Saudi Journal of Biological Sciences 28 (2021) 1203–1212

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Spectral studies of *Amaranthus tristis Linn.* in Bioremediated Silk dyeing effluent with mixed biofertilizer inoculants

Sumayya Rehaman [a,*], Mohamed A. El-Sheikh [b], Ahamed H. Alfarhan [b], U. Ushani [a]

[a] Department of Biotechnology, Karpagam Academy of Higher Education, Coimbatore 641 021, Tamil Nadu, India
[b] Department of Botany & Microbiology, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia

ARTICLE INFO

Article history:
Received 20 October 2020
Revised 14 November 2020
Accepted 15 November 2020
Available online 24 November 2020

Keywords:
Silk dyeing effluent
Amaranthus tristis
HPLC
FTIR
GCMS
Bioremediation
Soil analysis

ABSTRACT

Microbial degradation as a treatment, with the combination of mixed inoculants of the Biofertilizer of *Pseudomonas* sp., *Azospirillium* sp. and *Rhizobium* sp., was employed for the remediation of Silk dyeing effluent. Remediating studies was undertaken to assess the feasibility of the mixed biofertilizer inoculant source for degradation of the Azodye effluent from the Silk dyeing Industry. The Green leafy vegetable (GLV), *Amaranthus tristis Linn* used as investigational prototypical plant species is selected for examining the phytochemicals, functional groups and its compounds grown in the effluent and biotreated environment and compared. The laboratory scale investigation showed that leaves, stem and root of the *Amaranthus tristis Linn* was qualitatively analysed for 20 phytochemicals which was grown in the different treatments of raw effluent and the biotreated effluent and the results showed the phytochemicals on the effluent's influence reduced from strong positive to trace amounts while recovered on the biotreated environment. The FTIR analysis of the GLV grown in effluent and biotreated environments on comparison resulted in the functional group Alkene rescued in the biotreated effluent environment compared to the effluent contaminated area. The HPLC analysis of methanolic extracts of *A. tristis* grown in fresh water has 6 peaks of retention time of 2.6, 3, 3.9, 4, 4.2, and 4.6 RT whereas GLV effluent had only one peak of retention time of 4.1 RT. In the GLV from biotreated environment have 4 peaks were found with the maximum percentage area of 95.2% which proves that the compounds are rescued in the biotreated environment and few active compounds were confirmed in GCMS analysis. The Soil analysis results also indicate that the biotreatment of mixed inoculant of biofertilizers in the biotreated soil had influence resulting in improved levels of Ca, N, P and K with 114, 213, 10.5, 268 kg/ha respectively in the mixed inoculant biotreated soil. Similarly the micronutrients suchas Fe, Mn, Cu and Zn ranges to 4.1, 20.22, 2.13, 1.13 ppm respectively in the mixed inoculant biotreated soil within the optimal range. The study revealed that mixed biofertilizer inoculant has the recovery effect on the Silk dyeing (Azodyes) effluents effective reducing the pollutant capacity thereby meeting the discharged standards.

1. Introduction

In contemporary years there has been growing interest in using plants with microbes for decontamination. On the other hand, water, soil and air are increasingly contaminated. Huge toxic waste has been dispersed in hundreds of contaminated sites and it is spread all over the globe (Prakash and Karthikeyan, 2013). New modern civilization is the xenobiotics with heavy metals contaminated the environment which is spread all over the Earth. The contaminants are of various types comes from varies industries with toxicity and pollution absorbed by the animals and plants. These toxic substances emerge from the anthropogenic sources which is released from mining, industrial activities and automobile industries' exhaust. So for the removal of the pollutants are very important scenario for the conservation of our mother earth. The irreplaceable solution for rescuing by remediating the pollutant is by nature's gift of Bioremediation by the microbial process. Biodegradation is easy with cost effective technology and can be

* Corresponding author.
E-mail addresses: bio.sumay@gmail.com, drsumyya.rr@kahedu.edu.in (S. Rehaman), el_sheikh_eg@yahoo.co.uk (M.A. El-Sheikh), alfarhan@ksu.edu.sa (A.H. Alfarhan).

Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2020.11.057
1319-562X/

applied in large scale (Sivaramakrishnan et al., 2020a). As many microorganisms possess an enzyme azoreductase which can be able to degrade the substrate dyes. (Chen et al., 2010) and decolorise the dyewater (Bizuneh, 2012) is non toxic to the aquatic fishes (Sumayya and Srinivasan, 2014). The biofertilizers *Pseudomonas fluorescence, Azospirillium* sp., *and Rhizobium* sp., has capacity of remediating the dyewater (Gałązka et al., 2012; Sumayya and Srinivasan, 2018a,b) and also fertilizes the plant in their biometric growth (Anandaraj and Delapierre, 2010). Since 5000 years the dyeing is carried out with known archeological evidence. The dye whether Natural (Organic) or synthetic with a mordant for fixation and imparting the color to the material in aqueous medium (Chaube et al., 2010). Thousands of synthetic dyes nowadays are replaced with less cost and vast color variation (Thiripurasundari et al., 2013).

The Silk industry effluents of large volume from the illegal units with lot of dyes generating various metric tons per annum, let out into the environment. They contaminate the soil, plants which take up the groundwater, absorbed by the animals when they intake (Nupur, 2009). The aquatic animals are mutated by the dye effluents affects the organs and the skins causing allergies, dermatitis, skin irritation or different changes in tissues (Börnick and Schmidt, 2006) carcinogenic and mutagenic (Mathur and Bhatnagar, 2007). The dyes used for coloring the Silk are usually Azo dyes than acid dyes, mordant dyes, reactive dyes, disperse dyes, pigment dyes, vat dyes, basic dyes, direct/substantive dyes, ingrain dyes, solvent dyes and sulfur dyes (Kiernan, 2001).

The Azodyes are the ecotoxic hazards, a carcinogen (Lins et al., 2010) which variates itself from different colors of purple, brown, blue, red and so on persist with real problem to the environment.. The Environmental protection act (EPA, 2010) and their authorities has targetted on the illegal units of textile industry for the wastewater discharge into the streams. The Colored azodyes with the raw residual dye content directly is given out in the environment (Zaharia et al., 2012) affects the plant tissues (Sumayya and Srinivasan, 2018a,b). This is common in the various districts of Tamilnadu including Salem, Erode and Coimbatore. The Slender entrepreneurs are unable to afford a Common Effluent Treatment Plant (CETP) or else to have a their own plant due to lack of electricity and related expenses. On the otherhand, they discharge to the environment without meeting the guidelines of Zero discharge norms when let out in the environment (Jayarajan et al., 2011) meets the various plant species especially Green Leafy vegetables (GLV) grown in these lands near the Silk dyeing units.

The GLV is a boon for the Mankind and its nutritional knowledge has been conceded over generations without any documentation rather merely by practicing in our daily life. With modern civilization the dropdown of GLV with medicinal usage is further more. The medical properties with essential compounds are considered as the Antiaging wonders in Nature (Gupta and Prakash, 2009). The Green Leafy vegetables (GLVs) have recorded for the major phytochemicals with impending Antioxidants properties with the high nutritional importance of both seeds and leaves. (Khanam et al., 2012). One such GLV is *Amaranthus tristis Linn.*, (Fig. 1) as likely food crop called as Slender Joy weed are rich in Macronutrients and Micronutrients with varying micrograms are bioactive compounds to the diets of populations with increased biological value (Gamel et al., 2005) suggested to the lactating mothers (Grubben and Denton, 2004) on consumption can improve the nutritional level of Underweight children and adults (Asare-Marfo et al., 2013). The vegetable group of this family has most similar resembles to the weeds group.

Genus *Amaranthus* belongs to order, family, sub-family suchas Caryophyllales, Amaranthaceae, Amaranthoideae respectively. They are recorded with about 60–70 sp., (Xu and Sun, 2001) in which almost 17 species are reported for the edibility of its seeds and leaves (Grubben and Denton, 2004) others are usually weeds. The *Amaranthus sp*., are able to withstand in the stress environments mostly with resistance to heat, drought, diseases and pests (Wu et al., 2000). It has various phytoactive compounds like Amarantin, Isoamarantin, Betaine, Aminoacids, Flavonoids, and Phytosterols with therapeutic action.

1.1. *Amaranthus tristis Linn.*

Scientific classification

Kingdom	: Plantae
Phylum	: Tracheophyta
Class	: Magnoliopsida
Order	: Caryophyllales
Family	: Amaranthaceae
Genus	: *Amaranthus*
Species	: *Amaranthus tristis Linn.*

Usually plants respond to the environmental stress conditions and change or alter their functional group of the bioactive compounds and on recovery it responds to the regular pattern of Physiological mechanism. The capillary action of the plants in the textile effluent environment are toxic to the compounds and negative impact on its development. In this article, an attempt was made to check the *Amaranthus tristis* grown in the fresh water, 100% Silk effluent and Biotreated effluent environment (treated with the mixed inoculants of biofertilizers) to analysis the changes attained in the functional groups and the bioactive compounds using the preliminary phytochemical and spectroscopic analysis.

2. Methodology

Silk dyeing effluent was collected in the sterile containers from the small scale industrial effluent (Azodyes) located at Seelanayakanpatti, Salem district, Tamilnadu, Latitude 11.6252° N and Longitude 78.1474° E with technical details from the Entrepreneur was obtained. The 2% inoculum $2*10^8$ cells per ml of each biofertilizer *Rhizobium* sp., *Pseudomonas fluorescens* and *Azospirillum* sp.,grown separately in 250 ml Erlenmeyer flasks treated with the 50 mg/l if azodye effluent at optimum temperature (Nachiyar and Rajkumar, 2003). The collected crude effluent was subjected to degradation by mixed inoculants of *Rhizobium* sp., *Pseudomonas*

Fig. 1. Amaranthus tristis Linn.

fluorescens and *Azospirillum* sp.,(Obtained from Tamilnadu Agricultural University, Coimbatore District, Tamilnadu) kept for incubation for 1 month in the shade area. The GLV *Amaranthus tristis Linn* were grown in normal soil (ATNS), different concentrations of 25%, 50%, 75% and 100% of silk dyeing effluent (ATES) and biotreated soil (ATTS).

2.1. Selection and collection of GLVs for the study

The Green leafy vegetable *Amaranthus tristis* (Famously known as Araikeerai) have been selected and their seeds were obtained from Superseeds Nursery, Coimbatore, Tamilnadu, India to grow them in different environments set as treatment groups (ATN: *Amaranthus tristis,* were grown in fresh water as control soil, ATE: *Amaranthus tristis*, were grown in (25%, 50%, 75% and 100%) Silk dyeing effluent, ATT: *Amaranthus tristis*, were grown in biotreated effluent.

2.2. Harvest methodology

The GLVs were harvested from three treatment groups on their 45th day as Green leafy vegetables reach stage III maturity (Khader and Rama, 2003) by removing the adhering soil particles with water gently and blotting with the filter paper. Then GLVs were subjected to analysis.

2.3. Soil analysis

The soil of above treatment groups named as (ATNS – Rhizosphere Soil sample of *Amaranthus tristis,* were grown in fresh water as control soil, ATES- Rhizosphere Soil sample of *Amaranthus tristis,* were grown in 100% crude effluent, ATTS: Rhizosphere Soil sample of *Amaranthus tristis,* were grown in biotreated effluent ;ATTS) were collected separately in the Containers and analysed of following parameters.

Soil texture denotes to the relative proportion of sand, loam, silt and clay present in the soil. Based on these proportions, the soil used in this study were ATNS; ATES; ATTS was classified and identified into various textural classes on Visual observation. **Clayey soil** has a larger percent of clay considered with more fertile than the sandy soil which are less than 0.002 mm in diameter referred as soil colloids. **Sandy soil** is easy to work with low water retention capacity of large particles which are coarse and individual particles are visible with 0.02–2 mm in diameter but less fertile. **Loamy soil** is in between sandy and clayey soils which is best airable cropping. **Silt soil** has medium-sized particles which are 0.002–0.02 mm in diameter. The pH and EC (Jackson, 1962), Estimation of macronutrients Calcium(Cheng and Bray, 1951), Nitrogen (Johan Kjeldahl, 1990), Phosphorus (Olsen, 1954), Potassium (Toth and Prince, 1949) and micronutrients such as, Iron (Fe), Manganese (Mn), Copper (Cu) and Zinc (Zn) (Jackson, 1962) in the soils of different treatment groups were evaluated.

2.4. Phytochemical analysis

2.4.1. Preparation of aqueous extract

The plant material (5 g) were homogenated with 5 times of its weight of water and was heated in a waterbath for 30 min to 1 h, cooled and filtered. **Preparation of alkaline extract**: A portion of the plant material (5 g) was covered with 5 times the volume of methanol and was allowed to stand for 24 h. It was then filtered and the filtrate was evaporated to dryness.It was further filtered and phytochemicals were tested using the aqueous and alkaline filtrate for the phytochemical analysis using Standard procedure with the positive control.

2.5. FTIR, HPLC and GCMS analysis

2.5.1. FT-IR analysis of selected GLV

FT-IR (Fourier Transform Infrared) analysis provides a molecular fingerprint for functional groups as spectral information, of the unknown compound with libraries' with best match resulted in FTIR spectra of GLV grown in control soil, crude effluent and biotreated effluent to identify the functional group presence. The samples were subjected to IR Affinity- 1S Model of Shimadzu make (Sivaramakrishnan and Incharoensakdi, 2020b).

2.5.2. HPLC analysis of selected GLV

Sample preparation: The 20 g of each dried powdered sample of *Amaranthus tristis Linn.* grown in fresh water (ATN), crude effluent (ATE) and biotreated effluent (ATT) were packed with Whatmann Filter paper subjected to Soxhlet apparatus .and run with HPLC grade methanol was purchased from Fischer Scientific & Co.

2.5.2.1. HPL chromatographic conditions. C18 column equipped with 3 µl particle size (50 × 4.6 mm I.D) and detector UV– VIS model SPD 20A at specific nanometer at a flow rate of 1 ml/min in Shimadzu LC-10 HPLC model were used for sample analysis with solvent HPLC methanol was used with the stream of liquid N2 until it reached nearly 0.5 ml and mobile phase was added to reach 1 ml. Then 20 µl of the methanolic extract of the each ATN, ATE, ATT were injected into HPLC column. The presence of compounds was determined by comparison of peak area of the samples with each other. (Sivaramakrishnan et al., 2020c).

2.5.3. GC–MS analysis of selected GLV in biotreated effluent

The GLV grown in the biotreated effluent were subjected to GC–MS (Make - Perkin Elmer, GC Model - Clasrus 680, Mass spectrometer – Clarus 600 (EI), with helium (He) as carrier gas at a constant flow of 1 ml/min. During the chromatographic run, the injector was set with the temperature 260 °C. The mass detector conditions were with line temperature 230 °C; ion source temperature 230 °C; and ionization mode electron impact at 70 eV, a scan time 0.2 sec and scan interval of 0.1 sec The fragments from 40 to 600 Da were separated with fused silica column detected with Elite-5MS (5% biphenyl 95% dimethylpolysiloxane, 30 m × 0.25 m m ID × 250 µm df) and chromatogram were compared with the GC–MS National institute of standard and technology NIST 2008 Library (Stein, 1990).

3. Results

3.1. Qualitative phytochemical screening

The GLV *Amaranthus tristis* was grown in control soil (ATN); in crude effluent (ATE - different concentration 25%, 50%, 75%, 100%), and in biotreated effluent (ATT) as shown in the Figs. 2, 3a, b, c and 4. The qualitative phytochemical analysis of extracts of Leaf, stem and seed of GLV biomass revealed that the presence of various phytochemical in aqueous and methanolic extracts as shown in Table 1. Presence of phytochemical was indicated by the + sign.

The prominent strong presence of carbohydrate, protein, cellulose, glycosides, free aminoacids and quinone were observed in the leaves, the stems and the seeds of the methanolic extract of the GLV of the control soil (ATN) and it is reduced to trace amount in the GLV of crude effluent environment (ATE) vice versa results were obtained in the GLV extracts of biotreated soil was able to prove that its phytochemicals were recovered. The cyanogenic glycosides, catechol, saponin and lignin were completely absent in all parts of the selected GLVs. The phenol was present prominently in

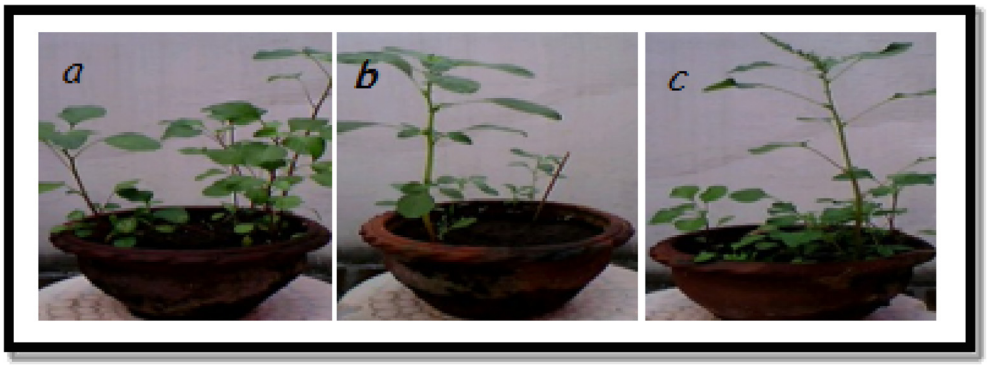


Fig. 2. a, b, c Growth of the *Amaranthus tristis Linn.* (Araikeerai) GLV grown in control soil.

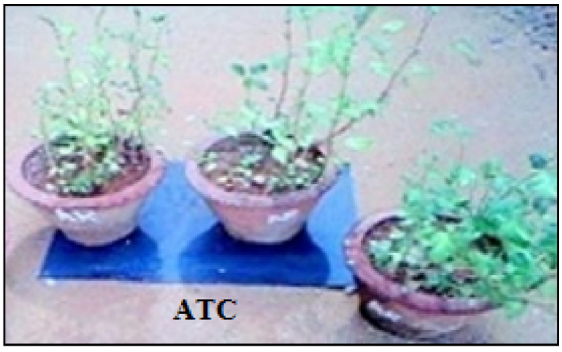


Figure 3a *Amaranthus tristis* **in 25% effluent (ATA)**
3b *Amaranthus tristis* **in 50% effluent (ATB)**

Figure 3c *Amaranthus tristis* **in 75% effluent (ATC)**

Figure 3d *Amaranthus tristis Linn* **in 100% effluent (ATD)**
Figure 3a,b,c,d *Amaranthus tristis* **grown in different concentrations of the silk dyeing effluent**

Fig. 3. a, b, c, d *Amaranthus tristis* grown in different concentrations of the silk dyeing effluent.

Fig. 4. *Amaranthus tristis Linn* (ATT) GLV grown in biotreated effluent (treated with Mixed Inoculants).

the stem and the seed of *A. tristis* grown in control soil (ATN) and biotreated environment (ATT). The sterol was in trace amounts in the leaf, stem and the seed of *A. tristis* in all environment (ATN, ATE, ATT). The alkaloid and flavonoid was present in all parts except in the seeds of the *A. tristis* after the biotreatment the GLV was unable to synthesis the phytochemical.

The leucoanthocyanidine was present in the leaf and seed extracts of *A. tristis* grown in the control soil (ATN) and trace amounts were detected in the GLV grown in crude effluent (ATE) whereas in biotreated GLV (ATT), only leaf extract of the GLV was able to get synthesized. The tannin was present in the leaf and the stem extracts whereas absent in the seed of *A. tristis* grown in all treatment groups. The volatile oil was absent in all parts of the GLVs except the stem of *A. tristis* as trace amounts. The ter-

Table 1
Preliminary phytochemical screening of methanolic extract of *A. tristis* GLV grown in different treatment groups.

Name of the Nutrient	*Amaranthus tristis* (ATN)			*Amaranthus tristis* (ATE)			*Amaranthus tristis* (ATT)		
	Leaf	Stem	Seed	Leaf	Stem	Seed	Leaf	Stem	Seed
Carbohydrate	++	++	++	Tr	Tr	Tr	+	+	+
Proteins	++	++	++	Tr	Tr	Tr	+	+	+
Phenol	–	++	++	–	Tr	Tr	–	+	+
Catechol	–	–	–	–	–	–	–	–	–
Sterols	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr
Glycosides	++	++	++	Tr	Tr	Tr	+	+	+
Saponin	–	–	–	–	–	–	–	–	–
Quinones	++	++	++	Tr	Tr	Tr	+	+	+
Cynogenic glycosides	–	–	–	–	–	–	–	–	–
Alkaloids	++	++	–	Tr	Tr	–	Tr	Tr	–
Flavonoids	++	Tr	++	Tr	Tr	Tr	Tr	Tr	Tr
Leucoanthocyanidines	++	–	+	Tr	–	Tr	Tr	–	+
Tannins	++	++	–	+	+	–	+	+	–
Anthocyanins	+	+	+	+	Tr	+	+	+	+
Volatile oils	–	Tr	–	–	Tr	–	–	Tr	–
Lignin	–	–	–	–	–	–	–	–	–
Terpenoids	Tr	–	+	Tr	–	+	Tr	–	+
Cellulose	++	++	++	+	Tr	+	+	+	+
Free aminoacids	++	++	++	–	Tr	–	–	+	–
Starch	–	–	++	–	–	+	–	–	+
Reducing sugars	–	–	++	–	–	+	–	–	+

++ Strong positive + Presence – Absence Tr- Trace presence; ATN: *Amaranthus tristis,* were grown in control soil, ATE: *Amaranthus tristis,* were grown in crude effluent. ATT: *Amaranthus tristis,* were grown in biotreated effluent. Note: The post harvested on the 45th day without any damage and were the plant material were further analyzed.

penoid was present in the seed extract and in trace amounts in the leaf extracts of GLV while absent in the stem of *A. tristis*. The starch and reducing sugar was present only in the seeds of *A. tristis* grown in control soil (ATN) and slight presence were seen in the GLV grown in crude (ATE) and biotreated soil (ATT).

3.2. Soil Analysis

3.2.1. Physiochemical characterization of control soil, Silk effluent contaminated soil and Bioremediated soil

The Soil analysis performed with the effluent infested and remediated revealed that the pH of 9.5 and EC of 1.5 were noticed in ATES (effluent contaminated) soil was above the permissible limits whereas on remediation the results were similar to the control, the pH and EC of biotreated soil which was able to recover and lie within the standard limits. The characterization of physicochemical parameters of untreated and biotreated the Silk dyeing effluent is shown in Table 2. The soil texture was observed to be S1- type sandy clay and loam in both treatment groups. The Macronutrients, Calcium (Ca), Nitrogen (N), Phosphorous (P) and Potassium (K) from 100, 105, 10, 245 kg/ha of Control soil (ATNS) has decreased to the level of 95, 57, 3, 134 kg/ha respectively of the effluent soil (ATES) and in turn improved in the biotreated soil (ATTS) with 114, 213, 10.5, 268 kg/ha respectively in the biotreated effluent soil. The macronutrient N, P, K were drastically affected in the Silk effluent soil (ATES). The micronutrients Iron (Fe), Copper (Cu), Zinc (Zn) and Manganese (Mn) ranges from 1.4, 15, 0.6, 0.7 ppm of effluent contaminant soil and increased to 4.1, 20.22, 2.13, 1.13 ppm in the biotreated effluent soil (Table 2) were detected to be lower than the optimal range.

3.2.2. Spectral study for A. tristis GLV grown in different treatment groups

The FTIR carried out (Fig. 5) with the *A. tristis* plant powder grown in control soil (ATN), Crude effluent (ATE) and Biotreated soil (ATT) and compared. The FT-IR spectrum of *A. tristis* of control soil (ATN) has significantly 12 absorption bands. The band with strong broad width intensity at 3425 cm^{-1} corresponds to alcohol group. The bands 2924 cm^{-1} and 2847 cm^{-1} wavenumber with C—H stretch belongs to alkane group. The alkynes group with

Table 2
Physicochemical Characterization of Control soil, Silk effluent contaminated soil and Biotreated soil.

Parameters	Optimal range in Normal soil*	Control soil (ATNS)	Effluent soil (ATES)	Bioremediated soil (ATTS)
pH	6–7.5	6.7	9.5	7.5
EC	<1.0	0.1(Good condition)	1.5	0.2
Texture	–	S1 –Type sandy loam (Red soil: sand) (3:1)	S1- Type sandy clay loam (Red soil: sand) (3:1) with Dyeing effluent contaminants	S1- type sandy clay loam (Red soil: sand) (3:1) with mixed inoculant
Calcium (kg/ha)	150	100	95	114
Available Nitrogen (kg/ha)	170–220	105	57	213
Available Phosphorus (kg/ha)	10–20	10	3	10.5
Available Potassium (kg/ha)	230–360	245	134	268
Iron (ppm)	5–20	4.4	1.4	4.1
Manganese (ppm)	>1.5	25	15	20.22
Copper (ppm)	>0.6	2.10	0.6	2.13
Zinc (ppm)	>1.0	2.002	0.7	1.13

ATNS: *Control Soil*, ATES: Crude Silk dyeing effluent Soil, ATTS: Bioremediated Soil. The optimal range in normal soil was taken from 'Soil Test Interpretation Guide', EC 1478, Oregon State University, 2011.

Table 3
Functional groups detected in the FT-IR spectra of the GLV in different treatments.

FunctionalGroup of *A. tristis*	Alcohol	Alkane	Alkene	Alkynes	Carboxylic acid	Esters	Ethers	Isocyanide	Phosphine	Aromatic	Nitro groups	Amide	Aldehyde	Amine	Alkyl halide
ATN	+	+	+	–	+	+	+	–	–	+	+	–	–	+	+
ATE	+	+	–	–	+	+	+	–	–	+	+	–	–	+	+
ATT	+	+	+	–	+	+	+	–	–	+	+	–	–	+	+

+ Presence – Absence.
Treatment groups: ATN: *Amaranthus tristis,* were grown in fresh water; ATE: *Amaranthus tristis,* were grown in crude effluent; ATT: *Amaranthus tristis,* were grown in biotreated effluent.

C═C stretch indicated at wavenumber 2121 cm^{-1} peak. The N—H bend symbolized at 1635 cm^{-1} peak reveals the amide group. The absorption bands at wavenumber 1381 and 1319 cm^{-1} peak with N—O symmetric stretch related to nitro groups. The wavenumber 1111 cm^{-1} peak of absorbance with C—O stretch has resemblance to alcohols, carboxylic acids, esters and ethers. All the other peaks such as 825, 779, 671, 617 and 524 wavenumber cm^{-1} with small peaks related to alkyl halide.

The FT-IR spectrum of *A. tristis* grown in crude effluent (ATE) has some peaks at 3425 wavenumber cm^{-1} of alcohol group, 2924 and 2854 wavenumber cm^{-1} of alkane group, 1319 wavenumber cm^{-1} of alcohols, carboxylic acids, esters, ethers and 532 wavenumber cm^{-1} of alkyl halide. The aromatic group was found at the peak 1435 wavenumber cm^{1}. Some spectral bands were viewed at 1627, 1033, 918 wavenumber cm^{-1} belong to primary amine, alcohols/carboxylic acids/esters/ether, primary and secondary amines respectively. The alkyl halide spectrum were noted at 779, 686 and 532 wavenumber cm^{-1}. Irrespective of the dye contamination, the GLV *A. tristis* (ATE) was able to synthesis the functional group analogous to the GLV of control soil (ATN). It shows that the silk dyewater is less interactive with the functional groups of GLV *A. tristis*.

Moreover, spectral study of *A. tristis* grown in biotreated effluent water (ATT) as depicted in the Fig. 5 resemble to some important absorption bands of already discussed functional groups of wavenumber 3410, 2924 and 2854 cm^{-1} corresponds to alcohol and alkane groups. The indefinite spectral bands of 2376 and 2330 wavenumber cm^{-1} were also noted. The nitro groups of 1543 and 1381wavenumber cm^{-1} correlates with N—O stretch. The amine group was identified at wavenumber 1327 and 1249 cm^{-1}. The bands of 1103 and 1033 wavenumber cm^{-1}, corresponds to alcohols/carboxylic acids/esters/ether group of different stretch of C—O and C—N respectively. The alkyl halide group was confirmed at the spectral bands of 779, 617 and 501 wavenumber cm^{-1}.

3.2.3. Identification of active components by HPLC

HPLC is extensively used to detect the components present in the plant extracts as a confirmatory test. The HPLC results of methanolic extracts of *A. tristis* grown in control soil has found to

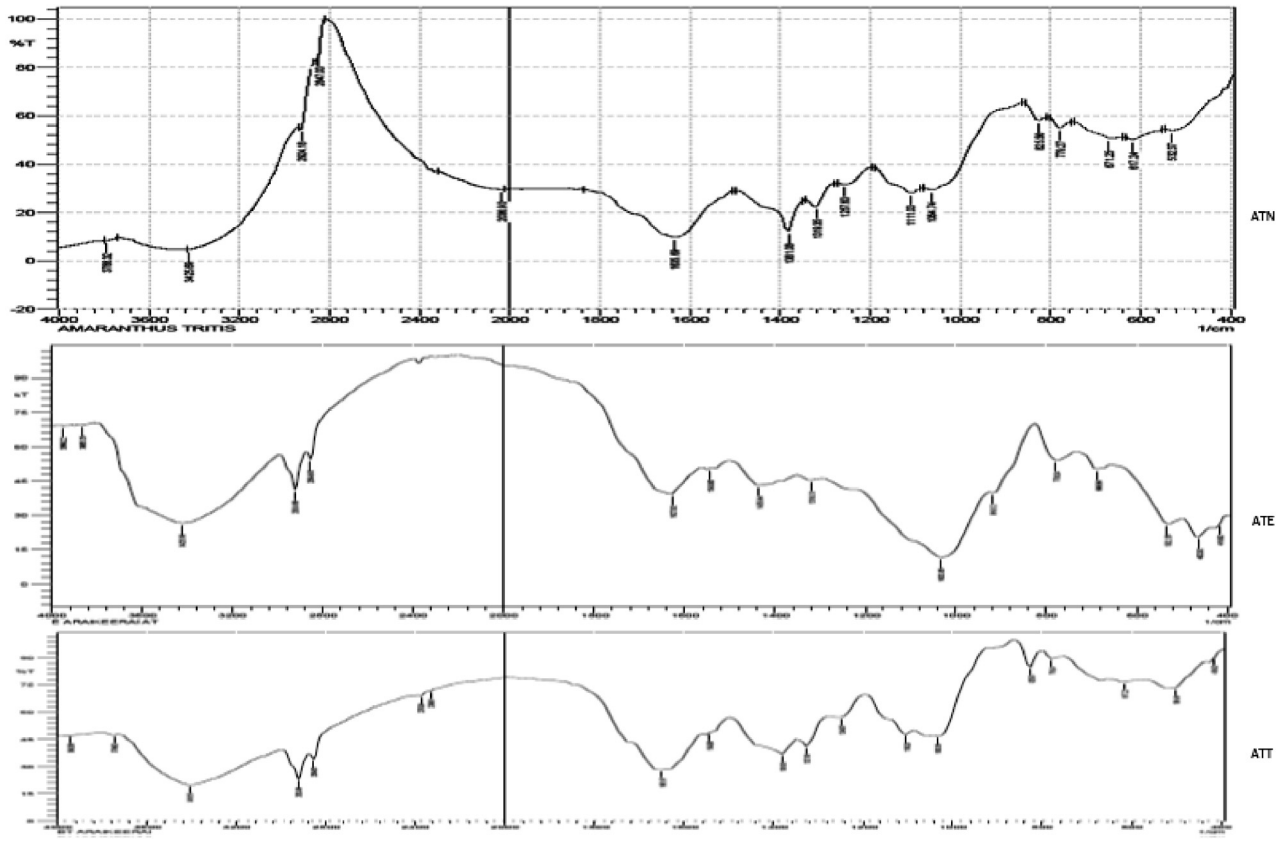


Fig. 5. FTIR Spectra of active components in ATN: *Amaranthus tristis,* were grown in control soil; ATE: *Amaranthus tristis,* were grown in crude effluent; ATT: *Amaranthus tristis,* were grown in biotreated effluent.

have six peaks with retention time of 2.6, 3, 3.9, 4, 4.2, and 4.6 RT whereas the effluent exposed GLV had only one peak of retention time of 4.1 RT inspite with same conditions was shown in the Fig. 6 and mentioned in Table 4.In the biotreated GLV among four peaks, the major peak was found with the maximum percentage area of 95.2% with the peak area of 54,244 with the reclamation of disappeared peak at 2.6 RT.

3.2.4. Identification of active components by GCMS analysis

The results concerning to GC–MS analysis of methanolic extract of *Amaranthus tristis* ATT to the identification of active compounds identified in the mass spectrometry with the Gas chromatography. The various compounds in the GLV grown in biotreated effluent were detected as in Table 5 and Fig. 7. The metabolites like N-Acetyl-3-Methoxyamphetamine, Bicyclo-3-Heptene, 2-Isoprope nyl-5-Isopropyl-7,7-Dimethyl, Tetracyclo nonane, 3,3,6,6,9,9-Hexamethyl-, Cis, Cis, Trans, n-hexadecanoic acid, BicycloHeptane, 7-Pentyl, Benz[E]Azulene-3,8-Dione -Octahydro-3a,10a-Dihydr, 1-monolinoleoylglycerol trimethylsilyl ether were the identified active compounds with the exact match with the NIST library.

4. Discussion

The phytochemical constituents of different parts of the *A. tristis* under treatment groups were characterized by qualitative screening of 20 metabolites where the constituents of the GLVs grown in control soil and biotreated effluent did not differ significantly. On the other hand absence or trace amount of presence seen in the GLV extracts of effluent contaminated soil, on biotreatment a subsequent presence were observed proves the effect of biotreatment. The parallel study investigated the presence of phytochemicals in *Taraxacum officinale* woodpecker observed by Mir et al. (2013) and the industrial effluent affects the phytochemical constituents observed in *Phaseolus mungo* and *Triticum aestivum* was reported by Chaube et al. (2010). The healthy soil is the primary constituent for the plant and its growth success relay on remediating it when contaminated. The effluent contaminated soil after bioremediating, needs to be analysed completely in all aspects assuring the removal of the contaminants then it can be recommended for the purpose of future usage in agriculture (Rajeswari, 2015). The alkaline pH of the Silk effluent dyebath cause the heavy metals to get precipitated as their salts at high pH are deposited as sediments in the soil as similarly reported by the study done by Rao and Patnaik (2000). Comparable to the results obtained Joshi and Kumar (2011) also reported in the effluent soil sample collected in the Sanganer region and Rena and Kanika (2013) study proves that the application of industrial effluent affects the physico-chemical properties of the soil as well as the fertility of soil. Thus the characteristics of soil particles showed that the silk dye contaminants can affect the macro and micronutrients of soil (Garg and Tripathi, 2011) and thereby remediation of contaminated soil reported by Das et al. (2009) proved that the available N, P and K contents improved with the application of the biofertilizers in the soil as well as in plants. Calcium can be reduced in contaminated soil and its deficiency symptoms impacts at the growing point, abnormally dark green foliage, weakened stems, shedding flowers as according to Larry (2011).Nitrogen deficiency in the soil affects the cellular metabolism and may lead to dwarfism of the plant, decrease of chlorophyll content of the cells and finally may lead to plant death as reported by Raymond et al. (2004). Analogous to these reports, in the present soil study, the Silk effluent treated soil drastically affect the macronutrients and micronutrients inturn the growth of the GLV is affected. The results also indicated that the biotreatment improves the nutritional status of the soil within the optimal range which strongly supports the growth of GLV.

The spectral bands of C—H, C=O, C=C, N—O, C—N stretches which were observed in all the samples of the treatment groups was correspondent to the studies of Hanson et al., 2016; Sumayya et al., 2019 through the adjacent range of spectrum

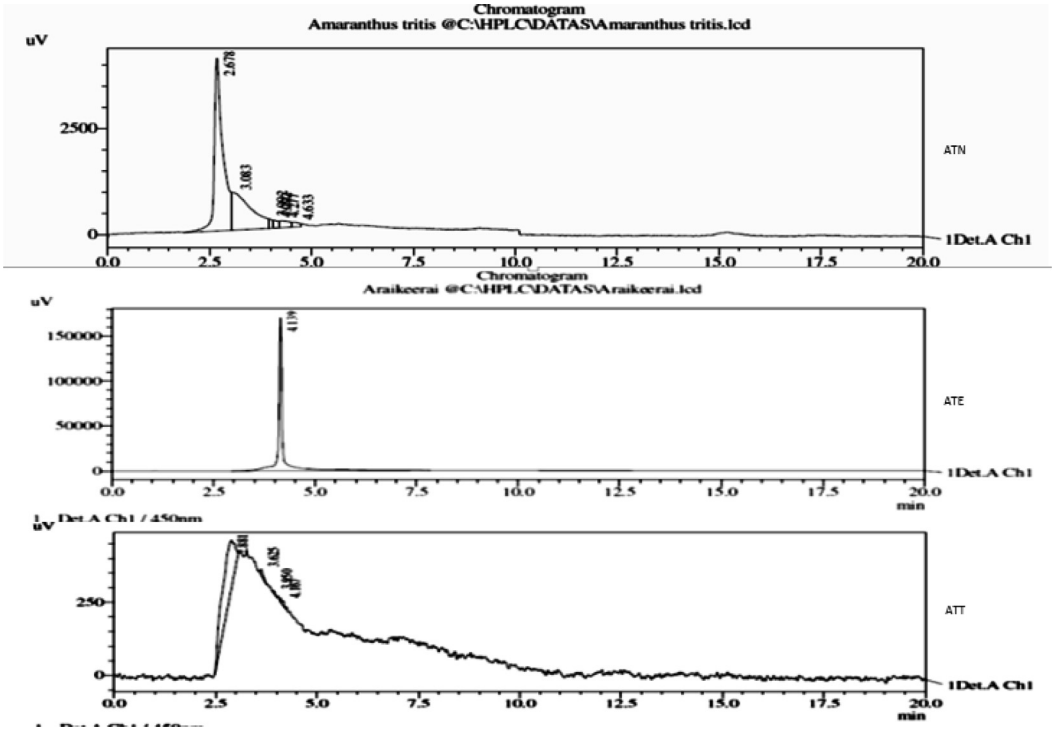


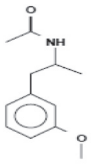
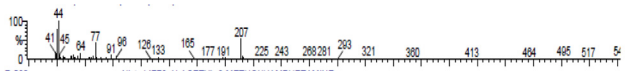
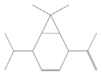
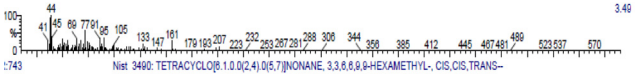
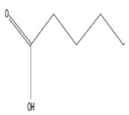
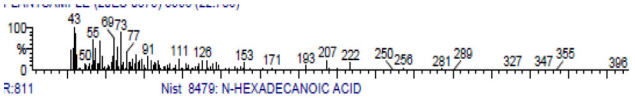
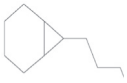
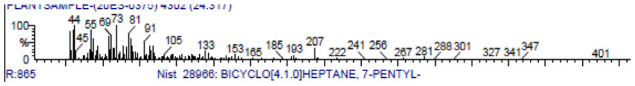
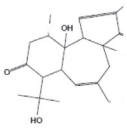
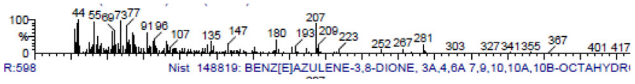
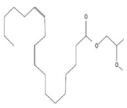
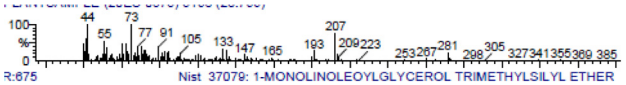
Fig. 6. HPLC Spectrum of ATN: *Amaranthus tristis,* were grown in control soil; ATE: *Amaranthus tristis,* were grown in crude effluent; ATT: *Amaranthus tristis* were grown in biotreated effluent.

Table 4
Comparison of HPLC spectral parameters of all treatment groups.

Treatment Groups	Peak No.	Retention Time (RT)	Peak Area	Peak Area %
ATN	1	2.6	60,799	63.284
	2	3	28,559	29.726
	3	3.9	1393	1.450
	4	4	1390	1.447
	5	4.2	2549	2.653
	6	4.6	1383	1.440
ATE	1	4.1	1,197,561	100
ATT	1	2.8	54,244	95.219
	2	3.6	1192	2.069
	3	3.9	1125	0.570
	4	4.1	2295	2.141

Treatment groups: ATN *Amaranthus tristis,* were grown in control soil; ATE: *Amaranthus tristis,* were grown in crude effluent; ATT: *Amaranthus tristis,* were grown in biotreated effluent.

Table 5
GCMS spectral analysis of *Amaranthus tristis* grown in the Biotreated effluent.

RT (Retention Time)	Name of the compound	Molecular formula	Molecular weight	Molecular structure	Hit spectrum
9.101	N-Acetyl-3-Methoxyamphetamine	C13H13O2N4Cl3S	394		
16.94	Bicyclo-3-Heptene, 2-Isopropenyl-5-Isopropyl-7,7-Dimethyl	C15H2	204		
18.83	Tetracyclo Nonane, 3,3,6,6,9,9-Hexamethyl-, Cis,Cis,Trans	C15H2	204		
22.78	n-hexadecanoic acid	C16H32O2	256		
24.31	BicycloHeptane, 7-Pentyl	C12H22	166		
26.83	Benz[E]Azulene-3,8-Dione, 3a,4,6a 7,9,10,10a,10b-Octahydro-3a,10a-Dihydr	C20H28O6	364		
28.79	1-monolinoleoylglycerol trimethylsilyl ether	C27H54O4Si2	498		

obtained in representing the same functional groups. Similar studies of FTIR analysis of *Tylophora pauciflora* wight by Starlin et al., 2012 showed the same functional groups mentioned in *A. tristis* grown in the biotreated effluent. On Comparative study of FT-IR spectrum of all treatment groups, the functional group studies revealed that the alcohol, alkane, alkyl halide, amine groups, esters, ethers and carboxylic acids were found in the GLV irrespective of the treatments, even in crude effluent, where the GLV has managed to produce these organic compounds. The amide, aldehyde, alkyne, Isocyanide and phosphine group was completely absent in GLVs of all the treatments as mentioned in the Table 3. The alkene group was not detected in the GLV of crude effluent (ATE) whereas detected in *A. tristis* of biotreated effluent (ATT) shows the influence of the mixed inoculants of biofertilizers. The FTIR spectral studies is reaffirmation of components obtained on the microbial remediation.

The HPLC spectral analysis done by Gong and Liu, 2017 investigated that the absence of the active compounds observed under abiotic stress and also few compounds were dropped in the HPLC analysis of Cowpea under drought stress was also reported by

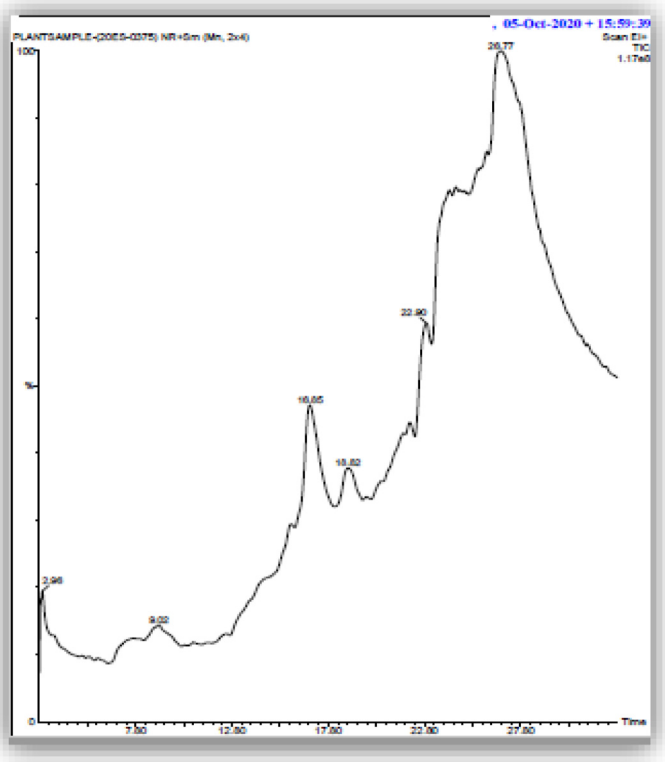


Fig. 7. GCMS chromatogram of methanolic extract of *Amaranthus tristis* grown in the Biotreated effluent.

Wei Lei et al., 2016. With the above evidence, the studies shows that GLV grown in the crude effluent was not able to synthesis some compounds which on biotreatment few compounds were obtained in the methanolic extract of *A. tristis* (ATT) proves the influence of microbial treatment on the effluent. The GC–MS spectral analysis of methanolic extract of *A. tristis* (ATT) revealed 7 active components shows evidence that the GLV was able to synthesize the metabolites in the biotreated effluent water. Similar tolerance were recorded in GC–MS spectrum of the rice tolerant varieties for salinity stress investigated by Gupta and De (2017).

From the above observations the outcome of the study revealed that the mixed inoculants of biofertilizers were with remediating effect of biotreated effluent in *A. tristis* against the abiotic stress caused by the Silk dyeing effluent. This mixed biofertilizer inoculant can act as an effective bioremediator for the Silk dye contamined soil and on the persistent application it supports the cultivation of GLV *A. tristis* in silk dyewater lands without affecting the major components.

5. Conclusion

Most of the small scale Silk dyeing units stretch out million liters of the effluent with hazardous chemicals or dyes inturn affects the biota. The Common treatment plant is not affordable for a slender entrepreneur. In the present study, the mixed inoculants of biofertlizers which are cost effective were observed to degrade the dyewater and applied to the GLV *Amaranthus tristis Linn* with optimum growth without affecting the plant metabolites. This methods were compared with their controls and found promising for the meagre investors of Silk dyeing units as it is easy, ecofriendly and cleanse the environment in a period of time towards attaining a sustainable environment.

Declaration of Competing Interest

The authors declare that there are no potential conflicts of interest regarding the publication of this paper.

Acknowledgement

Authors are thankful to the Researchers Supporting Project Number (RSP-2020/182), King Saud University, Riyadh, Saudi Arabia.

References

Anandaraj, B., Delapierre, L.R.A., 2010. Studies on influence of *bioinoculants (Pseudomonas fluorescens, Rhizobium sp., Bacillus megaterium*) in green gram. J. Biosci. Technol. 1 (2), 95–99.

Asare-Marfo, D., Birol, E., Gonzalez, C., Moursi, M., Perez, S., Schwarz, J., Zeller, M., 2013. Prioritizing countries for biofortification interventions using country-level data. International Food Policy Research Institute (IFPRI).

Bizuneh, A., 2012. Textile effluent treatment & decolorization techniques. Chem. Bulg. J. Sci. Edu. 21, 434–456.

Börnick, H., Schmidt, T.C., 2006. Amines. Organic pollutants in the water cycle: properties, occurrence, analysis and environmental relevance of polar compounds, pp.181–209.

Chaube, P., Indurkar, H., Moghe, S., 2010. Biodegradation and decolorisation of dye by mix consortia of bacteria and study of toxicity on Phaseolus mungo and Triticum aestivum. Asiatic J. Biotech. Res 1, 45–56.

Chen, H., Feng, J., Kweon, O., Xu, H., Cerniglia, C.E., 2010. Identification and molecular characterization of a novel flavin-free NADPH preferred azoreductase encoded by azoB in Pigmentiphaga kullae K24. BMC Biochem. 11 (1), 13.

Cheng, K.L., Bray, R.H., 1951. Determination of calcium and magnesium in soil and plant material. Soil Sci. 72 (6), 449–458.

Das, P., Choudhari, A.R., Dhawan, A., Singh, R., 2009. Role of ascorbic acid in human seminal plasma against the oxidative damage to the sperms. Indian J. Clin. Biochem. 24 (3), 312–315.

Environmental Protection Agency, EPA, 2010. Environment (Protection) Third amendment rules, Ministry of Environment and Forests, Johnstown castle estate, Wexford, Ireland, pp, 5–7.

Gałązka, A., Król, M., Perzyński, A., 2012. The efficiency of rhizosphere bioremediation with Azospirillum sp. and Pseudomonas stutzeri in soils freshly contaminated with PAHs and diesel fuel. Polish J. Environ. Stud. 21 (2).
Gamel, T.H., Linssen, J.P., Mesallem, A.S., Damir, A.A., Shekib, L.A., 2005. Effect of seed treatments on the chemical composition and properties of two amaranth species: starch and protein. J. Sci. Food Agric. 85 (2), 319–327.
Garg, S.K., Tripathi, M., 2011. Strategies for decolorization and detoxification of pulp and paper mill effluent. Reviews of Environmental Contamination and Toxicology, vol. 212. Springer, New York, NY, pp. 113–136.
Gong, X., Liu, J.H., 2017. Detection of free polyamines in plants subjected to abiotic stresses by high-performance liquid chromatography (HPLC). In: Plant Stress Tolerance. Humana Press, New York, NY, pp. 305–311. https://doi.org/10.1007/978-1-4939-7136-7.
Grubben, G.J.H., Denton, O.A., 2004. Plant resources of tropical Africa 2. Vegetables. Plant resources of tropical Africa 2. Vegetables.
Gupta, P., De, B., 2017. Metabolomics analysis of rice responses to salinity stress revealed elevation of serotonin, and gentisic acid levels in leaves of tolerant varieties. Plant Signaling Behav. 12 (7), e1335845.
Gupta, S., Prakash, J., 2009. Studies on Indian green leafy vegetables for their antioxidant activity. Plant Foods Hum. Nutr. 64 (1), 39–45.
Hanson, R.K., Spearrin, R.M., Goldenstein, C.S., 2016. Spectroscopy and optical diagnostics for gases. Springer International Publishing, Cham, pp. 227–253. ISBN-13: 978-3319232515.
Jackson, M.L., 1962. Soil Chemical Analysis. Prentice Hall of India Pvt. Ltd., New Delhi, pp. 1–483.
Jayarajan, M., Arunachalam, R., Annadurai, G., 2011. Agricultural wastes of jackfruit peel nano-porous adsorbent for removal of rhodamine dye. Asian J. Appl. Sci. 4 (3), 263–270.
Joshi, N., Kumar, A., 2011. Physico-chemical analysis of soil and industrial effluents of Sanganer region of Jaipur Rajasthan. Res. J. Agric. Sci. 2 (2), 354–356.
Khader, V., Rama, S., 2003. Effect of maturity on macromineral content of selected leafy vegetables. Asia Pacific J. Clin. Nutrit. 12 (1), 45–49.
Khanam, U.K.S., Oba, S., Yanase, E., Murakami, Y., 2012. Phenolic acids, flavonoids and total antioxidant capacity of selected leafy vegetables. J. Funct. Foods 4 (4), 979–987.
Kiernan, J.A., 2001. Classification and naming of dyes, stains and fluorochromes. Biotech. Histochem. 76 (5–6), 261–278.
Kjeldahl, Johan, 1900. Obituary. Ber. Dtsch. Chem. Ges., 33, 3881–3888.
Larry, O. 2011. Secondary Plant Nutrients: Calcium, Magnesium, and Sulfur, Mississippi State University, 1–4.
Lei, Wei, Huang, Shian, Tang, Shaohu, Shui, Xiaorong, Chen, Can, 2016. Determination of abscisic acid and its relationship to drought stress based on cowpea varieties with different capability of drought resistance. Am. J. Biochem. Biotechnol. 12 (1), 79–85. https://doi.org/10.3844/ajbbsp.20.
Lins, G.M., Cruz, W.S., Vieira, Z.M., Neto, F.A., Miranda, É.A., 2010. Determining indicators of urban household water consumption through multivariate statistical technique. J. Urban Environ. Eng. 4 (2), 74–80.
Mathur, N., Bhatnagar, P., 2007. Mutagenicity assessment of textile dyes from Sanganer (Rajasthan). J. Environ. Biol. 28 (1), 123–126.
Mir, M.A., Sawhney, S.S., Jassal, M.M.S., 2013. Qualitative and quantitative analysis of phytochemicals of Taraxacum officinale. Wudpecker J. Pharmacy Pharmocol. 2 (1), 001–005.
Nachiyar, C.V., Rajkumar, G.S., 2003. Degradation of a tannery and textile dye, Navitan Fast Blue S5R by *Pseudomonas aeruginosa*. World J. Microbiol. Biotechnol. 19 (6), 609–614.
Nupur, B., 2009. Low cost effluent treatment plants for small scale industries-Need for experience of dyes and Inputs, http://ndiawaterportal.org/questions/solution-exchange-consolidated-reply-low-cost-effluent-treatment-plants-small-scale.
Olsen, S.R., 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate (No. 939). US Department of Agriculture.
Prakash, P., Karthikeyan, B., 2013. Isolation and purification of Plant Growth Promoting Rhizobacteria (PGPR) from the rhizosphere of *Acorus Calamus* grown soil. Indian Streams Res. J. 3, 1–13.
Rajeswari, A., 2015. Evaluation of phytochemical constituents, quantitative analysis and antimicrobial efficacy of potential herbs against selected microbes. Evaluation 8 (2), 2–4.
Rao, L.M., Patnaik, R.M.S., 2000. Heavy metal accumulation in the cat fish *Mystus vittatus* (Bloch) from Mehadrigedda stream of Visakhapatnam, India. Pollut. Res. 19 (3), 325–329.
Raymond, J., Siefert, J.L., Staples, C.R., Blankenship, R.E., 2004. The natural history of nitrogen fixation. Mol. Biol. Evol. 21 (3), 541–554.
Rena, M., Kanika, Y., 2013. Soil contamination due to Textile Effluent- Case study on the Printing cluster of Jaipur. J. Textile Associat., 367–370
Sivaramakrishnan, R., Incharoensakdi, A., 2020b. Plant hormone induced enrichment of Chlorella sp. omega-3 fatty acids. Biotechnol. Biofuels 13 (1), 7.
Sivaramakrishnan, R., Ramprakash, B., Ramadoss, G., Suresh, S., Pugazhendhi, A., Incharoensakdi, A., 2020a. High potential of Rhizopus treated rice bran waste for the nutrient-free anaerobic fermentative biohydrogen production. Bioresour. Technol. 319, 124193.
Sivaramakrishnan, R., Suresh, S., Pugazhendhi, A., Pauline, J.M.N., Incharoensakdi, A., 2020c. Response of Scenedesmus sp. to microwave treatment: Enhancement of lipid, exopolysaccharide and biomass production. Bioresour. Technol., 123562
Starlin, T., Arul, R.C., Ragavendran, P., Gopalakrishnan, V.K., 2012. Phytochemical screening, functional groups and element analysis of *Tylophora pauciflora wight* and arn. Int. Res. J. Pharm 3 (6), 180–183.
Stein, S.E., 1990. National institute of standards and technology (NIST) mass spectral database and software.
Sumayya, A.R., Saranya, R.S., Rafiqkhan, M., Brindha, P.S., Sangeetha, S., 2019. Fourier Transform Infrared (FTIR) Spectroscopy and High-Performance Liquid Chromatography Analysis of Brassica juncea (Mustard) and Silk dye-ing effluent's impact on the spectral studies. *International Journal of Research*. Pharmaceut. Sci. 10 (2).
Sumayya, A.R., Srinivasan, Sivagami, 2014. Biotoxicity analysis of fishes in fresh water, untreated and biotreated silk dyeing effluent. J. Chem. Biolog. Phys. Sci. 4 (3), 2258–2264.
Sumayya, A.R., Srinivasan, Sivagami, 2018a. Functional Groups and Compounds Analysis Using Chromatographic and Spectroscopic Techniques of Untreated and Bio Remediated Silk Dyeing Effluent. Int. J. Current Res. 1 (10), 74083–74091.
Sumayya, A.R., Srinivasan, Sivagami, 2018b. Histological studies of the leaf tissues in selected Glv's of fresh water, crude silk dyeing effluent and biotreated effluent. Int. J. Innovat. Sci. Res. 7 (08), 1208–1212.
Thiripurasundari, N., Vinodhkumar, T., Ramanathan, G., Karthik, G., 2013. Screening of Dye degrading bacteria from Textile effluents. Int. J. Res. Rev. Pharmacy Appl. Sci. 3, 848–857.
Toth, S.J., Prince, A.L., 1949. Potassium determination in plant digests by flame photometer. Soil, Plant and water Analysis by PC Jaiswal, pp. 275–279.
Wu, H., Sun, M., Yue, S., Sun, H., Cai, Y., Huang, R., Brenner, D., Corke, H., 2000. Field evaluation of an Amaranthus genetic resource collection in China. Genet. Resour. Crop Evol. 47 (1), 43–53.
Xu, F., Sun, M., 2001. Comparative analysis of phylogenetic relationships of grain amaranths and their wild relatives (Amaranthus; Amaranthaceae) using internal transcribed spacer, amplified fragment length polymorphism, and double-primer fluorescent intersimple sequence repeat markers. Mol. Phylogenet. Evol. 21 (3), 372–387.
Zaharia, C., Suteu, D., Muresan, A., 2012. Options and solutions for textile effluent decolorization using some specific physico-chemical treatment steps. Environ. Eng. Manage. J. 11 (2), 493–509.

Further Reading

Horneck, D.A., Sullivan, D.M., Owen, J.S., Hart, J.M., 2011. Soil Test Interpretation Guide (EC 1478). Oregon State University Extension Service. ir.library.oregonstate.edu/xmlui/bitstream/handle/1957/22023/ec1478.pdf.