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Toxicological Communication

On the Phytotoxicity of Waste-Water from Textile Industry on Selected Crops Seed Germination and its Treatment Using Bacteria and Zinc Oxide Nanoparticles

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ABSTRACT

Industrialization plays a major role for the economic development of any nation. In spite of various positive aspects of industrialization, the foremost negative aspect is pollution by discharge of more waste water in to the environment. So, the aim of the present work is to analyse the phytotoxic effect of textile industry waste water on seed germination and treating the waste water using Bacterial Species and Zinc Oxide Nanoparticle. Physico-chemical parameters of dying industry waste water such as color, temperature, pH, conductivity, turbidity, total dissolved solid, total hardness, COD, BOD, oil and greases, chloride, sodium, potassium, chromium, copper, total alkalinity and zinc were analysed as per standard methods. To confirm the harmfulness, an investigation was made to study the degree of toxicity of dyeing industry effluent on seed germination and growth of *Sorghum bicolor* (white sorghum) *Vigna unguiculata* (cow pea) were selected for this study and it was placed in soil containing pots and watering with untreated industry waste water and treated waste water. The waste water was treated by two methods, one by bacterial degradation, using *Pseudomonas fluroscence* and the secondly, by chemical oxidation, using zinc oxide nanoparticles. After treatment their efficiency was tested with above two plants. All the above said parameters were found to be high in untreated waste water. There was a gradual decrease in the percentage of seed germination and seedling growth due to higher concentration of effluent, when compared with control. In this comparative study, dye degradation(methyl orange) degradation by the chemical oxidation using zinc oxide nanoparticle was more effective and faster than the biological oxidation of bacterial species.

KEY WORDS: DYE DEGRADATION, INDUSTRIALIZATION, MICROORGANISM, NANOPARTICLE, PHYTOTOXICITY.

INTRODUCTION

Water contamination is a current environmental concern that has caught a lot of attention throughout the world. Textile dyeing and finishing industry effluents are the major source of water contamination. The textile industry faces a challenge in using large quantities of dyes and auxiliaries that are required for current textile processes due to substantial changes in client demands (Mamatha and Beena 2018). Factories discharged a wide range of pollutants, many of which contain chemical elements in higher concentrations to harm the surface and ground water supplies (Khan and Malik 2018; Bharagava et al. 2020).

The waste water treatment system in Indian companies was substantially established to fulfil the waste water discharge regulations, but only 10% of the waste water is now treated, and the rest of the untreated water discharged into neighbouring water bodies. The major industrial pollutants are industrial effluents, which contain organic and inorganic chemicals, acids, alkalies, suspended particles, and other recalcitrant compounds (Mehta and Bhardwaj 2012). However, dumping of untreated effluents into the environment affects the ecological niches of living organisms (Bharagava et al. 2020). Municipalities from government sector, even private entities face a serious of difficulty with wastewater disposal, particularly in large urban areas with limited space for land-based treatment and disposal. But still, they are taking corrective steps to make safer (Naveenraj et al. 2021).

The textile mills daily discharging millions of litters of untreated effluents loaded with synthetic dyes into public

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wastewater stream that eventually drain into water bodies. This can lead to acute toxicity of aquatic ecosystem. This also impacts water quality by changing the pH and increasing the Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) (Imtiazuddin and Tiki 2020). Natural dyes come from a variety of sources, including vegetables, plants, minerals, insects, and animals. But synthetic dyes are made from organic molecules; they may be produced in a consistent manner. These dyes will linger in the environment for a long time if they are not properly treated (Olukanni et al. 2006). Among other dyes, Azo dyes are the greatest group of colorants in terms of number and production volume, accounting for 60-70 percent of all organic dyes produced worldwide (Carliell et al. 1998; Bafana et al. 2011; Imtiazuddin and Tiki 2020).

The success of azo dyes is owing to their ease and low cost of synthesis when compared to natural dyes, as well as their structural variety, high molar extinction coefficient, medium to high light and moisture fastness qualities. They're employed in a variety of industries, including textiles, pharmaceuticals, and cosmetics, as well as food, paper, leather and paints industries (Chung et al. 1992; Mansour et al. 2007; Seesuriyachan et al. 2007). Azo dyes have an azo bond (–N=N–) in their basic structure, which is difficult to breakdown naturally. Depending on the number of azo groups, this class of dye is divided into mono azo, di azo, tris azo, and poly azo dyes (Engel et al. 2008). Reactive azo dye is resistant due to their stability, xenobiotic nature, and it is not completely destroyed by standard treatments such as light, chemicals, or activated sludge. (Grekova-Vasileva et al. 2009; Imtiazuddin and Tiki 2020). Hence in the present study focused on treatment of textile industry waste water containing Azo dyes by using bacteria and Nanoparticles.

Waste water, on the other hand, is a resource that can be used for productive purposes because it includes nutrients that can be used in agriculture and aquaculture as well. As a result, wastewater can be viewed as a resource on one hand and a problem on the other. The use of industrial and domestic sewage effluents for irrigation has recently become a popular method of repurposing waste water, owing to the availability of significant amounts of N, P, K, and Ca, as well as other critical nutrients (Niroula 2003, Ali et al 2020).

Its repurposing could have a positive impact on the rural community. Another advantage of reusing wastewater for irrigation is water conservation; due to water shortage, industrial effluents are employed as a source of irrigation for crops; however, indiscriminate effluent uses, ignores the fact that untreated effluent can have severe effects on crop development and quality. Therefore, it is necessary to study the impact of these effluents on crop system before they are recommended and used for irrigation (Hari et al. 1994; Thamizhiniyan et al. 2009). The present investigation has been carried out to study the degree of toxicity of dyeing industry effluent on seed germination and development of *Sorghum bicolor* (white sorghum) *Vigna unguiculata* (cow pea). These crops we are commonly cultivating in agriculture land.

MATERIAL AND METHODS

The effluent samples were collected from dyeing industry in plastic container located at Srivilluputhur, Tamil Nadu (Fig-1 20L water sample). After collection, the effluent was immediately transported to the laboratory for analysis. Physico-chemical parameters such as colour, temperature, pH, conductivity, turbidity, total dissolved solid, total hardness, COD, BOD, copper, chloride; chromium, sodium, total alkalinity, potassium and zinc were analyzed as per the standard methods (APHA 2012).

The zinc oxide nanoparticle was obtained by the precipitation method. In a beaker 7.4g of zinc nitrate was taken in 50ml of water. In another beaker 2.65g of Sodium carbonate was taken in 50ml of water. Then sodium carbonate was added drop by drop to the solution of zinc nitrate. This mixture was stirred nearly 15-20min to get mixed together. Then it was filtered using the Whatman filter paper to filter the precipitate. The precipitate thus formed was washed with the water for 2 to 3 times to remove the minute particles. After washing with water, the precipitate was again washed with ethanol to remove the moisture present. Then it was dried in a hot air oven for 90-100 degree for overnight period of 4-5 hrs. Thus the moisture was fully removed and obtained a powdered substance. Finally powered substance was kept in a muffle furnace at 300°C for 2hrs (Fig -2).

For the characterization of zinc oxide nanoparticles, four main methods were considered.

- FT-IR (Fourier transform infrared spectroscopy)
- XRD (x-ray diffraction)
- SEM (scanning electron microscope)
- UV-DRS (UV-Vis diffuse réflectance spectroscopy)

The methyl orange was degraded by using the zinc oxide nanoparticle. The zinc oxide nanoparticle was taken as a catalyst for the degradation process. 250 ml of textile dyeing waste water was taken into a conical flask with the addition of 0.5g catalyst zinc oxide nanoparticle. This was kept under the sunlight for 24hrs and the stirring done after each 15min which it undergoes with the photo catalytic reaction. The zinc oxide nanoparticle added to the waste water degrades and decolorizes the compounds present in it. Then the absorbance of degraded waste water was measured by using UV-visible spectrophotometer. The degradation of methyl orange was done by bacteria. Bacterial species was cultured (*Pesudomonas fluroscence*) using minimal media. Then the cultured bacteria were added in to the waste water taken in a different concentration as 1, 2, 3, 4,5µl in 5ml. Thus it was kept in an incubator for overnight and the absorbance was measured for initial, 3rd and 6th day concentration. From the absorbance efficiency of degradation was noted.

To study the effect of untreated and treated waste water on seed germination and growth, the healthy and uniform seeds of *Sorghum bicolor* (White sorghum) and *Vigna unguiculata* (cow pea) were selected and surface sterilization done with 0.1% Hgcl2 and thoroughly washed with distilled water to avoid surface contamination. Germination experiments were carried out (Fig-3) in two set of soil containing pots (one pot for white sorghum and other pot for cow pea).

One set irrigated with untreated waste water and another set of pots irrigated with treated industrial waste water. The germination was observed and recorded after 48hours.

RESULTS AND DISCUSSION

It is important to study all features of the textile effluent to improve environmental performance and also to sustain considerable quality of the individual companies. Below are the images of nanoparticles collected with the help of precipitation method and seed germination done to initiate the experiment.

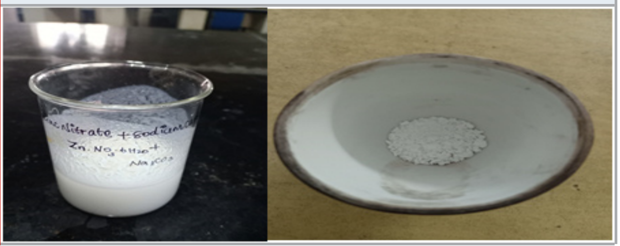
The Physico- Chemical parameters of textile effluent have been analysed and the experimental results were compared with standard BIS limits. The results of the analysis are presented in table -1.

Effect of effluents on seed germination: Experiment on *Sorghum bicolor* and Vigna unguiculata showed (Fig -4 a, b) an inhibitory effect of untreated industrial effluents on seed germination and its early growth.

Figure 1: Waste water collected from industry

Waste water treated with Zinc oxide nanoparticle (Fig -5a, b) and bacterial species (Fig -6a, b) used for irrigating the seed containing pots (*Sorghum bicolor* and *Vigna unguiculata*).

Figure 2: Nanoparticle obtained from precipitation method



The colour of the effluent was key issue in textile industry, and it was widely recognized as a important pollutant in wastewater. In fact, even at low concentrations, colour in effluent is easily visible to human eyes, and no one likes the appearance of unclean water (Ibrahim et al. 1996; Wijetunga et al. 2010). The effluent selected for the present study was very dark brown to black color, with observed value 70 hazen, it was higher than value of BIS (25 hazen). This value indicates that the effluent was highly colored due to presence of different dyes, colour producing compounds, metals, pH of the effluent, temperature during dyeing and bleaching of fabrics. Color reduces aquatic life's photosynthetic activity and has an impact on other metrics such as DO, BOD and others. In general, traditional treatment methods have a hard time to removing colours (Forgacs et al. 2004; Przystaś et al. 2012; Hubbe et al. 2012; Imtiazuddin and Tiki 2020).

Figure 3: Seed germination study

Table 1. The Physico-chemical parameters of dyeing industry effluent

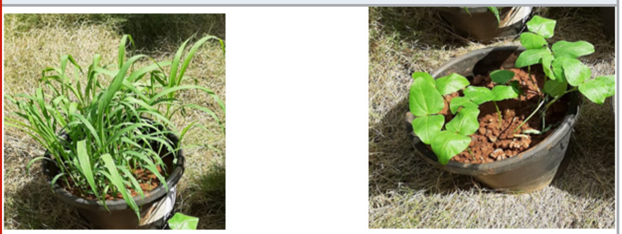
Parameters	Observed value	BIS Value
Colour , Hazen	70	25
Temperature	29.1	50
pH@25°C	6.75	5.5-9
Conductivity, mS/cm	2822	300
Turbidity, NTU	266	10
Total dissolved solid , mg/ L	3404	2,100
Total hardness , mg/L as $CaC0_3$	188	500
Chemical oxygen demand, mg/L	300	250
Biological oxygen demand, mg/L	128	100
Oil and greaces, mg/L	16	10
Chloride , mg/L as Cl	616	600
Sodium, mg/L	234	200
Potassium, mg/L	43.8	12
Chromium , µg/L	BDL(DL1:0.5)	100
Copper , µg/L	0.047mg/L as cu	3000
Total alkalinity	138 mg/L as $CaCO_3$	
P-Alkalinity	nil mg /l as $CaCo_3$	
Zinc, µg/L	BDL(DL1:0.02) mg/l as Zn	5000

The waste water temperature observed to be 29.1°C. The acidic and basic nature of the effluent can be identified by pH value and also it determines the presence or absence of various ionic species of the textile effluent. The pH value of the dyeing industry effluent was found to be little acidic pH 6.75 but it was within the permissible limit prescribed by BIS (pH 5.5-9). Although no health-based guideline was proposed for pH but sometimes, eye irritation and other skin disorders are associated with values of pH greater than 11, but in our result found within the BIS permissible limit. Joshi and Kumar (2011) observed the pH of textile effluent ranged from 7.6-7.9 whereas another study reported the pH of dye industry effluent ranged between 8.2 and 9.0 (Joshi and Kumar 2011; Ahmad et al. 2012; Farid et al. 2012; Imtiazuddin and Tiki 2020).

Figure 4: Seeds containing pots irrigated with untreated waste water

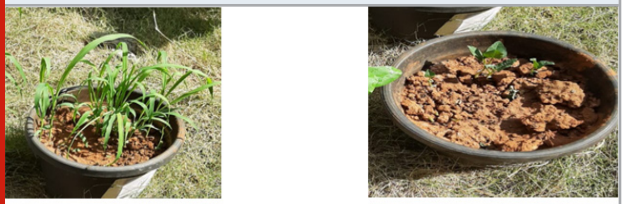
a) White sorghum b) cow pea

Figure 5: Seed containing pot irrigated with Zinc oxide nanoparticle treated waste water



a) White sorghum b) cow pea

Figure 6: Plants irrigated with *Pseudomonas fluorescens* treated waste water



a) White sorghum b) cow pea

Electrical Conductivity is an important physical parameter to measure the sodium hazard of water quality. The Electrical Conductivity found in the effluent (2822 mS/cm) was greater than that of the permissible limit of BIS (300 mS/cm) this may be due to the continuous discharge of the chemicals and salts used along with dyes in the industries. Our results in accordance with Saravanan et al. (2012) they reported that electrical conductivity of tannery industry effluent was above the accepted limits (Saravanan et al. 2012). Turbidity is to measure the degree, in which the water loses its transparency due to the presence of suspended particulates. High intensity of scattered lights it means that higher turbidity. The value of turbidity was recorded as 266 NTU which have been found to be much higher than the BIS limit (10 NTU). This may be due to more colour, more total suspended solids and oily scum incorporate with colloidal matter increases the turbidity which gives bad appearance and foul smell. Thus, turbidity should be measured and treated carefully before disposal (Elango 2017; World Health Organization 2017; Bharagava et al. 2020).

Inorganic salts (bicarbonates, chlorides, and sulphates of calcium, magnesium, potassium and sodium) and minor amount of soluble organic matter make up Total Dissolved Solids (TDS). As a result, TDS is equal to the total number of cations and anions in water. Although TDS is not generally regarded a main pollutant, it is utilised as an indicator of aesthetic features of drinking water as well as an aggregate signal of the presence of a wide range of chemical pollutants. The value of TDS ranges from 2320 to 9840, indicating the presence of high amount of inorganic salts. From this study we observed the TDS was about 3404 mg/L, it was higher than the BIS value (2100mg/L) (Islam and Guha 2013). The hardness of the analysed water sample was 188 mg/L as CaC03. The maximum limit of total hardness recommended by WHO and American Public Health Association (APHA) are 500 and 250 mg/L respectively. Samples evaluated possess hardness within the permissible limits according to APHA and WHO. Low hardness value may be due to water softening steps carried out in textile industries. Our findings are similar to study report published in the past. (Prasad 2011; Kumari et al. 2011; Joshi and Shrivastava 2012; Bharagava et al. 2020).

Chemical Oxygen Demand recorded in the effluent was 300 mgl^{-1} exceeded the permissible limit prescribed by BIS (250 mgl^{-1}). Saravanan et al. (2012) recorded that chemical oxygen demand of tannery industry effluent was above the acceptable limits. Increases in COD can be due to huge amount of industrial wastes such as detergents, softeners, non-biodegradable dyeing chemicals, formaldehyde based dye fixing agents etc. Higher concentration of COD in water implies toxic conditions and the presence of biologically resistant organic substances (Saravanan et al. 2012; Elango 2017; Bharagava et al. 2020).

The excess BOD due to the presence of more organic contaminants of textile effluents in to a water body. The low or nil BOD shows good quality water, whereas a high BOD indicates the water is highly contaminated and not suggested for drinking purposes. The experimental data of present work shows 128 mg/L BOD. It was found to be higher than the permissible limit of BIS. Increased level of BOD leads to microbial oxygen demand causes reducing DO which may induce hypoxia conditions with subsequent adverse effects on aquatic biota (Goel 1997). Oil and grease value was recorded as 16 mg/L which was quite higher than BIS limits (10 mg/L). Presence of oil and grease in water bodies leads to the formation of oil layer, which causes significant environmental problems like it prevent the transfer of oxygen from atmosphere to water medium as a result reduces the amount of dissolved oxygen (DO) at the bottom of the water bodies (Central Pollution Control Board 1990; Bharagava et al. 2020).

In the present study the amount of chloride was recorded as 616mg/L as Cl which exceeds the BIS limits (600 mg/L). Mainly high chloride content in water destroys the microorganisms which are necessary to maintain food chains in aquatic life and it was found to be favour the EC, TDS, TSS, alkalinity and sulphate (United States Environmental Protection Agency 1999). The studies of Sajani and Muthukkaruppan (2011) revealed that sugar mills effluent contains high amount of chloride (United States Environmental Protection Agency 1999; Samuel and Muthukkaruppan 2011). Sodium recorded in the effluent was 234 mgl¯1, this level was higher than the value set by BIS is 200 mgl¯. Potassium content recorded in the effluent was 43.8 mg/l found to be higher than the permissible limit of BIS (12 mgl¯1). But other compounds like copper, chromium, alkalinity and zinc levels are found to be trace value (BDL). Our results are accordance with Falah and Hussein (2013) who observed that no significant changes were observed in the concentration of zinc, iron, cadmium, copper, nickel, cobalt and manganese before and after treatment of real and simulated textile industrial wastewater. Moreover, the concentrations of most of these metals were very low or non-detective (Falah Hussein 2013; Naveenraj et al. 2021).

Effect of effluents on seed germination: Experiment on *Sorghum bicolor* and *Vigna unguiculata* showed (Fig -4 a, b) an inhibitory effect of untreated industrial effluents on seed germination and its early growth. Inhibition of seed germination may be due to greater number of dissolved solids that increases the salinity and conductivity of the absorbed solute by seed before germination moreover higher salt content also changes the osmotic potential outside the seed thereby reducing the amount of water absorbed by the seed which results in retardation of seed germination (Mehta and Bhardwaj 2012; Naveenraj et al. 2021).

Similar result reported by David Noel and Rajan (2015), was made investigation to study the degree of toxicity of dyeing industry effluent on seed germination and early growth of Lady's finger. Germination experiments were carried out in sterilized petri dishes containing 25, 50, 75 and 100% concentrations of untreated dyeing industry effluent. The germination percentage, growth parameters like plumule and radicle length, relative toxicity, percentage of phyto toxicity and tolerance index on the seed germination in response to dyeing effluent at various concentrations were also calculated. There was a gradual decrease in the percentage of seed germination and seedling growth with higher concentration of effluent. Suppression of germination at higher concentrations of effluent may be due to high levels of total dissolved solids which enhance the salinity and conductivity of the solute absorbed by the seeds before germination (Lav and Jyoti 2012; David Noel and Rajan 2015; Naveenraj et al. 2021).

Waste water treated with Zinc oxide nanoparticle and bacterial species used for irrigating the seed containing pots (*Sorghum bicolor* and *Vigna unguiculata*). By this comparative study of both processes, the dye degradation by the chemical oxidation of zinc oxide nanoparticle was more effective and faster than the biological oxidation of bacterial species (Fig -5a, b). The zinc oxide nanoparticle has an Advanced Oxidation Process (AOP). The zinc oxide nanoparticle also has a property of photo catalytic activity thus it possesses the higher seed germination and growth, when compared with waste water treated with *Pesudomonas flurosceence* (Fig – 6 a, b). The research finding of Hussein (2013) reported that Zinc oxide was most effective catalyst with all types of dyes the extent of decolourization reaches 100 %. The extent of decolourization was also 100 % when anatase was used as photocatalyst but that need more time than zinc oxide was used (Falah Hussein 2013; Naveenraj et al. 2021).

CONCLUSION

The findings of present study suggest that the physico-chemical parameters were relatively high in dyeing industry effluent and it was toxic to plants, severely affected the seed germination and seedling growth. The waste water treated by chemical oxidation of zinc oxide nanoparticle was more effective and fast than the biological oxidation of a bacterial species in seed germination and growth. The study further concluded that the plant *Sorghum bicolour* was more suitable to grow when compare to *Vulgna ungcuilata*. The future will concentrate on cost analysis of the treatment techniques and also on treatment of complex dyes. In Tamil Nadu, particularly in Tirupur, Erode, Karur, Srivilluputhur and other districts, the textile industries are growing very fast due to its several advantages but on the other hand it is one of the root causes for environmental pollution.

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Conflict of Interests: Authors declare no conflict of interests to disclose.

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