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Treatment of Tannery Effluent using *Lyngbya* sp. and Coir pith and application of treated effluent on Seed Germination of *Vigna radiata* L.

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ABSTRACT

A study was carried out using marine cyanobacterium Lyngbya sp. BDU 90901 and coir pith for analysis of physico-chemical parameters (pH, EC, OD, calcium, magnesium, chloride, *ammonia*, alkalinity, nitrate and nitrite) and Bio-chemical parameters (chlorophyll a and protein) in the treated tannery effluent. The treated tannery effluent was studied for seed germination of Vigna radiata L. at different concentrations. Results revealed that combined treatment of Lyngbya sp. with coir pith at dilution of 25% showed effective seed germination. Protein content was also estimated during germination period.

KEYWORDS Tannery effluent, cyanobacteria, decolourisation, seed germination

INTRODUCTION

The pollutants from the large number of tanneries have caused considerable damage in drinking water supply, irrigation and when allowed to stagnate gives rise to odour nuisance, unsightly appearance, creating water pollution (Baskar, 1992). The tannery industries in India (Vaniamvadi, Ambur, Trichy and Dindugul) process mostly goat or sheep skins while the tanneries in Ranipet, Pernampet, Erode and Pallavaram under take tanning of skin hides. The tannery industrial effluent contains several types of chemicals such as dispersants, and are mainly characterized by high salinity, high organic loading and specific pollutants such as chromium (Saritha *et al.*, 2013).

The coir pith contains high carbon to nitrogen (C/N) ratio, 75 to 186, together with 35% to 54% lignin content (Abad *et al.,* 2002). The spongy structure of coir pith facilitates retention of water (Neethi and Subramanian, 2006). The cyanobacteria can degrade the coir pith by using their lignin content (Henciya *et al.,* 2013).

Cyanobacteria are photosynthetic prokaryotes that capture sunlight for energy using chlorophyll *a* and various accessory pigments (Vincent, 2009). Cyanobacterial treatment method is a feasible technique for bioremediation of tannery effluents (Sonil *et al.*, 2010). Microbial bioremediation can be a cost competitive strategy and beneficial bio-resource for removing many hazardous contaminants from tannery and other industrial wastes (Garg *et al.*, 2012).

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; Selivanovskaya and Latypova, 2006; Yu and Gu, 2007; Giannis *et al.*, 2008). Hence, the present study was made to find the effects of tannery effluent, both treated and untreated on the germination of *Vigna radiata* L. seeds.

2. MATERIALS AND METHODS:

2.1. Samples collection

Tannery effluent was collected from a tannery industry located in Trichy, Tamilnadu, India. Coir pith was collected from coir industries near Sri Rangam, Tamilnadu, India.

2.2. Cyanobacterium

Marine cyanobacterial strain *Lyngbya* sp. 90901 BDU was obtained from the germplasm collections of National Facility for Marine Cyanobacteria (NFMC), Department of Marine Biotechnology, Bharathidasan University, Thiruchirappalli, Tamilnadu, India.

2.3. Inoculum Preparation

Marine Cyanobacterium (*Lyngbya* sp.) were grown and maintained in ASN III medium (Rippka *et al.*, 1979) respectively under white fluorescent light of 1500 lux at 25± 2^0C for light/dark cycle of 14/10 hrs.

2.4. Determination of Physiochemical parameters

All the parameters were determined based on standard methods (APHA, 1998). Color was assayed at wavelength 580nm by (Colorimetric method). The pH was determined by pH meter (Potential metric method), EC was measured by a conductivity meter (Rayment and Higginson, 1992), calcium, magnesium, alkalinity, ammonia, nitrate, nitrite and chloride concentrations were measured according to standard methods (APHA,1992). Protein has been estimated by (Lowry *et al.,* 1951) method.

2.5. Seed germination

Seeds of *Vigna radiata* were obtained from the local market and were surface sterilized in 3% (v/v) sodium hypochlorite for two minutes to avoid microbial contaminations. After that seeds were washed by running distilled water and placed on Petri dishes (100mm. Diameter) with a layer of filter paper (Whatman no. 1) wetted with 5ml of different working treated effluent concentrations with *Lyngbya* sp. and coir pith (viz. 25, 50, 75, 100% along with distilled water as control i.e 0% effluent. 10 seeds/petridishes were placed and kept

at room temperature for 72 hours (h) and the tests were performed in triplicates. The observations were made at an interval of 24 h to determine the germination percentage.

2.6. Calculations
Seed germination percentage at various treated effluent concentrations was calculated by using formula.

G = (n/N) ×100
G- Germination percentage, n- number of seeds germinated, N- Total number of seeds in each petri dishes.

2.7. Experimental set up
For individual treatments 0.6g dry weight of coir pith and 0.3g of wet weight *Lyngbya sp.* in 100 ml of tannery effluent separately and in combined treatment both *Lyngbya sp.* and coir pith were inoculated together and these were incubated for 25 days. Physiochemical parameters and colour reduction were carried out during the study and culture filtrate was applied on seed germination measurement at different concentrations.

2.8. Statistical analysis
The effect on each parameter were studied in triplicate and the data were graphically presented as the mean ± S.D. of triplicates (n = 3) and all the graphs have been prepared using Microsoft Excel 2007.

3. RESULTS AND DISCUSSSION
3.1. Estimation of colour reduction and

physiochemical parameters
Decolourising efficiency based on optical density reading was determined. Tannery effluent was decolourised more with cyanobacterium when compared with coir pith and combined treatment **(Fig. 1a).** UV spectral reading at the end of 30th day confirmed degradation of tannery effluent residues **(Fig. 1b).** The adsorption of colored particles may involve different mechanisms like active metabolism, passive transport or diffusion of adsorbed solute to the surface of the microbial cell (O' Mahong *et al*., 2002; Asku and Tezer 2000; Veglio and Beolchini, 1997).

Fig 1a. Effect on decolourising efficiency of Lyngbya sp. with coir pith in tannery effluent at 30th day

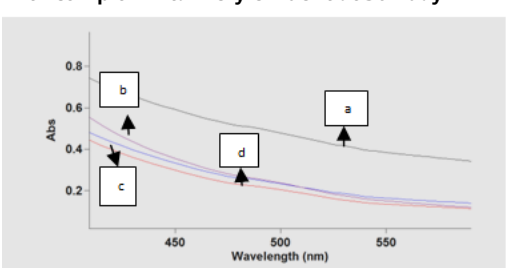
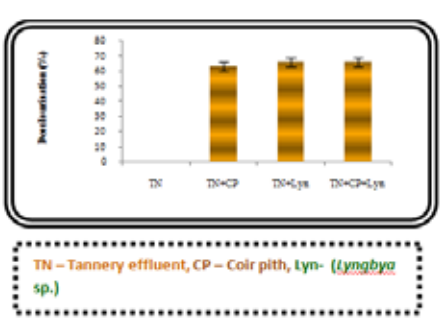


Fig 1b. UV spectrophotometric analysis at 30th day



Marine cyanobacterium *Lyngbya* sp. along with coir pith renders the properties such as adsorption of particles present in the dye, decolourisation of dye and degradation of coir pith (Jenny and Malliga, 2013). The other parametres such as EC, calcium, magnesium, nitrite, nitrate and chloride were also found to be decreased with all the treatments **(Fig 2-5).**

Fig 2. Effect on EC of Lyngbya sp. with coir pith in tannery effluent at 30th day

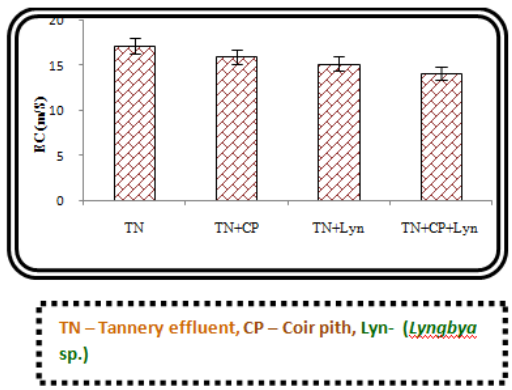


Fig. 3. Estimation of calcium, magnesium and alkalinity of tannery effluent treated with *Lyngbya* sp. and coir pith at 30th day

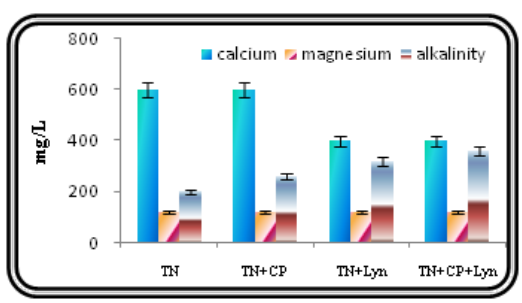


Fig 4. Estimation of Ammonia, Nitrate and nitrite of tannery effluent treated with Lyngbya sp. and coir pith at 30th day

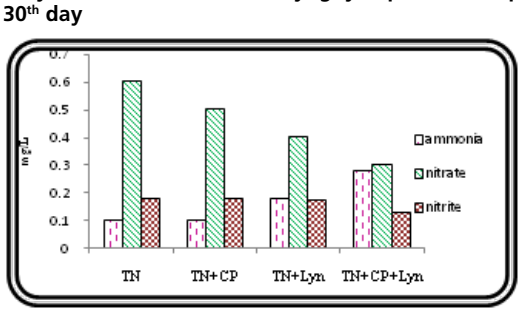
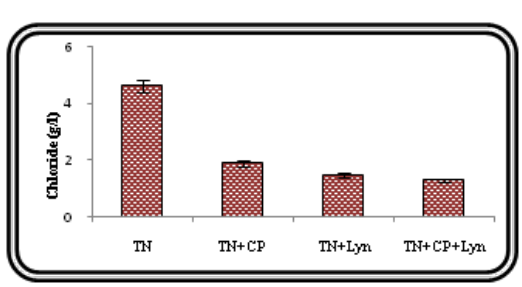


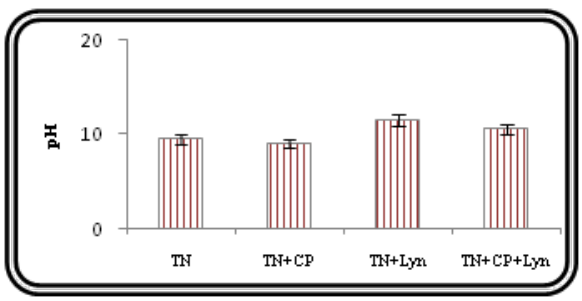
Fig 5. Estimation of chloride of tannery effluent treated with Lyngbya sp. and coir pith at 30th day



This might be due to the absorption of particles on the coir pith surface and consumption as nutrient source by the *Lyngbya* sp. Evidence given by Sonil *et al*, (2010) also states that the

calcium content was decreased when it was employed for the bioremediation of paper mill effluent. Similarly, magnesium content was found to be low in the physio chemical analysis of textile dye treated with *Lyngbya* sp. (Henciya *et al*., 2013). Most cyanobacteria can use nitrite and ammonia as primary N-sources, although urea and organic N-compounds can also be used in some cases (Flores and Herrero, 1994). The specific use of cyanobacteria in the efficient removal of combined nitrogen has also been reported (Chan *et al*, 1979, Shi Jing *et al*, 2007). Apte *et al*, (2011) have studied the suitability of *Parthenium* sp. as a sorbent for chloride removal and achieved 40% reduction with the dried biomass. On the contrast alkalinity, ammonia and pH were increased with *Lyngbya* sp. treatment and combined treatment when compared to control *(Fig 3, 4, 6*).

Fig 6. Effect on pH of Lyngbya sp. with coir pith in tannery effluent at 30th day

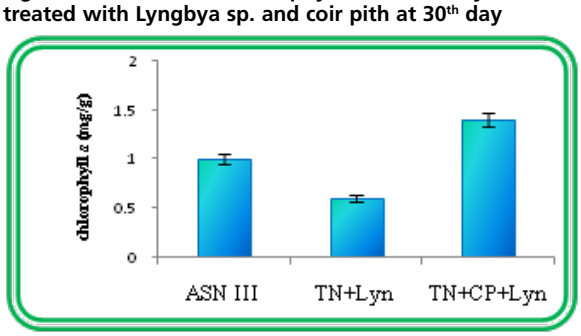


Carbon dioxide and bicarbonate removed from the water by the photosynthetic process of aquatic plants raises the pH. Reports confirmed pH of the water sample treated with *Chlorella vulgaris* and *Synechocystis salina* showed a drift towards alkalinity from 6.0 to 8.1 and pH has been changed from 6.0 to 7.9 in the water sample treated with *Gloeocapsa gelatinosa* (Dominic *et al*., 2009). Untreated tannery effluent showed higher level of electrical conductivity which could reflect the presence of organic and inorganic substances and salts that would have increased the conductivity (Marwaha *et al*., 1998; Krishna Priya, 2010).

3.2. Estimation of chlorophyll a

The chlorophyll *a* content was found to be higher in the combined treatment (coir pith with *Lyngbya* sp.) followed by individual treatment of *Lyngbya* sp. *(Fig 7).*

Fig 7. Estimation of chlorophyll a in tannery effluent treated with Lyngbya sp. and coir pith at 30th day

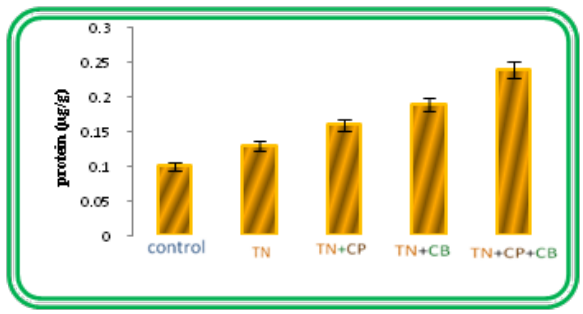


The treated tannery effluent enhances the growth of the inoculated *Lyngbya* sp. because, the presence of organic compounds in tannery effluent, which act as nutrient source and stimulates the growth of *Lyngbya* sp. Moreover addition of coir pith along with *Lyngbya* sp. gave luxuriant growth of cyanobacterium as these organisms are able to degrade the coir pith and hence utilize the available nutrients from the surroundings medium, there by showing profuse growth in biomass and therefore increased chlorophyll *a* content is evident. According to Henciya *et al.* (2013) chlorophyll *a* is an essential pigment which is present in cyanobacteria due to the activity of photosynthesis. It plays a significant role in electron transport system and this is an important factor for growth analysis.

3.3. Protein estimation in germinated Seeds

Protein content was reduced in the tannery effluent. Little increase of protein was found to be in tannery effluent by coir pith treatment followed by cyanobacterium treatment. High protein content was found to be in a combined treatment of coir pith with *Lyngbya* sp. *(Fig 8).*

Fig 8. Estimation of protein content in germinated seed of Vigna radiata L. in treated tannery effluent (72hrs).

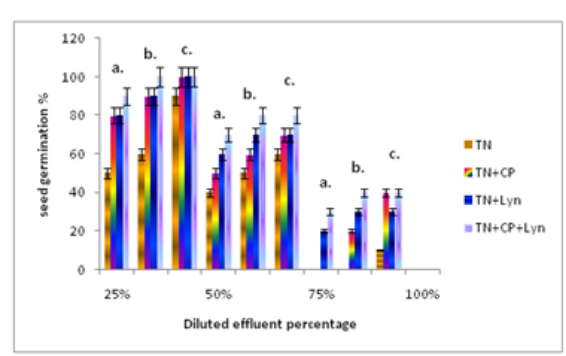


Germinated fenugreek (*Triogonella-foenum-graecum L.)* seed had significantly higher contents of total protein (29%) and total lysine (6.48/100gm protein) compared to unprocessed seeds. There was significant (P<0.05) increase in protein (25.90 to 29.00%) in germinated fenugreek (*Triogonella-foenum-graecum L.)* flour (Shalini and Sudesh, 2002). Germinated endosperm is rich in protein; whereas germinated seed coat is rich in dietary fiber, poly unsaturated fatty acids (PUFA) and mono saturated fatty acids (MUFA) content when compared to un germinated endosperm and seed coat respectively (Sathyanarayana *et al.,* 2011).

3.4. Seed germination

The extent of germination inhibition depends on treated tannery effluent concentration. Percentage of seed germination was found to be 100% with combined treatment of *Lyngbya* sp. and coir pith shell at concentration of 25% during 48th hrs. and 72 hrs. respectively. A close concurrence was noticed with the individual treatments using coir pith and *Lyngbya* sp. during 72 hrs. But at higher concentration of 50-100% reduction in germination percentage was exhibited *(Fig 9).*

Fig. 9. Seed germination of Vigna radiata L. seeds in tannery effluent treated with *Lyngbya* sp. and coir pith at different concentrations



TN – Tannery effluent, CP – Coir pith, Lyn- (*Lyngbya* sp.), a- 24th hrs., b- 48th hrs., c- 72 hrs.

This selective growth could be ascertained to growth promoting effect of low concentration of mineral elements present in the diluted effluent. Aklilu *et al*. (2012) conducted study to determine the effect of tannery effluent on seed germination of six different vegetable crops at 0, 25, 50, 75, and 100% concen-

trations of effluent. The results indicated that, application of effluent did not show any inhibitory effect on seed germination and other morphological parameters at low concentration except in onion, carrot and tomato which were found to be susceptible with 75 and 100%. Garg and Kaushik (2008) reported sorghum cultivars seed germination in 100% effluent, but, did not survive for longer period whereas, 6.25% effluent concentration did not show any inhibitory effect. Ramana *et al*, (2002) experimented to study the effect of different concentrations (0, 5, 10, 15, 20, 25, 50, 75 and 100%) of distillery effluent (raw spent wash) on seed germination percentage in some vegetable crops. In onion the germination was much higher at 10% concentration and the germination of kidney bean (*Phaseolus aureus*) and Lady's finger (*Abelmoschus esculentus*) seeds were affected adversely at 75% and 100% effluent concentrations compared to control (water). But, no progressive effect was evident upto 50% effluent concentration (Mohammad, 1985). Whereas, Bengal gram (*Cicer arietinum*) seeds germination was adversely affected even as low as 5% textile effluent concentrations (Dayama, 1987).

3.5. Effect on root and shoot length

The effect of untreated effluent showed slender variation on radical length and shoot length suggesting presence of essential organic compounds in the textile effluent supporting the growth of seedlings. The difference extended in treated effluent with coir pith and *Lyngbya* sp. evidently at 25% at 72 h but deleterious effect was initiated at higher concentrations (*Fig. 10 and 11*).

Fig. 10. Effect on radical length of Vigna radiata L. seeds in tannery effluent treated with *Lyngbya* sp. and coir pith at different concentrations

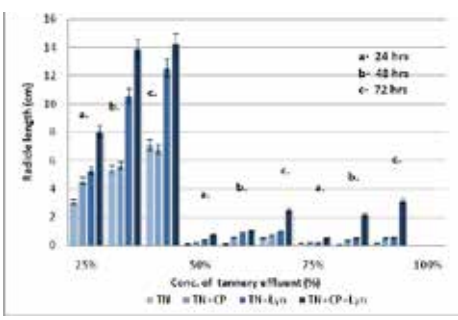
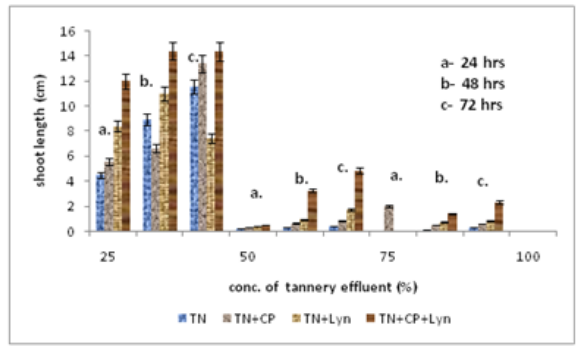


Fig. 11. Effect on shoot length of Vigna radiata L. seeds in tannery effluent treated with *Lyngbya* sp. and coir pith at different concentrations



TN – Tannery effluent, CP – Coir pith, Lyn- (*Lyngbya* sp.)

These results are in concurrence with the findings of Garg and Kaushik (2008) which indicated that the treated effluent had lesser detrimental effects on sorghum than untreated effluent. Jahidol *et al*, (2013) also observed highest value of shoot length and root length of country bean with the treatment of neutralization and 2nd wash after soaping of dyeing effluent compared to control.

CONCLUSION

The effect of tannery water is a challenging process to both tannery industry and the waste treatment. The result of this finding suggests a great potential of marine cyanobacterium *Lyngbya* sp. to be used to remove the turbidity from the tannery effluent. The main objective of seed germination study was to determine the seed germination promoting ability of *Vigna radiata* using treated tannery effluent by coir pith, cyanobacterium *(Lyngbya* sp.) and combined treatment. The seed germination percentage and protein content were highly improved in the combined treatment of tannery effluent by coir pith and cyanobacterium when compared to different concentration of individual treatment of coir pith, cyanobacterium (*Lyngbya* sp.) and tannery effluent.

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