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EFFICACY OF MARINE CYANOBACTERIUM *LYNGBYA* SP. 90901 WITH GROUND NUT SHELL IN TEXTILE DYE INDUSTRY EFFLUENT

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ABSTRACT

Marine cyanobacterium *Lyngbya* sp. was selected to check its bioremediation ability in textile dye effluent. Ground nut shell was used as an adsorbent in the current study. The biochemical parameters like Nitrate, Nitrite, EC, TDS, Ammonia, Alkalinity, Calcium, and Magnesium were studied. The parameter analysis was carried at end of 60th day of incubation for the future application of degraded products on plants. 77.55% decolourisation was attained in the treatment of textile effluent with *Lyngbya* sp.

The heavy metals like Zinc, Mercury, Nickel, Cadmium, Chromium and Iron present in textile Effluent was found to be decreased as it was adsorbed on the filament surface of *Lyngbya* sp. The presence of metabolites released during degradation was also analyzed by FTIR.

KEYWORDS: Textile Effluent, *Lyngbya* Sp., Decolourisation, Agricultural Waste, Heavy Metals

INTRODUCTION

It is estimated that 10,000 different types of dyes and pigments are produced worldwide annually (**Walker and Weatherly, 1997**). Synthetic textile dyes used each year are lost during manufacture and processing operation and 20% of these dyes enter the environment through effluents that result from the treatment of residual industrial waters. Waste water from printing and dyeing units is often rich in color, containing residues of reactive dyes and chemicals, and requires proper treatment before being released into the environment.

Interest in ecologically friendly, wet-processing textile techniques has increased in recent years because of increased awareness of environmental issues throughout the world. Consumers in developed countries are demanding biodegradable and ecologically friendly textiles (**Chavan, 2001**). Metals found as integral parts of the dye chromophores (e.g., phthalocyanine) comprise mainly cobalt, copper, and chromium. However, some dyes have low-level metal impurities that are present incidentally, rather than necessity in terms of functionality and color.

When mercury based compounds are used as catalysts in dye manufacturing, there is a possibility of its presence as trace residue. Very few (e.g., only 2% commercial direct dyes) have metal as an integral part of the dye chromophore (**Al-Ghouti *et al.*, 2003; Andrady, 2003**). Unless textile effluent is treated properly, as a result of extensive use of dyes and pigments throughout the world, toxic metals associated with the dyes and pigments inevitably reach to aquatic environments, and pose serious threats to aquatic lives and the system (**Waranusantigul *et al.*, 2003**).

Microalgae, the primary producers of the aquatic food chains have been found to be very effective in removing pollutants from wastewater. Although microalgae have been successfully used as biomass for wastewater treatment systems because of photoautotrophic growth properties, very few have been investigated to determine their dye removal abilities **(Shah *et al.*, 2001).** Previous studies on removing pollutant by microalgae were mainly focused on heavy metal removal (**Malik, 2004)**. Hence, the present study explores treatment of textile dye industry effluent with *Lyngbya* sp. and cheap available agrowaste (ground nut shell) and application of treated effluent on the seed germination.

MATERIALS AND METHODS

Effluent Collection

A textile effluent containing water soluble dyes was collected from Karur district, Tamil nadu, India to address the present study.

Cyanobacterium and Culture Conditions

The strain of *Lyngbya* sp. BDU 90901 was obtained from the germplasm of National Facility for Marine Cyanobacteria (NFMC), Bharathidasan University, Tiruchirappalli, Tamilnadu, India, previously maintained in Artificial Sea Nutrient (ASNIII) medium (**Rippka *et al*., 1979**). The culture was maintained at temperature of 27±2˚C and illuminated with day light fluorescent tubes under 1500 lux with light/dark cycle of 14/10 hrs.

Adsorbent Used

Locally sourced ground nut shell was washed, dried and crushed into fine powder of mesh size 2mm**.**

Physico-Chemico Analysis

The physico-chemical parameters were determined using analytical methods **(APHA, 1989).** The parameters such as OD, pH, Nitrate, Nitrite, Alkalinity, Calcium, Magnesium, EC, Ammonia, and TDS were estimated to analyze bioremediation efficiency of cyanobacterium. Protein **(Lowry *et al.*, 1951)** and Carbohydrates **(Hedge and Hofreiter, 1962)** were estimated in the seeds treated with effluent. The colour intensity was measured at maxima absorption of 580 nm.

Heavy Metal Analysis

Heavy metals like Zinc, Cadmium, Nickel, Mercury, Copper and Chromium were determined in the treated textile effluent supernatant (culture filtrate) obtained from the degradation process by **AOAC, (2000)** method and the presence of degradative compounds were analyzed in the pellet using FTIR.

Analytical Procedure

The treated biomass was removed after decolourisation by centrifugation (5000 rpm for 15 min at room temperature) dried and the sample (KBr: Sample, 5:95) was finely ground and fixed in the sample holder of FTIR. Fourier transform infrared spectroscopy (FTIR) analysis was done in the mid IR region of 400-4000 cm^{-1} with 16 scan speed.

Statistical Analysis

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

RESULTS AND DISCUSSIONS

Decolourisation

Decolourising efficiency based on optical density reading was determined at the end of 60th day treatment. Textile effluent control did not demonstrate any colour change on 60th day, whereas, textile effluent treated with ground nut shell showed declourisation (Figure 1). Adsorption of dye particles on the surface of ground nut shell may lead to decolourisation of textile effluent. **Shanmugapriya and Malliga** (**2013**) reported that dye particles get easily adsorbed by the addition of coir pith and it showed reduction in optical density of the treated effluent on 14th day**.** Maximum decolourisation percentage was observed in treatment with *Lyngbya* sp. alone due to adsorption and absorption of colour residues by the filaments of cyanobacterium *Lyngbya* sp. when compared to ground nut shell treatment (Figure 1).

The adsorption of coloured 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; Aksu and Tezer, 2000; Veglio and Beolchini, 1997)**. **Vijayakumar and Manoharan (2012)** investigated the efficiency of cyanobacteria on the removal of textile dye industry effluents and reported that both in *Oscillatoria* and *Westiellopsis* and reduction in colour from the dye effluent in both free and immobilized conditions significantly. **Koushar *et al*, (2000)** reported that *Aspergillus niger* and *Trichoderma viride* proved to be efficient in decolourisation of scarlet red upto 80%.

Marine cyanobacterium *Lyngbya* sp. along with coir pith renders the properties such as adsorption of particles present in the dye, decolourization of the dye and degradation of coir pith **(Jenny and Malliga, 2013).**

Effect on pH

The initial pH of textile dye effluent was alkaline which was slightly reduced with ground nut shell treatment but augmented with all other treatments and with all the treatments the pH obstinated in alkaline condition itself (Figure 2).

Cyanobacteria during growth significantly increase the H^+ ions concentration which is directly proportional to pH level. Supporting evidence showed that the *Phormidium valderianum* could remove more than 90% textile dyes acid red 119 and direct black 115 from the solution in the pH range higher than pH 11 **(Shah *et al*., 2001).**

Estimation of Physico-Chemical Parameters

There were no transformations in physico-chemical parameters in textile effluent control on 60th day while ground nut shell treated effluent showed minor reductions in all parameters such as Nitrate, Nitrite, Ammonia (Figure 3), Alkalinity, Calcium, Magnesium and TDS (Figure 4).

Evidence given by **Sonilnanda *et al*, (2010)** also states that the calcium content was decreased when it was treated with *Nostoc* which was employed for the bioremediation of paper mill effluent. Similarly, magnesium content was found to be low where the decolourisation and physico chemical analysis of textile azo dye with *Bacillus* Sp. was also carried out **(Arul Jayan *et al*., 2011).**

The TDS in wastewater shows pollution strength which may result into alteration in taste or odors. TDS in wastewater to be used for agriculture activity may be as high as 1850 - 2000 mg/l, as but not more than this limit **(Kumar, 1989).** Though in the current study TDS of textile effluent does not exceeded the permissible limit but it was notably reduced by the treatments.

Almost all the physico- chemical parameters exhibited reduction on 60th day significantly with *Lyngbya* sp. as well as *Lyngbya* sp. with groundnut shell treatment when compared to ground nut shell treatment alone. This can be explained as intake of nutrients present in textile by the cyanobacterium for their growth and also adsorption of some particles by ground nut shell. Most cyanobacteria can use nitrate, nitrite, and ammonia as primary N-sources, although urea and organic N-compounds can also be used in some cases **(Flores and Herrero, 1994)**

Estimation of EC

After 60 days EC was consequently increased with *Lyngbya* sp. and combination of *Lyngbya* sp. with ground nut shell when compared to all other treatments (Figure 5). This might be due to the degradation of textile effluent as well as ground nut shell by *Lyngbya* sp. which resulted in dissociation of certain ions into the culture filtrate**.**

The EC is total parameter for dissociated dissolved substances and it depends upon degree of dissociation of ions and their concentration, temperature, and migration of ions in the electric field but does not give idea about type of ions present **(Rump and Krist, 1992)**.

Estimation of Heavy Metals

Heavy metals were estimated both from treated culture filtrate obtained after treatment. Maximum removal was found to be with the combined treatment of textile with *Lyngbya* sp. and ground nut shell for the heavy metals like mercury, cadmium, nickel, zinc and iron except chromium (Table 1). This was followed by cyanobacterial and then ground nut shell treatment emphasizing that *Lyngbya* sp. along with ground nut shell becomes more effective in binding and concentrating heavy metals from wastewater.

Reduction in chromium, zinc, Mercury, nickel and cadmium content of textile effluent was observed on 14th day with addition of coir pith and *O. Subuliformis* **(Shanmugapriya and Malliga, 2013).** Other type of bio sorbents, such as the biomass of marine dried green alga (biological materials) **(Gupta *et al*., 2006, Fenga and Aldrich, 2004, El-Sikaily *et al*., 2007, Gupta and Rastogi, 2008 and Ahmady-Asbchin *et al*., 2008),** were investigated for up-taking of some heavy metals from aqueous solution. Some of the used alga wastes were; *Spirogyra* species **(Gupta *et al*., 2006), (Fenga and Aldrich, 2004)**, *Ulva lactuca* (**El-Sikaily *et al*., 2007),** *Oedogonium* sp. and *Nostoc* sp. **(Gupta and Rastogi, 2008),** and brown algae *Fucus serratus* **(Ahmady-Asbchin *et al*., 2008).** The mechanism of up-taking heavy metal ions can take place by metabolism-independent metal-binding to the cell walls and external surfaces **(Deliyanni *et al*., 2007).**

FTIR Analysis

FTIR analysis was conceded to centralize degradation of groundnut shell as well as residues present in textile effluent by *Lyngbya* sp. The novel peaks in treated effluent with *Lyngbya* sp. with ground nut shell depicts produced metabolites after degradation by *Lyngbya* sp. Degradation of coir pith was carried out by variety of enzymes released by cyanobacteria which is having lignin degrading ability and thereby using lignocellulosic coir pith as an excellent and inexpensive carrier for cyanobacterial inoculam **(Malliga *et al*., 1996; Malliga and Viswajith, 2008).** Coir pith degradation by *Oscillatoria annae* under laboratory conditions were observed by **Malliga et al, (2012).** 30th day of degradation showed the presence of compounds using TLC, spectral and biochemical analysis.

FTIR of control ground nut shell resulted in peaks at 3411.92 cm^{-1} for O-H and H bond stretching of alcohols and phenols, a peak at 2924.84 cm^{-1} for C-H stretching of alkanes, 1637.87 cm^{-1} N-H bending of 1° amines, peak at 1423.80 cm^{-1} for C-C stretch of aromatic compound, peak at 1035.55 cm^{-1} for C-O stretch of carboxylic acids, esters and ethers, 619.98 cm^{-1} for C≡C-H showing presence of alkynes (Figure 6.a).

FTIR analysis of textile effluent treated with ground nut shell displayed a minor increased peak at 3419.46 cm^{-1} and 1424.22 cm^{-1} for O-H and H bond stretching of alcohols and phenols and for C-C stretch of aromatic compound respectively due to the chemical nature of effluent whereas slight decrease in the peaks at 2919.83 cm^{-1}, 1636.62 cm^{-1} and 1032.10 cm^{-1} for for C-H stretching of alkanes, N-H bending of 1° amines and for C-O stretch of carboxylic acids, esters and ethers respectively (Figure 6.b).

FTIR analysis of textile effluent treated with *Lyngbya* sp. exhibited highest peak at 3422.95 cm^{-1} than all other treatments for O-H and H bond stretching of alcohols and phenols which might be due to the release by *Lyngbya* sp. On addition all other peaks at 2925.07 cm^{-1}, 1645.27 cm^{-1} and 1039.61 cm^{-1} were elevated for C-H stretching of alkanes, N-H bending of 1° amines, C-O stretch of carboxylic acids, esters and ethers respectively (Figure 6.c).

FTIR analysis of textile effluent treated with *Lyngbya* sp. and ground nut shell demonstrated reduced peak at 3401.43 cm^{-1} O-H and H bond stretching of alcohols and phenols, 2922.70 cm^{-1} for C-H stretching of alkanes, 1033.75 cm^{-1} for C-O stretch of carboxylic acids, esters and ethers, peak at 609.80 cm^{-1} for C≡C-H of alkynes suggesting degradation of ground nut shell by *Lyngbya* sp. Moreover, new peaks at 1514.13 cm^{-1} and 1264.56 cm^{-1} for N-O stretch of nitro compounds and C-N stretch of aromatic amines respectively implied release of these compounds (Figure 6.d).

FTIR analysis of the produced metabolites after degradation by *Penicillium ochrocloron* displayed a peak at 2,962/cm for C-H stretching of alkanes, a peak at 1,261/cm for NO_2 vibrations of nitrates, peaks at 1,095 and 1,022/cm for S=O stretching of sulphoxides and sulphonic acid respectively, and a peak at 864/cm for C-H deformation of benzene, all of which indicate the formation of alkanes, sulphoxides, sulphonic acids and nitrates **(Utkarsha and Jyoti, 2011).**

CONCLUSIONS

Lyngbya sp. is potent to declourise textile effluent, degrade residue particle along with ground nut shell and able to grow in the same. The biochemical parameters like Nitrate, Nitrite, EC, TDS, Ammonia, Alkalinity, Calcium, Magnesium of textile effluent was carried out and checked with the treatments simultaneously. Alkalinity and Ammonia was found to be increased in textile with ground nut shell, textile with *Lyngbya* sp. but decreased in combined treatments.

Nitrate and Nitrite were found to be decreased in all treatments and also the heavy metals were found to be diminished as it was adsorbed on the filament surface of *Lyngbya* sp. FTIR analysis indicated the degradation of effluent residue and ground nut shell as well as release of some compounds in the culture filtrate.

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APPENDICES

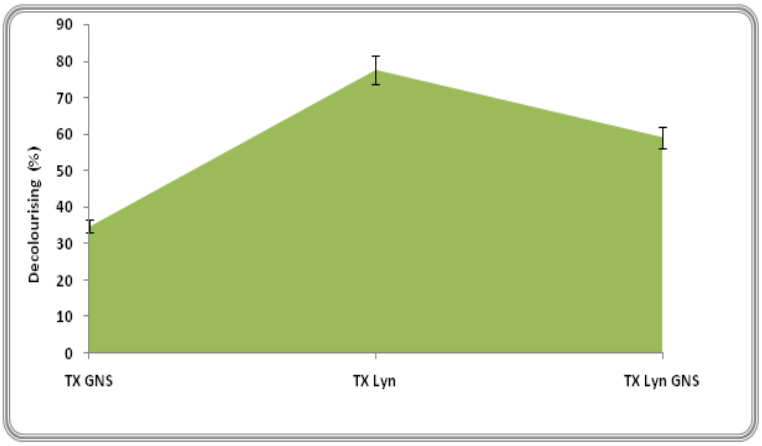


Figure 1: Effect on Decolourising Efficiency of *Lyngbya* Sp. with GNS in Textile Effluent (Culture Filterate) at 60th Day

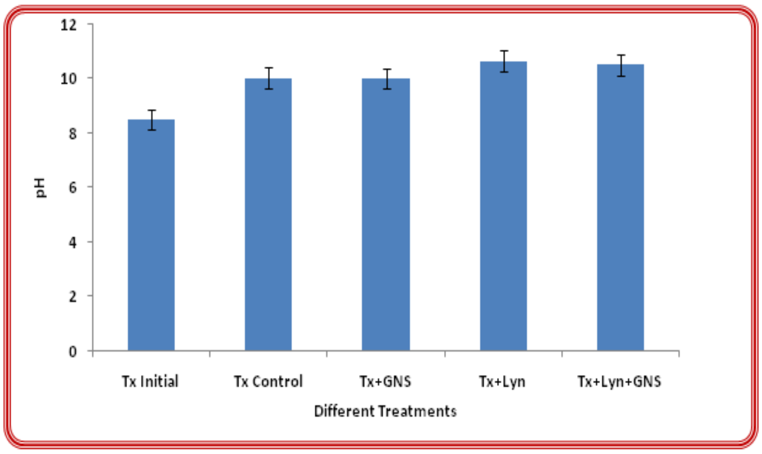
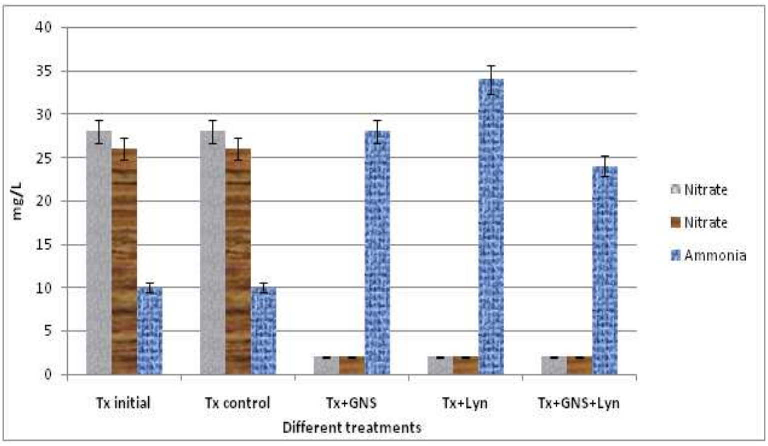
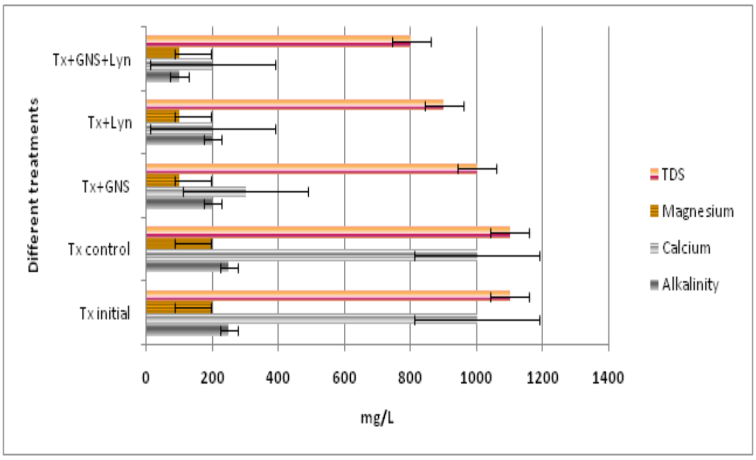


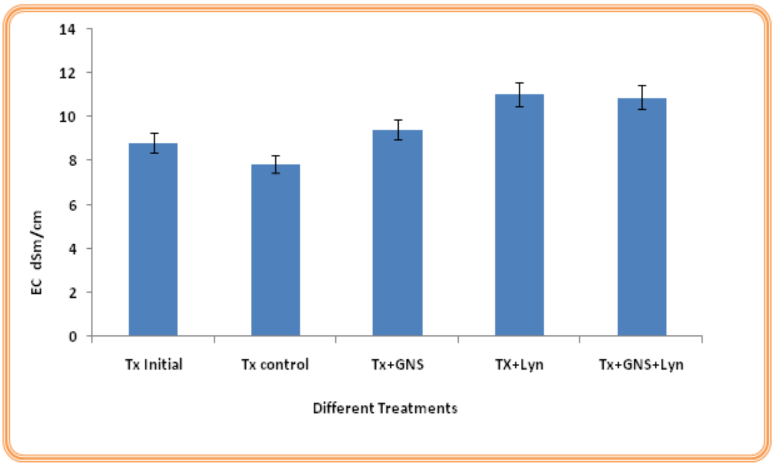
Figure 2: Effect on pH of Textile Effluent Treated with *Lyngbya* Sp. and GNS (Culture Filterate) at 60th Day



TX-Textile Effluent, GNS-Ground Nut Shell, Lyn-*L*yngbya Sp.
Figure 3: Effect on Nitrate, Nitrite and Ammonia Content of Textile Effluent (Culture Filtrate) Treated with *Lyngbya* Sp. and GNS at 60th Day



TX-Textile Effluent, GNS-Ground Nut Shell, Lyn-*L*yngbya Sp.
Figure 4: Effect on Alkalinity, Calcium, Magnesium and TDS Content of Textile Effluent (Culture Filtrate) Treated with *Lyngbya* Sp. and GNS at 60th Day

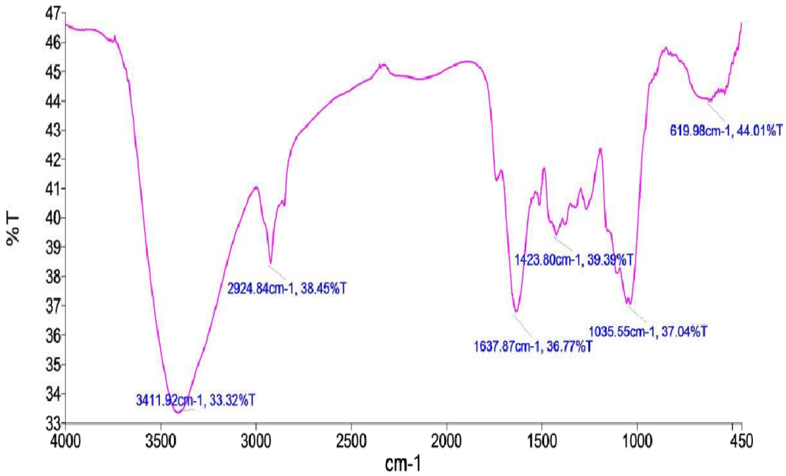


TX-Textile Effluent, GNS-Ground Nut Shell, Lyn- *L*yngbya Sp.
Figure 5: Effect on EC of Textile Effluent (Culture Filtrate) Treated with *Lyngbya* Sp. Andgns at 60th Day

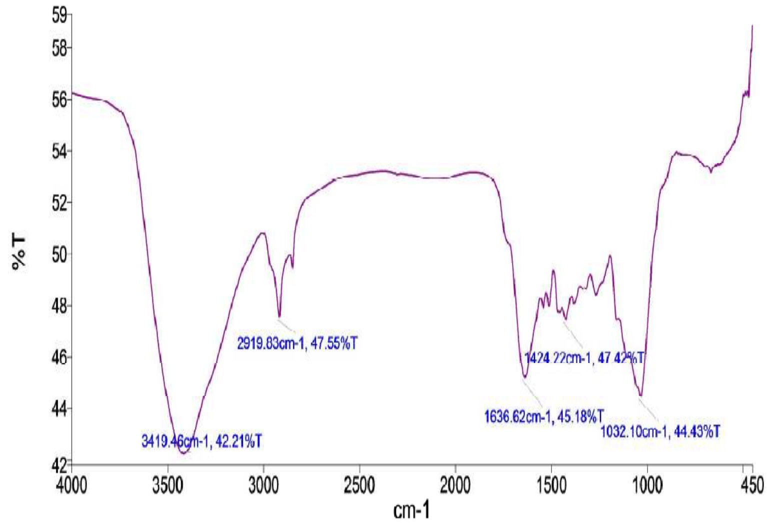
Table 1: Estimation of Heavy Metals and Textile Effluent Treated with *Lyngbya* Sp. and GNS (Culture Filtrate) on 60th Day

Heavy Metals(ppm)	TX	TX+ GNS	TX+LYN	TX+LYN+GNS
Mercury	1.652 ±0.001	0.37 ±3.0	0.28 ±0.00	0.20 ±5.5
Cadmium	0.432 ±4.16	0.35 ±4.61	0.14 ±5.77	0.11 ±6.0
Nickel	0.097 ±0.002	0.037 ±0.001	0.047 ±1.6	0.037 ±0.00
Chromium	0.09 ±0.00	0.011 ±0.00	0.029 ±0.00	0.045 ±0.00
Zinc	0.68 ±0.02	0.58 ±0.02	0.28 ±0.02	0.29 ±0.02
Iron	0.00461 ±0.001	0.00326 ±0.005	0.00126 ±0.003	0.00112 ±0.007

TX-Textile Effluent, GNS-Ground Nut Shell, Lyn- *Lyngbya Sp.*

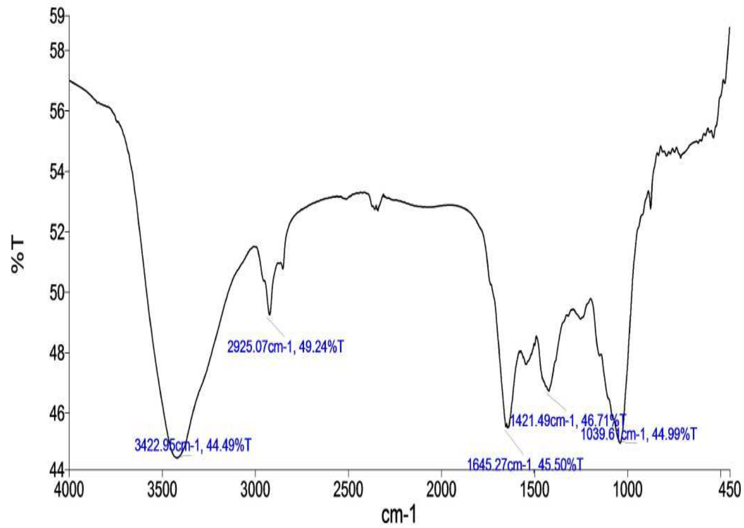


(a) Ground Nut Shell

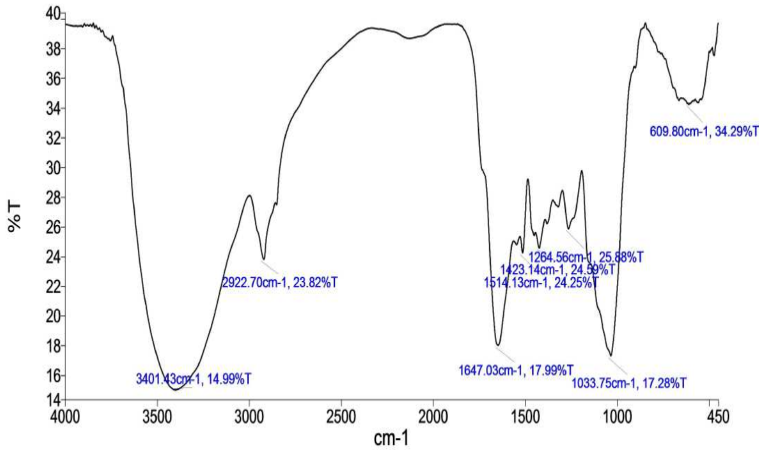


% T- Transmission, Frequency- cm^{-1}

(b) Textile Effluent Treated with Ground Nut Shell



(c) Textile Effluent Treated with *Lyngbya* Sp.



% T- Transmission, Frequency- cm^{-1}

(d) Textile Effluent Treated with Ground Nut Shell and *Lyngbya* Sp.

Figure 6: FTIR Spectral Analysis of Textile Effluent Treated with *Lyngbya* Sp. and Ground Nutshell