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Explicating Bacterial Detoxification of Textile Azo dye Acid Red-88 by Acute Toxicity in *Danio rerio*, Genotoxicity, Cytotoxicity and Phytotoxicity Studies

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A B S T R A C T

The discharge of toxic effluents from various textile industries adversely affects the water resources, soil fertility, aquatic organisms, and ecosystem integrity. Toxic effluents containing azo dyes are discharged from various industries, and they adversely affect water resources, soil fertility, aquatic organisms, and ecosystem integrity. They pose toxicity (lethal effect, genotoxicity, mutagenicity, and carcinogenicity) to plants, aquatic organisms, as well as animals. Considering the potential applications of bioremediation processes in wastewater treatment, the present investigation targets the complete detoxification of Acid Red-88 by acute toxicity in *Danio rerio*, genotoxicity in *Allium cepa*, cytotoxicity in CHO cell lines, and phytotoxicity studies on *Macrotyloma uniflorum* and *Sorghum vulgare*. The study further emphasizes the urgent need for sustainable treatment strategies to minimize the ecological footprint of industrial effluents. It also highlights the role of biological indicators in assessing environmental safety. Moreover, the findings are expected to contribute towards developing eco-friendly methods for azo dye degradation. Ultimately, this research aims to provide a scientific basis for integrating bioremediation into large-scale wastewater management systems.

Keywords

Danio rerio, Allium cepa, CHO cell lines, phytotoxicity studies

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Introduction

The Indian textile industry has been enduring a hasty transformation and is in the process of integrating with the World textile trade and industry. The influence of textile industries to the Indian economy is demonstrated in terms of its involvement to the industrial production, employment generation and foreign exchange earnings. It endorses 20 % of industrial production, 18 % of employment, 9 % of excise collections in the industrial subdivision, nearly 20 % to the country's total export incomes and 4 % to the gross inland product (Hemapriya and Vijayanand, 2013). The first synthetic dye, Mauevin was manufactured in the year 1856, since then, more than 1,00,000 new synthetic dyes have been generated. These dyes were used in different industries, with a yearly consumption of about 0.7 million tons worldwide (Saratale *et al*., 2006).

Environmental pollution by textile effluents has been

recognized as one of the major problems of the modern world. The increasing demand for water and the dwindling supply has made the treatment and reuse of industrial effluents as an attractive option. Textile effluents are of global concern because they color the drains and ultimately the receiving water bodies (Barathi *et al.,* 2020a and Barathi *et al.,* 2020b). Toxicants generated from textile industry effluent get into aquatic organisms, pass through the food chain and ultimately reach humans, leading to various physiological disorders like hypertension, sporadic fever, renal damage, cramps, etc., Bioaccumulation of toxicants depends on the availability and persistence of the contaminants in water, food and physico-chemical properties of the toxicants (Puvaneswari *et al.,* 2006).

Various methods have been established for the removal of artificial dyes from waters and wastewaters to decline their impact on the environment (Bavani *et al.,* 2021; Aulprakash *et al.,* 2022). Bioremediation of textile effluents has been of substantial significance since it is low-priced, eco-friendly and produces a reduced amount of mud. The efficacy of bacteriological decolorization be contingent on the adaptableness and the commotion of selected microorganisms including bacteria, actinomycetes, fungi, yeasts and algae accomplished of degrading azo dyes (Hemapriya *et al.,* 2010; Vijayanand and Hemapriya, 2013; Vijayanand *et al.,* 2017; Barathi *et al.,* 2020a; Das *et al*., 2024a; Das *et al*., 2024b). In view of the advantages and probable applications of bioremediation processes in effluent treatment, the present study targets the comprehensive detoxification of Acid Red-88, by phytotoxicity and cytotoxicity studies.

Materials and Methods

Bioremediation of Acid Red-88

The effluent samples were serially diluted and incubated over basal nutrient agar medium containing 50 ppm of Acid Red-88 at 37 °C for 5 days. Colonies surrounded by halo (decolorized) zones were picked and streaked on nutrient agar plates containing azo dyes. Different colonies of dye decolorizing bacteria were picked and restreaked several times to obtain pure cultures.

Decolorization extent of the isolates were determined by measuring the absorbance of the culture supernatant using UV-visible spectrophotometer (Hitachi U 2800), according to Hemapriya *et al.,* (2010). Following visible decolorization, the sample is subjected for toxicity analysis to ensure complete detoxification of Acid Red-88.

Detoxification Assays

Detoxification of the decolorized Acid Red-88 was monitored by the following detoxification assays

Acute Toxicity in Fish samples

(a) Experimental Fish - Zebra fish (*Danio rerio*)

The zebra fish (*Danio rerio*) used in this study is a fresh - water fish belonging to the minnow Family (Cyprinidae) of the Order Cypriniformes. The healthy and active specimens of *Danio rerio* were initially treated with 0.2 % $KMNO_4$ solution to get rid of any dermal infection. After disinfection, they were acclimatized to lab conditions in the aquarium provided with dechlorinated tap water, aerators, and filters for a week.

(b) Toxicity Assay

Fish fingerlings were divided into 2 groups viz control group in which fish fingerlings were grown in water containing Acid Red-88 dye solution (100 ppm in 500 ml) and the test group in which fishes were grown in water containing treated dye sample. After 48 h incubation fishes were sacrificed and the skin samples were subjected for histopathological studies. The skin samples were fixed in 4 % paraformaldehyde in 0.1 M phosphate-buffered solution (pH 7.4) at 48 ºC, dehydrated in ethanol, and embedded in paraplast. The histological sections (5 mm thick) were cut with a rotary automatic microtome and sections were mounted on glass slides. Finally, the slides were stained with hematoxylin /eosin to visualize typical morphological features.

Genotoxicity in Plants (*Allium cepa*)

Medium-sized *Allium cepa* was acquired from the local market in Tiruvannamalai, Tamilnadu. 3 sets of Fresh and healthy onion bulbs were selected and its base was suspended in a 50 ml beaker consisting of (i) 200 ppm Acid Red-88 dye in 25 ml of distilled water (negative control); (ii) 25 ml of treated (decolorized) dye solution (test); and (iii) 25 ml of distilled water alone (Positive control) respectively and was incubated for 12 days in the dark. At the end of the exposure period, about 5 root

tips (minimum 10 mm length) per onion were cut using forceps and placed into a petridish containing 2 ml acetic acid and hydrochloric acid solution. The root tips were then heated for 5 min at 50 ºC. The heated root tips were placed on a petridish containing safranin solution for 5-10 min. Thereafter, the stained root tips were placed on glass slides, squashed with a coverslip by pressing slightly with the thumb, and viewed under a microscope.

Cytotoxicity in Chinese Hamster Ovary (CHO) Cells

(a) Cell line and culture

Chinese Hamster Ovary (CHO) cell line selected for the study was acquired from the National centre for cell sciences, Pune (NCCS). The cells were maintained for its viability in DMEM media supplemented with 10 % Foetal Bovine Serum, penicillin (100 µ/ml), and streptomycin (100 µg / ml) in a humidified atmosphere of 50 µg / ml CO2 and incubated at 37 °C.

(b) In Vitro assay for Cytotoxic Activity (MTT Assay)

CHO Cells (1 x 10^5/well) were plated in 24 well plates and incubated at 37 °C with 5 % CO_2 condition. At once the cell reaches the confluence; various concentrations of the samples (were added and incubated for 24 h. After incubation, the sample was removed from the well and washed with phosphate-buffered saline (pH 7.4) or DMEM without serum. 100 µl / well (5 mg/ml) of 0.5 % 3- (4, 5- dimethyl-2-thiazolyl) -2, 5-diphenyl-tetrazolium bromide (MTT) was added and incubated for 4 h. After incubation, 1 ml of DMSO was added to all the wells. Then, 50 µl Acid Black 1 dye was added to the test wells, and 50 µl media was added to the positive control wells and incubated overnight. After incubation, the absorbance at 570 nm was measured with UV-Spectrometer using DMSO as the blank. Measurements were performed and the concentration required for a 50 % inhibition (IC_{50}) was determined graphically.

% Cell viability = A_{570} of treated cells / A_{570} of treated cells /A_{570} of control cells x 100

Phytotoxicity studies on Macrotyloma uniflorum and Sorghum vulgare

Phytotoxicity tests were performed in order to assess the toxicity of the untreated and treated dye samples. The ethyl acetate extracted products of degraded azo dyes were dried and dissolved in 5 ml sterile distilled water to make a final concentration of 100 ppm. Phytotoxicity tests were carried out on *Macrotyloma uniflorum* and *Sorghum vulgare.* 10 healthy plant seeds were treated separately with 5 ml of control dye and degraded products respectively/per day. Control sets were carried out using distilled water at the same time. Germination percentage as well as the length of plumule and radical was recorded after 7 days (Saratale *et al*., 2009; Shyamala *et al*., 2014).

Results and Discussion

Histopathological studies of fish skin (*Danio rerio*)

The histopathology of skin samples revealed that in the control group (treated with decolorized Acid Red-88), exhibited the epithelial shifting, enlargement and swelling, accompanied with haemorrhagic primary lamellae, whereas in the test group (treated with Acid Red-88) the cells possessed a normal central axis, primary and secondary lamellae with protuberant squamous epithelium. Projecting stratified epithelial cells have also been witnessed (Fig 1).

Genotoxicity, in *Allium cepa* (Onion bulbs)

Genotoxicity study of Acid Red-88 solution (control) and treated Acid Red-88 solution (Experimental) was carried out in *Allium cepa* onion bulbs. The results displayed pigmentation in control plant grown in the presence of Acid Red-88 solution whereas the *Allium cepa* grown in the presence of treated dye solution (Experimental) were found to be normal without any pigmentation. In the control sample, the epidermal layer was entirely liquefied with a damaged nucleus. Giant cells presented multiple aberrations with cellular disintegration, cellular lesion, and cellular breakage, whereas in the test sample, the epidermal layer was evidently visible with the appropriate nucleus (Fig 2).

Cytotoxicity in a cell line (MCF-7 cell line)

MCF-7 cells treated with control dye sample displayed misrepresentation of cells and decrease in cell viability equated to those treated with treated Metanil Orange solution (Experimental). MCF-7 cells exposed to treated dye solution exhibited no substantial damage to the cells.

MTT assay on MCF-7 cell lines revealed that in the control group, the cell viability diminished with an upsurge in concentration whereas, in the test sample, the cell viability was steady even in increasing concentrations of treated dye solution (Table 1 & Fig 3). Similar results were reported by Das *et al*., (2024b).

Phytotoxicity study

(a) Phytotoxicity study on Macrotyloma uniflorum

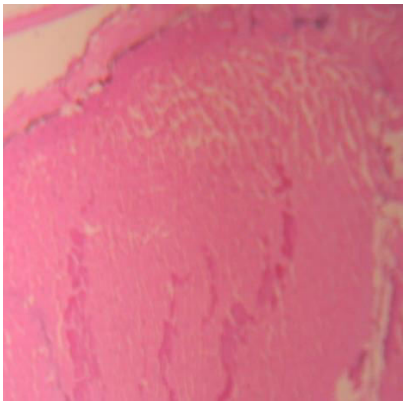
Macrotyloma uniflorum seeds treated with tap water showed 100 % germination, the mean plumule length of 19 ± 0.5 cm and the mean radical length of 7 ± 0.6 cm. *M. uniflorum* seeds treated with control sample (untreated dye) showed 60 % germination, the mean plumule length of 10 ± 0.4 cm and the mean radical length of 4 ± 0.2 cm. Interestingly, *M. uniflorum* seeds treated with test sample (treated dye) showed 100 % germination, the mean plumule length of 18 ± 0.5 cm and the mean radical length of 5.5 ± 0. 2 cm (Table 2).

(b) Phytotoxicity study on Sorghum vulgare

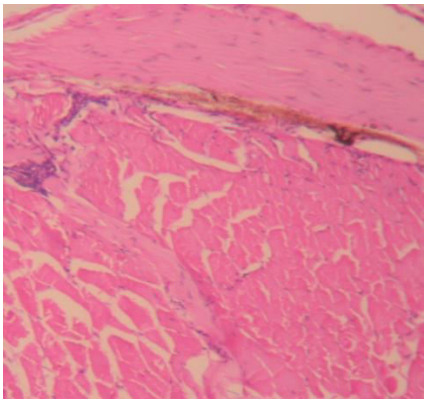
Sorghum vulgare seeds treated with tap water showed 100 % germination, the mean plumule length of 18 ± 0.7 cm and the mean radical length of 6 ± 0.5 cm. *Sorghum vulgare* seeds treated with control sample (untreated dye) showed 80 % germination, the mean plumule length of 7± 0.4 cm and the mean radical length of 3 ± 0.2 cm. Interestingly, *Sorghum vulgare* seeds treated with test sample (treated dye) showed 100 % germination, the mean plumule length of 17 ± 0.5 cm and the mean radical length of 5.0 ± 0. 4 cm (Table 3).

The above-mentioned results clearly demonstrated the detoxification of Acid Red 88. Similar results were recorded by many researchers (Saratale *et al*., 2009; Shyamala *et al*., 2014).

Fig. 1 Histopathology of the Fish skin section



Test Fish



(b) Control Fish

Fig. 2 Microscopic view of onion root tips grown in a) Water containing dye b) Water containing treated dye c) water alone

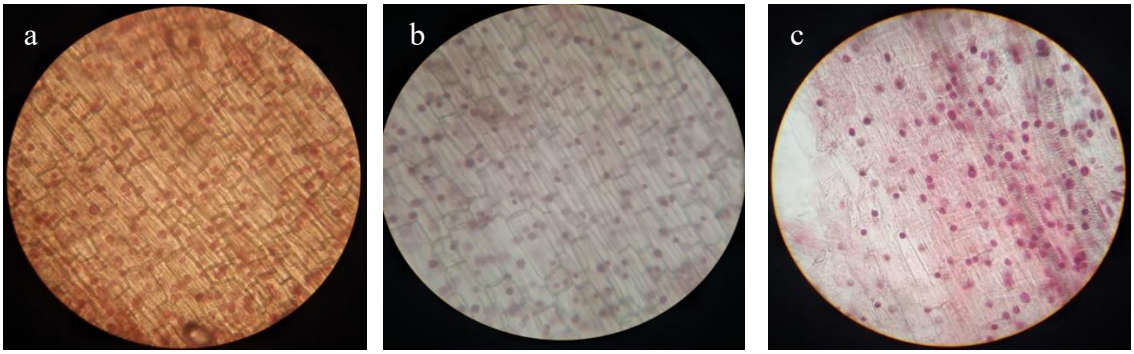


Table.1 Cytotoxicity Assay on CHO Cells

S. No	Dye Concentration (µg/ µl)	Cell Viability (%)	
		Treated Dye	Untreated Dye
1.	20	90	78
2.	40	90	54
3.	60	90	32
4.	80	90	12
5.	100	90	08

Table 2 Phytotoxicity Study of Acid Red-88 and its Degradation Products on *Macrotyloma uniflorum*

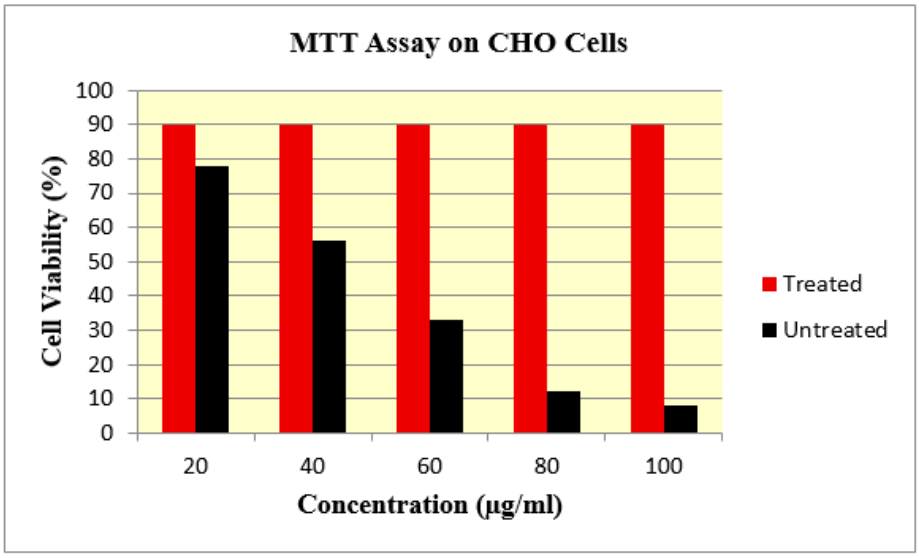
Sl. No	Parameters Studied	Tap Water	Acid Red-88 (100 ppm)	Treated sample (100 ppm)
1	Germination (%)	100	60	100
2	Plumule (cm)	19 ± 0.5	10 ± 0.4	18 ± 0.5
3	Radical (cm)	7 ± 0.6	4.0 ± 0.2	5.5 ± 0.2

Table.3 Phytotoxicity Study of Acid Red-88 and its Degradation Products on *Sorghum vulgare*

Sl. No	Parameters Studied	Tap Water	Acid Red-88 (100 ppm)	Treated sample (100 ppm)
1	Germination (%)	100	80	100
2	Plumule (cm)	18 ± 0.7	7 ± 0.4	17 ± 0.5
3	Radical (cm)	6 ± 0. 5	3 ± 0.2	5 ± 0.4

Each value is an average of three parallel replicates. ± indicates standard deviation among the replicates

Fig.3 Cytotoxicity Assay on CHO Cells using MTT Assay



Author Contributions

M. Thanigaimalai: Investigation, formal analysis, writing—original draft. R. Elaiyaraja: Validation, methodology, writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

References

Arulprakash, A., K. Sathishkumar, M. S. AlSalhi, S. Devanesan, P. Mani, S. Kamala Kanan, S. Vijayanand, A. Rajasekar (2022). Integrated approach of photo-assisted electrochemical oxidation and sequential biodegradation of textile effluent. Environmental Pollution. 307 (15), 119412. https://doi.org/10.1016/j.envpol.2022.119412.

Aswinkumar, S., N. Arunagirinathan, S. Vijayanand, J. Hemapriya and Indra, V. (2017). Bioremediation and Detoxification of a Textile Azo Dye-Evans Blue by Bacterial Strain AKIP2. Int. J. Curr. Microbiol. App. Sci. 6(5): 2687 - 2694. http://dx.doi.org/10.20546/ijcmas.2017.604.313.

Barathi, A., J. Hemapriya, Ramya Gunasekaran, Kayeen Vadakkan, A. Shyamala, Aswini Ravi and Vijayanand, S. (2020a). Bioremediation of Textile and Tannery Effluents – An Overview. Int. J. Curr. Microbiol. App. Sci. 9(11): 3782- 3790. https://doi.org/10.20546/ijcmas.2020.911.454.

Barathi, A., J. Hemapriya, Ramya Gunasekaran, Kayeenvadakkan, A. Shyamala, Aswini Ravi and Vijayanand, S. (2020b). Biodegradation and Detoxification of Congo Red by Intuitive Bacterial Strain TVU-CR4. Int. J. Curr. Microbiol. App. Sci. 9(11): 3772-3781. https://doi.org/10.20546/ijcmas.2020.911.453.

Bavani, T., J. Madhavan, S. Prasad, M. S. AlSalhi, M. A L Jaffreh and S. Vijayanand. (2021). Fabrication of novel AgVO3/BiOI nanocomposite photocatalyst with photoelectrochemical activity towards the degradation of Rhodamine B under visible light irradiation. Environmental Research 200 (2021) 111365. https://doi.org/10.1016/j.envres.2021.111365.

Das, H.S., Reshma Girirajan, Rajalakshmi Balaji, S. Vijayanand and Hemapriya, J. 2024. Sustainable Approach for the Bioremediation of Textile Azo Dye – Metanil Orange by Textile Effluent Adapted Bacterial Strain JHP-1. *Int.J.Curr.Microbiol.App.Sci.* 13(6): 278-285. https://doi.org/10.20546/ijcmas.2024.1306.030.

Hemapriya, J and S. Vijayanand. (2013). Bioremediation of Structurally different textile dyes by a novel bacterial consortium. *Int.J.Curr.Microbiol. Appl.Sci.,* 2(11):212- 226.

Hemapriya, J., Rajesh Kannan and S. Vijayanand. (2010). Bacterial decolorization of textile azo dye Direct Red-28 under aerobic conditions. J. Pure Appl. Microbiol., 4(1):309-314.

Hemapriya, J., Rajesh Kannan and S.Vijayanand. (2010). Bacterial decolorization of textile azo dye Direct Red-28 under aerobic conditions. J.Pure Appl.Microbiol., 4(1):309-314.

Puvaneswari, N., J. Muthukrishnan and P. Gunasekaran. (2006). Toxicity assessment and microbial degradation of azo dyes. Ind. J. Exp. Biol., 44:618-626.

Saratale, G.D., S.D.Kalme and S.P.Govindwar. (2006). Decolorization of textile dyes by *Aspergillus ochraceus* (NCIM-1146). Ind. J. Biotechnol., 5:407- 410.

Saratale, R. G., G. D.Saratale, D. C. Kalyani, J. S. Changand S. P. Govindwar. (2009). Enhanced decolorization and biodegradation of textile azo dye Scarlet R by using developed microbial consortium GR. Bioresour. Technol., 100: 2493-2500.https://doi.org/10.1016/j.biortech.2008.12.013

Shyamala, A., J. Hemapriya, Kayeen Vadakkan and S.Vijayanand. (2014). Bioremediation of Methyl Orange, a Synthetic azo dye by a Halotolerant Bacterial strain. Int. J. Current Research and Academic Review. 2(8):373- 381.

Vijayanand, S. and J. Hemapriya. (2013). Bacterial

bioremediation of textile azo dyes A Review. Ind. J. Appl. Res., 3(12): 480-482.

Vijayanand, S., Kayeen Vadakkan and Hemapriya, J. (2017). Ecofriendly Bioremediation of Acid Orange - An Electron Deficient Xenobiotic Chromogen by Haloalkaliphilic Bacterial Consortium TVU- AO 64. *Int.J.Curr.Microbiol.App.Sci.,* 6(4): 956- 963. http://dx.doi.org/10.20546/ijcmas.2017.601.113.

Das, H.S., Reshma Girirajan, Rajalakshmi Balaji, S. Vijayanand and Hemapriya, J. 2024. Sustainable Approach for the Bioremediation of Textile Azo Dye – Metanil Orange by Textile Effluent Adapted Bacterial Strain JHP-1. *Int.J.Curr.Microbiol.App.Sci.* 13(6): 278-285. doi: https://doi.org/10.20546/ijcmas.2024.1306.030

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