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Efficient decolorization and detoxification of textile industry effluent by *Salvinia molesta* in lagoon treatment

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ABSTRACT

Salvinia molesta, an aquatic fern was observed to have a potential of degrading azo dye Rubine GFL up to 97% at a concentration of 100 mg/L within 72 h using 60 ± 2 g of root biomass. Both root as well as stem tissues showed induction in activities of the enzymes such as lignin peroxidase, veratryl alcohol oxidase, laccase, tyrosinase, catalase, DCIP reductase and superoxide dismutase during decolorization of Rubine GFL. FTIR, GC-MS, HPLC and UV–visible spectrophotometric analysis confirmed phytotransformation of the model dye into smaller molecules. Analysis of metabolites revealed breakdown of an azo bond of Rubine GFL by the action of lignin peroxidase and laccase and formation of 2-methyl-4-nitroaniline and N-methylbenzene-1, 4-diamine. Anatomical tracing of dye in the stem of *S. molesta* confirmed the presence of dye in tissues and subsequent removal after 48 h of treatment. The concentration of chlorophyll pigments like chlorophyll a, chlorophyll b and carotenoid was observed during the treatment. Toxicity analysis on seeds of *Triticum aestivum* and *Phaseolus mungo* revealed the decreased toxicity of dye metabolites. *In situ* treatment of a real textile effluent was further monitored in a constructed lagoon of the dimensions of 7 m × 5 m × 2 m (total surface area 35 m^2) using *S. molesta* for 192 h. This large scale treatment was found to significantly reduce the values of COD, BOD_5 and ADMI by 76%, 82% and 81% considering initial values 1185, 1440 mg/L and 950 units, respectively.

1. Introduction

One fourth of the Indian urban and suburban population is presently engaged in textile industries which contribute about 25% of total foreign currency (Goyal et al., 2009). Dye processors routinely use about 3500 different dyes, out of which, 84% is contributed by sulphonated azo dyes. Around 10–15% of the total wastewater is discharged to the environmental sink by these industries (Sarayu and Sandhya, 2009). Dye effluents contain organic compounds, metals, salts directly affect water color, chemical oxygen demand (COD), biochemical oxygen demand (BOD), total dissolved solid (TDS), total suspended solid (TSS) and pH (Kabra et al., 2013). In most of the developing countries, many textile industries are small scale and house hold whose treatment processes are less efficient and releases the wastewater directly to river and other natural water resources (Rane et al., 2014). These wastewaters are highly toxic to the living beings like humans and flora and fauna of various habitats (Sharma et al., 1999).

There are several physico-chemical methods like filtration, flocculation, coagulation, adsorption, chemical oxidation, photodegradation designed for the treatment of textile effluents containing dyes. These processes are costly and less efficient, produce secondary waste materials and thus possess limitations in treating effluents with multiple types of dyes (Kabra et al., 2013). Although, color removal from the effluent is achieved with these systems, the toxicity of secondary waste remains a major threat. Therefore, there is an urgent need to design unique systems which are cost effective, eco-friendly and efficient in textile wastewater treatment. Because of these reasons needs modern biological treatment using microorganisms were tried for the treatment of wastewater which also has shortcomings when on site administration is concerned (Khandare and Govindwar, 2015; Patil et al., 2016).

A number of reports on phytoremediation of textile dyes have been published in the last decade describing the use of plants from various habitats. However, the large scale demonstrations still

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remain scares. The present study deals with decolorization and degradation of customarily used dye Rubine GFL, a simulated dye mixture and a real textile effluent by *Salvinia molesta*. Further, *S. molesta* which is an aquatic fern, with dense root system spreading over water was explored in a constructed lagoon for large scale treatment. The treatment pond system can be controlled and planted with aquatic vegetation to achieve effective dye removal. *S. molesta*, if grown once, it does not need to monitor in future for its growth as it grows naturally. It is also known as kariba weed which belongs to the family Salviniaceae. It is native to south-eastern Brazil. The favourable condition for growth requires 20–30 °C temperature and pH range in between 4 and 9. It can be grown in high salt concentration (Upadhyay and Panda, 2005). *Sesuvium portulacastrum*, a halophyte was shown to treat Green HE4B (50 mg/L) dye up to 70% (Patil et al., 2012). *S. molesta* has a potential to grow in dye containing water and requires less time period for adaptation (Sukumaran, 2013). Use of *S. molesta* was for treatment of dye effluents has earlier been successfully tried for removal of heavy metals such as lead, arsenic and cadmium (Sukumaran, 2013). Halophytic plants could be explored for treatment of a variety of industrial effluents.

2. Material and methods

2.1. Chemicals

2, 2-Azino-bis (3-ethylbenzothiazoline)-6-sulphonic acid (ABTS) and riboflavin were purchased from Sigma-Aldrich (St Louis, MO, USA). 2, 6-dichlorophenol indophenol (DCIP), nicotinamide adenine dinucleotide (di-sodium salt), n-propanol, catechol and veratryl alcohol were bought from Sisco Research Laboratories, Mumbai, Maharashtra, India. Tartaric acid was obtained from BDH chemicals (Mumbai, Maharashtra, India). The textile dyes Rubine GFL, Remazol Black B, Red RBL were obtained from Mahesh dye processors, Ichalkaranji and effluent was from CETP, Kagal, Maharashtra, India. The seeds of *T. aestivum* (monocot) and *P. mungo* (dicot) were purchased from a local grain market and *S. molesta* plant was procured from wastewater creek Ambewadi, Kolhapur, Maharashtra.

2.2. Decolorization studies with S. molesta

Well grown *S. molesta* plants with root biomass of 60 ± 2 g was used for phytoremediation studies. The *S. molesta* plants roots were carefully washed with running tap water and submerged in 500 mL dye solutions in 1000 mL beaker. Dyes namely Rubine GFL, Remazol Black B, Red RBL, Bottle Green No.9, Navy Blue Rx and Scarlet RR were used at concentration of 100 mg/L separately.

Similarly, plant were exposed to 500 mL of the simulated dye mixture containing Rubine GFL, Remazol Black B and Red RBL at a concentration of 100 mg/L. Aliquots of 2 mL were withdrawn from dye solution at intervals of 12 h, over the period of 72 h. This aliquot was centrifuged at $4561 \times g$ for 10 min to separate any solid matter if present. The absorbance of the clear solution of Rubine GFL was measured at its wavelength maxima of 530 nm using a spectrophotometer method. The percent of decolorization was calculated using following equation.

$$\%\text{Decolorization} = (\text{Initial absorbance} - \text{Final absorbance}/\text{Initial absorbance}) \times 100.$$

2.3. Characterization of dye, dye mixture and textile effluent

Rubine GFL, dye mixture and textile effluent were characterized by using parameters such as American Dye Manufactures Institutes (ADMI), biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), total suspended solid (TSS) and total dissolved solid (TDS) (APHA, 1995) before and after treatment. Heavy metals were estimated using Atomic Absorption Spectrophotometer (Thermofisher AA203) (Lindsay and Norvell, 1978). BOD_5 was estimated using following procedure of Winkler's iodometric method (APHA, 1995). The sample was collected and stored at 4 °C till used. Known diluted sample using distilled water was transferred to the BOD bottle (300 mL). One mL each phosphate buffer, $MgSO_4$, $CaCl_2$ and $FeCl_3$ solution was added into diluted sample and mixed thoroughly. The sample was neutralized to pH 7 by using 1N NaOH or H_2SO_4. One set of BOD bottle was incubated in BOD incubator at 20 °C for 5d. Other set was used to determine the DO content at the same time. One mL of alkaline KI and $MnSO_4$ was added into diluted solution. 2 mL concentrated H_2SO_4 was added and stirred well to dissolve precipitate. Aliquot of 50 mL sample was taken from BOD bottle into conical flask to titrate against sodium thiosulphate using starch as an indicator. After 5d incubation, DO was measured using same procedure. Distilled water was kept as blank. BOD was calculated using following formula.

$$BOD_5\ (\text{mg/L}) = (D_0 - D_5) - (B_0 - B_5) \times \text{dilution factor.}$$

where, D_0 – 0 d DO, D_5 – after 5 d DO, B_0 – 0 d blank and B_5 – after 5 d blank.

2.4. Anatomical studies of stem during dye degradation

The transverse sections of stem were taken at 12, 24, 36 and 48 h time interval after the exposure of Rubine GFL. Then these sections were mounted in glycerine after overlaying with cover slip and result were micro-photographed with a Zeiss Axio Imager 2 Upright Trinocular Microscope with attached camera at 100 magnifications (Rane et al., 2014). The plants again transfer to fresh water after 48 h experiment and were again studied anatomical changes at 60 h.

2.5. Analysis of photosynthetic pigments

Five gram of each control and treated plants leaves were taken in separate mortar and pestle. 50 mL of acetone (80%) was added at the time of crushing along with a pinch of $MgCO_3$ powder. After crushing, extract was filtered and then centrifuged at $2000 \times g$ for 10 min. For the estimation of chlorophyll content, absorbance of supernatant was measured at wavelength 663 and 645 nm; while carotenoids were estimated at 470 nm (Arnon, 1949).

2.6. Preparation of crude extracts of root and stem tissue and enzyme assay

Two gram of each root and stem of *S. molesta* were excised and cut into fine pieces. They were then separately suspended in 50 mM potassium phosphate buffer of pH 7.4. The fine pieces of root and stem were crushed using a mortar and pestle, then homogenized using glass homogenizer and centrifuged for 20 min at $8481 \times g$ at 4 °C. The obtained clear supernatant was used as an enzyme source (Kagalkar et al., 2009).

Activities of dye degrading enzymes like lignin peroxidase (LiP), laccase, veratryl alcohol oxidase (VAO), tyrosinase, azo reductase, DCIP reductase, catalase, superoxide dismutase (SOD) and riboflavin reductase were assayed spectrophotometrically. The lignin peroxidase assay was performed by using n-propanol as substrate ($20\ M^{-1}\ cm^{-1}$) at 300 nm (Shanmugam et al., 1999) and activity of laccase was measured by oxidation of ABTS as substrate at 420 nm (Hatvani and Mécs, 2001). Activity of veratryl alcohol oxidase was determined by earlier reported method (Jadhav et al., 2009). Enzyme activity was calculated by using extinction coefficient of oxidised veratryl alcohol ($9.5\ M^{-1}\ cm^{-1}$) at 300 nm. Activity of

tyrosinase was measured by previously quoted method (Zhang and Flurkey, 1997). The total volume of 3.0 mL reaction mixture contains 2.7 mL potassium phosphate buffer (50 mM, pH 6.8), 0.1 mL catechol (1.5 mM), 0.1 mL L-ascorbic acid (2.5 mM), 0.1 mL crude extract and absorbance was measured at 265 nm. The DCIP reductase activity was performed using DCIP as substrate (Salokhe and Govindwar, 1999) and measured spectrophotometrically at 620 nm. Azo reductase and riboflavin reductase activity were measured by previously reported method (Kurade et al., 2011). Activities of antioxidant enzymes catalase and superoxide dismutase were determined as reported earlier by Vafaei et al. (2013).

All enzyme assays were carried out at room temperature with reference blank containing all components except crude extract. The protein concentration was measured by Lowry method (Lowry et al., 1951). All enzyme sets were done in triplicate, average rate of test calculated.

2.7. Analysis of metabolites

Decolorization of the Rubine GFL was examined by UV–visible spectrophotometric analysis (Hitachi U-2800; Japan) using crude extract, whereas metabolites were examined using High Performance Liquid Chromatography (HPLC), Fourier Transform Infrared Spectroscopy (FTIR) and Gas Chromatography-Mass Spectroscopy (GC-MS). For the extraction of the metabolites after the dye decolorization, plants were removed from dye solution and then centrifuged to separate any solid particles like root hairs and dust particle if present. The solution was extracted with equal volume of ethyl acetate. The extract was evaporated and dried. Obtained residue was redissolved in small quantity of HPLC grade methanol and used for analytical study.

HPLC analysis was performed using Water 2690 equipped system (Water Corporation, UK) on C18 column (4.6 mm × 250 mm, 3.5 μm symmetry) and for mobile phase, HPLC grade methanol was used with a flow rate of 1 mL min^{-1} at 280 nm. FTIR was used to determine changes in surface functional group of control and phytotransformed dye product. The analysis was carried out using Shimadzu 8400 S FTIR spectrometer (Shimadzu Corporation, Japan) in mid infra red region of 400–4000 cm^{-1}. The stereoscopically pure KBr mixed with extracted residues in 95:5 ratios was used as sample (Rane et al., 2014).

GC-MS analysis was used to identify the produced metabolites after phytotransformation using a Shimadzu 2010 MS Engine (Shimadzu Corporation, Japan) by earlier reported process. The temperature was enhanced to 200 °C with a rate 10 °C min^{-1} and then linearly raised to 280 °C at a rate of 20 °C min^{-1}. Helium was used as sample carrier gas with a flow rate of 1 mL min^{-1}. The initial temperature of column was stabilised at 80 °C for 2 min by oven. Produced metabolite was identified using mass spectra and structure was determined by NIST library.

2.8. Analysis of phytotoxicity and total bacterial count before and after treatment

Toxicity of effluents containing textile dyes has direct effects on the photosynthetic reaction and also arrests the growth of the plant. Fifty seeds of each *T. aestivum* (monocot) and *P. mungo* (dicot) were taken separately in petri plate containing blotting paper and phytotoxicity assay was done at 30 ± 2 °C. Daily application of 5 mL untreated and treated Rubine GFL, dye mixture and textile effluent on separately above seeds to assess their phytotoxicity. Distilled water was kept as control at the same time. The germination (%) of seed was compared to control. Plumule (shoot length) and radicle (root length) were measured after 6 days.

One mL sample of textile effluent was collected before and after treatment. This sample was diluted using 0.9% saline (NaCl) and a serial dilution up to 7 times. This diluted sample was spread on a nutrient agar medium and incubated at 37 °C. Bacterial count was measured in terms of colony forming units (CFUs). The composition of nutrient agar medium was as follows (g/L) Yeast extract 1.5, peptone 5, NaCl 5, beef extract 1.5% and 1.5% agar.

2.9. Phytotreatment in lagoons

Initial laboratory level studies showed potential of *S. molesta* fern to degrade textile dyes; hence, it was further used for phytoremediation treatment process of textile effluent at lagoon scale on HRTS (High rate transmission system), Kagal, India. The initial root length of the plant was 5–8 cm at the time of collection. These were stored in a lagoon for 15 days containing normal tap water. During this period, plants were stabilised and root lengths grew up to 10–12 cm at the time of treatment. The dimension of lagoon was 7 m × 5 m × 2 m of surface area 35 m^2. This lagoon was mulched properly using mulching paper for reducing loss of effluent. The lagoon was provided an inlet as well as an outlet channel opposite to each other for passing effluent. At the time of treatment, lagoon was filled with effluent through inlet up to a height of 1.5 m (52,500 L) and well grown plants of *S. molesta* were spread on the lagoon. The sample was collected from inlet, noted as 0 h; while after treatment sample was collected at 192 h. The lagoon was stirred and with steel rod before collection of the samples so that homogeneity was maintained and sampling errors minimized. During effluent treatment process parameters like ADMI, pH, COD, BOD_5, TDS, TSS and heavy metals were analysed up to 8 days (192 h) with 24 h intervals.

2.10. Statistical analysis

Data were analysed by one way analysis of variance (ANOVA) with Tukey-Kramer multiple comparisons test.

3. Results and discussion

3.1. Decolorization of dye Rubine GFL by S. molesta

Wild plants of *S. molesta* was observed to decolorize various screen dyes such as Remazol Black B, Red RBL, Bottle Green No.9, Navy Blue Rx, Scarlet RR and Rubine GFL up to 48%, 61%, 58%, 64%, 69% and 76%, respectively within 60 h. Looking at maximum decolorization with Rubine GFL it was taken for further studies. Absorbance of withdrawn supernatant was measured at 530 nm which is the wavelength of its maximum absorbance. The percent decolorization of the dye was observed to be 58%, 63%, 75%, 87%, 92% and 97%; with 166.5, 180.8, 215.3, 249.7, 264.0 and 278.4 μmole removal at 12, 24, 36, 48, 60 and 72 h, respectively. Scarlet RR (50 mg/L) and Remazol Red (70 mg/L) were reported to be decolorized previously by *Ipomoea hederifolia* and *Alternanthera philoxeroides* within 72 h up to 96% and 100%, respectively (Rane et al., 2014, 2015). *Typha angustifolia* was shown to decolorize Reactive blue 19 up to 70% within 6 d (Mahmood et al., 2014). *Bouteloua dactyloides* was also shown to decolorized textile effluent up to 92% within 24 h (Vijayalakshmidevi and Muthukumar, 2014).

3.2. Involvement of S. molesta in treatment of dye mixture and textile effluent

S. molesta could also treat a simulated mixture of dyes and real textile effluent. ADMI removal values after treatment of the dye mixture and effluent were found to be decreased 5.4 and 6.5 fold, respectively. COD, BOD_5, TDS, TSS, hardness and turbidity of dye

Table 1.
Characterization of dye mixture and textile effluent before and after their treatment at lab scale.

Parameter	Dye mixture		% reduction	Textile effluent		% reduction
	Untreated	Treated		Untreated	Treated	
ADMI	534	98	81	694	107	84
BOD_5 (mg/L)	1490	492	66	1845	573	68
COD (mg/L)	1367	418	69	1652	576	65
pH	8.5	7.2	15	9.9	7.6	23
TDS (mg/L)	18	4	77	4380	794	81
TSS (mg/L)	25	12	52	640	235	63
Turbidity (NTU)	34	18	47	278	54	80
Hardness	280	110	60	540	190	64

mixture after treatment by *S. molesta* were reduced by 3.2, 3.0, 4.5, 2.1, 2.5 and 1.9 fold, respectively. The textile effluent also showed reductions in the values of COD, BOD_5, TDS, TSS, hardness and turbidity 2.9, 3.2, 5.5, 2.7, 2.8 and 5.1 fold, respectively. The TSS of textile effluent was decreased because it might be absorbed by plant root. A significant decrease in the pH of textile effluent as well as dye mixture was observed after treatment using *S. molesta* (Table 1).

In an earlier study, textile effluent was treated using *I. hederifolia* which revealed the reduced ADMI value, BOD and COD up to 88%, 63% and 68%, respectively within 60 h (Rane et al., 2014). Plants like *Typhonium flagelliforme, Blumea malcolmii* and *Phragmites australis* have been used for treatment of textile industry effluent and achieved noteworthy BOD, COD, TOC, TDS and TSS removal (Davies et al., 2005; Kagalkar et al., 2009, 2010; Ong et al., 2010).

3.3. Anatomical analysis of stem tissue of S. molesta during Rubine GFL decolorization

Stem tissue of *S. molesta* grown in a beaker was harvested at 12 h intervals up to 60 h for histochemical analysis to understand the movement of dye in tissue and location of degradation. Stem tissue of control plant did not show the presence of dye color in any cell (Fig. 1a) and cells were observed to be in normal position. Accumulation was seen to be spreads to neighbouring cortical cells with the increase in the period of dye exposure. During first 12 h of dye exposure accumulation of the dye was observed in the epidermal cells (Fig. 1b). Further it spread to cortical cells at 24 h of dye exposure (Fig. 1c). It is observed that as cortical region was saturated with Rubine GFL and accumulated dye at epidermal cells gradually decolorized (Fig. 1d). Dye degradation was observed in both epidermal and cortical cells at 48 h (Fig. 1e). These results indicate the biotransformation of dye in both the regions. When exposed plants were transferred to the normal tap water after 48 h experiments, some epidermal cells were observed distorted and contained small amount of residual dye after 60 h (Fig. 1f). Shapes of xylem and phloem were also found distorted.

3.4. Alteration of photosynthetic pigment during decolorization

Chlorophylls and carotenoid concentrations in the leaves were measured after 72 h of the dye exposure to *S. molesta*. During decolorization process, chlorophyll a, chlorophyll b and carotenoid were found to be increased up to 11%, 19% and 29%, respectively when compared to control (Table 2). *S. molesta* is a floating aquatic fern which upon exposure to dyes might synthesize more chlorophyll (a, b) and carotenoid for fulfilment of energy requirement.

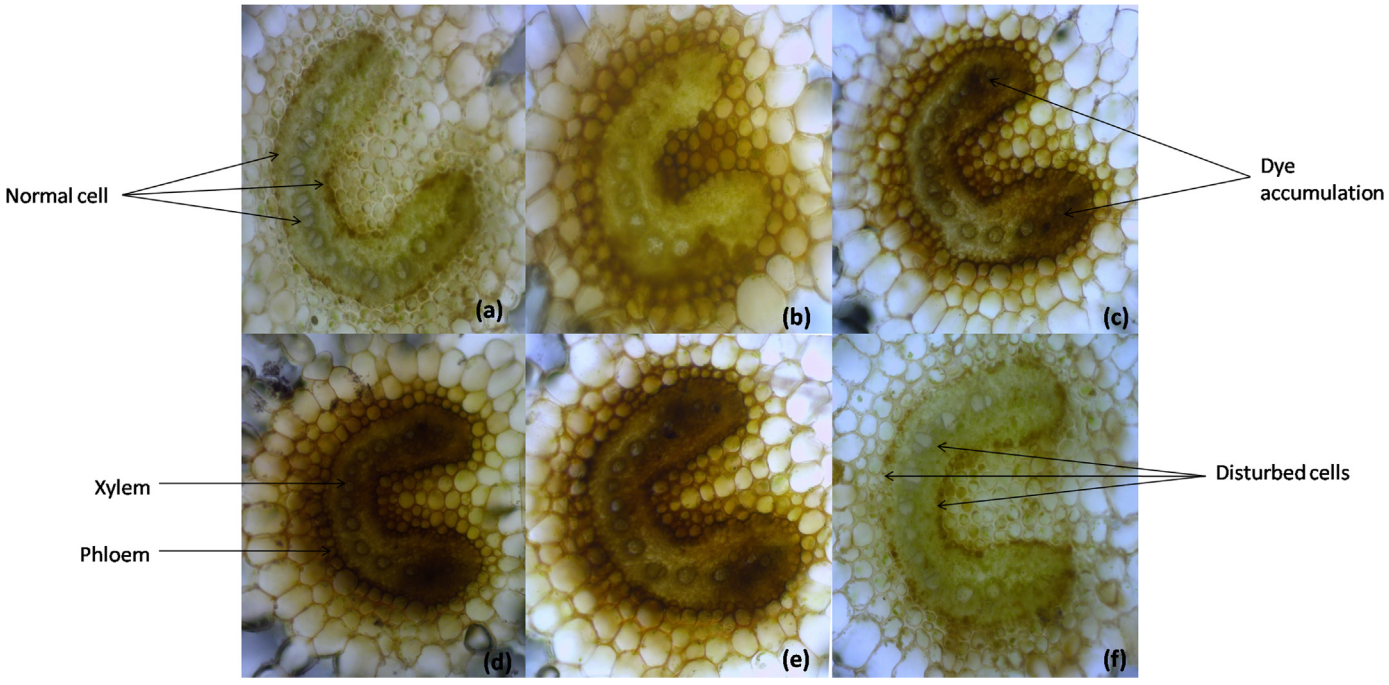


Fig. 1. Anatomy of stem of *S. molesta* a) control plant, exposed to Rubine GFL b) 12 h, c) 24 h, d) 36 h, e) 48 h, f) after 48 h plant exposed to normal water and observed anatomical changes at 60 h.

Table 2.
Chlorophyll and carotenoid content of *S. molesta* leaves before and after exposure to 100 mg/L with Rubin GFL over a period of 72 h.

Sample	Chlorophyll a (mg/mL)	Chlorophyll b (mg/mL)	Total chlorophyll (mg/mL)	Carotenoid (mg/mL)
Control	24.48 ± 0.36	8.01 ± 0.25	32.76 ± 0.38	12.52 ± 0.34
Test	27.73 ± 0.26	10.01 ± 0.23	37.52 ± 0.31	17.79 ± 0.27

Similar, increase in the contents of chlorophyll pigment was observed in the leaves of *I. hederifolia* after scarlet RR exposure (Rane et al., 2014).

3.5. Analysis of enzyme activities of S. molesta during dye degradation

Various oxido-reductive enzymes like LiP, VAO, SOD, laccase, tyrosinase, catalase, DCIP and azo reductase in root and stem tissues are well known to be involved in dye phytotransformation processes. In present study, specific activities of oxido-reductive enzymes were observed in root tissues of *S. molesta;* like LiP, VAO, laccase, tyrosinase, catalase, DCIP reductase and SOD activities were observed to be induced by 8.1, 2.9, 1.3, 2.0, 2.9, 1.6 and 1.7 fold, respectively. Similarly, stem tissues of *S. molesta* showed induction in the activities of enzymes such as LiP, VAO, laccase, tyrosinase, catalase, DCIP reductase and SOD up to 5.1, 3.6, 4.9, 1.9, 2.5, 1.8 and 1.7 fold, respectively. Both root and stem tissues showed a considerable decrease in activity of riboflavin reductase; while specific activity of azo reductase was observed to be induced in root cell and decreased in stem tissues (Table 3). Similar induction in the activities of peroxidase enzyme was also noted in *I. palmate and P. australis* during dye removal process (Shaffiqu et al., 2002; Carias et al., 2007). Similar set of oxido-reductive enzymes were have been shown to be induced during decolorization of dyes Direct Red 5 B, Scarlet RR and Remazol Red using plant *B. malcolmii, I. hederifolia* and *Aster amellus,* respectively (Kagalkar et al., 2009; Khandare et al., 2011; Rane et al., 2014).

3.6. Analysis of phyto-degradation products

The treated Rubine GFL, dye mixture and textile effluent showed different FTIR spectra from their respective controls. This confirmed the conversion of dye compound into different metabolites (Fig. S1) (Table 6). The HPLC spectrum of untreated dye Rubine GFL, dye mixture and textile industry effluent were significantly differed from spectrum of their respective metabolites produced after treatment. The obtained HPLC spectra of treated and untreated sample of dye, dye mixture and textile effluent showed difference in retention time indicating degradation of dye compound (Fig. S2) (Table 7).

The GC-MS analysis of treated dye Rubine GFL sample was used to predict chemical structure and nature of the obtained products. Two different peaks with m/z value of 278 and 149 were observed after treatment. The obtained metabolites and increased level of enzymes in root and stem tissue of *S. molesta* during decolorization were used to predict degradation pathway of Rubine GFL. Rubine GFL underwent the laccase or lignin peroxidase activity of and cleaved the dye asymmetrically. Subsequent demethylation further resulted to form 2-{(*E*)-[4-(methylamino) phenyl] diazenyl}-5-nitrobenzonitrile. This formed intermediate was then cleaved by azo reductase to form two products such as 2-methyl-4-nitroaniline and N-methylbenzene-1, 4-diamine (Fig. S3) (Table 8). Asymmetrical and/or oxidative cleavage by the action of enzymes such as veratryl alcohol oxidase and lignin peroxidase and laccase has been shown to be the key mechanism behind the dye structure breakdown (Kabra et al., 2011; Khandare et al., 2011, 2013; Patil and Jadhav, 2013). Azo bond cleavage is known as the function of azo reductase enzyme (Leelakriangsak and Borisut, 2012).

3.7. Phytotoxicity monitoring

In many developing countries, textile effluents have been observed to be released into agricultural farms. Thus, it is important to study the toxic effect of treated and untreated effluent on agricultural crop seedlings. Effect of untreated Rubine GFL, dye mixture and textile effluent showed reduced germinations of *T. aestivum* and *P. Mungo* seeds. Decrease in the root and shoot lengths were observed in all untreated samples. After 8 days of treatment, seeds in untreated dye, dye mixture and effluent showed toxicity in the form of decreased the length of plumule and radicle in comparison with the seeds grown in water. Dye samples treated with *S. molesta* showed enhanced germination percentages and shoot and root lengths indicating reduced toxicity of formed metabolites (Table 4). If retention time of treatment is increased, proper dye removal could be achieved after which the water be reused for agriculture.

3.8. Study of decolorization of dye containing textile effluent in lagoon using S. molesta

Plants of *S. molesta* with long and dense roots were prepared in the lagoon containing tap water for 15 d before the treatment. *S. molesta* plants were well grown in broad pH range of 3–9 in effluent, however wilting of whole plants was observed beyond pH

Table 3.
Enzyme activities in root and stem of *S. molesta* plant control tissue at 0 h and after 72 h for Rubine GFL dye.

Enzymes	*Salvinia molesta* root cell			*Salvinia molesta* stem cell		
	Control	Test	% Induction	Control	Test	% Induction
Lignin peroxidase	$4.0 \pm 0.01 \times 10^{-7}$	$3.26 \pm 0.28 \times 10^{-8***}$	716	$2.72 \pm 0.01 \times 10^{-7}$	$1.39 \pm 0.05 \times 10^{-8*}$	411
Veratryl alcohol oxidase	$2.91 \pm 0.26 \times 10^{-8}$	$8.56 \pm 1.16 \times 10^{-8*}$	193	$2.89 \pm 0.48 \times 10^{-8}$	$1.07 \pm 0.71 \times 10^{-7**}$	269
Laccase	$1.99 \pm 0.75 \times 10^{-9}$	$2.67 \pm 0.90 \times 10^{-9*}$	34	$1.98 \pm 0.21 \times 10^{-8}$	$9.77 \pm 0.90 \times 10^{-8**}$	392
Tyrosinase	$3.03 \pm 1.50 \times 10^{-9}$	$6.28 \pm 6.87 \times 10^{-9*}$	106	$3.14 \pm 1.17 \times 10^{-9}$	$5.99 \pm 1.32 \times 10^{-9*}$	90
Catalase	$5.92 \pm 0.04 \times 10^{-7}$	$1.74 \pm 0.14 \times 10^{-8*}$	194	$7.52 \pm 0.11 \times 10^{-7}$	$1.88 \pm 0.14 \times 10^{-8*}$	151
Riboflavin reductase	$6.72 \pm 0.48 \times 10^{-8}$	$3.71 \pm 0.27 \times 10^{-8**}$	−44	$4.11 \pm 0.14 \times 10^{-8}$	$9.12 \pm 0.14 \times 10^{-7**}$	−77
NADH-DCIP reductase	$3.73 \pm 12.22 \times 10^{-10}$	$6.32 \pm 34.91 \times 10^{-10**}$	69	$4.54 \pm 13.53 \times 10^{-10}$	$8.38 \pm 22.09 \times 10^{-10***}$	84
Superoxide dismutase	$3.44 \pm 0.44 \times 10^{-8}$	$6.09 \pm 0.06 \times 10^{-8**}$	77	$2.76 \pm 0.12 \times 10^{-8}$	$4.86 \pm 0.20 \times 10^{-8*}$	75
Azo reductase	$2.27 \pm 0.28 \times 10^{-8}$	$4.97 \pm 0.31 \times 10^{-8*}$	119	$9.32 \pm 0.19 \times 10^{-8}$	$4.83 \pm 0.53 \times 10^{-8**}$	−48

Values are a mean of three experiments ± SEM. Significantly different from control (0 h) at $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ by one-way ANOVA with Tukey–Kramer comparison test. Enzyme activity unit in katal.

Table 4.
Phytotoxicity testing of Rubine GFL, mixture of dyes and textile effluent before and after 72 h treatment in initial experiments using *S. molesta* in glass beakers.

Parameters	Water	Rubin GFL	Dye metabolite	Untreated dye mixture	Treated dye mixture	Untreated textile effluent	Treated textile effluent
Triticum aestivum							
Germination %	100	50	90	40	80	30	70
Plumule (cm)	9.11 ± 0.46	$2.98 \pm 0.35^{*}$	$6.88 \pm 0.11^{\$}$	$2.66 \pm 0.16^{*}$	$4.88 \pm 0.18^{\$}$	$2.28 \pm 0.09^{*}$	$4.66 \pm 0.24^{\$}$
Radicle (cm)	5.95 ± 0.27	$2.33 \pm 0.21^{*}$	$4.56 \pm 0.33^{\$}$	$1.96 \pm 0.37^{*}$	$4.31 \pm 0.23^{\$}$	$1.46 \pm 0.20^{*}$	$3.68 \pm 0.14^{\$}$
Phaseolus mungo							
Germination %	100	40	90	30	90	40	80
Plumule (cm)	8.23 ± 0.40	$3.11 \pm 0.34^{*}$	$6.28 \pm 0.16^{\$}$	$2.30 \pm 0.11^{*}$	$4.70 \pm 0.32^{\$}$	$2.11 \pm 0.28^{*}$	$4.63 \pm 0.19^{\$}$
Radicle (cm)	6.43 ± 0.30	$1.93 \pm 0.32^{*}$	$5.10 \pm 0.23^{\$}$	$2.08 \pm 0.10^{*}$	$3.93 \pm 0.08^{\$}$	$1.53 \pm 0.22^{*}$	$3.60 \pm 0.15^{\$}$

Values are a mean of three experiments ± SEM. Shoot and root lengths of plants grown in textile dye Rubine GFL, mixture of dyes and textile effluent, respectively, are significantly different from that of plants grown in distilled water by $^{*}P < 0.001$. Shoot and root lengths of fifty plants grown in the degraded treated Rubine GFL, mixture of dyes and textile effluent, respectively, are also significantly different from that of plants grown in untreated Rubine GFL, mixture of dyes and textile effluent by $^{\$}P < 0.001$.

Table 5.
Characterization of untreated and treated textile effluent in lagoon grown with *S. molesta*.

Parameter	Untreated effluent	Treated effluent
pH	9.0	7.2
COD (mg/L)	1185	283
BOD_5 (mg/L)	1440	249
ADMI	950	180
TDS (mg/L)	7560	2480
TSS (mg/L)	4730	1720
Cadmium (ppm)	0.03	0.01
Mercury (ppm)	0.0	0.0
Chromium (ppm)	3.45	0.80
Lead (ppm)	0.30	0.15
Nickel (ppm)	0.0	0.0
Arsenic (ppm)	1.70	0.49
Bacterial count (CFUs)	28×10^{-7}	93×10^{-7}

9. The old plant parts were detached with the newly regenerated plant after 15 d. Well grown plants were used for treatment of textile effluent in lagoon for 8 d. The values of ADMI, COD, BOD_5, TDS and TSS were reduced by 81%, 76%, 82%, 67% and 63%, respectively after the treatment. Additionally, Cadmium (Cd), chromium (Cr), lead (Pb) and arsenic (As) were decreased by 66%, 76%, 50% and 71%, respectively after treatment. Bacterial count of untreated effluent (28×10^{-7} CFUs) was increased in treated effluent (93×10^{-7} CFUs). Increase in bacterial population can be attributed towards the reduced toxicity and favourable conditions for growth. The pH of untreated effluent was 9 which decreased after

Table 7.
HPLC analysis data of dye Rubine GFL, dye mixture and textile effluent before and after treatment.

Peak number	Untreated Rubine GFL (min)	Treated Rubine GFL (min)	Untreated dye mixture (min)	Treated dye mixture (min)	Untreated textile effluent (min)	Treated textile effluent (min)
1	0.824	2.064	2.561	2.064	0.891	2.192
2	1.099	2.225	3.219	2.255	1.048	2.415
3	1.426	2.455	8.221	2.432	1.248	2.668
4	1.653	2.862		2.62	1.424	2.848
5		3.001		2.869		3.065

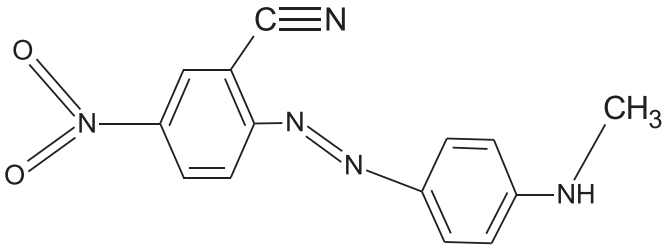
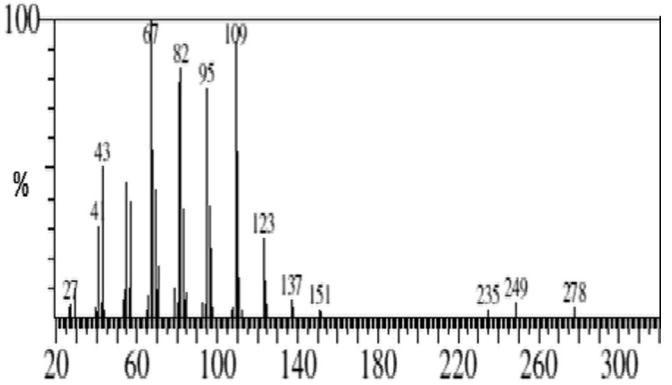
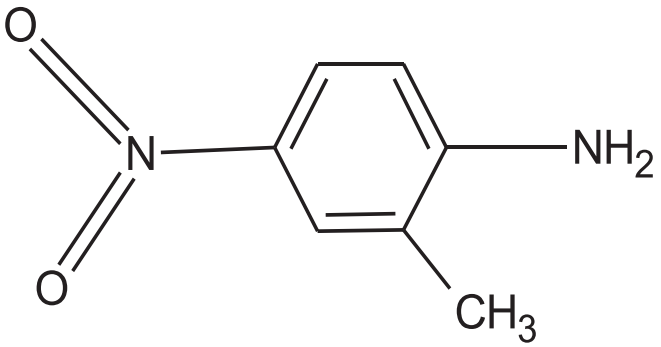
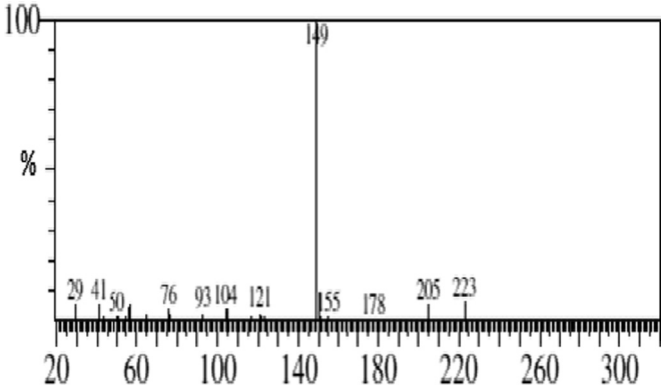
treatment to 7.2 (Table 5) (Table S1). The pH of dye mixture and textile effluent was shown to be decreased from 7.9 to 7.5 and 10.2 to 8.1, respectively after treatment by *I. hederifolia* (Rane et al., 2014). In another study, treatment of dye effluents of varying pH were shown to be neutralized (Rane et al., 2015). *P. australis* was explored in vertical flow constructed wetland for treatment of real dye effluent and a decrease in color and COD of 89% and 90%, respectively was observed (Ferreira et al., 2014). Horizontal bed reactor constructed using *Typha domingenesis* was reported to reduce BOD, COD, TSS and TDS of an effluent up to 77%, 79%, 27% and 59%, respectively after a treatment of 72 h (Shehzadi et al., 2014). In another study with a static reactor using *Pogonatherum crinitum* also showed effective removal of ADMI, COD, BOD, TSS and TDS up to 93% ,78% ,70% ,13% and 70%, respectively (Watharkar et al., 2015).

Table 6.
FTIR analysis of dye Rubine GFL, dye mixture and textile effluent before and after treatment.

Untreated Rubine GFL		Treated Rubine GFL		Untreated dye mixture		Treated dye mixture		Untreated textile effluent		Treated textile effluent	
Peak (cm^{-1})	Bond	Peak (cm^{-1})	Bond	Peak (cm^{-1})	Bond	Peak (cm^{-1})	Bond	Peak (cm^{-1})	Bond	Peak (cm^{-1})	Bond
3373.61	N–H Str	3383.26	N–H Str	3367.82	C═O Str	3375.54	N–H Str	3365.90	N–H Str	3367.82	NH_2 Str
2916.47	C–H Str	2914.54	O–H Str	2912.61	O–H Str	2951.19	C–H Str	2914.54	O–H Str	2914.54	O–H Str
2744.80	C–H Str	2746.73	O–H Str	2748.65	O–H Str	2922.25	C–H Str	2750.58	O–H Str	2746.73	O–H Str
2245.22	C≡N Str	2553.84	S–H Str	2347.45	NH^{+} Str	2339.73	NH^{+} Str	2341.66	NH^{+} Str	2345.52	NH^{+} Str
2000.25	NH^{+} Vib	2347.45	NH^{+} Str	1610.61	N═N Str	1998.32	NH^{+} Vib	2088.98	NH^{+} Vib	1614.47	NH Def
1583.61	N═N Str	1610.61	NH_3^{+} Def	1398.44	S═O Str	1857.51	C═O Str	1899.95	NH^{+} Vib	1417.73	O–H Def
1348.29	NO_2 Str	1427.37	C–H Def	1284.63	O–NO_2 Vib	1616.40	NH Def	1618.33	C═C Str	1053.17	C–N Vib
1105.25	C–OH Str	1055.10	S═O Str	1051.24	C–S Str	1419.66	C–H Def	1423.51	S═O Str		
1051.24	S═O Str	856.42	C–H Def	831.35	C–H Def	1057.03	C–OH Str	1049.31	S═O Str		
860.28	C–H Def	644.25	C–S Str								
771.55	N–O Str										
534.30	C–N Str										

Str – Stretching; Def – Deformation; Vib – Vibrating.

Table 8.
GC-MS data of obtained metabolite after degradation of Rubine GFL by *Salvinia molesta.*

Peak	RT (min)	m/z	Mol. weight	Structure and name of metabolite	Mass spectrum
1	23.542	278	281	 2-{(*E*)-[4-(methylamino)phenyl]diazenyl}-5-nitrobenzonitrile	
2	24.367	149	152	 2-methyl-4-nitroaniline	

4. Conclusion

An aquatic fern *S. molesta* (60 ± 2 g root biomass) could completely remove color and degrade the dye Rubine GFL within 72 h because of its tolerance to dye conditions. *S. molesta* showed the potential to decolorize and degrade textile effluent at laboratory scale as well as on field application in a constructed lagoon of a total surface area of 35 m^2. The enzyme induction patterns suggest involvement of oxido-reductive enzymes for dye degradation. Anatomical pattern of decolorization of dye Rubine GFL confirmed the entry of dye into the tissue and further degradation. The developed lagoon efficiently removes the dye as well as metals and reduces the COD, BOD_5, TSS, TDS and pH of real textile industrial effluent. After treatment of dye Rubine GFL, dye mixture and textile effluent showed unaffected shoot and root development after germination of *T. aestivum* and *P. mungo* seeds. Further research on treating textile effluents on actual dye disposal site is in progress.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.envres.2016.05.047.

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