Desalination and Water Treatment
www.deswater.com

doi: 10.1080/19443994.2012.748637

51 (2013) 4556–4565
June

Taylor & Francis
Taylor & Francis Group

Toxicity analysis of azo Red BS and Methyl Red dye solutions on earthworm (*Pheretima phosthuma*), micro-organisms, and plants

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Received 6 September 2012; Accepted 8 November 2012

ABSTRACT

Azo dyes containing effluent from various textile industries adversely affects water resources, soil fertility, aquatic organisms as well as animals. Pure cultures of bacteria or bacterial consortia have been successfully applied for the biodegradation of toxic dye effluents. In this view, toxicity analysis of Red BS and Methyl Red dyes and their biologically decolorized solutions were studied on earthworm (*Pheretima phosthuma*), plants, and micro-organisms. Different types of morphological symptoms were observed upon exposure of dye solutions on earthworm. Mortality rate in terms of LD_{50} value was determined for both the dye solutions. The LD_{50} of untreated Red BS and Methyl Red dye solution was 120.22 and 218.77 mg l^{-1}, respectively. Alteration in the protein content was observed in various organs, head, clitella, and abdomen of earthworms on exposure of dye solutions and the presence of proteins under stress condition was studied using Sodium dodecyl sulphate-Polyacrylamide Gel Electrophoresis. Microbial toxicity study revealed the impact of toxicity to be dependent on dye concentration and biologically decolorized dye samples are less toxic. Phytotoxicity study suggests that, percent germination, length of shoot, and leaf of *Phaseolus mungo* and *Triticum aestivum* are adversely affected by the exposure of dye solutions in comparison to the decolorized dye solutions.

Keywords: Azo dye; Earthworm (*Pheretima phosthuma*); *Phaseolus mungo*; *Triticum aestivum*; Red BS; Methyl Red

1. Introduction

In the past few decades, environmental pollution has become one of the world's major concerns. Azo dyes are synthetic organic compounds characterized by the presence of one or more azo bonds (–N=N–) in association with one or more aromatic systems. These synthetic dyes are extensively used in the textile and dyestuff industries. They are also widely used in paper, food, cosmetics, and pharmaceutical industries. Especially, dyes have been identified as one of the most problematic compounds in textile effluents as around 30% of the dye quantity remains in the aqueous phase, mainly in hydrolyzed form

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thus imparting color to the resulting effluent stream apart from their negative esthetic effects. Certain azo dyes have been proved to be highly toxic [1] and in some cases these compounds have also shown to be mutagenic and carcinogenic [2]. Several physical and chemical methods have been proposed for the treatment of dye loaded waste streams, but generation of sludge cause subsequent disposal problems. One of the promising approaches is to promote the use of microbes for the degradation of these compounds in waste water treatment systems. Use of microbes/biological methods provide a more natural and a complete clean up of the pollutants in a more economical way [3]. Thus, in the present study, bacterial consortium-*Pseudomonas.aeruginosa, Escherichia hermanii* and *Stenotrophomonas maltophilia* (PES) was used for the biological treatment of two azo dyes.

Among various hazardous compounds, accumulation of dyes in the soil causes multiple problems such as alteration in physico-chemical nature of the soil, decrease in soil fertility, and causes adverse effect on microbial fauna and flora and on soil invertebrates. Therefore, it is necessary to investigate the toxic impacts of dyes on environment using various living organisms or cells as "analytical devices" [4]. One of the common soil invertebrate is the earthworm. Earthworms play an important role in the enhancement of soil structure and fertility so beneficial to plant growth and nutrient recycling [5]. Moreover, earthworms ingest soil contaminants and absorb them through the skin, thus mortality of earthworms may be considered as a valuable and sensitive biomarker to diagnose adverse effect of dyes in soil environment [6]. Dissolved substances in industrial effluents even create an immediate environment concern with regard to water quality which directly influences the growth and productivity of plants. Thus, plant growth parameters, namely percent germination, seedling survival, and seedling height, have been taken as criteria to assess plant response to dye solutions by various researchers [7–9]. Similarly, microbes are ubiquitously present in soil and water, their growth is adversely affected by the dye solutions. The toxicity of a chemical to micro-organisms is normally measured in terms of growth inhibition [10], oxygen consumption [11], adenosine triphosphate level [12], enzyme activity [13], and agar diffusion plate assay [14–16]. Thus, the present study describes the effect of untreated (original) and decolorized dye solutions on earthworm, soil flora as well as on plants. The comparative study determines the significance of biological treatment in terms of decolorized sample which is less toxic than native dye solution.

2. Materials and methods

2.1. Earthworm toxicity

2.1.1. Test organism

Earthworm (*Pheretima phosthuma*) was used as a test organism in the present work.

2.1.2. Methods for collection

Earthworm is commonly found in the upper layer of the earth, up to the depth of 30–50 cm from the surface. The suitable appropriate time for the collection of earthworm was found to be early morning in the summer and noon time during the winter. The collected live earthworms were stored in the plastic bags filled with wet compost soil. Earthworms were placed at 25°C in the laboratory conditions. The experiments were carried out as prescribed in *the Organization for Economic Cooperation and Development* test protocol [17]. Prior to the use of earthworm for experimental work they were acclimatized for seven days under the laboratory conditions. A wide mouth jar covered with a muslin cloth having moist soil compost manure was used as a feed medium for the earthworms. Adult earthworms weighing 300–600 mg having clitellum were used in experiments.

2.1.3. Test solution/compound

Untreated and decolorized solutions of Red BS and Methyl Red azo dyes were used in the present study. These dye samples were procured from CAB Chemicals GIDC, Ankleshwar, Gujarat.

2.1.4. Preparation of the test compound

Stock solutions of Red BS and Methyl Red were prepared having a concentration of 25,000 $mg\,l^{-1}$. From this stock solutions four different concentrations 100, 200, 300, 400 $mg\,l^{-1}$ and 100, 300, 500, and 700 $mg\,l^{-1}$ were prepared for Red BS dye and Methyl Red dye solutions, respectively.

2.1.5. Toxicity determination

2.1.5.1. Method for treatment. Contact feeding method was used for the exposure of the earthworms to the untreated dye and decolorized solutions. Three air-dried plastic beakers containing 500 g of soil were amended with suitable dose of the untreated and decolorized solutions of Red BS and Methyl Red, respectively. The plastic beakers were marked for their respective concentration, whereas one beaker

was kept as a reference to determine the environmental effect. Five adult earthworms of same age and size were released in each of the beakers. At regular time intervals, weight of the earthworms and morphological as well as pathological symptoms were monitored. Percent mortality was determined on the basis of data obtained on earthworms exposed to untreated dye solutions. If mortality was found to be more than 20% in the control set, then the entire experiment was repeated.

2.1.5.2. Determination of the lethal dose (LD_{50}). The LD_{50} of Red BS and Methyl Red dyes was determined by Probit-mortality curves [18]. Probit analysis was used for assessing the acute toxicity of contaminants in soil to earthworm. Oneway ANOVA ($p < 0.05$) and correlation analysis was used for assessing the effects of contaminants on growth and reproduction.

2.1.6. Determination of the total protein content

The effect of Red BS and Methyl Red dye solutions was studied on the total protein content of the earthworms. Five adult earthworms were exposed to LD_{50} dose of respective dye solutions. Thereafter, the exposed earthworm's clitellum, head, and abdomen were removed and suspended in 2 ml of deionized water and homogenized for 15 min. The homogenate thus obtained was centrifuged at 5,000 *g* for 10 min at 4°C and the supernatant was used for the estimation of protein. Protein estimation was carried out using bovine serum albumin as a standard [19].

2.2. Microbial toxicity

The microbial toxicity of the untreated and decolorized dye solutions was determined against test microbes *such as Escherichia coli*, *Azotobacter vinelandii*, and *Azospirillum brasilence*. Microbial toxicity study was determined by agar well diffusion assay. Actively grown micro-organisms were seeded on the nutrient agar plates. Four wells of 8 mm diameter each were bored in nutrient agar media. The wells were filled with 100 µl of different concentrations of untreated and decolorized solutions of Red BS and Methyl Red dyes. The plates were incubated at 30°C for 48 h and zone of inhibition (in mm) was measured representing the index of the toxicity.

2.3. Phytotoxicity

Phytotoxicity test was performed in order to assess the toxicity of untreated and decolorized dye solutions on plant growth. The phytotoxicity study was carried at room temperature on two important crops *Phaseolus mungo* and *Triticum aestivum*. Small plastic beakers were first labeled as per the concentration of Red BS and Methyl Red dye solution. Ten seeds of *P. mungo* and *T. aestivum* were sown in the beakers containing soil solution. At regular time interval different concentrations (100 and 400 mg l^{-1}) of Red BS and Methyl Red untreated and decolorized dye solutions (10 ml) were added and a beaker was supplied with distilled water served as control for 7 days [20]. The toxicity effect was measured in terms of percent germination and lengths of shoot/stem and leaves.

3. Results and discussion

Azo dyes Red BS and Methyl Red dye solutions were decolorized using bacterial consortium-PES. Bacterial consortium-PES consists of three bacterial cultures which were identified by using Bio Log 1420 system as well as on the basis of 16S rRNA sequence. The phylogenetic tree was constructed by the neighbor-joining method on the program Mega 3.1 and the bacterial strains were identified as: *Pseudomonas aeruginosa* (Gene Bank Accession No. GQ884172), *Escherichia hermanii* (Gene Bank Accession No. GQ884173), and *Stenotrophomonas maltophilia* (Gene Bank Accession No. GQ884174). The consortium had ability to decolorize the azo dyes (Red BS and Methyl Red) within 30 and 24 h, respectively. In order to study the effect of untreated and decolorized dye solutions on different biological systems, toxicity studies were done on earthworm, plants, and micro-organisms.

3.1. Determination of earthworm toxicity

The earthworms which were selected for experiment weighed more than 600 mg and were about 10–13 cm long. It has been reported that immature earthworms below 5 cm are generally susceptible to rapid death regardless of exposure to concentration of toxic compound [21]. The toxic effect of dye solutions were recorded on earthworms between 7 and 14 days of exposure through direct contact cum feeding method.

Upon exposure to untreated Red BS and Methyl Red solutions, the morphological symptoms of toxicity in the earthworm (*P. phosthuma*) were observed. Sluggish movement of earthworms was observed which may be due to excessive mucus secretion within three days of incubation.

Upon further incubation, the disappearance of pigmentation was observed in the posterior region of the earthworms exposed to untreated Methyl Red dye (700 mg l^{-1}) and Red BS dye (400 mg l^{-1}) solution on fourth and fifth days (Fig. 1(a)). However, in reference earthworms no such changes were observed. Similar

observations were also reported in the case of azodrin pesticide exposed earthworms (*Eisenia foetida*) [22]. The exposed earthworms to untreated Methyl Red and Red BS solutions also developed multiple lesions, swelling of the clitellum, fragmentation, and subsequent autolysis (Fig. 1(b) and (c)) upon incubation of 5–6 days. Such degeneration of earthworms indicates the complete drain of utilizable level of energy reserves and lead to the subsequent autolysis of its own tissues to meet the energy requirements. The results thus obtained could be an adaptive strategy tide over the adverse conditions. Inherent capacity of regeneration, the utilization of its own tissue from the posterior region sparing anterior region containing vital organs, thus arrest energy drain beyond its storage capacity without affecting its survival. In earthworms (*Polypheretima elongate*), 50% detachment of one or two of their posterior parts was observed after three days of exposure to textile dyes [23]. Body ruptures, bloody lesions, and internal excessive formation of glandular cell mass and disintegration of circular and longitudinal muscles, which failed to regulate the internal coelomic pressure, leading to fragmentation in earthworms like morphological changes were observed in earthworms (*E. foetida*) upon exposure of an organ phosphorous pesticide [24]. Furthermore, in the case of earthworms exposed to dye solutions, coiling with weight loss was observed which may be due to the muscular alterations and thereby leading to difficulties in locomotion and their related ability to feed themselves [25]. The findings are in agreement with the observation of other researchers [25–27].

3.1.1. Mortality study of earthworm (*P. posthuma*)

Toxicity of Red BS and Methyl Red dye was determined using four different concentrations of test solutions against *P. phosthuma*. The effect of dye solution was concentration dependent with an increase in concentration of the dye, the percentage survival decreased. The increase in mortality rate was observed after 7 days of incubation with respect to the concentrations of dyes applied. The mean mortality values were plotted against applied concentration of the dye and LD_{50} was calculated according to the method described by other researchers [18,28]. The LD_{50} obtained was 120.22 and 218.77 $mg\,l^{-1}$ for the untreated dye solution of Red BS and Methyl Red solutions, respectively (Table 1(a) and (c) and Fig. 2(a) and (c)). The LD_{50} for decolorized Red BS and Methyl Red dye solutions was 229 and 331.1 $mg\,l^{-1}$,

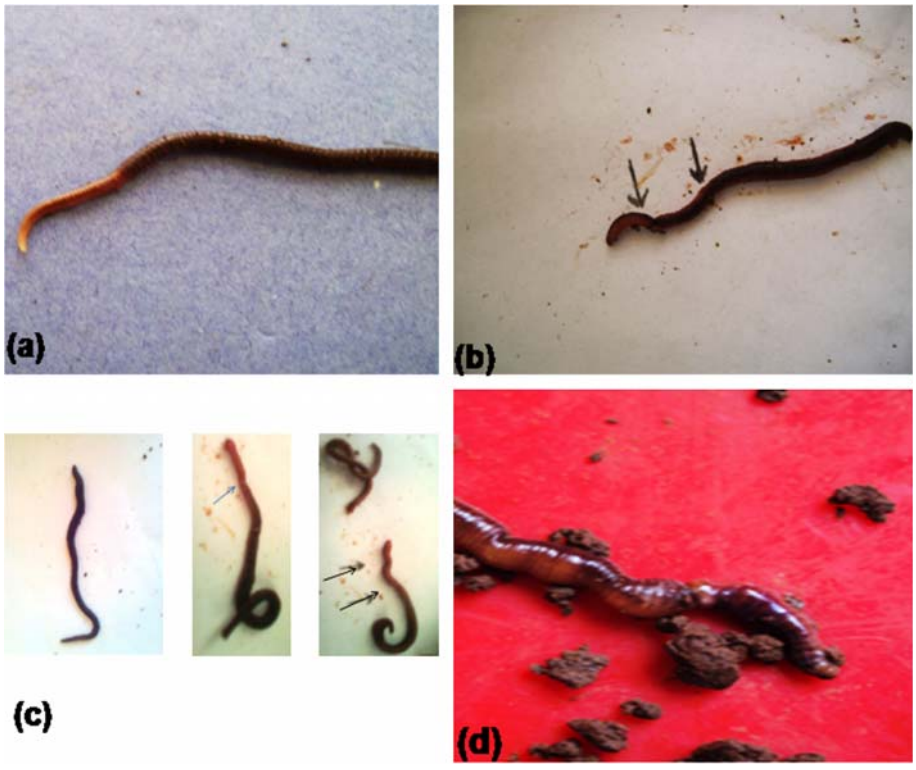

Fig. 1. Morphological symptom due to adverse effect of Red BS and Methyl Red dyes exposure (a) loss of pigmentation in the posterior region; (b) extrusion of coelomic fluid from the posterior; (c) fragmentation in the posterior end; and (d) multiple lesion.

respectively (Table 1(b) and (d) and Fig. 2(b) and (d)). Low mortality values have also been reported in earthworms by other researchers [21,26,27]. The death of the earthworms was observed in reference was with respect to the time.

3.1.2. Effect of dyes on total protein content

The amount of protein present varies in different organs/body parts of the earthworm. The protein content of head, clitellum, and abdomen was measured on 9th and 11th days in the earthworms exposed to LD_{50} dose of dyes and in the reference (control) earthworm. The total protein content was found to be $1{,}212 \pm 0.1\,\mu g\,ml^{-1}$ in the head; $2{,}145 \pm 1.1\,\mu g\,ml^{-1}$ in the clitellum, and $4{,}570 \pm 0.56\,\mu g\,ml^{-1}$ in the abdomen region. For earthworms exposed to untreated Red BS dye solution, total protein content was found to be $958 \pm 0.11\,\mu g\,ml^{-1}$ in the head; $1{,}045 \pm 0.15\,\mu g\,ml^{-1}$ in the clitellum, and $1{,}950 \pm 0.2\,\mu g\,ml^{-1}$ in the abdomen region, whereas in the case of decolorized Red BS dye exposed earthworm total protein content was found to be $1{,}067 \pm 0.25\,\mu g\,ml^{-1}$ in the head; $1{,}546 \pm 0.12\,\mu g\,ml^{-1}$ in the clitellum; and $3{,}410 \pm 0.6\,\mu g\,ml^{-1}$ in the abdomen region. The total protein content in earthworms exposed to untreated Methyl Red solution was found to be $1{,}000 \pm 0.01\,\mu g\,ml^{-1}$ in the head; $1{,}212 \pm 0.12\,\mu g\,ml^{-1}$ in the clitellum, and $2{,}400 \pm 0.4\,\mu g\,ml^{-1}$ in the abdomen region, while in earthworm exposed to decolorized Methyl Red dye solution the total protein content was found to be $1{,}170 \pm 0.02\,\mu g\,ml^{-1}$ in the head; $1{,}610 \pm 0.06\,\mu g\,ml^{-1}$ in the clitellum, and $2{,}764 \pm 0.5\,\mu g\,ml^{-1}$ in the abdomen region. There was reduction in protein content in all parts of the earthworm exposed to dye solutions as compared to the reference (control) earthworms (Fig. 3). The decrease in protein content may be attributed to catabolism of protein and glycogen in response to worm energy demand and to overcome the stress situation [29–31]. Reduction in total protein content upon long-term exposure to chemical fertilizer and this decrease in protein content were due to a mechanical lipoprotein formation which may be used to repair the damages to various tissues and organs [32]. Even reduction of worm protein content was one of the primary toxic effects of various pesticides and the decrease may be due an

Table 1
Percent mortality of (a) untreated Red BS, (b) decolorized Red BS dye and percent mortality of, (c) untreated Methyl Red, and (d) decolorized Methyl Red dye solution on the earthworm

No.	Concentrations ($mg\,l^{-1}$)	Log dose	Earthworm exposed	% killed	% corrected[a]	Probit
(a)						
1	100	2	5	40	38	4.75
2	200	2.3	5	60	57	5.25
3	300	2.47	5	80	76	5.84
4	400	2.6	5	100	95	6.64
(b)						
1	100	2	5	0	5	3.36
2	200	2.3	5	20	19	4.16
3	300	2.5	5	60	57	4.69
4	400	2.6	5	80	76	5.18
(c)						
1	100	2	5	20	19	4.16
2	300	2.5	5	60	57	5.25
3	500	2.7	5	80	76	5.84
4	700	2.9	5	100	95	6.64
(d)						
1	100	2	5	0	5	3.36
2	300	2.5	5	20	19	4.75
2	500	2.7	5	80	76	5.85
3	700	2.9	5	100	95	6.64

[a]% Corrected values are with 95% confidence limit.

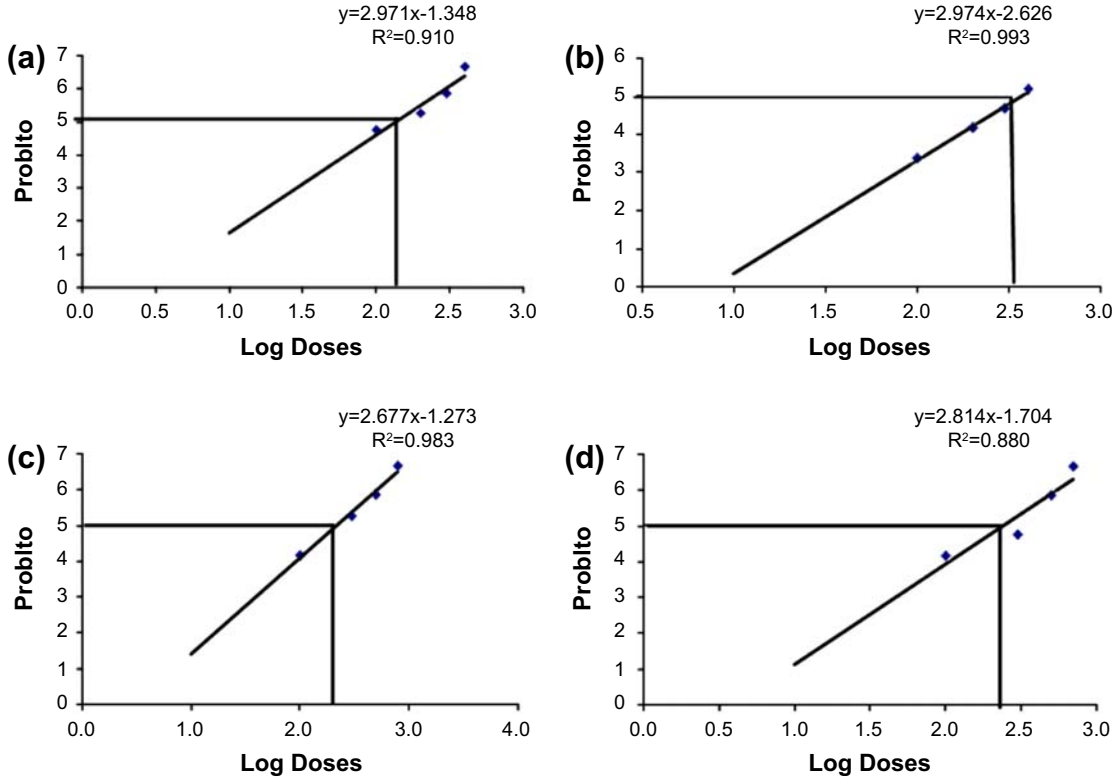

Fig. 2. Mortality curve of earthworm *P. posthuma* exposed to (a) untreated Red BS dye; (b) decolorized Red BS dye; (c) untreated Methyl Red; and (d) decolorized Methyl Red.

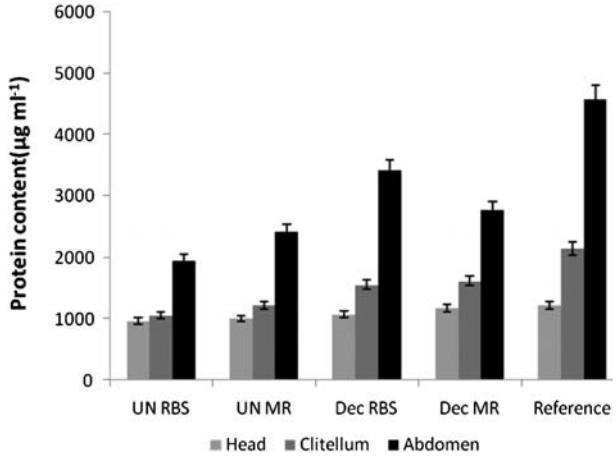

Fig. 3. Effect of Red BS and Methyl Red untreated and decolorized dye solution on the head, clitellum, and abdomen protein content of earthworm. UN RBS: Untreated Red BS, UN MR: Untreated Methyl Red, Dec RBS-Decolorized Red BS, Dec MR-Decolorized Methyl Red.

early defense reaction to the pesticides stress [33]. Similar kind of studies and effect of pesticide on proteins and enzymes of different organisms have been also cited by various researchers [34–36].

3.1.3. SDS-PAGE

Upon exposure of earthworms to untreated Red BS and Methyl Red dyes, change in protein content was observed. The crude protein content of head, clitellum, and abdomen of earthworm showed the distinct bands from low to high molecular weight. Fig. 4 represents protein profile of clitellum, head, and abdomen of earthworms exposed to Methyl Red and Red BS dyes and reference earthworm. The result determines less numbers of bands were seen in head, clitella, and abdomen content of Methyl Red and Red BS dye exposed earthworms in contrast to reference earthworms. This pattern indicates the level of expression of protein differs under stress conditions.

3.2. Microbial toxicity

Microbial toxicity was determined by an agar well diffusion assay. Different concentrations, 100–400 $mg\,l^{-1}$ of untreated and decolorized Red BS and 100–700 $mg\,l^{-1}$ of Methyl Red dye solutions, were used and tested against certain microbial cultures such as *E. coli, A. vinelandii, and A. brasilence*. Zone

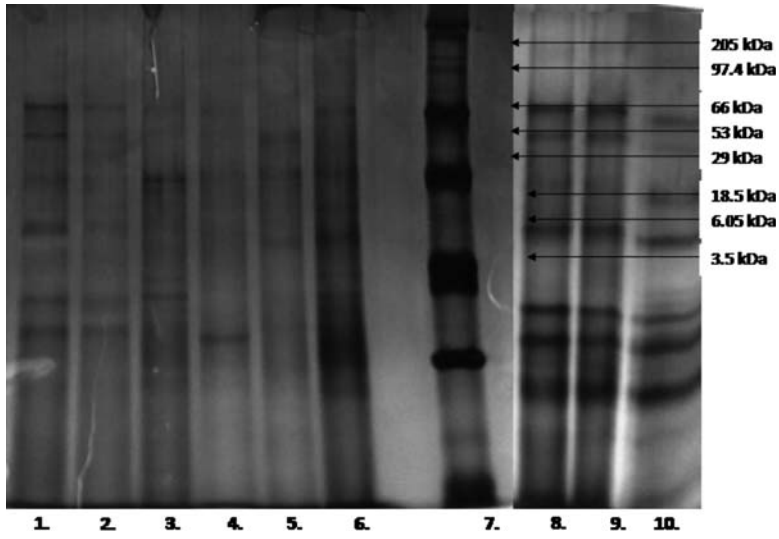

Fig. 4. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis analysis of earthworm protein. Lane 2 and 3 Corresponds to proteins extracted from clitellum of earthworms exposed to Methyl Red and Red BS dyes; Lane 1, 6, and 8: Reference-clitellum, head, and abdomen, respectively; Lane 4 and 5 corresponds to proteins extracted from head of earthworms exposed to Methyl Red and Red BS dyes; Lane 7: Marker; and Lane 9 and 10 corresponds to proteins extracted from abdomen of earthworms exposed to MR and Red BS dyes.

of inhibition was obtained with untreated Red BS and Methyl red dye solutions and in contrast no zone of inhibition was obtained with the decolorized samples as well as reference (Table 2). *A. vinelandii* was found to be the most sensitive among the various test cultures used. In the case of microbial toxicity study of dye Navy blue Rx, the native dye was found to be more toxic in comparison to decolorized sample using *A. vinelandii* and *P. aeruginosa* as test organisms [37]. In accordance to obtained results, similar kinds of microbial toxicity study of dye solutions against various soil microbes were also investigated and decolorized samples have shown less toxicity [8,38].

Table 2
Microbial toxicity of untreated Red BS and Methyl Red dye solutions (values are mean ± SD from three measurements)

Name of dye sample	Concentration ($mg\,l^{-1}$)	*E. coli* Diameter of zone of inhibition (mm)	*A. vinelandii* Diameter of zone of inhibition (mm)	*A. brazilence* Diameter of zone of inhibition (mm)
Untreated Red BS Dye solution	100	10 ± 0.0	13.33 ± 0.33	10.3 ± 0.7
	200	11 ± 0.58	14 ± 0.58	11.3 ± 0.3
	300	11.7 ± 0.88	19.3 ± 0.88	12 ± 0.6
	400	12.3 ± 0.33	21.3 ± 0.33	14 ± 0.0
Decolorized Red BS dye solution	400	0.0	0.0	0.0
Untreated Methyl Red dye solution	100	10 ± 0.0	10 ± 0.0	10 ± 0.67
	300	10.3 ± 0.6	11 ± 0.58	11.3 ± 0.0
	500	11 ± 0.6	11.7 ± 0.88	12 ± 0.58
	700	12.0 ± 0.0	13 ± 1	13 ± 0.33
Decolorized Methyl Red dye solution	700	0.0	0.0	0.0

Table 3
Effect of untreated and decolorized Red BS and Methyl Red dye solutions on *P. mungo* and *T. aestivum* (values are mean ± SD from three measurements)

Plant species	Treatment	Concentration ($mg\,l^{-1}$)	Seed germination (%)	Shoot length (cm)	Mean length of leaf (cm)
	Control		100	18	2.5
P. mungo	Untreated Red BS	100	43 ± 0.02	12 ± 0.58	11.3 ± 0.03
		400	10 ± 0.0	6.97 ± 0.55	1.5 ± 0.07
	Decolorized Red BS	100	100 ± 0.00	14.7 ± 0.1	2.3 ± 0.2
		400	83 ± 0.03	14.7 ± 0.37	1.8 ± 0.06
P. mungo	Untreated Methyl Red	100	60 ± 0.0	6.9 ± 0.58	1.2 ± 0.03
		400	13 ± 3.33	5.33 ± 0.22	1.1 ± 0.07
	Decolorized Methyl Red	100	100 ± 0.0	14.7 ± 1.53	1.9 ± 0.29
		400	43 ± 0.02	6 ± 0.0	1.3 ± 0.1
T. aestivum	Untreated Red BS	100	60 ± 0.1	11.33 ± 0.2	—
		400	43 ± 3.0	5.5 ± 0.79	—
	Decolorized Red BS	100	70 ± 0.2	14.7 ± 0.0	—
		400	57 ± 3.0	15.4 ± 0.48	—
T. aestivum	Untreated Methyl Red	100	23.3 ± 0.04	7.9 ± 0.1	—
		400	13 ± 0.3	4 ± 0.56	—
	Decolorized Methyl Red	100	73.3 ± 0.05	12.5 ± 0.4	—
		400	40 ± 0.0	10 ± 0.57	—

3.3. Phytotoxicity

Aromatic sulfonic azo group and their metabolic intermediates (sulfonated and unsulfonated aromatic amines) represent an important group of environmental pollutants having a toxic nature [39,40]. Improper disposal of the dye effluents containing reactive groups cause a serious problem to environment and health. Such effluents when disposed in the water bodies are of major concern particularly when such water is to be used in irrigation.

3.3.1. Effect of Red BS and Methyl Red untreated and decolorized dye solutions on P. mungo

Effect of untreated and decolorized dye solutions on seed germination and shoot and leaf length was studied on *P. mungo* seeds. The 43 and 10% germination and 60 and 13% germination of *P. mungo* seeds were observed in the presence of 100 and $400\,mg\,l^{-1}$ untreated Red BS and Methyl Red dye solutions, respectively (Table 3). However, 100% seed germination was observed with $100\,mg\,l^{-1}$ of Red BS and Methyl Red decolorized dye solutions and 83 and 43% seed germination was observed with a concentration of $400\,mg\,l^{-1}$ of Red BS and Methyl Red decolorized dye solutions, respectively. In decolorized solutions of both dyes ($400\,mg\,l^{-1}$), 16 and 28% reduction in shoot and leaf length was observed whereas 70 and 56% reduction in the shoot and leaf length was observed in plants exposed with $400\,mg\,l^{-1}$ untreated and decolorized dye solutions of Red BS and Methyl Red, respectively. Thus, the obtained results demonstrates that decolorized Red BS and Methyl Red dye samples are less toxic than untreated dye samples. Maximum shoot and leaf length was obtained in the reference plants (Table 3). Similarly, maximum leaf and shoot height was observed in reference plants in comparison to dye exposed rice (*Oryza sativa*) plants [9]. Negative influence of textile effluent was reported on seed germination and shoot length of peanuts plants [41] and similar kinds of negative impacts on the length of shoot and root of *P. mungo* and *Sorghum vulgare* plants as compared to the metabolites obtained after degradation of Remazol Red and Golden yellow dyes were also described [42]. Toxicity in terms of germination and growth of seeds irrigated with extracted metabolites (color less) less than native dye compound was demonstrated with the phytoxicity study of antraquinone and azo dyes [43].

3.3.2. Effect of Red BS and Methyl Red untreated and decolorized dye solutions on T. aestivum

Effect of untreated and decolorized dye solutions on percent germination and shoot height was analyzed in *T. aestivum* seeds. The 60 and 43% germination and 23.3 and 13% germination of *T. aestivum* seeds were observed in the presence of 100 and 400 $mg\,l^{-1}$ untreated Red BS and Methyl Red dye solutions, respectively (Table 3). However, 70 and 73.3% seed germination was observed with 100 $mg\,l^{-1}$ of Red BS and Methyl Red decolorized dye solutions, whereas 57 and 40% seed germination was observed with a concentration of 400 $mg\,l^{-1}$ of Red BS and Methyl Red decolorized dye solutions, respectively. Similarly, 100% germination inhibition of *T. aestivum* was observed at 150 $mg\,l^{-1}$ of untreated dye solution and only 10% inhibition was observed by decolorized solution [44]. Moreover, 37 and 56% and 67 and 78% reduction in shoot length was observed with both decolorized dye solutions, whereas 16 and 28% and 16 and 54% reduction in the shoot length were observed in plants exposed with (100 and 400 $mg\,l^{-1}$) concentration containing untreated dye solutions of Red BS and Methyl Red, respectively. The outcome of results demonstrate lesser toxicity of metabolize obtained upon decolorization of dyes decolorized Red BS and Methyl Red dye samples and maximum shoot length were reported in the reference plants in comparison to dye exposed plants (Table 4). Similar kinds of effect on growth, shoot length, and seed germination have also been reported upon exposure of tetrazine dye and distillery effluent, respectively, in *T. aestivum* plants [45,8].

4. Conclusion

The untreated dyes were found to be highly toxic and adversely affected the growth of the earthworms, test bacterial cultures as well as *T. aestivum* and *P. mungo* seeds and its growth. Moreover, the untreated dye solutions also showed different physiological and observable changes in earthworms and plants, whereas toxicity was significantly reduced for same concentration containing decolorized dye solutions after the biological treatment using bacterial consortium-PES. Overall study revealed the potential role of biological treatment in reduction of toxicity which is essential for safe disposal of dye-contaminated waste waters.

References

[1] R. Anlinker, E.A. Clarke, P. Moser, Use of the partition coefficient as an indicator of bio-accumulation tendency of dyestuffs in fish, Chemosphere 10 (1981) 263–274.

[2] K.T. Chung, S.E. Stevens, C.R. Cerniglia Jr., The reduction of azo dyes by the intestinal microflora, Crit. Rev. Microbiol. 18 (1992) 175–190.

[3] A. Moutaouakkil, Y. Zeroual, F.Z. Dzayri, Decolorization of Azo dyes with *Enterobacter agglomerans* immobilized in different supports by using Fluidized Bed Bioreactor, Curr. Microbiol. 48 (2004) 124–129.

[4] R.M. Logar, M. Vodovnik, The applications of microbes in environmental monitoring, in: A. Menez-vilas (Ed.), Communicating Current Research and Educational Topics and Trends in Applied Microbiology, Formatex, Badajoz, 2007, pp. 577–585.

[5] N. Puvaneswari, J. Muthukrishnan, P. Gunasekaran, Toxicity assessment and microbial degradation of azo dyes, Indian J. Exp. Biol. 44 (2006) 618–629.

[6] D. Yang, J. Zhu, R. Fu, W. Wang, X. Guo, Z. Wang, H. Yao, Enchytraeidae *Fridericia bulbosa* as a new test species for soil ecotoxicity assessment, Chemosphere 88(4) (2012) 501–506.

[7] U.V. Mane, P.N. Gurav, A.M. Deshmukh, S.P. Govindwar, Degradation textile Dye reactive navy—blue Rx (Reactive blue—59) by isolated Actinomycetes *Streptomyces krainskii* SUK—5, Malaysian J. Microbiol. 4(2) (2008) 1–5.

[8] P. Chaube, H. Indurkar, S. Moghe, Biodegradation and decolorization of dye by mix consortia of bacteria and study of toxicity on *Phaseolus mungo* and *Triticum aestivum*, Asiatic J. Biotechnol. Res. 1 (2010) 45–56.

[9] D. Konwar, D.K. Jha, Response of Rice (*Oryza sativa* L.) to contamination of soil with refinery effluents under natural conditions, Assam University, J. Sci. Technol. 5 (2010) 14–22.

[10] N. Norkis, C. Zur, Toxicity test accompanied biodegradation test of anionic surfactants, Bull. Environ. Contam. Toxicol. 22 (1979) 448–456.

[11] J.L. Slabbert, W.O.K. Brabow, A rapid water toxicity screening test based on oxygen uptake of *Psuedomonas putida*, Toxic. Assess. 1 (1986) 10–15.

[12] C.R. Parker, E.J. Pribly, Assessment of bacterial toxicity screen procedure using bacterial system, in: E. Liu, B.J. Dutker (Eds.), New York, NY, 1984, pp. 283–293.

[13] G. Bitton, T. Khafif, J.B. Chatinger, C.M. Coste, A direct INT-Dehydrogenase Assay (DIDHA) for chemical toxicity, Toxic. Assess. 1(1) (1986) 1–12.

[14] A.C. Aderson, A.A. Abdelghani, Toxicity of arsenical compounds is short term bacterial bioassay, Bull. Environ. Contam. Toxicol. 24 (1980) 124–127.

[15] G. Cenciet, G. Caldini, G. Morozzi, Chlorinated phenol toxicity by bacterial and biochemical test, Bull. Environ. Contam. Toxicol. 38 (1987) 868–875.

[16] O. Liu, Y.K. Chau, B.S. Dutka, Rapid toxicity assessment of water soluble chemical using a modified agar plate method, Water Res. 23 (1989) 333–339.

[17] Organisation for Economic Co-operation and Development (OCED), Guidelines for testing of chemicals. Test no. 207: Earthworm acute toxicity test. Organisation for Economic Co-operation and Development (OECD), Paris, France, 1984.

[18] L.C. Miller, M.L. Tainter, Estimation of LD50 and its error by means of log-probit graph paper, Proc. Soc. Exp. Bio. Med. 57 (1944) 261–264.

[19] O.H. Lowry, N.J. Rosebrough, A.L. Farr, R.J. Randall, Protein measurement with folin phenol reagent, J. Biol. Chem. 193 (1951) 265–275.

[20] V.V. Dawkar, U.U. Jadhav, G.S. Ghodake, S.P. Govindwar, Effect of inducers on the decolorization and biodegradation of textile azo dye Navy blue 2GL by *Bacillus* sp. VUS, Biodegradation 20 (2009) 777–787.

[21] P.T.E. Ozoh, Effect of dyestuff effluent on Nigeria macro — benthic invertebrates *Hippopera nigeriae*, Bull. Environ. Contam. Toxicol. 33 (1984) 210–214.

[22] J.V. Rao, P. Kavitha, Toxicity of azodrin on the morphology and acetylcholinesterase activity of the earthworm *Eisenia foetida*, Environ. Res. 96 (2004) 323–327.

[23] N. Ramaswami, V. Subburam, Effect of selected textile dyes on the survival, morphology and burrowing behaviour of the earthworm *Polypheretima elongate*, Poll. Environ. Contam. Toxicol. 48 (1992) 249–252.
[24] N. Chakra Reddy, J. Venkateswara Rao, Biological response of earthworm, *Eisenia foetida* (Savigny) to an organophosphorous pesticide, Ecotoxic. Environ. Saf. 71(2) (2008) 574–582.
[25] S. Yasmin, D.D. Souza, Effect of pesticide on the growth and reproduction of earthworm: A review, Appl. Environ. Soil. Sci. (2010) 1–9.
[26] J.V. Rao, C.H.S. Rani, P. Kavitha, R.N. Rao, S.S. Madhavendra, Toxic effects of Chlorpyrifos to the fish *Oreochromis mossambicus*, Bull. Enviorn. Contam. Toxicol. 70 (2003) 985–992.
[27] M. Faheem, M.F. Khan, Toxicity of imidacloprid (nicotinamide) against earthworm, *P. posthuma* with reference to its effect on protein, J. Basic Appl. Sci. 6 (2010) 55–62.
[28] M.A. Randhawa, Calculation of LD_{50} values from the method of Miller and Tainter, 1944, J. Ayub. Med. Coll. Abbottabad. 21(3) (2009) 184–185.
[29] J. Granett, N.C. Leeling, Trehalose and glycogen depletion during DDT poisoning of American cockroach *Periplaneta Americana*, Ann. Ent. Soc. Am. 64 (1971) 785–789.
[30] G.L. Orr, R.G.H. Dower, Effect of lindane (Thexachlorocyclohexane) on carbohydrates and lipid reserve in the American cockroach *Periplaneta Americana*, L. Pestic. Biochem. Physiol. 17 (1982) 89–95.
[31] P. Singh, R. Sanghi, A. Pandey, L. Iyengar, Decolorization and partial degradation of monoazo dyes in sequential fixed-filmed anaerobic batch reactor (SFABR), Bioresour. Technol. 98 (2007) 2053–2056.
[32] C. Xiang, P. Zhang, D. Pan Qiu, Q. Chu, Changes in diversity, protein content, and amino acid composition of earthworms from a paddy soil under different long-term fertilizations in the Tai Lake Region, China, Acta Ecologica. Sin. 26(6) (2006) 166–167.
[33] S. Ribeiro, J.P. Sousa, A.J.A. Noguerira, A. M.V.M. Soares, Effect of endosulfan and parathion on energy reserves and physiological parameters of the terrestrial isopod *Porcellio dilatatus*, Ecotoxicol. Enviorn. Saf. 49 (2001) 131–138.
[34] N. Yasmin, S.M.H. Jafri, M.F. Khan, Toxic effects of cyfluthrin on protein patterns of American cockroach, Pak. J. Entomol. 9 (1994) 79–82.
[35] S.N.H. Naqvi, K.H. Temuri, S.M. Nurulain, Toxicity and effect of neem fractions (RBU-9, RB-B and Margosan-O) on phophatases and protein pattern of *Culex fatigans* (K.U. strain), Pak. J. Pharm. 12(2) (1995) 49–52.
[36] K. Suganthi, S. Bragadeswaran, N. Sri Kummaram, Biological and pharmacological activities of jelly fish *Crambionella stuhalmanni* (Chun, 1896) and *Chrysaora quinquecirrha* (Deror, 1848), Int. J. Pharma. Sci. 3 (2011) 230–236.
[37] U.V. Mane, P.N. Gurav, A.M. Deshmukh, S.P. Govindwar, Degradation of textile dye reactive navy—blue Rx (Reactive blue–59) by an isolated Actinomycete *Streptomyces krainskii* SUK—5, Mayaysian J. Microbiol. 4(2) (2008) 1–5.
[38] R.G. Saratale, G.D. Saratale, J.S. Chang, S.P. Govindwar, Decolorization and biodegradation of reactive dyes and dye wastewater by a developed bacterial consortium, Biodegradation 21 (2010) 999–1015.
[39] N. Junnarkar, D.S. Murthy, N. Bhatt, D. Madamwar, Decolorization of diazo dye direct Red 81 by a novel bacterial consortium, World J. Microbiol. Biotechnol. 22 (2006) 163–168.
[40] M.S. Gottlieb, J.K. Carr, J.R. Clarkson, Drinking water and cancer in Louisiana: A retrospective mortality study, Am. J. Epidemiol. 116 (1982) 652–667.
[41] M.D. Saravanamoorthy, B.D. Ranjitha Kumari, Effect of textile waste water on morphophysiology and yield on two varieties of peanut (*Arachis hypogaea* L.), J. Agri. Technol. 3 (2) (2007) 335–343.
[42] T.R. Waghmode, M.B. Kurade, S.P. Govindwar, Time dependent degradation of mixture of structurally different azo and nonazo dyes by using *Galactomyces geotrichum* MTCC 1360, Int. Biodeter. Biodeg. 65 (2011) 479–487.
[43] C.A. Hsu, T.N. Wen, Y.C. Su, Z.B. Jiang, C.W. Chen, L.F. Shyur, Biological degaradation of antraquinone and azo dyes by a novel laccase from *Lentinus* sp., Environ. Sci. Technol. 46(9) (2012) 5109–5114.
[44] A. Pourbabae, A. Ali, F. Malekzadeh, Decolorization of Methyl Orange (model dye) by the newly discovered *Bacillus* sp, Iran. J. Chem. Eng. 24(3) (2006) 41–45.
[45] N. Srivastava, R. Sahai, Effects of distillery wastewater on the performance of *Cicer arientium* L., Environ. Pollut. 43 (1987) 91–102.