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RESEARCH ARTICLE

EFFECTS OF TANNERY EFFLUENTS ON BIOCHEMICAL GROWTH PARAMETERS OF *ABELMOSCHUS ESCULENTUS*. L (LADIES FINGER)

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ABSTRACT

This experimentation was carried out the effect of tannery effluent on seed germination of *Abelmoschus esculentus* .L (Ladies finger) seeds under the tannery spiked soil condition. The treatments were made by mixing measured amount of tannery effluents in ground water i.e.5%,10%,15%,20%,25%,and 100% concentrations on seed germination of *Abelmoschus esculentus* .L (Ladies finger). The seed germination experiments were carried out in completely randomized design (CRD) with six effluent concentrations (treatments), each experiment carried out in triplicates. The seeds were grown in different effluent concentrations and the effects of different concentrations of effluents were compared to that of ground water (control). After the experiments, 80th day, the seedlings were collected and growth parameters like germination percentage, shoot length, number of leaves and auxillary buds were observed as decreased with higher amount of 25%(v/v) up to 100% (v/v) concentrations. The same trend also observed in biochemical parameters like protein, carbohydrate and chlorophyll content respectively. In conclusion, the *Abelmoschus esculentus* .L growth and biochemical parameters were minimum effect with 20%(v/v) concentration comparing to control. As a result the optimum concentration of tannery effluent contaminated soil was found to be 20%(v/v). The toxic chromium Cr(III) was transferred to different parts of the plant as non-toxic Cr(III). Finally it was observed that the effluent can be used for irrigation purpose after appropriate dilution with ground water.

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INTRODUCTION

The Ranipet Town is located at 12 56° Northern latitude and 79.20° eastern longitude and is 93 KM west of Chennai. It is geographically 25 Km away in the North East of Vellore, the District Headquarters of Vellore District. Ranipet has been selected by the SIDCO & SIPCOT to establish the estates/complexes, since it is situated at a distance of 3.5 Km from River Palar and adjoining Chennai-Chittoor Bangalore Road (NH-4). Palar river is one of the major water sources running West to East located at downstream of the Industrial complex and other water bodies namely Puliyankannu and Karai Eri located in the down stream of the site. The SIPCOT industrial complex, Ranipet was established during the year 1973.The industrial complex has Phase-I and Phase-II where Petro- chemical, Bulk drugs & Pharmaceuticals, Heavy Engineering, Foundry, Chemicals, Tanneries and miscellaneous industries are located. Ranipet SIPCOT Industrial Complex lies in the North-West of Ranipet town. The area of the SIPCOT Industrial Complex comprising of Phase I & II is 862.91 Acres. *Abelmoschus esculentus*. L (Ladies finger).is one of the mainly accepted, delicious and jellylike vegetable and it play a very important responsibility in human diet. Which contains protein, carbohydrates and vitamin C. It also has antioxident and anti –diabetic properties. More than a few workers have reported that *Abelmoschus esculentus* .L (Ladies finger).has found medicinal application as a plasma substitute or blood level expander (Lengsfeld *et al*., 2004,Adetuyi *et al*.,2008,Kumar *et al* .,2010). *Abelmoschus esculentus* .L (Ladies finger) is susceptible to many pathogens. Many fungicides and insecticides are used to control them. Use of Agricultural fertilizers, herbicides, insecticides and pesticides and migration of contaminates into a non contaminated land as vapours and leach at though soil, or as dust, or spreading of sewage sludge contributes towards contamination of the ecosystem. The polluted river water are mostly used for irrigation of crop plant. water is an important natural resources essential for all living organism. The important water resources and ponds, lakes, rivers, stream, etc., These water bodies provide water for drinking, irrigation, cooling industrial machines and also for other, domestic purpose. The waste water released from leather tannery .They are produce large amount waste water effluent and create water pollution. The contamination of the flora and fauna has been increased as a result of the industrial revolution and urbanization of the landscape.

The stage of metals in soil may also be prominent by natural sources or by agricultural, industrial, mining and waste disposal content. The soil and sediments are considered as sinks for metals, which are accumulated in high concentrations. The disposal of sewage and industrial sludge is becoming a severe problem in many countries including India. Among industries, tannery industry occupies a place of prominence in Indian economy, but it contributes significantly towards the contamination of the ecosystem (Shraddha Sing, Sarita Sinha, 2003). The effects of industrial waste, containing high concentrations of metals on the growth of the mature plants and its surface are meager (Pandey *et al*.1994; Khan and Khan, 1996; Chandra *et al*.,1997; Suseela *et al*., 2002).Heavy metals do not biodegradable during composting process and can be concentrated due to the loss of carbon and water from the compost due to compost due to microbial respiration(Richard,1992). Tannery effluents contribute mainly in this regard, India ranks 6th in the world for leather production and at present, there are about 3000 tanneries processing about 600 million kg of raw skin and hide per annum generating around 50 million liters per day of liquid waste and 305 million kg solid waste (Ahamed *et al*.,2014).Tannery sludge enhanced the productivity of crop and metal accumulation occurred in roots with a meager translocation to shoots, hence it can be used as photo stabilizer (Janhvi Pandey *et al*.,2015).

Production of tannery effluent is increasing day by day as a result of waste water treatment, which is generally massive with high moisture contents, and form highly organic mineral depending on their origin in nature. There is an increasing interest in agricultural application of sludge obtained from waste water treatment plants due to possibility of recycling voluble components (Wong *et al*., 2001). It is impossible to visualize a soil without trace level of heavy metal. Further, anthropogenic behavior have determined several of these elements in certain areas up to dangerous levels for living organism (Amit k *et al*., 2006). In view of above, the proposed work was aimed to study the different amendment of tannery effluent and the potential of the vegetable crops *Abelmoschus esculentus*. L (Ladies finger).for the removal of metals from tannery effluent contaminated soil

MATERIALS AND METHODS

Collection of samples and Preparation: The uncontaminated garden soil were collected from University Botanical Garden, Thiruvalluvar University, Serkkadu, Vellore -632 115,Tamil Nadu ,India was used to make the various amendments for experimental studies. Liquid of leather tannery effluent were collected from Common Effluent Treatment Plant Phase –II at Ranipet, Vellore -632 406, Tamil Nadu, in plastic container and brought to the field laboratory. The leather tannery effluent (LTE) and soil were dried, finely powdered and sieved to 2mm mesh size before use. The seeds of *Abelmoschus esculentus* .L (Ladies finger) were sterilized with 0.1% mercuric chloride for 5 min to avoid fungal contamination, washed with distilled water for three times. The seeds of *Abelmoschus esculentus* .L (Ladies finger) were sterilized with 0.1% mercuric chloride for 5 min to avoid fungal contamination, washed with distilled water for three times.

Experimental setup: The various amendments of leather tannery effluent (LTE) (5%, 10%, 15%, 20%, 25% and 100%) were prepared using garden soil, which served as control. The soaked seeds evenly sown in polythene bags (12 in. diameter), which were filled with different amendments of leather tannery effluent (LTE) along with one set of control, each in three replicates. Ten seeds were sown each polythene bag to depth 0.5cm.The bag were watered daily till seed germination. When the seedlings have developed 5 or 6 leaves, they were thinned out to retain 6 uniform ones per bag and allowed to grow. Bags were placed in the field laboratory at an average diurnal temperature of 25- 45°C.The water level was made up as and when required using tap water. The plants were harvested after 80 d after sowing. All the plants were free any disease in whole of the experiment duration. The experiments were repeated twice, each time with three replicates.

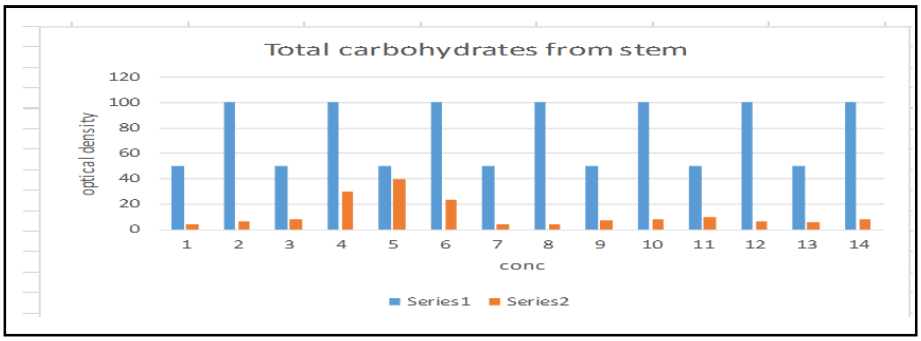
Physico-chemical characterization of leather tannery effluent (LTE): The effluent was collected in the glass containers, from the outlet of the tannery. Samples were analyzed for various physico-chemical properties by standard method of analysis as per the guidelines of American Public Health Association (APHA *1998).* pH was determined by pH meter, EC (Electrical conductivity) by EC meter, TDS (Total dissolved solids) by gravimetric method, BOD (Biological oxygen demand) by dissolved oxygen meter (incubator) and COD (chemical oxygen demand) by volumetric method (open reflex), AAS (atomic absorption spectrophotometer) were used to determine heavy metals such as Arsenic, Lead, chromium, cadmium respectively.

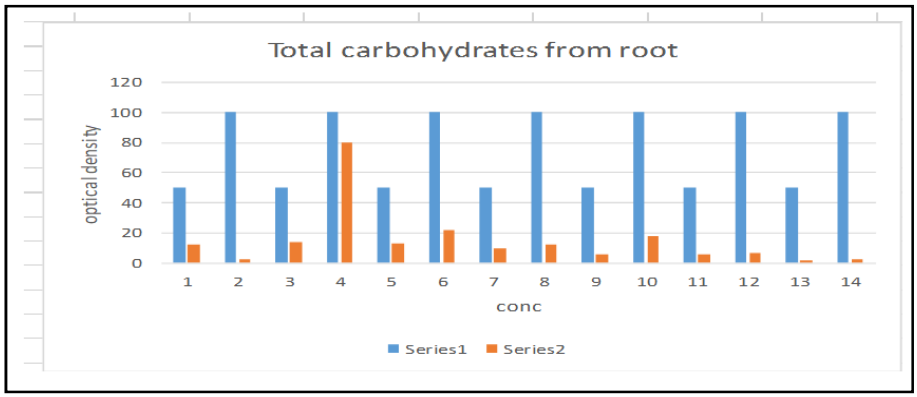
Estimation of carbohydrates: (Orthotoluidine reagent): Total carbohydrates were determined according to the method of Hedge and Hofreiter (1962). Weigh 100 mg of the sample (fresh roots and leaves) into a boiling tube. Hydrolyze by keeping it in a boiling water bath for 3 h with 5 mL of 2.5 N HCl and cool to room temperature. Neutralize it with solid sodium carbonate until the effervescence ceases and centrifuged at 6000 r/min. Collect the supernatant and take 0.5 and 1 Ml aliquots for analysis. Then a/dd 4 mL of anthrone reagent and heat for 8 min in a boiling water bath. Cool rapidly and read the green to dark green colour at 630nm using spectrophotometer.

***Estimation of protein*:** Alkaline copper reagent (2% sodium bicarbonate, 0.1N sodium hydroxide, 1.56gm copper sulphate, 2.37gm sodium potassium tartarate). Protein content in the roots and leaves (500 mg) were extracted with buffers used for enzymes assay, grind well the samples with a pestle and mortar in 5-10 mL of buffer and centrifuge, the supernatant for protein was measured at 660 nm by the method of Lowry *et al*. (1951) using bovine serum ^{albumin} as the standard protein. Protein content was expressed as mg g-1 fresh weight.

Estimation of chlorophyll: 80% acetone, whatman filter paper, Instrument: UV-spectrophotometer, centrifuge. A known amount of ladies finger leaf tissue 100mg was suspended in 10ml of 80% acetone, mixed well and kept at 4^{o}c over night in dark. Supernatant was withdrawn after centrifugation (5000 rpm) and absorbance was recorded at 663 and 645 nm in spectrophotometer. The amount of chlorophyll was calculated according to Arnon (1949)

The Physicochemical properties: The physicochemical properties of leather tannery effluent (LTE) are shown in (Table 2). Effluent colour is white and odorless. pH is 7.6, this value is compared to ISI (Indian Standard Institution) standards recommended for disposal of effluent on land for


Total carbohydrates from stem
optical density
conc
Series1
Series2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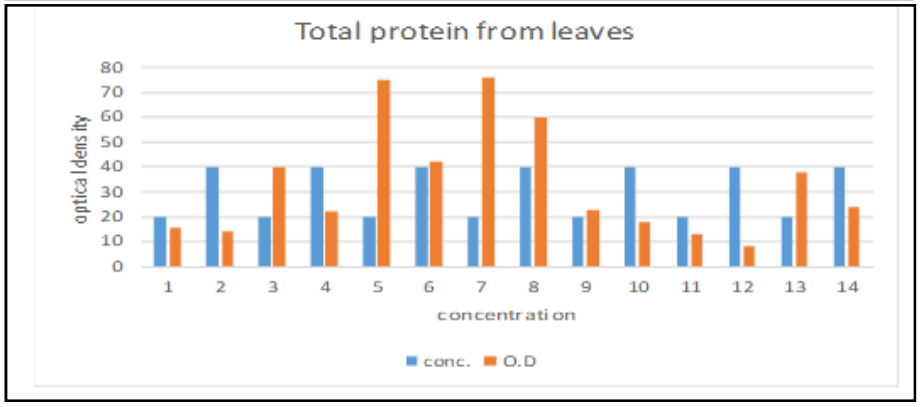
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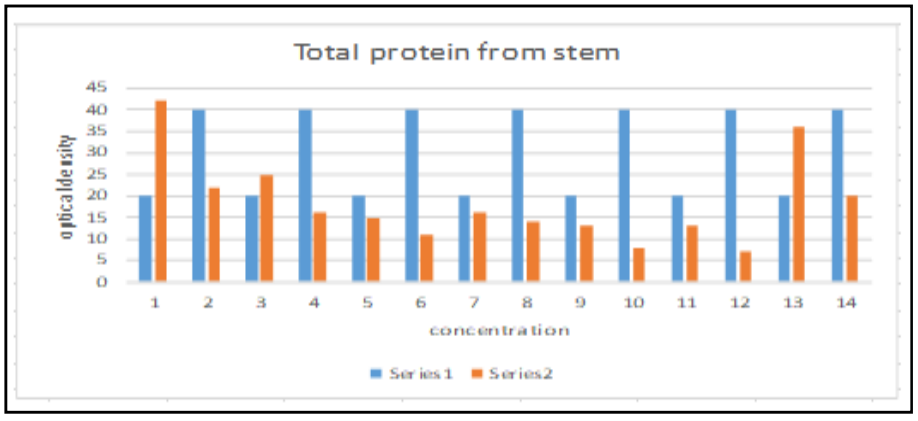
optical density
concentration
conc.
O.D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optical density
concentration
Series1
Series2

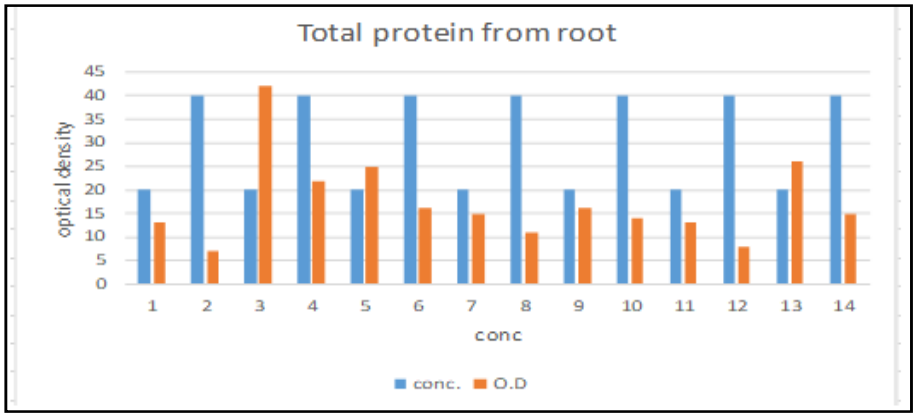
Total protein from root
optical density
conc
conc.
O.D

Table 1. Physico – Chemical properties of tannery effluent (TE)

Parameters	Concentrations/Units
PH	7.6
Total dissolved solids(TDS)	4828 mg/litter
Electrical conductivity(EC)	5160 mmhos/cm
Biochemical oxygen demand(BOD)	330mg/liter
Chemical oxygen demand(COD)	1560mg/litter
Total chromium	142 ppm/liter
Dissolved oxygen(DO)	6.9

Table 2. The effect of tannery effluent on growth parameters of *Abelmoschus esculentus*. L (Ladies finger)

Treatment	control	5%	10%	15%	20%	25%	100%
Germination (%)	17	14	13	11	6	6	4
Shoot length(cm)	13	12	11.4	15	16.5	14.6	15
Leaves (Nos.)	54	46	51	39	12	12	23
Auxillary buds (Nos)	2	1	1	2	2	Nil	Nil

Table 3.The effect of tannery effluent on carbohydrate content of *Abelmoschus esculentus*. L (Ladies finger)

Samples (After 80 days)	Treatment	Control	5%	10%	15%	20%	25%	100%
Leaves	T1	7mg/dl	14mg/dl	18mg/dl	14mg/dl	7mg/dl	7mg/dl	36mg/dl
	T2	4mg/dl	7.5mg/dl	9mg/dl	7mg/dl	6mg/dl	7.5mg/dl	24mg/dl
Stem	T1	4mg/dl	8mg/dl	40mg/dl	4mg/dl	7.5mg/dl	10mg/dl	6mg/dl
	T2	7mg/dl	30mg/dl	24mg/dl	4.5mg/dl	8mg/dl	7mg/dl	8mg/dl
Root	T1	12mg/dl	14mg/dl	13mg/dl	10mg/dl	6mg/dl	6mg/dl	2mg/dl
	T2	3mg/dl	80mg/dl	22mg/dl	12mg/dl	18mg/dl	7mg/dl	3mg/dl

Table 4. The effect of tannery effluent on protein content of *Abelmoschus esculentus*. L (Ladies finger)

Samples (After 80 days)	Treatment	Control	5%	10%	15%	20%	25%	100%
	T1	T1-16mg/dl	40mg/dl	75mg/dl	76mg/dl	25mg/dl	35mg/dl	38mg/dl
Leaves	T2	T2-14mg/dl	22mg/dl	42mg/dl	60mg/dl	18mg/dl	8mg/dl	24mg/dl
	T1	12mg/dl	35mg/dl	35mg/dl	14mg/dl	10mg/dl	9mg/dl	36mg/dl
Stem	T2	6mg/dl	18mg/dl	50mg/dl	75mg/dl	13mg/dl	10mg/dl	20mg/dl
Root	T1	13mg/dl	42mg/dl	25mg/dl	15mg/dl	16mg/dl	13mg/dl	26mg/dl
	T2	7mg/dl	22mg/dl	16mg/dl	16mg/dl	14mg/dl	8mg/dl	15mg/dl

Table 5 .The effect of tannery effluent on chlorophyll content of *Abelmoschus esculentus*. L (Ladies finger)

Treatment	Control	5%	10%	15%	20%	25%	100%
Chlorophyll(mg\g)	0.128	0.165	0.229	0.359	0.349	0.212	0.145

Table 6 .The effect of tannery effluent on Chromium accumulation of *Abelmoschus esculentus*. L (Ladies finger)

Samples after 80days	Chromium content						
	Control	5%	10%	15%	20%	25%	100%
Leaves	0.009	0.066	0.083	0.092	0.101	0.127	0.141
Stem	0.002	0.049	0.062	0.077	0.089	0.102	0.127
Root	0.012	0.081	0.105	0.121	0.144	0.153	0.191

irrigation purpose. EC value is (5160) msm-1. Total dissolved solids (TDS) values are 4828mg/liter respectively. BOD (biological oxygen demand) value is 330mg/liter; COD (chemical oxygen demand) value is 1560mg/liter. Chromium is142 ppm/litre, Dissolved oxygen 6.9.Microalgae exhibit phytoremediation capacity due to the presence of reactive groups with an active binding sites that complex with effluent resulting in flocculation which reduces the total solids in tannery effluent (Pandi *et al*. 2009)

Estimation of Protein: Protein in leaves and roots of (Table 4) *Abelmochus esculentus., sp* showed decreasing trend with increasing effluent concentrations (control, 5, 10, 15, 20, 25, and 100%) between 30 -80 days after sowing. At5% effluent concentration and between 30-80days, the values for protein in leaves, stem and roots are (40, 22, 35, 18, 42, 22) mg/dl; At 10% effluent concentration and between 30-80 days, the values are (75, 42, 35, 50, 25, 16) mg/dl; At 15% effluent concentration and between 30-80 days, the values are (76, 60, 14, 75, 15, 11) mg/dl; At 20% effluent concentrations 30-80 days, the protein values are (25, 18, 10, 13, 16, 14) mg/dl; At 25% effluent concentration and between 30-80 days, the protein values are (13, 5, 9, 10, 13, 8) mg/dl; At 100% effluent concentration and between 30-80 days, the protein values are (38, 24, 36, 20, 26,15) mg/dl.

Estimation of carbohydrate: Carbohydrates in leaves, stem and roots (Table 3) showed increasing trend with increasing effluent concentrations (5%, 10%,15%, 20%, 25%, 100%,) and between 30-80 days, At 5% effluent concentration and between 30-80 days, the values for carbohydrates in leaves, stem, and roots are (14, 75, 8, 30, 14, 80) mg/dl.

At 10% effluent concentration and between 30-80 days, the values of carbohydrates are (18, 9, 40, 24, 13, 22) md/dl; At 15% effluent concentration and between 30-80 days, the carbohydrates values are (14, 7, 4, 4.5, 10, 12) mg/dl; At 20% effluent concentration and between 30-80 days, the values of carbohydrates (7, 6, 7.5, 8, 6, 18) mg/dl; At 25% effluent concentration the values of carbohydrates (7, 7.5, 10, 7, 6, 7) mg/dl; At 100% effluent concentration the values of carbohydrates (36, 24, 6, 8, 2, 3) mg/dl.

Estimation of chlorophyll: Chlorophyll in leaves (Table 5) showed increasing trend with increasing effluent concentrations (5%, 10%,15%, 20%, 25%, 100%,) and between 30-80 days, At 15% effluent concentration and between 30-80 days, which was found rich amount of chlorophyll in leaves.

Conclusion

The present investigation clearly indicate that through Leather tannery effluent has stimulatory effect on all biochemical contents at lower concentration and have found that at higher concentrations they have deleterious effects. Plants growing in such type of polluted soil or irrigated water may accumulate these elements at high levels, and such plants or their product, if consumed, may have similar effects on live-stock or human beings. Proper care should be taken in disposal of leather tannery effluent to avoid soil pollution.

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