chromium reducing capability of the organism was identified based on the presence of chromium reductase gene in the organism using PCR technique. From the total organism two organisms namely *Bacillus cereus* and *Enterobacterium kobei* have the chromium reductase gene. To study the bioremediation *Enterobacterium kobei* was taken for further studies. The inoculum containing *E. kobei* was mixed with effluent water and used to water the egg plant and the physical and chemical characteristic of the plant was studied. The plant that watered with bioremediated water showed considerably reduced chromium concentration in all parts of the egg plant. The treatment of waste water should be easily affordable and safe that is highly recommended. Thus, the present study aims to explore the microorganims which are able to bioremediates the tannery effluent water and irrigates the cleaned water for agricultural purposes.

KEY WORDS : Tannery effluent, Egg plant, Chromium reducing Bacteria and Bioremediation.

INTRODUCTION

Bioremediation is the process to remove the toxic metals from the aquatic environment. Bioremediation can be defined as the capability of certain biomolecules or types of biomass to bind and concentrate selected ions or other molecules present in aqueous solutions. Bioremediation using microorganisms is a budding field for future development due to its environmental compatibility and possible cost-effectiveness. A wide range of microorganisms, including bacteria, fungi, yeasts, and algae, can act as biologically active methylators, which are able to at least modify toxic species (Shiomi, 2018).

Contamination of heavy metals from industry is the important problem which affects both flora and fauna and cause many deleterious effects to human health. These heavy metals posses a strong electrostatic and binding affinity to the same site therefore it strongly binds to the cellular structures and destabilizes the biomolecules in the living environment. Chromium is an important heavy metal and has been ranked seventh in the earth's crust. In recent years, pollution caused by the chromium is considered as a serious environmental threat (Jaishankar *et al.* 2014).

Heavy metal accumulated in the soil and water poses risk to mankind and the environment. In soil heavy metals causes toxicological effect on microbes, which may lead to decrease in their numbers and activities. Chromium in soil exists predominantly in the Cr (III) and Cr (VI) forms. Cr (III) applied in large amounts had no effect on plant growth or Cr uptake (Shewry and Peterson, 1974) and accumulated mainly in the plant roots (Barcelo *et al.*, 1985).

Microorganisms that are present in the soil have greater impact on the growth of the plant. Bioremediation is based on the co metabolism of one organism or group of organisms. Since the microorganism is not dependent on this metal for growth the transformation of contaminant is non-beneficial biotransformation. Various species including *Escherichia coli, Bacillus subtilis, Saccharomyces boulardii*, *Enterococcus faecium*, and *Staphylococcus aureus* were involved in the bioremediation of heavy metals (Shiomi, 2018).

The bioremediated water was used to grow the eggplant (*Solanum melongena* L.) belongs to the family of *Solanaceae* which is the important vegetable crop in tropical and sub-tropical region. In this plant, the unripe fruit is consumed as a vegetable and it shows wide varieties of shapes and colour. Starting from oval shape, the shape changes to egg-shaped or long club-shaped and the colour varies from white, yellow, green, through degrees of purple pigmentation to almost black. The fruit contains high amount of water, protein, carbohydrate, fibres, low fat and calories make this fruit as a good vegetable (Sidhu and Dhatt, 2006).

In the present study on the efficiency of bioremediation of microorganism was conducted under laboratory conditions, by setting up experiments with three pots in triplicates in which the germination and the growth ability of *Solanum melongena* was analysed by wattering the plant with tannery effluent water bioremediated with *Enterobacter kobei*, untreated effluent and tap water as control.

MATERIALS AND METHODS

The plant *Solanum melogena*, commonly known as the egg plant was selected for the study purpose. Healthy seeds of egg plant (*Solanum melogena*) were purchased from the National seeds corporation Ltd, Chennai, Tamil Nadu, India.

Preparation of Inoculum

- To prepare the inoculum, *E. kobei* colonies were picked from nutrient agar and inoculated in 100 ml of nutrient broth in a 500 ml conical flask and kept it in a shaker.
- The flask was incubated at 37 °C for 48 hours. The culture was grown until it reaches a mid log phase (0.6 *105 CFU/ml).
- This was used as an inoculum for future studies. The tannery effluent (500 ml) was taken in a 2 litre flask and 1% inoculum was added and kept it in a shaker at 37 °C.
- For every 48 hours, the bacterial count and the physico chemical parameter of the soil was determined.

Estimation of Bacterial growth

- Bacterial growth in the tannery effluent was estimated by colony forming units.

Physical parameter of the tannery effluent

- The physical parameter like TDS, BOD and COD were estimated at the time interval of 48, 72 and 96 hours.

Preparation of Soil

Agriculture soil was sterilized before the experiments to ensure no interference from other microorganisms. The seeds were sown in each pot at a depth of 1 cm. To perform the experiment in triplicate, 9 pots were choosen, 3 in each group. The groups are described as below;
Group A: seeds sowed on soil, watered with normal tap water as control.
Group B: seeds sowed on soil, watered with bioremediated water with Enterobacter kobei.
Group C: seeds sowed on soil, watered with tannery effluent water.

Estimation of heavy metals in different parts of the plant

Analysis of metal content

Chromium (Cr) was analyzed using Inductively Coupled Plasma - Optical Emission Spectrometer (ICPOES).

RESULTS AND DISCUSSION

Bacterial growth

Chromium reducing bacteria was found in both contaminated and non-polluted regions (He *et al.* 2010). In the present study such chromium reducing bacteria was found in tannery effluent isolated from Dindigul district. Two bacterial strains were isolated for chromium reducing property but we have chosen *E.kobei* based on the chromium reducing property for the further continuation of the study.

Colony forming unit for every 48 hours was calculated. On the 9th day the colony forming unit in the tannery effluent was 8.7x10^5 CFU/ml. in the control sample the initial culture concentration was 0.4x105 CFU/ml which was reduced in the following days (Figure 1). Number of colonies in the tannery effluent was increased day by day. *E.kobei* was able to grow in the contaminated water for 9 days with 8.7x10^5 colonies per ml. Previous studies also showed the similar result where *Enterobacter sp* can be able to grow in high chromium effluent water (Ashraf *et al.* 2018).

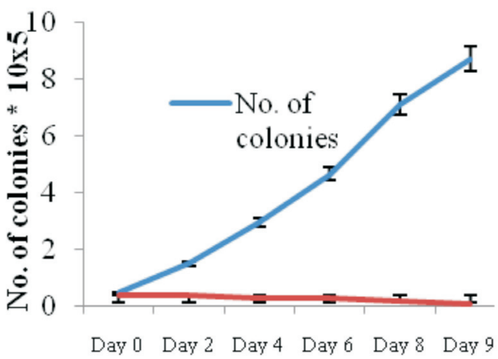

Fig. 1. Number of colonies in the tannery effluent with and without treatment of *E.kobei*.

Physical parameters of tannery effluent

Physical parameter such as Total dissolved solids BOD and COD of the tannery effluent with and without microorganism was calculated at every 48 hours of interval up to day 9.

Total Dissolved solids

Total dissolved solids in the tannery effluent treated with microorganism showed gradual decrease from 11,000 mg/l to 1,000 mg/l with increasing number of days (Figure 2). Tannery effluent treated with *E.kobei* showed considerable reduction in total dissolved solids when compared with the control during nine days of experimental work.

BOD and COD of the tannery effluent

BOD and COD is one of the important markers for pollution and the content is high in tannery effluent water. In the tannery effluent BOD of the sample is 550 mg/l. It was subsequently reduced in tannery effluent treated with *E.kobei* (Figure 3). Similarly COD in the control sample was 1500 mg/l.

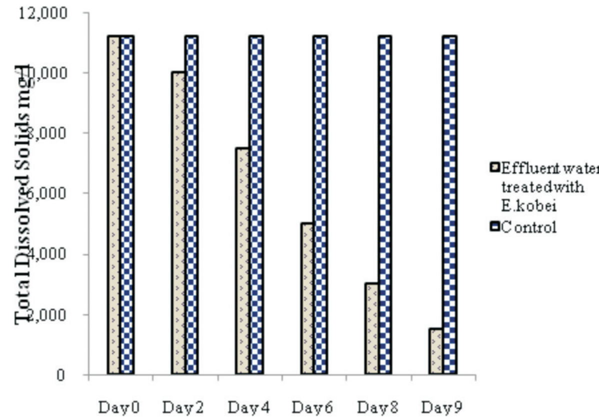

Fig. 2. TDS levels in the tannery effluent with and without treatment of *E.kobei*.

Treatment with *E.kobei* reduces the level of COD up to 992 mg/l in the tannery effluent (Figure 3).

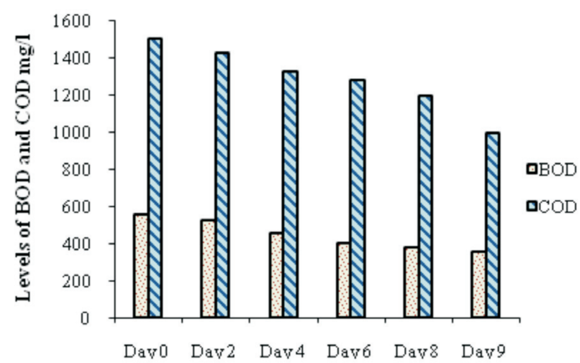

Fig. 3. BOD and COD levels in the tannery effluent with and without treatment of *E.kobei*.

This study also focused on the physical and chemical parameters like TDS, BOD and COD of the tannery effluent after treatment with *E.kobei*. Previous studies showed that *Pseudomonas* sp. decreased in the BOD (75%) and COD (65%) of tannery effluent after 9th day of incubation (Gidhamaari *et al.* 2012). Similarly, *Citrobacter freundi*i significantly reduced BOD and COD of tannery effluent and can be considered a prospective bioremediator for the safe disposal of tannery effluent to the environment (Ashraf *et al.*, 2018).

Physico chemical characteristics of the soil treated with tannery effluent

The soil sample was autoclaved to remove the other microorganism and the soil was watered using the normal tap water, tannery effluent treated with *Enterobacter kobei* and untreated tannery effluent. The physical and chemical characteristic of the soil namely Nitrogen, Phosphorus and Potassium was measured and the results were tabulated (Table 1). The nitrogen, phosphorus and potassium were significantly reduced in effluent water treated with *Enterobacter kobei* when compared with untreated tannery effluent and normal water as control.

Toxic metals including Cadmium and Lead was high in the soil treated with effluent water when compared with the bioremediated water. Previous study showed that the soil taken from the tannery region has high content of lead. Cadmium and lead in the soil affect the growth of microorganism by damaging the cell membrane and obliterate the DNA structure. In the present study lead and cadmium were reduced in the Bioremediated treated water. Studies showed *Vibrio harveyi* strain precipitated soluble divalent lead as complex lead phosphate salt (Igiri *et al.*, 2018). Some organism use the mechanism of methylation and increase the bioremediation process. Lead is methylated to dimethyl lead by some organisms. Mercury-resistant bacteria such as *Alcaligenes faecalis, Bacillus pumilus, Pseudomonas aeruginosa* and *Brevibacterium iodinium* are used for the removal of cadmium (Cd) and lead (Pb) from the soil.

Estimation of metals from the soil

In all the three samples various metals including copper, magnesium, lead, iron, zinc, cadmium, manganese and chromium was estimated. In the effluent of untreated soil all the metals were drastically increased. In the soil treated with bio-remediated water, metals were decreased significantly. Copper in the untreated effluent soil was 50.1 mg/kg but in the effluent treated watered soil it was reduced to 40.1 mg/kg of soil. Similarly, chromium content in the soil was 65.7 mg/kg and in the treated soil the chromium content was reduced. Magnesium in the effluent untreated soil was 88.7 mg/kg but it was reduced in the effluent treated soil. Iron in the soil gets reduced in the effluent untreated soil but it increased in soil treated with *E.kobei* (Table 2).

Heavy metal like chromium was quantified in the soil with effluent water and with bioremediated effluent water. In effluent watered soil chromium content was recorded as 65.7 mg/Kg. In previous studies samples near to tannery region in Ranipet in which the chromium content in the sample was more than that of the sample collected from Dindigul district (Gowd and Govil, 2008). Similarly, previous studies in Dindigul reported the chromium level of soil in tannery region was 50 µg/ml (Mondal, 2010). After eight years the chromium content in the soil is increased. Microorganism can be able to reduce the chromium from the soil effectively. *Bacillus subtilis, B. megaterium, Aspergillus niger*, and *Penicilliumsp* Effectively reduce the chromium content in the soil. Among the various organisms *Aspergillus niger*effectively reduce the chromium followed by *Penicillium sp.* In the present study *E.kobei* has effectively reduced the chromium in the soil.

Micronutrients such as manganese, magnesium,

Table 1. Physico chemical characteristics of soil treated with tannery effluent

5.5	Soil sample	Depth (cm)	Soil texture	Nitrogen (mg/kg)	Phosphorous (mg/kg)	Potassium (mg/kg)
1	Control	7.5	Clay	0.65	2.4	25
2	Soil treated with E.kobei	7.5	Clay	2.0	6.5	30
3	Untreated Soil	7.5	Clay	5.5	6.3	32

Table 2. Metal contents recorded in control, tannery effluent soil and bioremediated effluent soil

Parameters in (mg/kg)	Control soil (mg/kg)	Effluent untreated soil (mg/kg)	Effluent treated soil (mg/kg)
Copper	32.3	50.1	40.1
Magnesium	21.4	88.7	50.32
Lead	2.7	24.2	10.7
Iron	11.48	9.50	11.1
Zinc	24.1	46.6	30.1
Cadmium	10.2	16.4	12.32
Manganese	33.82	47.9	40.21
Chromium	41.3	65.7	45.3

iron, zinc and copper was estimated in the soil in all the three groups. In the effluent watered soil, all the metals were higher than the control. In the bioremediated water soil, all these metals were lower and it was almost equal to that of control. Thus the study showed *E.kobei* has effectively reduced the metal content in the soil.

Plate 1. Effect on the growth of the *Solanum melongena* with the tannery effluent treated, untreated and control water.

T1) Plant watered with effluent treated with *E.kobei*; T2) Plant water with effluent untreated water; Control as Tap water

Accumulation of chromium in the different parts of the plants

Chromium accumulation in the different parts of the plant including root, stem, leaf and fruit was estimated using ICPOES (Figure 4). The chromium

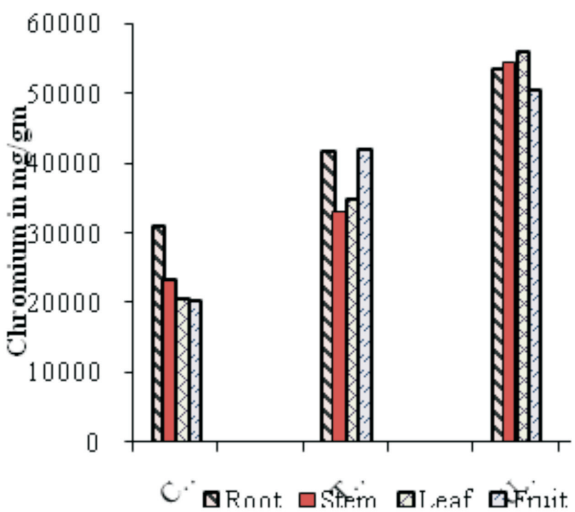

Fig. 4. Accumulation of chromium in different parts of the egg plant

accumulation in all parts of the plant was considerably high in effluent untreated water when compared with control plants. Treatment of water with *E. kobei* reduced the accumulation of chromium in the plant which suggested that the organism can be used in the bioremediation of tannery effluent water before bathing in to the agricultural runoff.

Seed germination is a sensitive stage of growth in plants. The total seed germination was determined. Seed germination can be used to determine the effect of pollutants on the plant growth (Banks and Schultz, 2005). Previous study showed reduced germination, in soil, contaminated by chromium and other heavy metals by Whani and Khan *et al.* (2010). Similarly, germination of green gram, *Vigna radiate L* was studied on petroleum hydrocarbon contaminated soil by Masakorala *et al.* (2013) and the result showed the inhibition of seed germination. Treatment of *E.kobei* increases the germination of seed when compared with the untreated effluent water. Using industrial waste water for an agricultural need is the cheapest mode of wastewater disposal and decreases the disposal pressure. Treating with *E.kobei* increased the seed germination process which showed that the tannery effluent can be used for agricultural process after bioremediation.

CONCLUSION

This study showed tannery effluents after bioremediation can be used for irrigation purpose in the Dindigul region which helps agriculture during the scarcity of water. It is beneficial for both the industrial sector and agriculture. The fresh water stream will not affect with the tannery effluent as it is in minimal concentration.

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