In situ textile wastewater treatment in high rate transpiration system furrows planted with aquatic macrophytes and floating phytobeds

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HIGHLIGHTS

- Remazol Red, dye mixture and textile effluent treated efficiently by *V. zizanioides*.
- Histological analysis confirmed entry and degradation of dyes in roots.
- Floating phyto-bed reactor treated textile wastewater effectively.
- Textile wastewater was noteworthily treated in plant cultivated furrows of HRTS.
- Plant consortium enhanced the potential of textile dye removal.

GRAPHICAL ABSTRACT
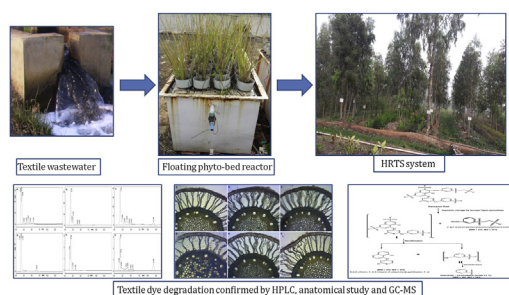
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ABSTRACT

Plants are known to remediate dyes, metals and emerging contaminants from wastewaters. *Vetiveria zizanioides,* a perennial bunchgrass showed removal of Remazol Red (RR, 100 mg/L) up to 93% within 40 h. Root and shoot tissues of *V. zizanioides* revealed induction in dye degrading enzymes viz. lignin peroxidase by 2.28 and 1.43, veratryl alcohol oxidase 2.72 and 1.60, laccase 6.15 and 3.55, and azo reductase 2.17 and 2.65-fold, respectively, during RR decolorization. Substantial increase was observed in the contents of chlorophyll *a*, chlorophyll *b*, and carotenoids in the plant leaves during treatment. Anatomical studies of roots, HPLC and GC-MS analysis of metabolites, and phytotoxicity assessment confirmed phytotransformation of RR into nontoxic metabolites. Floating phytobed with *V. zizanioides* treated textile wastewater (400 L) effectively and reduced ADMI, COD, BOD, TDS, and TSS by 74, 74, 81, 66 and 47%, respectively within 72 h. *In-situ* treatment of textile wastewater for 5 days in constructed furrows planted with semiaquatic plants, *V. zizanioides, Ipomoea aquatica* and its consortium-VI decreased ADMI by 68, 61 and 76%, COD by 75, 74 and 79%, BOD by 73, 71 and 84%, TDS by 77, 75 and 83%, and TSS by 34, 31 and 51%, respectively. This treatment was also useful to remove arsenic, cadmium, chromium and lead from wastewater. Overall observation suggests wise strategy to use this plantation in the furrows of high rate transpiration system and phytobeds in deep water for textile wastewater treatment.

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1. Introduction

The dyes are extensively used in a variety of industries like plastic, textile, pulp, rubber and paper industries. Among all industries, the textile is at first rank in the usage of dyes for coloration of fiber. The effluent containing textile dyes are the largest pollutant source of valuable water bodies due to its carcinogenic, allergic, cytotoxic and mutagenic nature. The present physico-chemical methods such as, flocculation, adsorption, filtration, coagulation, photo-degradation, membrane processes, reverse osmosis and chemical oxidation are efficient in color removal, but they have some limitations like high cost, limited applications and secondary pollution problems such as sludge formation, toxic gases etc. To comply with environmental laws, it is necessary to look for less sludge producing, eco-friendly, in situ applicable and low-cost methods for dye wastewater treatment.

Biological mode of the treatment mineralizes toxic compounds into an organic form which are nontoxic to life (Tahir et al., 2016). Presence of high amount of toxic dyes has become environmental concern that affect agriculture and impacting on accumulation and magnification of the components in food chain (Dipu et al., 2011). Phytoremediation used as a novel approach in dye degradation since last few years; it deals with the use of plant and rhizospheric microorganisms to exclude toxic compounds from the contaminated sites. Phytoremediation can be used for treatment of different kinds of contaminant, such as landfill leachates, explosives, chlorinated solvents, radionuclides, polyaromatic hydrocarbons, heavy metals, and pesticides (Khandare and Govindwar, 2015). Although, phytoremediation potential of many plants was studied in textile wastewater treatment, plenty of them remained at lab scale. Hydroponic phyto-tunnel (HPT) system has shown to be an efficient textile wastewater treatment (Khandare et al., 2013). Laboratory scale plant-bacterial consortia reactors such as vertical (VSbF) and horizontal subsurface flow, (HSbF) were built for textile wastewater treatment (Khandare and Govindwar, 2015). Some pilot scale systems with aquatic plants (Chrysopogon zizanioides, Typha domingensis and Phragmites australis) were reported to treat textile wastewater (Ong et al., 2010; Shehzadi et al., 2014). However, only a few studies have been recorded for actual dye contaminated site remediation. Constructed wetlands with individual plants (Salvinia molesta, Ipomoea aquatica, Typha angustifolia and Fimbristylis dichotoma) were proposed for dye wastewater management (Rane et al., 2016; Chandanshive et al., 2017; Kadam et al., 2017). Co-plantation was advantageous in waste wastewater remediation because of synergistic effect of oxido-reductases from the rhizospheric and individual plants.

This work describes on field application of Vetiveria zizanioides, I. aquatica and its consortium-VI when planted in constructed furrows of high rate transpiration system (HRTS), MIDC, Kagal, India for textile wastewater treatment. Both the plants are annual herb and able to sustain the presence of waterlogged condition. Textile wastewater was also treated with phyto-beds possessing these plants, which can further be used in furrows, where plantation is not possible.

2. Materials and methods

2.1. Plant material and chemicals

Aestivum sativum and Phaseolus mungo seeds were bought from a local grain market. V. zizanioides was recovered from Botany Lake, Shivaji University, Kolhapur; while I. aquatica was collected from Ambewadi, Kolhapur, Maharashtra. Riboflavin and 2, 2-azino-bis (3-ethylbenzothiazoline)-6-sulphonic acid (ABTS) were purchased from Sigma Aldrich (St Louis, MO, USA). Veratryl alcohol, nicotin-amide adenine dinucleotide (di-sodium salt), n-propanol, 2, 6-dichlorophenol indophenol (DCIP) and catechol were procured from Sisco Research Laboratories, Mumbai, India. The textile dyes Remazol Red, Scarlet RR, Rubine GFL, Methyl Orange, Disperse EMGR, Navy Blue Ex and Scarlet GDR were acquired from Mahesh dye processors, Ichalkaranji. The textile wastewater was acquired from Common Effluent Treatment Plant (CETP), Kagal, India. An analytical grade and highly purified chemicals were used for experiments.

2.2. Textile dye decolorization by V. zizanioides

Wild plants of V. zizanioides were used after removing soil from the roots for decolorization experiments. Two plants were subjected to Remazol Red, Scarlet RR, Rubine GFL, Methyl Orange, Disperse EMGR, Navy Blue Ex and Scarlet GDR solutions (100 mg/L) in Erlenmeyer flasks. Samples (1 mL) were collected at every 12 h. The samples were centrifuged for 10 min at 4561×g. Remazol Red concentration was assessed at 530 nm using UV−visible spectro-photometer. Remazol Red was used for further experiments, because it took less time for the decolorization than other dyes.

In another experiment, V. zizanioides was used for the decolorization of textile wastewater. Tristimulus filter method was used to measure the color before and after treatment of textile wastewater. Percent decolorization was computed using ADMI color removal. Textile wastewater and Remazol Red solution were characterized before and after treatment using parameters like chemical oxygen demand (COD), biological oxygen demand (BOD), color value (American dye manufacturers institute, ADMI) (Chandanshive et al., 2017), total suspended solid (TSS) and total dissolved solid (TDS) (APHA, 1998). Atomic absorption spectrophotometer (AAS) was used to estimate the concentration of heavy metals (Chandanshive et al., 2017). The average mean value of three experiments was considered for inference. Abiotic controls of dye and textile wastewater was devoid of the plant.

2.3. Analysis of photosynthetic pigments and plant histology

Analysis of chlorophylls and carotenoids is important to understand the health and energy requirement of the plants. Two gram leaves V. zizanioides plant (Control and treated) were homogenized using mortar and pestle, separately. Leaves were crushed in 50 mL of cold acetone (80%) with bit amount of MgCO₃. The obtained homogenate was filtered and centrifuged (2000×g) for 10 min. The chlorophylls were quantified spectrophotometrically at 663 nm and 645 nm; however, carotenoids were measured at 470 nm using acetone as blank (Chandanshive et al., 2017). Root transverse section mounted in glycerine was examined for accumulation of dye at 100X magnification using a Trinocular Microscope (Zeiss Axio Imager 2).

2.4. Preparation of enzyme extracts

Roots and shoots of V. zizanioides from control and after decolorization of the textile wastewater were taken to prepare crude enzyme extract (Chandanshive et al., 2017). Roots and shoots (2 gm) were chopped and grounded using mortar and pestle in 50 mM potassium phosphate buffer at pH 7.4. Obtained sample was centrifuged at 9000×g for 20 min in a cold centrifuge at 4 °C.

Enzyme assayes of dye degrading enzymes were performed at room temperature using spectrophotometer. Laccase activity was assessed at 420 nm using ABTS as a substrate (Hatvani and Mecs, 2001). Veratryl alcohol oxidase was assessed using a substrate, veratryl alcohol at 300 nm (Jadhav et al., 2009). Activity of lignin peroxidase was assessed at 300 nm by measuring propionaldehyde formation due to *n*-propanol oxidation (Shanmugam et al., 1999). Azo, riboflavin and DCIP reductase were estimated measuring at 430, 340 and 600 nm, respectively (Chandanshive et al., 2017). Triplicate experiments were performed for all enzymes. Lowry method was used to estimate protein concentration in the enzyme extract (Lowry et al., 1951).

2.5. Extraction and analyses of phytodegraded products

Phytodegraded product were extracted taking equal amount of ethyl acetate. The extracted samples were dried by evaporating at room temperature and dissolved in methanol (HPLC grade) (Chandanshive et al., 2016). Metabolites separation and identification were done using HPLC, GC-MS, and FTIR (Kagalkar et al., 2015). NIST library and mass spectram (m/z) was used to idetify the metabolites.

2.6. Toxicity analysis

Toxicity of Remazol Red and its products produced by *V. zizanioides* treatment were independently tested on *A. sativum* and *P. mungo* seeds. Fifty seeds were watered for 7 days with 5 mL of Remazol Red (100 mg/L) and metabolite solution separately. Control set of seeds was watered with distilled water. The root and shoot lengths were measured on 7th days.

2.7. Construction of floating phyto-bed reactor of V. zizanioides

Green-remediation process of textile wastewater was performed using floating phyto-bed in rectangle metal tank (400 L, dimensions: 1.2 m × 0.61 m x 0.61 m). The floating phyto-beds (1.0 × 0.58 m) were prepared using PVC pipes, elbows, thermocol sheet and aluminium metal wire gauze. The aluminium wire guaze holds the plants and allow roots to enter in the wastewater tank. Twenty-four *V. zizanioides* plants were planted in a PVC reducer along with 100 g of soil making appropriate holes on the thermacol sheet. Initially, floating phyto-bed was kept floating in tap water for 3 months to develop the root system. Then, developed floating beds were exposed to textile wastewater. The samples were withdrawn at 24 h interval and analysed for environmental parameters (APHA, 1998).

2.8. Textile wastewater treatment in planted furrows

Furrows (91.4 m × 1.2 m × 0.6 m) were constructed in HRTS to treat textile wastewater. This furrow was mulched correctly using mulching paper to reduce seepage of textile wastewater. Three furrows were planted independently with *V. zizanioides, I. aquatica* and in consortium. *V. zizanioides* was planted 15 cm away from each other at the bottom of furrow (49 plants/m^2), because it is a submerged rooted plant. Whilst, *I. aquatica*, a free-floating aquatic plant was planted on the upper edges of furrow which further covered surface of water. Initially, plants were grown watering tap water for three months to develop and sustain in the environment. The planted furrow was then independently supplied with textile wastewater in a stagnant state. The experiments were carried out in winter season (September to January) at atmospheric conditions such as, temperature range- 15—25 °C, light- 11/13 h (light/dark cycle), CETP textile effluent pH range 7—10, and without adding special nutrients. The samples were collected for 5 d at the interval of 24 h.

Samples (1 mL) of textile wastewater were withdrawn at 0 time and after phytoremediation. Bacterial count was measured using serial dilution (with 0.9% NaCl) technique. The obtained samples were grown independently in Petri dishes containing nutrient agar medium at 37 °C. The colony forming units (CFUs) were measured after 24 h.

2.9. Statistical analysis

Data were analysed using (ANOVA) one way analysis of variance and Tukey-Kramer comparisons test, considering significant level $P < 0.05$.

3. Results and discussion

3.1. Textile dyes decolorization using V. zizanioides

Azo dyes such as Scarlett RR, Green HE4B, Navy Blue HER and Rubine GFL decolorized by 45, 57, 39 and 62%, respectively; due to the treatment of long rooted wild plant of *V. zizanioides* within 40 h. However, Remazol Red was decolorized up to 93% within 40 h (data not shown). Variation in percent degradation of these dyes might be due to structure of the dyes. Wild plants such as *S. molesta, Ipomoea hederifolia, F. dichtomas* and *Asparagus densiflorus* were found to decolorize dyes Rubine GFL (97%), Scarlett RR (96%), Methyl Orange (91%) and Rubine GFL (91%) within 72, 60, 60 and 48 h, respectively (Kadam et al., 2017; Watharkar et al., 2018). *Bouteloua dactyloides* and *T. angustifolia* were found to efficiently decolorize the Reactive Blue 19 and textile effluent, respectively (Mahmood et al., 2005; Vijayalakshmidevi and Muthukumar, 2015). *V. zizanioides* also promote the biodegradation of organic compounds such as phenol, 2,4,6-trinitroluene, atrazine and benzo[a] pyrene (Danh et al., 2009). *A. philoxeroides* also displayed a decolourization potential of Reactive Green up to 3000 mg/L concentration within 60 days treatment, whereas, association of *Klebsiella* sp. VITAJ23 was found more efficient (Sinha et al., 2019). *Ceratophylum demersum* showed phytoremediation of Reactive Black, Reactive Red and Reactive Brown to the extent of 76—84% at 50 ppm concentration (Priyanka and Krishnaswamy, 2019). *Lemna minor* decolorized triphenylmethane dyes (Crystal Violet and Malachite Green) significantly during phytoremediation process (Törö;k et al., 2015). *Bacopa monnieri* (L.) Pennell showed potential to degrade fourteen azo dyes at 40 mg/L concentration by 90—100% within 2 weeks (Shanmugam et al., 2020).

Treatment of simulated dye mixture and textile wastewater by *V. zizanioides* decreased ADMI color value up to 74 and 73%, respectively within 40 h. COD, BOD, TSS, TDS and turbidity were reduced by 73 and 73%, 75 and 78%, 58 and 68%, 55 and 59%, 57 and 60% after 40 h treatment of simulated dye mixture and textile wastewater, respectively with *V. zizanioides*. The pH of the solution was decreased to 7.7 and 7.3 from 10.6 to 9.8, respectively, after the treatment of textile wastewater and simulated dye mixture by *V. zizanioides*. The electric conductivity of textile wastewater and simulated dye mixture was found to increase by 3.48 and 5.52-fold, respectively by the exposure of *V. zizanioides* (Table 1). Plants like *S. molesta* (in lagoon (52,500 L) treatment), *T. angustifolia* (planted in furrows of HRTS) and *I. hederifolia* (in flask studies) were found to significantly reduce ADMI, BOD, COD, TSS, TDS and electric conductivity after treatment of dye mixture and textile effluent (Rane et al., 2016; Chandanshive et al., 2016). Textile wastewater treatment with *Gaillardia grandiflora, Tagetes patula, Aster amellus,* and *Portulaca grandiflora* showed decolorization of dye after 30 d of treatment at HRTS (Patil and Jadhav, 2013; Chandanshive et al.,

2018). *C. zizanioides* L. treated textile industry wastewater and showed removal of BOD (98.47%) and COD (89.05%) (Tambunan et al., 2018). *Eichhornia crassipes*, *Pistia stratiotes* and *S. molesta* were found effective in removing pollutants, COD, TDS, nutrients and metals (Cd, Ni and Zn) from textile wastewater (Wickramasinghe and Jayawardana, 2018).

3.2. Enzyme induction in roots and stems of V. zizanioides

Phytoremediation possesses accumulation, degradation, adsorption and biotransformation of contaminant through metabolic processes of the plant. Root and shoot tissues were separately studied for a variety of oxido-reductive enzymes involved in the dye phytodegradation process. The activities of the degrading enzymes such as veratryl alcohol oxidase, lignin peroxidase, laccase and azo reductase were increased by 2.72, 2.18, 6.15 and 2.17-fold in root tissues; 1.6, 1.43, 3.55 and 2.65-fold in stem tissues of *V. zizanioides*, respectively, due to exposure of Remazol Red. Root and stem tissues demonstrated a reduction in the activity of riboflavin reductase and NADH-DCIP reductase (Table 2). The contribution of these enzymes is proposed in *I. aquatica* (Rane et al., 2016). Involvement of dye degrading enzyme was the key cause behind better dye degradation and removal. Additionally, it always showed induction in the enzyme activities of lignin peroxidase, veratryl alcohol oxidase, laccase and azo reductase during the decolorization process. These oxido-reductive enzyme plays an important role in biotransformation of textile dyes. Because of these reasons, *V. zizanioides* might have shown a better dye removal potential from the textile wastewater. The participation of these enzymes has been reported earlier in the degradation of several textile dyes like Scarlett RR, Rubine GFL, Methyl Orange and Congo Red by plants *I. hederifolia*, *S. molesta, F. dichotomas* and *T. angustifolia,* respectively (Rane et al., 2016; Kadam et al., 2017; Chandanshive et al., 2017). Similarly, *Ipomea palmata and Phragmites australis* induced peroxidase enzyme in decolorization process of textile dyes (Shaffiqu et al., 2002; Caries et al., 2007).

3.3. Photosynthetic pigment and anatomical analyses of plants

Photosynthetic pigments (Chlorophyll *a*, Chlorophyll *b* and carotenoid) are involved in the cell energy metabolism, hence, it is essential to know the effect of dyes on the level of these pigments. Plants *V. zizanioides* showed elevated levels by 41% in chlorophyll *a*; 24% in chlorophyll *b*, and 36% in carotenoids, as compared to untreated plants (control) (Suppl. Table 1). *V. zizanioides* is a long rooted macrophyte, might synthesize additional amount of chlorophylls and carotenoids to complete the energy requirement and metabolic activities during phytoremediation of Remazol Red.

Several reports showed an increase in pigments when exposed to textile effluent in phytoreactors/lagoons (Chandanshive et al., 2018).

Histological study of root tissues of *V. zizanioides* was performed at 10 and 50 h for understanding the association of dye Remazol Red in plant tissue and to locate phytodegradation site. Accumulation of dye was found at adjacent cortical cells with an extended time period. The plant of *V. zizanioides* revealed accretion and transformation of dye in plant tissues. Cells of control (untreated) plant root were normal in size and shapes without having any color (Suppl. Fig. 1a). Remazol Red was accumulated in epidermal cells of plant roots after 10 h of dye treatment (Suppl. Fig. 1b) and further exposure affected to the cortical region at 20 h (Suppl. Fig. 1c). Dye was accumulated at both the cortical and epidermal regions at 30 h (Suppl. Fig. 1d). Both cortical and epidermal cells showed degradation of dye at 40 h (Suppl. Fig. 1e); while phloem and xylem was containing some traces of dye. These results signify the phytodegradation of dye in the cortical and epidermal region. Further exposure of plants to the tap water for 10 h, histology of root did not find color in epidermal cells and found some distorted cells in cortical containing a minute traces of dye (Suppl. Fig. 1f). *T. angustifolia* and *Paspalum scrobiculatum* root epidermis as well as cortical tissue showed the presence of Congo Red after 24 h and subsequently phytotransformed at 48 h (Chandanshive et al., 2018).

3.4. Products analysis

Remazol Red indicated peaks at retention time of 1.565, 1.657, 1.846, 2.462, 3.426, 3.864, 4.556 and 5.554 min (Fig. 1a) in HPLC spectrum, while new peaks were observed at 1.498, 1.653, 1.748 and 6.636 min after treatment (Fig. 1b). Simulated dye mixture indicated peaks at 1.732, 2.118, 2.564, 4.018 and 4.452 min (Fig. 1c). Some distinct peaks were appeared at 1.552, 1.792, 2.532 and 3.428 min after the treatment of simulated dye mixture (Fig. 1d). Untreated textile wastewater indicated peaks at retention time of 1.741, 2.446, 3.087, 3.826, 4.475, 4.922 and 11.784 min (Fig. 1e). However, treated textile wastewater showed some distinct peaks at 1.568, 1.745, 1.921, 2.044, 3.602, 3.925, 4.933, 5.657 and 11.780 min (Fig. 1f). The HPLC peak pattern of untreated and treated dye, simulated dye mixture and textile wastewater demonstrated the variation in retention time suggesting dye degradation.

GC-MS analysis of treated Remazol Red was used to propose chemical nature and structure of extracted metabolites. The biotransformation of Remazol Red was projected based on the formed metabolites and enhanced enzyme activities in the root and stem tissues of *V. zizanioides*. Asymmetric break down of Remazol Red through the action of laccase or lignin peroxidase formed intermediate I and 2-[(3-diazenylphenyl) sulfonyl] ethanesulfonate

Table 1
Characterization of dye mixture and textile wastewater before and after treatment by *Vetiveria zizanioides* at laboratory scale (40 h), and using floating phyto-bed (72 h).

Treatment/Parameters	Laboratory scale (40 h)				Floating phyto-bed (72 h)	
	Dye mixture		Textile wastewater		Textile wastewater	
	Control	Test	Control	Test	Control	Test
ADMI	821 ± 12	215 ± 3***	1424 ± 8	380 ± 17***	1142 ± 21	295 ± 12**
pH	9.8	7.3	10.6	7.7	9.5	7.8
COD (mg/L)	1189 ± 22	325 ± 15***	1645 ± 22	491 ± 10***	1495 ± 20	395 ± 18**
BOD (mg/L)	832 ± 9	207 ± 3***	1309 ± 11	289 ± 12***	1135 ± 20	218 ± 7**
TDS (mg/L)	21 ± 2.3	9 ± 1.2***	4280 ± 5.7	1364 ± 6.4***	4706 ± 28	1580 ± 10**
TSS (mg/L)	35 ± 1.1	15 ± 0.2*	973 ± 14.4	392 ± 12.2***	734 ± 8	392 ± 7**
Electric conductivity	0.54 ± 0.02	2.98 ± 0.05***	0.89 ± 0.05	3.10 ± 0.06***	0.72 ± 0.07	4.18 ± 0.05*
Turbidity (NTUs)	42 ± 0.8	19 ± 2.2**	291 ± 3.5	118 ± 3.6***	305 ± 5.0	125 ± 2.9**

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.

Table 2
Enzyme activities of root and shoot of *Vetiveria zizanioides* at 0 h and 40 h for textile wastewater.

Enzyme	Root		Stem	
	Control	Test	Control	Test
Lignin peroxidase[a]	0.21 ± 0.02	$0.48 \pm 0.10^*$	0.07 ± 0.01	0.10 ± 0.02
Laccase[a]	29.6 ± 1.8	$182.2 \pm 7.0^{***}$	14.7 ± 1.9	$52.2 \pm 4.0^{**}$
Veratryl alcohol oxidase[a]	198.2 ± 6.8	$539.1 \pm 15.6^{***}$	78.5 ± 5.4	$125.5 \pm 2.2^*$
Azo reductase[b]	5.87 ± 0.47	$12.75 \pm 1.43^{***}$	1.16 ± 0.09	$3.08 \pm 0.07^*$
NADH-DCIP reductase[c]	24.15 ± 1.24	23.09 ± 0.95	0.28 ± 0.01	$0.10 \pm 0.01^*$
Riboflavin reductase[d]	0.77 ± 0.02	$0.03 \pm 0.01^{***}$	0.75 ± 0.03	$0.07 \pm 0.01^{***}$

Values are a mean of three experiments $\pm$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.
 [a] Enzyme activity unit in $min^{-1} mg^{-1}$.
 [b] µg of azo dye reduced min^{-1} mg protein^{-1}.
 [c] µg of DCIP reduced min^{-1} mg protein^{-1}.
 [d] µg of riboflavin reduced min^{-1} mg protein^{-1}.
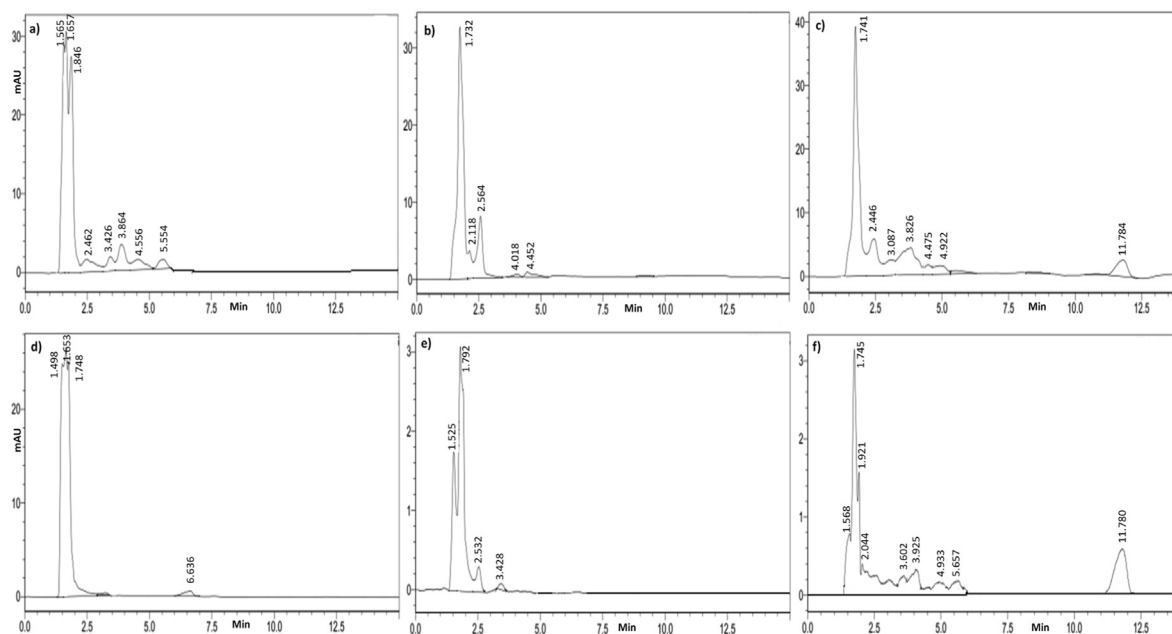
Fig. 1. HPLC analysis of a) untreated Remazol Red, b) untreated dye mixture, c) untreated textile wastewater, d) degraded product of Remazol Red, e) dye mixture after treatment, f) treated textile wastewater.

(mw = 277, m/z = 277). Further, intermediate I undergoes desulfonation to yield 8-[(4-chloro-1,3,5-triazin-2-yl)amino]naphthalen-1-ol (mw = 272, m/z = 271) and intermediate II. Intermediate II underwent denitrification to form 8-[(4-chloro-1,3,5-triazin-2-yl)amino]naphthalen-1-ol (mw = 142, m/z = 143) (Fig. 2). Oxidative and asymmetrical breakdown revealed to be an important mechanism for the cleavage of dye structure because of laccase, lignin peroxidase and veratryl alcohol oxidase enzymes action (Khandare and Govindwar, 2015).

3.5. Toxicity analysis of dye products

Treated textile wastewater is being used to irrigate agricultural field. Hence, it is imperative to study the harmful impact of treated wastewater on the plantlets of routinely grown crops. Remazol Red and textile wastewater demonstrated inhibition of *A. sativum* and *P. mungo* seed sprouting. The maximum germination was 50 and 55%; 40 and 50%, respectively. However, *V. zizanioides* treated dye Remazol Red and textile wastewater showed 85 and 90%; 80 and 80% seed germination of *A. sativum* and *P. mungo*, respectively. The lengths of root and shoot watered with formed metabolites of

Remazol red and textile wastewater after treatment by *V. zizanioides* were also increased when compared to plants germinated with normal water. While notable decrease in the length of shoot and root of the seedlings was observed when watered with untreated Remazol Red and textile wastewater (Table 3). *V. zizanioides* treatment confirmed significant reduced toxicity of produced metabolites.

3.6. Treatment of textile wastewater using floating phytobed

Treatment of textile wastewater by chemical precipitation and microbial treatment fails to remove color and reduce TDS. The irrigation with high TDS wastewater enhanced soil salinity, and increased TDS of surface and ground water. Hence, phytobeds were used to improve earlier treatment practices. Wild macrophytes *V. zizanioides* were grown with long, intense and strong root (45 cm, 180 g biomass) arrangement in normal tap water for two months on floating phytobed. *V. zizanioides* could grow healthily in an extensive range of pH 4–10. Textile wastewater treatment with fully grown macrophytes on floating phytobed in a reactor (400 L) for 72 h notably reduced the parameters like ADMI values, BOD,
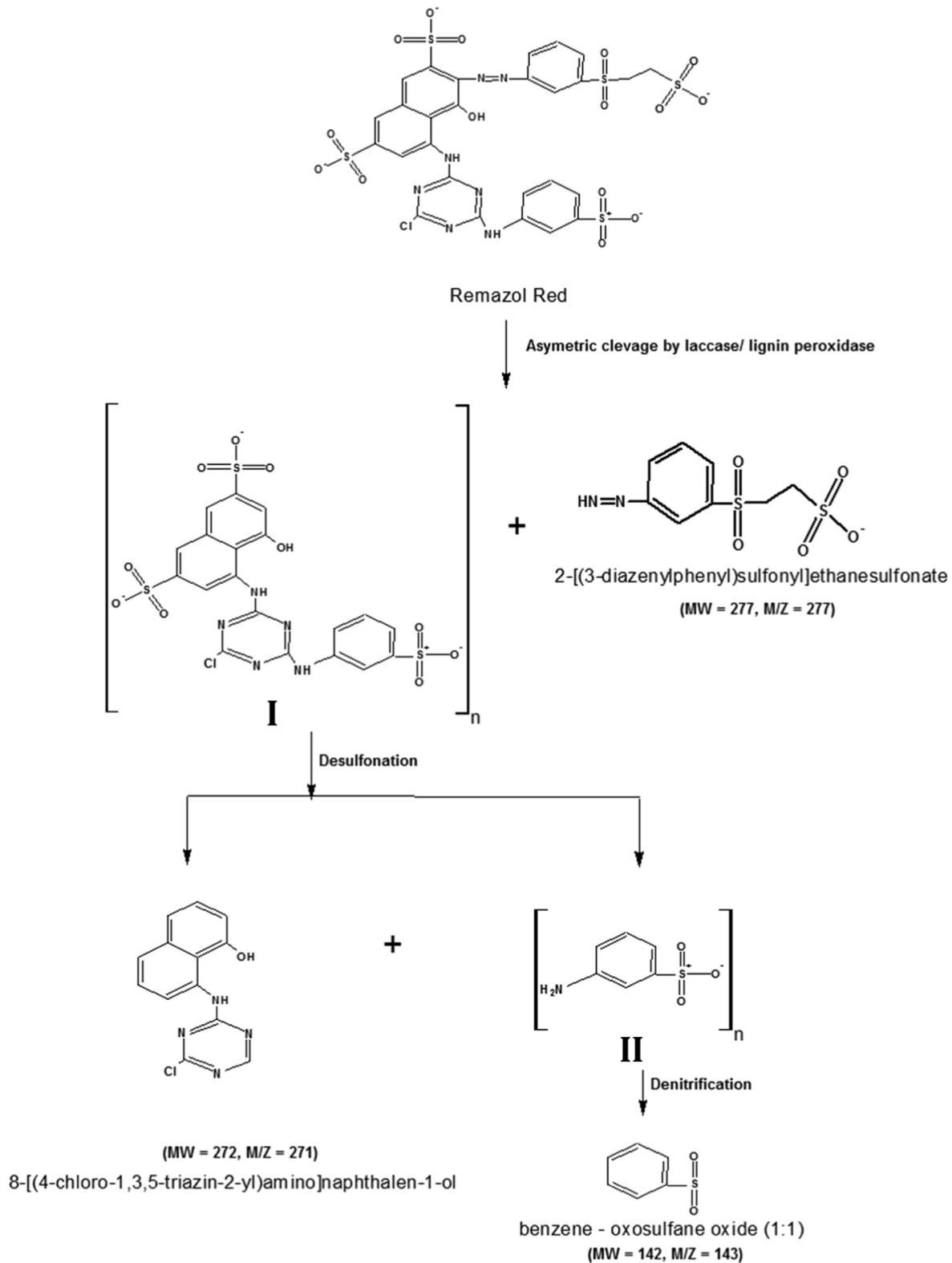
Fig. 2. Proposed pathways for metabolites of Remazol Red by *V. zizanioides*.

COD, TSS and TDS by 74, 81, 73, 47 and 66%, respectively (Table 1). Static reactor planted with macrophyte *Pogatherum orinithum* also revealed noteworthy performance and reduced ADMI, COD, BOD, pH, TSS and TDS (Watharkar et al., 2015). Pilot scale phytoreactor along with *P. grandiflora* as well showed efficient treatment of real

textile wastewater and achieved the BOD, COD, turbidity, TSS, TDS and TOC up to 38, 59, 41, 60, 71 and 37%, respectively (Khandare et al., 2013). Ornamental plant, *Glandularia pulchella* in static phytoreactor demonstrated significant reduction of BOD (70%), COD (70%) and TOC (74%) within 60 h (Kabra et al., 2013). It was also

Table 3
Phytotoxicity of Remazol Red and textile wastewater before and after (40 h) on *Aestivum sativum* and *Phaseolus mungo*.

	Water	Control dye	Treated dye	Untreated textile wastewater	Treated textile wastewater
Aestivum sativum					
Germination %	100	50	85	40	80
Root length	6.15 ± 0.37	$2.75 \pm 0.15*$	$5.45 \pm 0.12^{\$}$	$1.85 \pm 0.16*$	$4.92 \pm 0.51^{\$\$}$
Shoot length	10.50 ± 0.32	$4.89 \pm 0.06*$	$7.64 \pm 0.29^{\$\$}$	$3.40 \pm 0.19*$	$8.78 \pm 0.31^{\$\$}$
Phaseolus mungo					
Germination %	100	55	90	50	80
Root length	7.25 ± 0.24	$2.10 \pm 0.07*$	$5.96 \pm 0.14^{\$\$}$	$2.92 \pm 0.26*$	$6.00 \pm 0.48^{\$\$}$
Shoot length	12.50 ± 0.43	$3.58 \pm 0.19*$	$10.45 \pm 1.01^{\$\$}$	$4.21 \pm 0.37*$	$11.20 \pm 0.21^{\$\$}$

Values are mean of three experiment $\pm$ SEM. Shoot and root lengths of plants grown in dye Remazol Red and untreated textile wastewater are significantly different from that of plant grown in distilled water by $*P < 0.001$.
Shoot and root lengths of fifty plants grown in the treated dye Remazol Red and textile wastewater are also significantly different from that of plants grown in untreated Remazol Red and textile wastewater, respectively by $^{\$}P < 0.05$ and $^{\$\$}P < 0.01$.

noted the decrease the pH 7.8 from initial pH 9.5 due to the treatment of wastewater. It was also observed in the phyto-treatment (with *I. hederifolia*) of real textile wastewater and simulated dye mixture, that reduced pH to 7.5 and 8.1 from 7.9 to 10.2, respectively (Rane et al., 2016). While, *Alternanthera philoxeroides* exposed to effluent decreased pH from acidic to neutral (Rane et al., 2015). Electric conductivity of wastewater was found to enhance after treatment of *V. zizanioides* by 5.8-fold within 72 h (Table 1). Floating beds with *P. australis* and *T. domingensis* augmented with a bacterial consortium (*Acinetobacter junii, Pseudomonas indoloxydans* and *Rhodococcus* sp.) also resulted in the removal of colour, organic matter, toxicity, and heavy metals from textile wastewater (Tara et al., 2019).

3.7. On field treatment of textile wastewater in a constructed furrow planted with V. zizanioides, I. aquatica and their consortium

Individual furrow cultivated with *V. Zizanioides, I. aquatica* and consortium-VI in modified high rate transpiration system constructed at Kagal 5 star MIDC, Maharashtra, India when used to treat textile wastewater showed significant decrease in ADMI (67, 61 and 76%), BOD (73, 70 and 83%), COD (75, 74 and 79%), TDS (77, 75 and 83%) and TSS (34, 31 and 51%), respectively after 5 d (Table 4). Constructed lagoon (52,500 L) with floating plants like *S. molesta* reduced BOD, ADMI and COD of textile wastewater considerably by 82, 81 and 76% within 8 d treatment, respectively (Chandanshive et al., 2016). Developed vertical and horizontal constructed wetland planted with *P. australis* reduced color value, BOD, COD, TSS up to 90, 66, 84 and 93%, respectively of textile effluent (Bulc and Ojstrsek, 2008). Macrophytes *V. zizanioides, I. aquatica* and consortium-VI were observed to reduce pH of textile wastewater to 8.1, 7.9 and 7.4, respectively from pH 10.9 within 120 h (Table 4). Similar decrease in pH (8.8, 8.5 and 7.5, respectively

from 10.7, within 4 d treatment) was also observed when textile effluent treated with *P. scrobiculatum, T. angustifolia* and consortium-TP planted on ridges of HRTS (Chandanshive et al., 2018). Wastewater exposed to *V. zizanioides, I. aquatica* and consortium-VI planted furrows were found to enhance bacterial counts to 1.89, 1.87 and 1.93-fold, respectively within 120 h. This might be due to decrease in dye toxicity (Table 4). Lower bacterial population in untreated/unplanted textile effluent might be due to toxicity of dyes present in the effluents. Increased bacterial population by the plantation of *V. zizanioides, I. aquatica* and consortium-VI could be the result of release of exudates and carbon sources from dye metabolism, which ultimately supported the growth of bacteria. Eventually, increased bacterial population might have contributed for the degradation of textile dyes as well as decreased toxicity of effluent (Kabra et al., 2013; Hussain et al., 2018). *T. angustifolia, P. grandiflora, P. scrobiculatum, F. dichotoma, Ammannia baccifera, G. grandiflora, A. amellus*, and *T. patula* exposed to the textile effluent in a laboratory scale reactor also increased bacterial count after phytoremediation (Kadam et al., 2017; Chandanshive et al., 2018). Textile wastewater treated by *V. zizanioides* planted in furrow showed 18, 47, 64 and 56% reduction in cadmium (Cd), lead (Pb), arsenic (As) and chromium (Cr), respectively. *I. aquatica* grown in furrow also decreased in Cd, Pb, As and Cr up to 18, 50, 46 and 52%, respectively, within 120 h. Consortium-VI showed significant reduction of Cd (63%), Pb (74%), As (66%) and Cr (70%) after treatment of 120 h. A healthy root zone microflora is always advantageous in phytoremediation trials. Metals from textile effluent could have been absorbed/biotransformed by soil microbial community and further make available to the plants for easy uptake and accumulation, establishing synergistic removal of metals. Plants such as, *T. angustifolia, P. scrobiculatum* and consortium-TP also showed notably decreases of Pb (45, 50 and 69%), Cd (28, 28 and 71%), Cr (59, 63 and 77%) and As

Table 4
Characterization of untreated and treated textile wastewater (5 days) in furrows cultivated *Vetiveria zizanioides, Ipomoea aquatica* and their consortium.

Parameters	Textile wastewater	*Vetiveria zizanioides*	*Ipomoea aquatica*	Consortia VI
ADMI	1308 ± 11	$426 \pm 8***$	$512 \pm 5***$	$319 \pm 4***$
pH	10.9	8.1	7.9	7.4
COD (mg/L)	1794 ± 7	$452 \pm 3***$	$471 \pm 5***$	$382 \pm 7***$
BOD (mg/L)	1350 ± 13	$368 \pm 4***$	$397 \pm 8***$	$221 \pm 7***$
TDS (mg/L)	5143 ± 21	$1185 \pm 9***$	$1268 \pm 8***$	$859 \pm 5**$
TSS (mg/L)	1900 ± 15	$1249 \pm 12***$	$1310 \pm 10***$	$928 \pm 5**$
Bacterial count (CFUs)	$07 \pm 0.5 \times 10^{-7}$	$62 \pm 1.7 \times 10^{-7}**$	$55 \pm 2.3 \times 10^{-7}**$	$97 \pm 2.9 \times 10^{-7}***$
Cadmium (mg/L)	0.11 ± 0.01	0.09 ± 0.01	$0.09 \pm 0.01**$	$0.04 \pm 0.01***$
Lead (mg/L)	0.80 ± 0.09	$0.42 \pm 0.07*$	$0.38 \pm 0.09*$	$0.21 \pm 0.01**$
Arsenic (mg/L)	2.89 ± 0.03	$1.05 \pm 0.05***$	$1.57 \pm 0.10***$	$0.98 \pm 0.04***$
Chromium (mg/L)	1.93 ± 0.28	$0.85 \pm 0.07**$	$0.92 \pm 0.09**$	$0.58 \pm 0.01***$

Values are a mean of three experiments $\pm$ SEM.
Significantly different from control (untreated wastewater) at $*P < 0.05$, $**P < 0.01$ and. $P < 0.001$ by one-way ANOVA with Tukey-Kramer comparison test.

(60, 54 and 72%) within 96 h of textile effluent treatment (Chandanshive et al., 2017). Several aquatic and semi-aquatic plants have potential to eliminate heavy metals from textile wastewater (Miretzky et al., 2004; Alvarado et al., 2008; Rezania et al., 2015). Three *V. zizanioides* ecotypes also showed removal of Fe, Mn, Zn, Cu, Cd and Pb from wastewater of milk factory, an electric lamp plant, a battery manufacturing plant and ink manufacturing facility (Roongtanakiat et al., 2007; Kafil et al., 2019). *E. crassipes,* a macrophytes having extensive root system showed effective removal of heavy metal and color (85%) due to adsorption from wastewater (Roy et al., 2018; Tabinda et al., 2019). It also has a potential to decrease COD, TSS and color from batik textile effluents (Safauldeen et al., 2019). Simulated shallow pond system planted with *L. minor* was able to remove Basic Red 46 at low concentrations (Yaseen and Scholz, 2016; Ekperusi et al., 2019). A pilot-scale vertical flow constructed wetland with Brachiaria mutica demonstrated successful plant-dye degrading endophytic bacteria partnership showing efficient degradation of textile effluent and reduction in toxicity. Bacterial augmentation enhanced the remediation efficiency which decreased COD (81%), BOD (81%), TDS (32%), and color (74%) along with reduction of nitrogen (84%), phosphorous (79%), and heavy metals viz. Cr, Fe, Ni, and Cd by 72—97% (Hussain et al., 2018).

4. Conclusion

Significant decolorization and removal of Remazol Red, removal of dyes from textile wastewater and dye mixture, induction of oxido-reductive enzymes, significant reduction in environmental parameters such as COD, BOD, TDS, TSS, and ability to survive in unfavourable conditions suggests the potential of *V. zizanioides* for phytotransformation. Floating phytobeds of *V. zizanioides* could be used in a deep textile wastewater of the reservoirs. The combinatorial plantation of *V. zizanioides* and *I. aquatica* in furrows of existing HRTS could effectively treat textile wastewater than with individual plants. Plantation of aquatic plants consortium in furrows of HRTS will be intelligent approach to clean textile wastewater and management strategies.

Declarations of competing interest

None.

CRediT authorship contribution statement

Vishal Chandanshive: Data curation, Formal analysis, Investigation, Methodology, Resources, Software, Validation, Visualization, Writing - original draft, Writing - review & editing. **Suhas Kadam:** Data curation, Formal analysis, Investigation, Methodology, Resources, Software, Validation, Visualization. **Niraj Rane:** Data curation, Formal analysis, Investigation, Methodology, Resources, Software, Validation, Visualization. **Byong-Hun Jeon:** Conceptualization, Funding acquisition.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2020.126513.

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