

# Bioremediation of Tannery Effluent Using *Lyngbya* Sp. with Coir Pith

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**Abstract:** Aim of the investigation was to evaluate the treatment of tannery effluent using marine cyanobacterium (*Lyngbya* sp.) with coir pith. The physico-chemical parameters such as pH, OD, EC, BOD, COD, TDS, Cl<sub>2</sub> and removal of heavy metals such as Mercury, Cadmium, Iron, Copper and Lead contents were monitored on 30<sup>th</sup> day of incubation. The presence of metabolites released during degradation period was also analyzed by HPLC.

**Keywords:** Bioremediation, Coir pith, Heavy metals, *Lyngbya* sp.

## 1. Introduction

Tannery effluent is a major source of aquatic pollution in and around industries with high Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD) and hexavalent chromium. There are a large number of tanneries scattered all over the country. Chromium is one of the most toxic heavy metals, discharged into the environment through various industrial waste waters, such as leather tanning, electroplating, paints, pigment production and steel manufacture. The effluents of these industries contain chromium at concentrations ranging from tenths to hundreds of milligrams per litre (Sivaruban *et al.*, 2014).

Pollution of water resources is a serious and growing problem but, despite the existence of relevance legislation the pollution of the aquatic environment by toxic chemical continues to occur with domestic and industrial effluents being the main sources responsible for the contamination of aquatic environments (Claxton *et al.*, 1998; White and Ramussen, 1998).

Environmental contamination by heavy metals is a growing global problem, which is directly related to anthropogenic actions. For this motive, many techniques for environmental remediation of heavy metals are being studied (Ofer *et al.*, 2003; Bayramoglu *et al.*, 2006; Rai, 2008, 2010; Rawat *et al.*, 2011). Among these techniques, the application of microorganisms has been widely discussed, mainly in view of their capability to remove pollutants from aquatic environments with good efficiency and relatively low cost.

To survive under metal – stressed conditions, bacteria have evolved several types of mechanisms to tolerate and uptake of heavy metal ions. These mechanisms include the effect of metal ions outside the cell and reduction of the heavy metal ions to a less toxic state (Niles, 1999; Spain, 2003). Cyanobacteria and coir pith in the tannery effluent would be an ideal absorbent/adsorbent to remove heavy metals and other minerals. Hence, the present study is to investigate the growth of cyanobacterium along with coir pith and decolorization / degradation of tannery effluent.

## 2. Materials and Methods

### Collection and characterization of tannery effluent

Tannery effluent sample was obtained from a tanning industry in Sempattu near Bharathidasan University, Tiruchirappalli, Tamil Nadu, India.

### Organism and Source

Cyanobacterial strain *Lyngbya* sp. was obtained from the germplasm collections of National Facility for Marine Cyanobacteria (NFMCC), Department of Marine Biotechnology, Bharathidasan University, Tiruchirappalli, Tamil Nadu, India.

### Experimental set up

For individual treatments 0.5gm dry weight of coir pith and 0.3gm 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 30 days. Physicochemical parameters, color reduction and heavy metals were carried out during the study period.

### Determination of pH

pH of the sample was determined using pH meter which has been initially standardized by buffer solution of known value before analysis.

### Determination of EC

EC of the sample was determined using conductivity meter (Rayment and Higginson, 1992).

### Determination of physico-chemical parameters

The color was assayed at wavelength 580nm by (Colorimetric method), BOD, COD and Cl<sub>2</sub> concentrations were measured according to standard methods (APHA, 1992) and total dissolved solids (TDS) using filtration method (Valentine, 1996) was also analyzed.

### Estimation of heavy metals

Estimation of mercury, lead, cadmium, chromium, Iron, and copper has been carried out by (AOAC method, 2000). Separation of compounds using HPLC

The HPLC analysis (Water model no. 2690, USA) the treated samples were dried with control and subjected to HPLC (High Performance Liquid Chromatography) with C<sub>18</sub> column (symmetry, 4.6x 250nm) to find out the compounds present in it. The coir pith, cyanobacterium and combination of coir pith with cyanobacterium were allowed to dry under shade after incubation of 30 days. Then the dried pellets (1:10) were dissolved with HPLC grade methanol, each sample was centrifuged separately and 10ml of supernatant was extracted with equal volume of methanol. Then the compounds were injected in HPLC at 25°C, Eluent-acetonitrile: water (80:20v/v) flow rate was 20µl for 10 min. (Senan *et al.*, 2004).

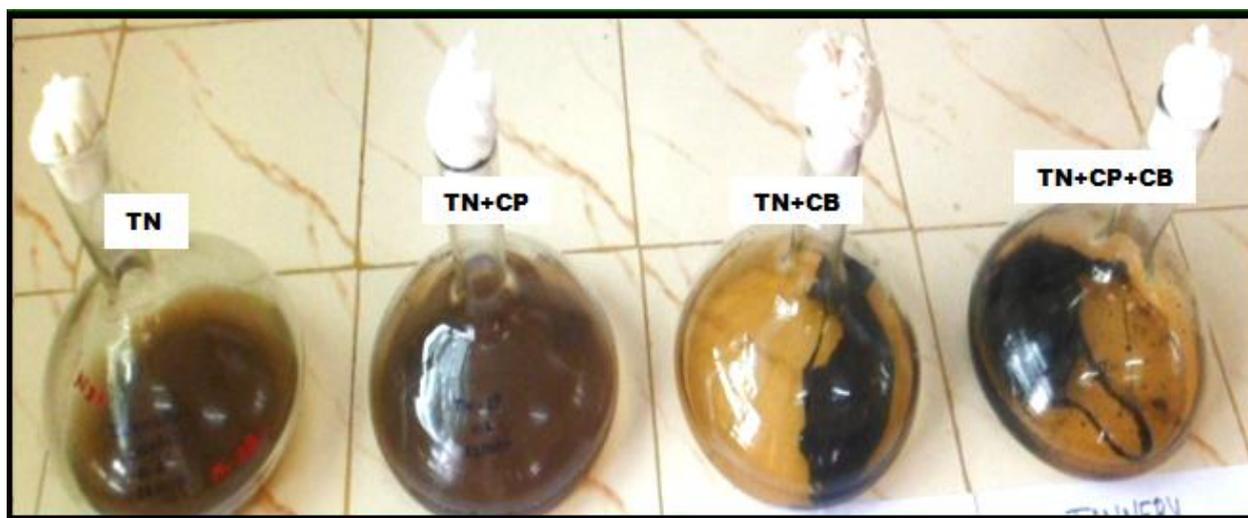
### 3. Results and Discussion

Effluent samples were analyzed for the color reduction before and after treatment with coir pith and cyanobacterium individually as well as in combinations. Plate-1 showed comparisons between all the treatments on 30<sup>th</sup> day observation. A change in color of the effluent was an initial indication of decolorization period. The initial effluent was dark brown in color and on 30<sup>th</sup> day treatment with coir pith exhibited pale brown in color. *Lyngbya* sp. treated effluent showed pale green in color, whereas, slight pale yellow

color was observed in combined treatment (coir pith with *Lyngbya* sp.). This could be as a result of individual and combined action of coir pith and cyanobacterium during treatment process. Investigation revealed that the tannery effluent was grey in color with disagreeable odour, which may be due to derivation of effluent from tannery sub process such as bathing, pickling, neutralization and dying of the treated skin (Panneerselvam *et al.*, 2003).

The color of industrial sample is blackish before degradation but after degradation using native *E. coli* and in non-native *Bacillus* sp. there is a color change from blackish to light brown ( native *E. coli*) and black to color less of the industrial effluent (Noorjahan *et al.*, 2014). The cyanobacterium *Lyngbya* sp. exhibited the higher percentage of decolorization under 1500 lux photoperiod, when compared to coir pith individual treatment (Henciya *et al.*, 2013). Combined treatment showed slightly brown in color since degradation of coir pith by *Lyngbya* sp. and release of degradative compounds to the surrounding medium. Shanmugapriya and Malliga (2013) reported that dye particles easily absorbed by the addition of coir pith and it showed reduction in optical density of the treated effluent on 14<sup>th</sup> day. Maximum decolorization percentage was observed in treatment with *Lyngbya* sp. alone due to adsorption and absorption of color residues by the filaments of cyanobacterium *Lyngbya* sp.

**Plate-1.** Effect of coir pith and *Lyngbya* sp. in tannery effluent on 30<sup>th</sup> day



TN = Tannery effluent; CP = coir pith; CB = cyanobacterium

Ramalingam and Suniti (2010) reported color generally; within the collection tank it is brown in color upon addition of the activated sludge (sent back from setting tank). It gets a thicker texture and acquires dark brown in color. The color is usually the first contaminant to be recognized in the waste waters that affects the aesthetics, water transparency and gets solubility of water bodies (Yuxing and Gain, 1999). Cyanobacterial interaction with tannery effluents effectively decreased their color intensity (Nantha *et al.*, 2010).

The pH of tannery effluent was alkaline in nature, but reduced with only coir pith treatment. With cyanobacterium as well as combined treatment pH levels were increased and

obstinated in alkaline condition (Fig. 1). Reports confirmed that the lower pH of the effluent indicates the acidic nature of the effluent. The pH of waste water color varies due to the presence of various tanning and coloring materials. This acidic pH of the effluent could affect the biological property on the receiving water body (Lalita *et al.*, 2010). Cyanobacteria during growth significantly increase the H<sup>+</sup> ions concentration which is directly proportional to pH level (Nanthini *et al.*, 2014). Srikumaran *et al.* (2011) reported that the pH plays a very critical role in microbial metal uptake by influencing the metal speciation and solution chemistry as well as surface properties of bacterial cells. The initial pH of waste water was 7.41, when the waste water

was treated with *Chlorella vulgaris* and *Scenedesmus quadricauda* the pH was increased as compared to control (Ayodhya and Kshirsagar, 2013).

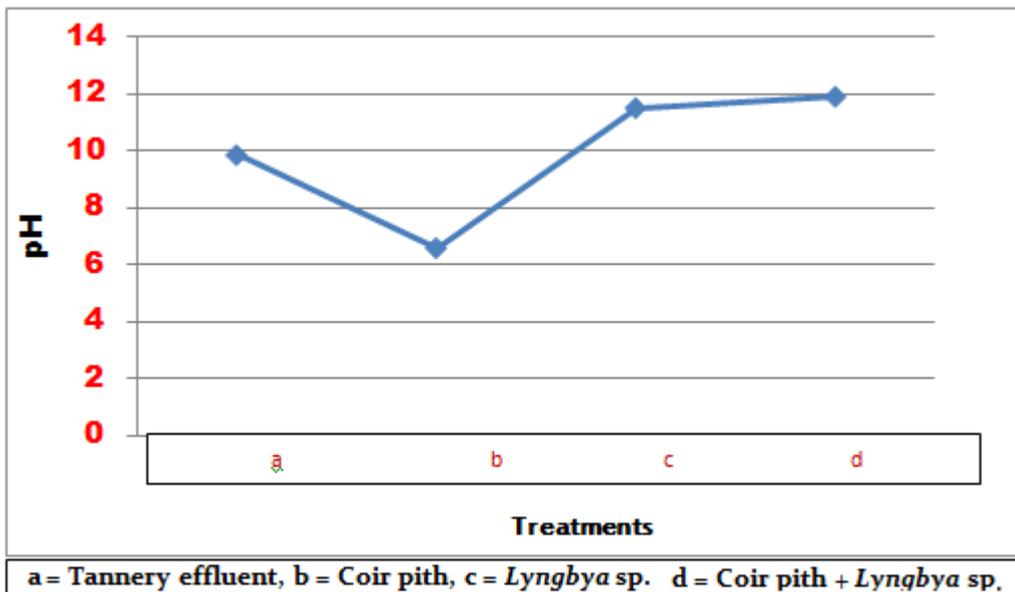


Figure 1: Effect of *Lyngbya* sp. with coir pith on pH content of tannery effluent

This correlates with the current study as evident in both treatments such as *Lyngbya* sp. as well as combined treatments. Tannery effluents were initially characterized with a high electrical conductivity and after treatment a satisfactorily decrease on 30<sup>th</sup> day was noticed in all the treatments, however maximum reduction was observed in combined treatment (Fig. 2.) Supporting evidences showed that the high electrical conductivity does not affect

cyanobacterial activity during bioremediation of tannery effluent (Nantha *et al.*, 2010). High level of conductivity may be due to the presence of inorganic substances and salts (Robinson and Strokes, 1959). According to Kataria and Jain (1995), high electrical conductivity level may be due to high concentration of acid – base and salt water.

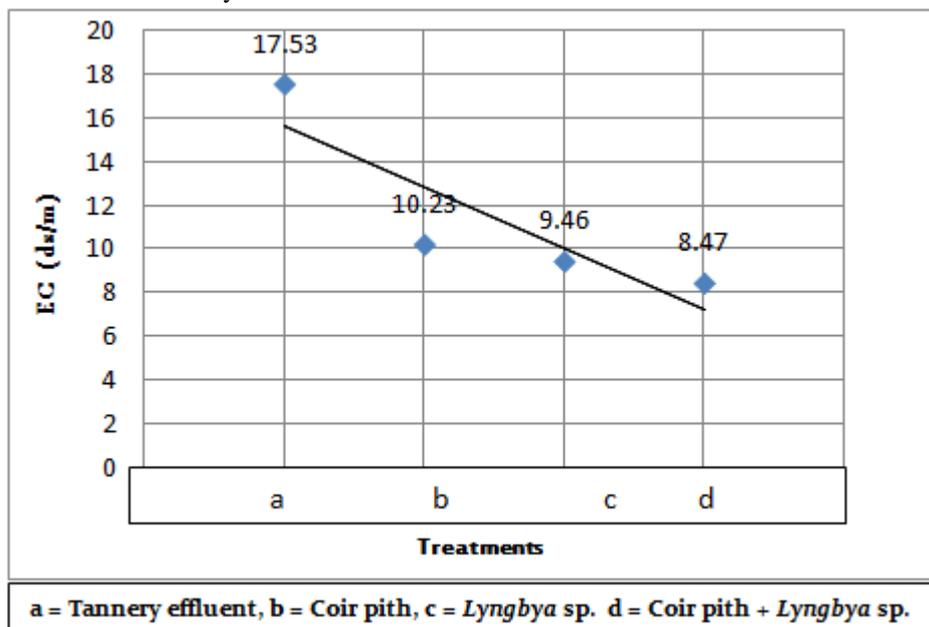


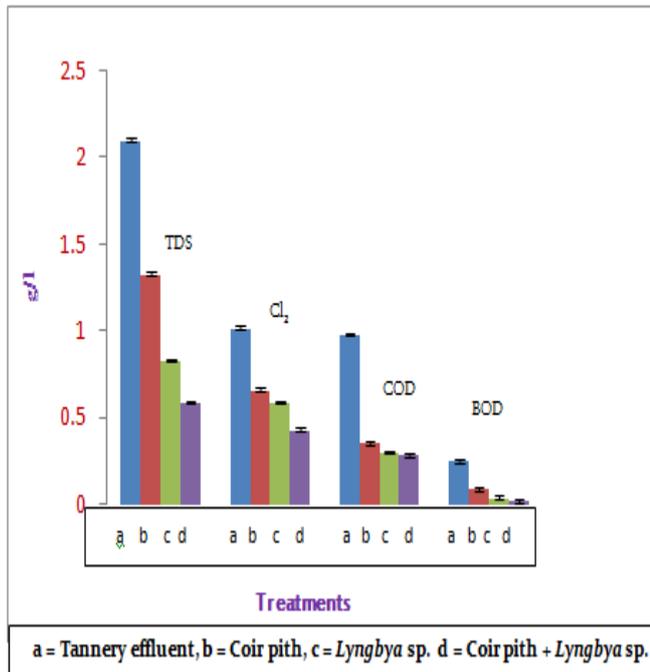
Figure 2: Effect of *Lyngbya* sp. with coir pith on EC of tannery effluent.

The chloride, BOD, COD and TDS content were found to be reduced in all the treatments but significant reduction was observed only with the combined treatment (Fig. 3). Seethalakshmi, (2004) studied on the absorption of chloride using microorganisms. The tannery effluents are the natural habitat of the microorganisms. Inoculations of the same, after enrichment in to the effluent, the growth of microbes were flourished and subsequent degradation was observed.

In *Parthinium* sp. dried biomass is capable of achieving up to 40 % reduction in chloride content at lab scale (Apte *et al.*, 2011).

In combined application as well as in individual treatment with *Lyngbya* sp. growth was more since it uptake nutrition, from tannery effluent thereby reducing in BOD and COD. The BOD show the decreased rate in the treated effluent of

cyanobacterium with coir pith compared to other individual treatments (Henciya *et al.*, 2013). Duangporn *et al.*, (2005) identified the reduction of biological oxygen demand in tannery effluents from 4967 mg/ml to 1010 mg/ml after inoculation of *Rhodospseudomonas blastica*. *Aspergillus* sp. was used at various concentrations to treat the effluent. Among that while 50% inoculum was given there was a decrease in the value of BOD 252 mg/l to 45 mg/l, COD 512 mg/l to 304 mg/l and chlorides 36.92 mg/l to 25.28 mg/l (Subramani *et al.*, 2012). Reduction of COD from 7328 mg/l to 3371 mg/l in tannery effluents was also noticed after inoculation of *Enterococcus casseliflavus* (Saranraj *et al.*, 2010).



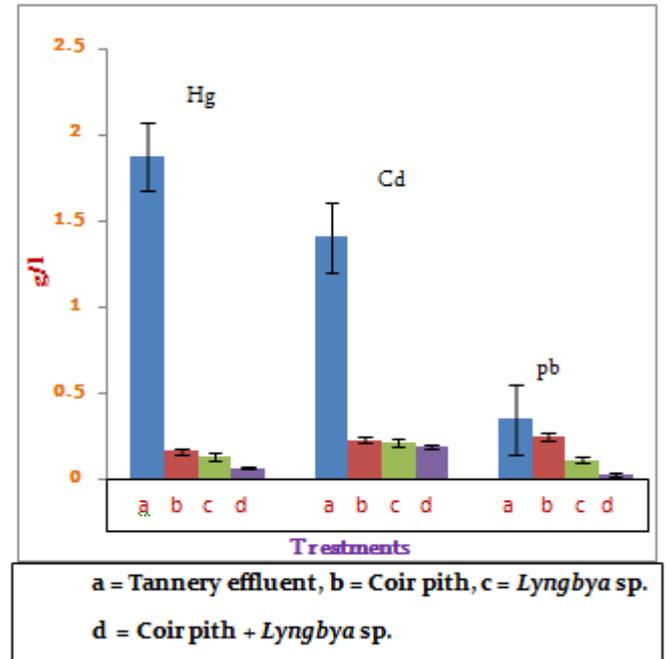
**Figure 3:** Effect of *Lyngbya* sp. with coir pith on TDS, Cl<sub>2</sub>, BOD and COD content of tannery effluent.

High levels of COD in the tannery effluent indicates that the effluent is not suitable for the existence of the aquatic organisms, due to the reduction in the dissolved oxygen content (Deepa *et al.*, 2011). *Botryosphaeria rhodina* was inoculated and after five days under agitation at 180 rpm at 28°C the COD was reduced by 91% (Maria *et al.*, 2011).

High levels of total suspended solids present in tannery effluents could be attributed to their accumulation during the processing of finished leather. TDS concentration also decreased from 25264 mg/l to 20788 mg/l (17.72% removal) by the extend aeration period of 24 hours (Khan, 2005). Presence of total suspended solids in water leads to turbidity resulting in poor photosynthetic activity in the aquatic system (Coal, 2000). Mythili and Karthikeyan (2011) reported TDS (406 mg/l), BOD (1260 mg/l) and COD (2035 mg/l) in the treated effluent to be reduced.

All heavy metals were found to be reduced with all the treatments notably with the combined treatment of *Lyngbya* sp. with coir pith in tannery effluent due to absorption of heavy metal particles on the surface of *Lyngbya* sp. and coir pith (Fig. 4 and 5). Cyanobacteria are present in environments that are polluted by heavy metals and have

been the object of research in to metals biosorption (Philippis *et al.*, 2003) Selvi *et al.* (2012) have also reported higher reduction of Lead (90%), Copper (85%), Zinc (80%), Chromium (70%) and Mercury (80%) from tannery effluent using isolated strains of *Pseudomonas* sp.

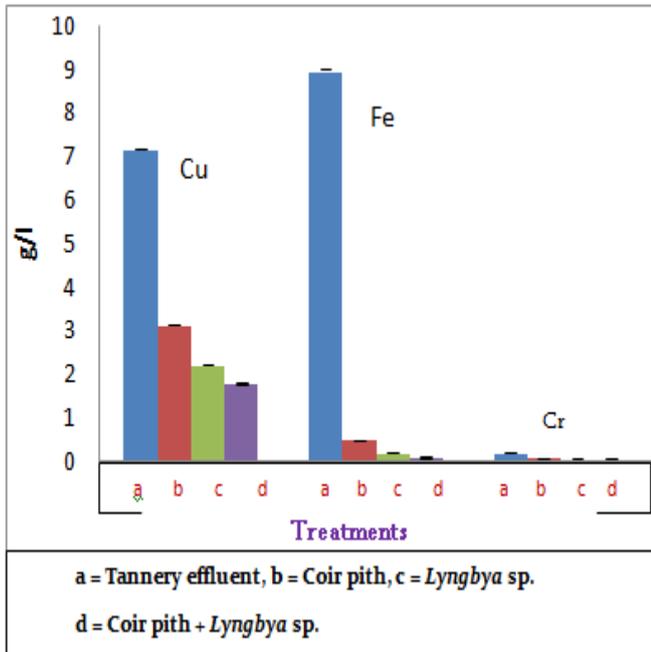


**Figure 4:** Effect of *Lyngbya* sp. with coir pith on Hg, Cd, and Pb contents of tannery effluent.

Surya *et al.* (2013) reported that isolated strains of *Enterobacter cloacae* AB6 reduced Lead 65- 68% at 200 mg/l and Copper 74.46% at 300mg l<sup>-1</sup> of initial metal ion concentration of these optimized conditions. Srivastava *et al.* (2013) reported that various kinds of biosorbents have been used including bacteria, yeast, fungi and algae. Fungi species such as *Trichoderma* sp. *Aspergillus niger*, *A. fumigatus*, *Rhizopus arrhizus*, *Penicillium crysogenum* and *Streptomyces pimprina* etc, have been tested for uptake of metals.

Kumar *et al.* (2010) reported lower reduction of heavy metals with acclimated bacterial strains that were used to remediate the waste by biosorption process in a Batch culture. *Pseudomonas* sp. and *Bacillus* sp reduced at (68% / 56%) and Ni (65% / 48%). Earlier studies done by Vijayaraghavan and Yun (2008) and Suryan and Ahulwali (2012) on removal of heavy metals revealed that biomass is the key factor, which affects the biosorption process for heavy metal concentrations. In the current study, biomass increases with the combined treatment. Oves *et al.* (2013) reported that *Bacillus thuringiensis* strain OSM29 showed an obvious removing potential for Cu, Pb and Ni. *Aspergillus*, *Penicillium*, *Alternaria*, *Geotrichum*, *Fusarium*, *Rhizopus*, *Monilia* and *Trichoderma* were found resistance to Cadmium (Zafar *et al.*, 2007). From the study it is evident that in the cell suspension technique, using *Brevibacterium casei* showed 84% of Chromium (VI) reduction within 48 hours (Venkatesaperumal *et al.*, 2011). The results indicated that the biomass of *Spirogyra* sp. is suitable for the development of efficient biosorbent for the removal and recovery of Mercury from wastewater (Razee *et al.*, 2006).

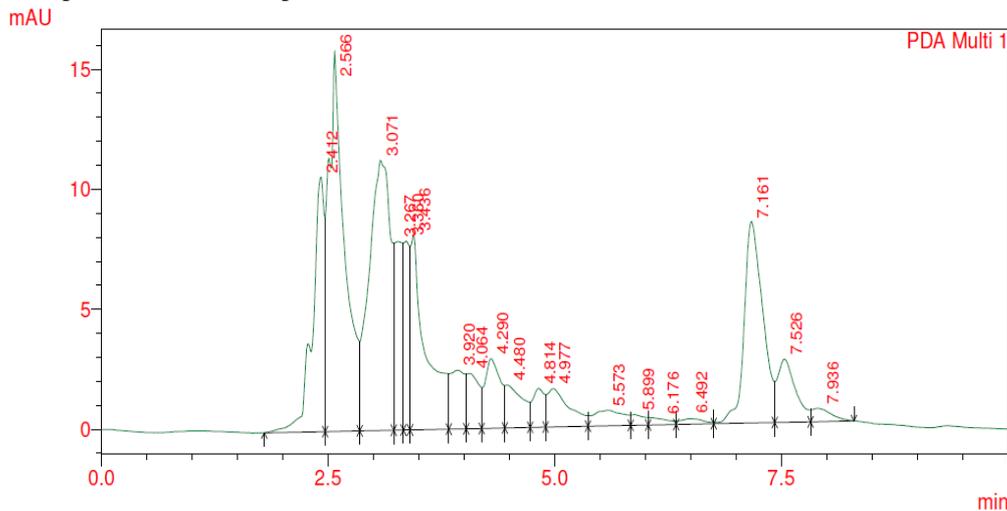
Rani *et al.* (2010) reported that biosorption of immobilized cells had potential for reduction of different heavy metals from electroplating industrial effluent samples using different strains of bacteria such as *Bacillus sp.* for Cd (90.4%) and Cu (69.34%), *Pseudomonas sp.* for Cd (90.4%) and *Micrococcus sp.* for Pb (84.27%). Selvi *et al.* (2012) have also reported higher reduction of Pb (90%), Cu (85%), Zn (80%), Cr (70%) and Hg (80%) from tannery effluent using isolated strains of *Pseudomonas sp.* An unicellular cyanobacterium *Synechococcus sp.* had been showed to have the ability of binding metal ion lead (Yee *et al.*, 2004)



**Figure 5:** Effect of *Lyngbya sp.* with coir pith on Cu, Fe, and Cr content of tannery effluent.

**HPLC Analysis**

HPLC (High Performance Liquid Chromatography) analysis depicts that raw tannery industry effluent exhibited 19 number of peaks (Fig. 6). After decolorization of Tannery effluent with coir pith 15 number of peaks were obtained



**Figure 6:** Tannery Effluent

(Fig. 7). This might be due to the adsorption of tannery effluent particles to the surface of coir pith. Very less number (12) of peaks were present in the Tannery Effluent with cyanobacterium treatment (Fig. 8). This could be due to the absorption and adsorption of tannery residues on to the filaments of *Lyngbya sp.* and also showed profused growth of cyanobacterium and this could also be the reason behind obtaining the number of peaks when compared to tannery control and all other treatments. Approximately 14 numbers of peaks were analyzed in the tannery effluent treated with cyanobacterium with coir pith (Fig. 9) and (Plate-1). Most evidently it is lesser number of peaks when compared to control and coir pith treated effluent but more when compared to the cyanobacterial treatment. This can be well explained as degradation of coir pith by *Lyngbya sp.* could release some intermediate compounds in the effluent which could increase the number of observed peaks.

Supporting evidences showed that peak of TECHQ was increased in 3<sup>rd</sup> day sample and subsequently decreased in 7<sup>th</sup> day sample, indicating conversion of PCP in Tetrachloroquinone (TECHQ) (Indu and Srivastava, 2011). Cyanobacteria are capable of abating various kinds of pollutants and have advantages as potential biodegradation organism (Subramanian and Uma, 1996). These organisms degrade various aromatic hydrocarbons and are useful for metal removal from polluted water (Cerniglia *et al.*, 1980). The degradation of phenol by the marine cyanobacteria *Phormidium valaderianum* was reported by Shashirekha *et al.* (1997). Anantharaj *et al.* (2012) the spectral results showed that the selected strain *Oscillatoria annae* has the ability to degrade the lignocellulosic waste coir pith within a short period of incubation (7 days). The higher degradation rate of lignin, when compared to the holocellulose by cyanobacteria may be due to photoautotrophic nature of the organism (Viswajith *et al.*, 2009). In HPLC analysis the reduction of IAA area (purity) of the extract compared to the standard chemical should be due to the presence of other natural compounds from cyanobacterial biomass (Varalakshmi *et al.*, 2011)

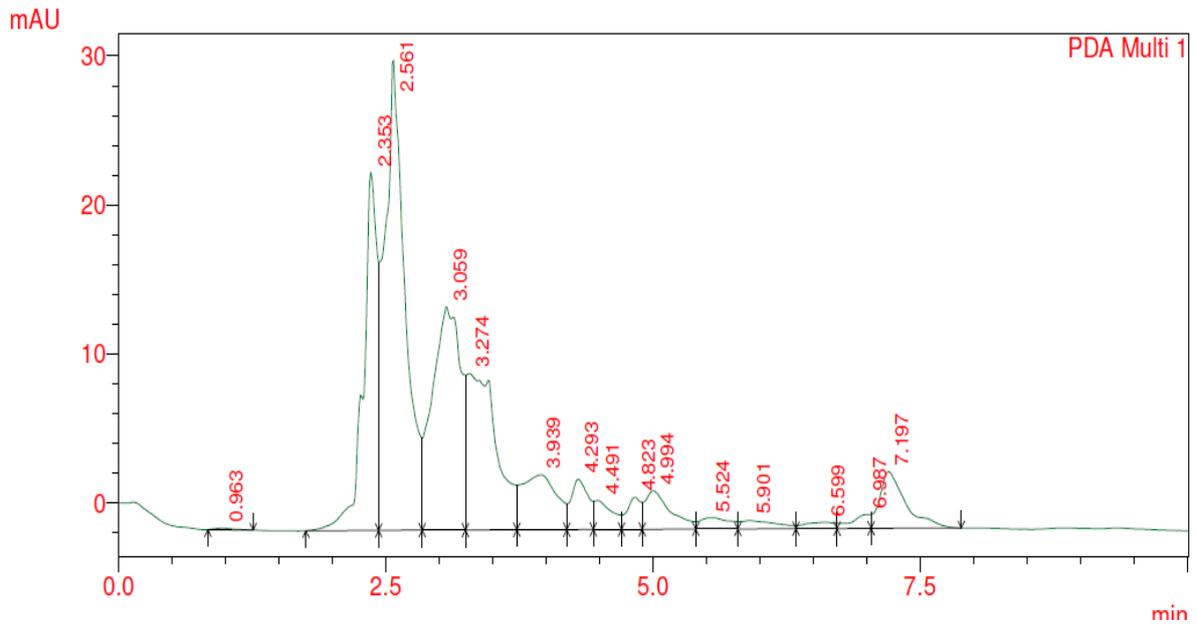


Figure 7: Tannery Effluent + Coir Pith

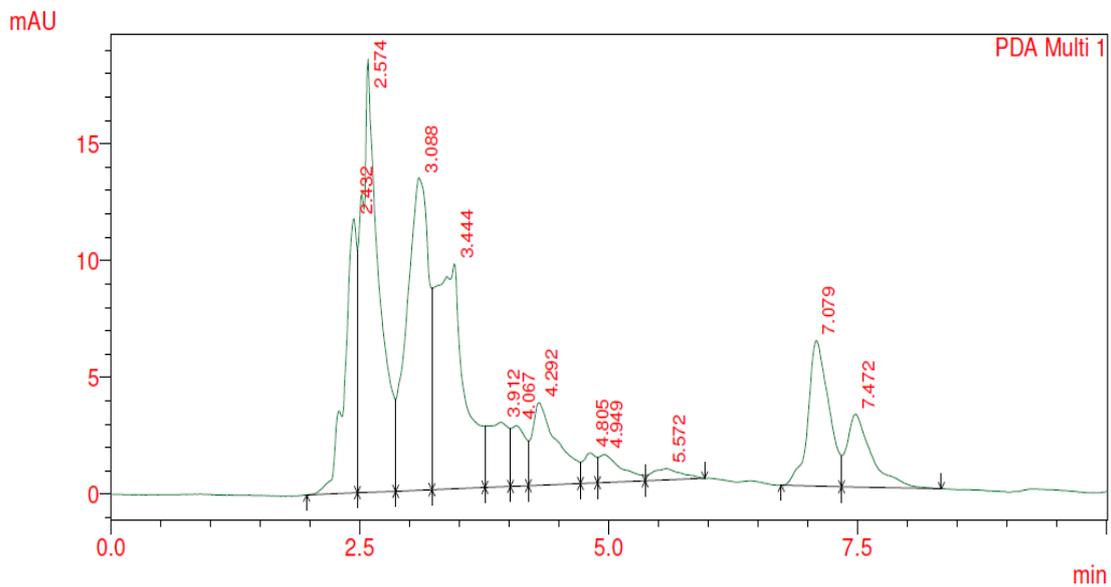


Figure 8: Tannery Effluent + *Lyngbya* sp.

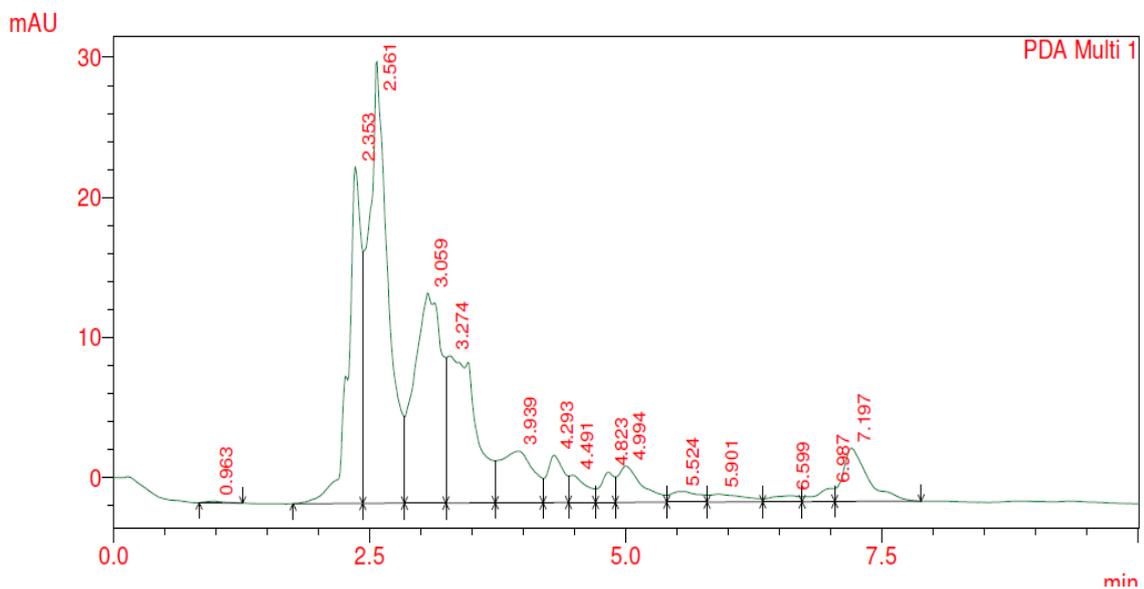


Figure 9: Tannery Effluent + coir pith + *Lyngbya* sp.

#### 4. Conclusion

From this study it was clear that the *Lyngbya* sp. with coir pith showed the best reduction capacity of all physicochemical parameters. The results indicate that cyanobacterial treatment method is a feasible technique for bioremediation of tannery effluent. Therefore, it was found that the remediation using *Lyngbya* sp. with coir pith provides an effective and environmentally acceptable option for waste water remediation.

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