Research Article

# Augmentation of *Arachis hypogeae* L. Seeds Germination using Cyanobacterial Treated Effluent

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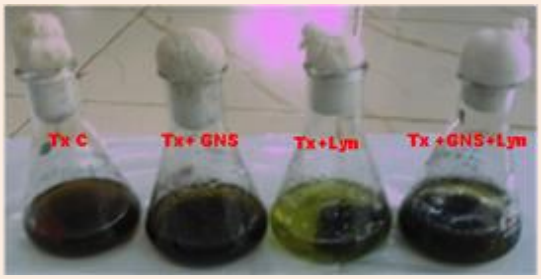
**Abstract**

The current study investigated on decolourisation and degradation of textile effluent using cyanobacterium *Lyngbya* sp. BDU 90901 along with adsorbent Ground nut shell (GNS) and application of treated effluent to inspect its effect on the seed germination of *Arachis hypogeae* L. Degradation of effluent with GNS was confirmed by HPLC analysis. Seed germination was studied in the presence of varying concentrations of treated effluent with GNS and *Lyngbya* sp. Certain degree of inhibition was effectively noticed with reduction in seed germination and radical length at higher concentration. The findings indicate tolerance level of the seed to the treated and untreated effluent during early growth phase of the seedlings. Protein content was estimated during germination period.




**Keywords:** Degradation, HPLC, germination, radical length

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## Introduction

Textile dyes are one of the most prevalent type chemicals in use today. Around 10,000 different dyes with an annual production of more than 7x105 metric tones worldwide are commercially available [1]. Synthetic textile dyes used each year are lost during manufacture and textile coloration process and 20% of these dyes enter the environment through effluent discharge during treatment of residual industrial water [2]. Water pollution control is at present one of the major areas of scientific activity. Moreover though the quality of treated effluent may not violate the discharged limits, such wastewater could show toxicity to humans and the environment [3]. Since living materials respond to the total effect of actual and potential disruptions, biological assays emerged as an important tool in assessing harmful chemical activities. The ability of bacteria and fungi to metabolize azo dyes and release of compounds using LCMS and HPLC analysis has been investigated by a number of research groups [4, 1, 5, 6, 7].

A number of studies have focused on the toxicity assessment of industrial effluent using plant system [8, 9, 10] [11] evaluated the suitability of different industrial effluents (textile mill, oil refinery, soap and detergent mill, hydrogenated oil mill and rubber industry) for irrigation purposes in wheat crop. It was concluded that wastewaters should not be discharged in agricultural crops, water stream etc. There has been a very few literature that confirms the accurate impact of these effluent on seed germination after biological treatment. Therefore, this study focused the influence of cyanobacterial with agro waste treated effluent on seed germination of *Arachis hypogeae* L.

## Experimental

### Materials and Reagents

***Effluent collection and experimental design****:* Textile industry effluent was collected from Karur ditrict, Tamil nadu, India and inoculated with *Lyngbya* sp and GNS independently and combination of both in 100 ml Erlenmeyer flasks. After 30 days of incubation time under favourable conditions [12], experiments were conducted from the resulting supernatant.

***HPLC****:* High performance liquid chromatography (HPLC) analysis (Waters model No. 2690, USA) of Textile control effluent, treated effluent with Ground nut shell, *Lyngbya* sp. and combination of both was carried out with C18 column (Symmetry, 4.6 × 250 mm) using isocratic method with 10 min run time. The mobile phase used was HPLC grade methanol with a flow rate 1ml/min. The products were monitored by their absorbance at 254 nm with a UV detector [13].

***Seed germination****:* Seeds of *Arachis hypogeae* 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 Groundnut shell (viz. 1, 2.5, 5, 10, 12.5, 25, 50, 75, 100% along with distilled water as control i.e 0% effluent.15seeds/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 hours to determine the germination percentage.

***Calculations****:* Seed germination percentage at various treated effluent concentrations was calculated by using formula [14].

$$G = (n/N) \times 100$$

G- Germination percentage, n- number of seeds germinated, N- Total number of seeds in each petri dishes

***Protein estimation*****:** Germinated seed of *Arachis hypogea* at 72 hrs. was grinded with 5ml of 0.2 mol of phosphate buffer (pH 7) and subjected to centrifuge at 5000rpm for 10 mins. The filterates were subjected to protein estimation by Lowry method [15] with absorbance with 660nm.

***Statistical analysis:*** The data generated in the experiment was subjected to statistical analysis by applying one way analysis of variance (ANOVA) to confirm viability.

## Results and Discussions

***Degradative analysis using HPLC*****:** The chromatogram of control effluent textile sample showed 12 peaks with retention time (2.375, 2.568, 2.803, 3.053, 3.340, 4.533, 4.941, 5.588,6.071, 6.674, 7.268, 9.690) (**Figure. 1a**). Alterations in stature of the peaks were observed in textile effluent treated with GNS. It indicated the release of compounds by the agrowaste during treatment (**Figure. 1b**) whereas, treatment with *Lyngbya* sp. showed reduction in number (8) of peaks than all other treatments. This could be improvised as adsorption of dye particles on the filaments of cyanobacterium **(Figure. 1c**). New peaks were demonstrated with combined treatment of *Lyngbya* sp. and ground nut shell indicating degradation of ground nut shell and dye particles present in the effluent by cyanobacterium suggesting formation of intermediate compounds (**Figure. 1d**). [16] also reported biodegradation of Rubin GFL using HPLC technique. The effluent treated with newly developed bacterial- yeast consortium BL-GG confirmed biotransformation of dyes mixture by HPLC analysis [17]. Localization of the compounds of interest for purification using HPLC was studied in *Lyngbya majuscula* for bioprocess intensified production of cyclic and linear lipopeptides [18].

**Figure 1** HPLC chromatogram of textile effluent treated with *Lyngbya* sp. and ground nut shell - 30th day

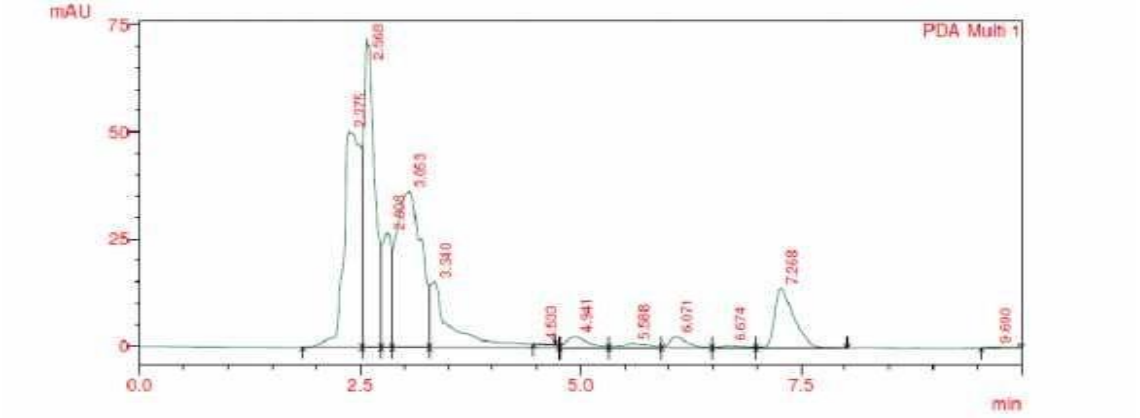


**Figure a** HPLC chromatogram of textile effluent control

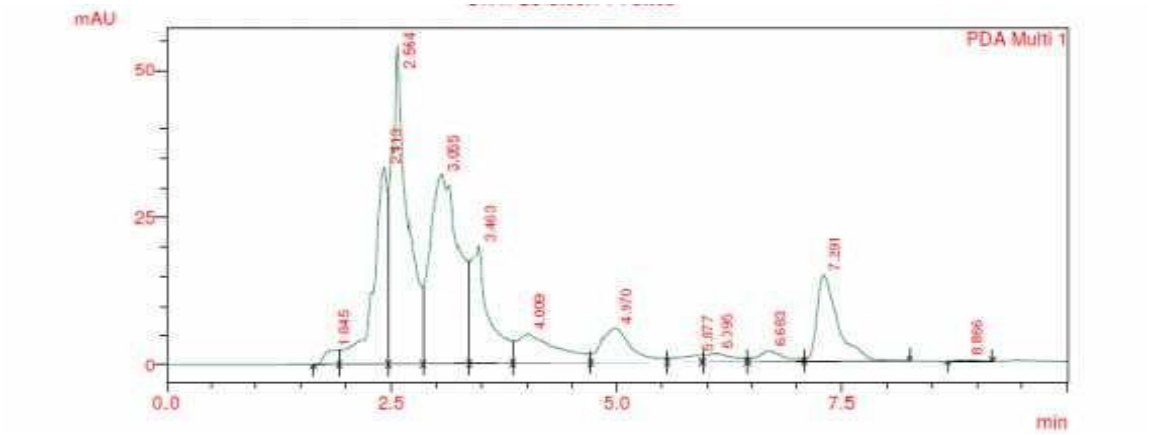


**Figure b** HPLC chromatogram of textile effluent treated with ground nut shell

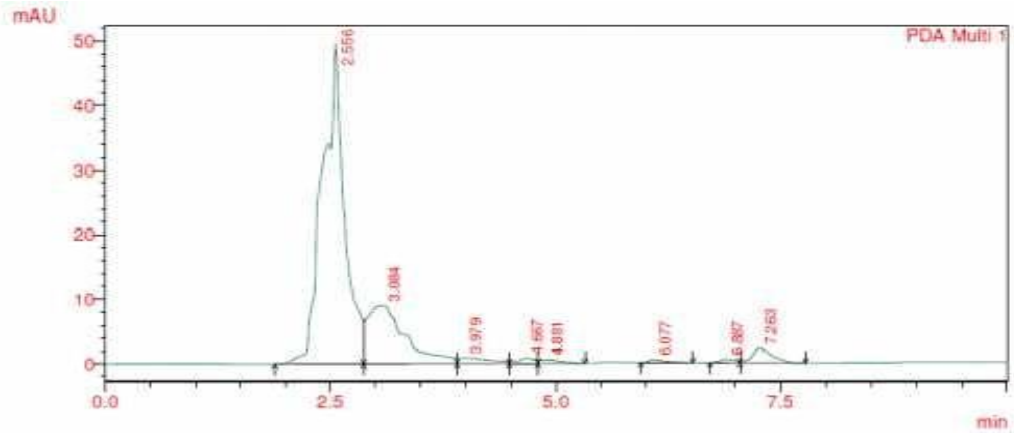


**Figure c** HPLC chromatogram of textile effluent treated with *Lyngbya* sp.

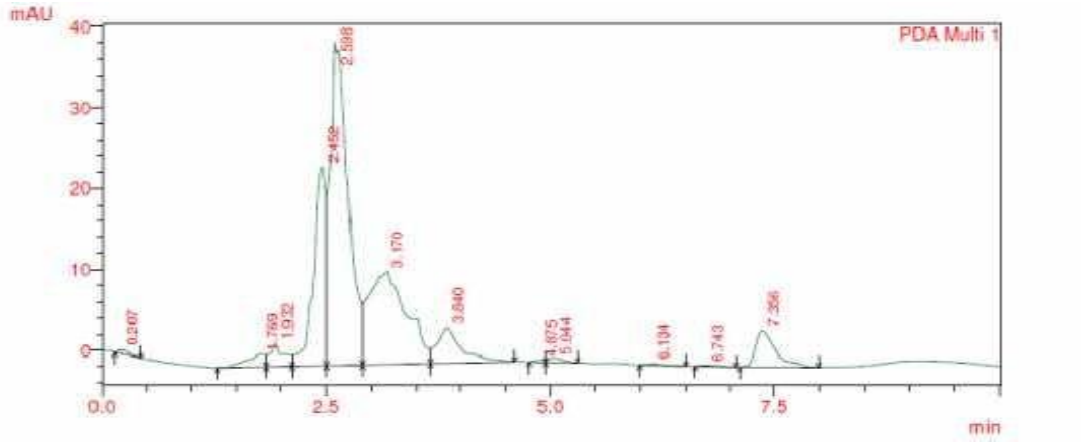


**Figure d** HPLC chromatogram of textile effluent treated in combination with ground nut shell and *Lyngbya* sp.

***Seed germination:*** The extent of germination inhibition depends on treated effluent concentration. It was observed that the seeds showed varied germination rate at different concentrations (**Figure. 2**). Percentage of seed germination was found to be 100% with distilled water control and combined treatment of *Lyngbya* sp. with ground nut shell at concentration of 12.5% and 25% respectively. But at higher concentration of combined treatment 40-60% reduction in germination percentage was exhibited. This selective growth could be ascertained to growth promoting effect of low concentration of mineral elements present in the diluted effluent.

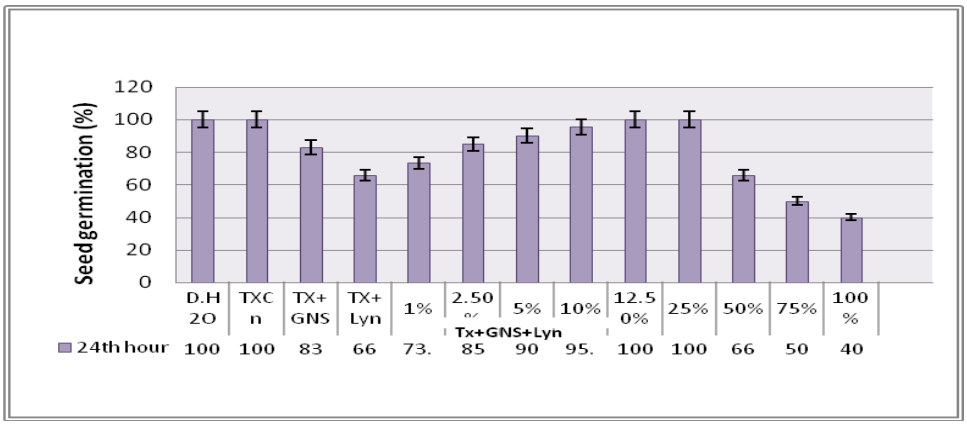


**Figure 2** Seed germination of *Arachis hypogeae* L. seeds in textile dye effluent treated with *Lyngbya* sp. and groundnut shell in different concentrations at 24th hour

[19] 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. [20] 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, acme value and germination value 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 [21]. Whereas, Bengal gram (*Cicer arietinum*) seeds germination was adversely affected even as low as 5% textile effluent concentrations [22].

***Effect on radical length:*** The effect of untreated effluent showed slender variation on radical length in comparison with control suggesting presence of essential organic compounds in the textile effluent supporting the growth of seedlings. The difference extended in treated effluent with GNS and *Lyngbya* sp. evidently at 12.5% and 25% when

compared to control and textile effluent at 72 hrs but deleterious effect was initiated at higher concentrations (**Figure. 3**). These results are in concurrence with the findings of [19] which indicated that the treated effluent had lesser detrimental effects on sorghum than untreated effluent. [23] 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.

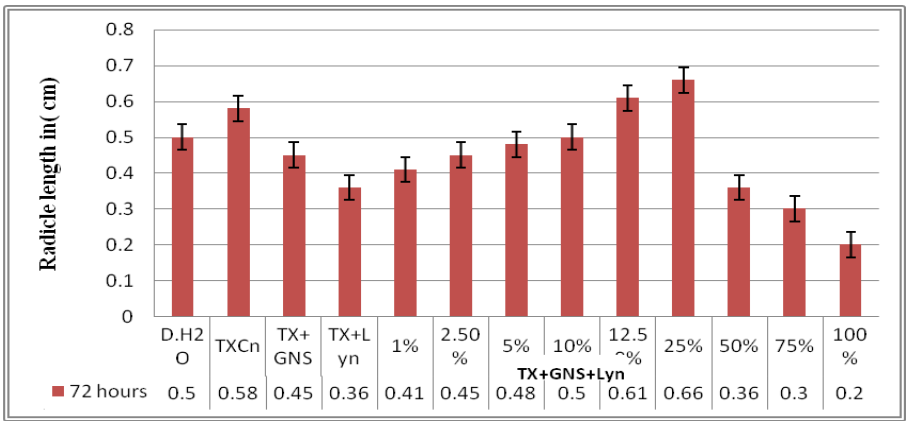


**Figure 3** Effect on radical length of *Arachis hypogeae* L. seeds in textile dye effluent treated with *Lyngbya* sp. and groundnut shell at different concentrations (72 hours)

***Protein estimation***: As the seeds of higher plants accumulate large amounts of storage proteins during seed development and seed maturation, which are mobilized to provide building blocks and energy for seed germination and early seedling growth upon seed germination [24]. In the present investigation, the effect of treated textile effluent and diverse concentrations of textile effluent with GNS and *Lyngbya* sp. on protein content were analyzed at the 72 hr of germination (**Figure. 4**).

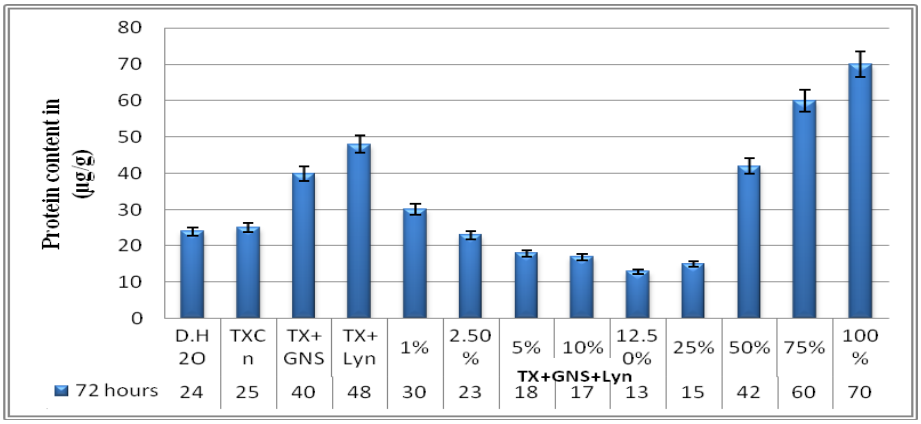


**Figure 4** Estimation of Protein content in germinated seeds of *Arachis hypogaea* L. in effluent treated with *Lyngbya* sp. and Ground nut shell at 72 hour

Protein content was found lowest at lower dilution of 12.5% in combined treatment whereas highest at 100% due to poor germination. Approximately same divergence of protein content was established between distilled water control and untreated textile effluent control whereas with treatment by GNS and *Lyngbya* sp. independently there was roughly 2 fold increase in the protein content when compared to distilled water control and textile effluent control. Moreover, higher dilutions of combined treatment displayed more protein content when compared to lower dilutions. This emphasized that lower concentrations favoured seed germination in assessment of all other treatments. The loss of proteins from growing radicle could be due to the transport of amino acids to the growing axis. The reports are

similar to the results of [25] in germinating *A. tristis* where lower concentration of Bijamrita, Cyanospray and combination illustrated less content of protein when compared to control. Similarly, the protein level decreased during germination in *Lupinus leteus* L [26], Australian sweet lupin [27], *Ceiba pentandra* [28], Horse gram [29] and chickpea seeds [30].

## Conclusion

The work revealed that the treated effluent on proper dilutions can be utilized for invigorating the seeds and further the agricultural crops. The dilution evades the lethality of the pollutants. Depending upon the availability of effluent specific to site can give way to utilize the waste material for betterment of mankind without causing ill effects to surrounding environment, human and animals.

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