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IMPACTS OF TEXTILE DYE EFFLUENTS ON THE MICROBIAL AND PHYSICOCHEMICAL QUALITIES OF SURROUNDING SOIL IN OSHOGBO METROPOLIS, NIGERIA

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AUTHORS' CONTRIBUTIONS

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ABSTRACT

This study was carried out to investigate the microbiological and physicochemical properties of textile dye effluent contaminated soil collected from selected production discharge units within Oshogbo metropolis using standard techniques. The mean pH of the textile effluents was 8.4. The levels of the other parameters investigated were 96.50 ± 0.02 mg/l, 10.08 ± 0.05 mg/l, 3.65 ± 0.21 mg/l, and 6.35 ± 0.21 mg/l, for total dissolved solids (TDS), total suspended solids (TSS), biochemical oxygen demand (BOD) and chemical oxygen demand (COD) respectively. The level of Chloride (204.20±0.14 mg/l) was significantly high when compared with Sulphate that had 7.21 ± 0.08 mg/l. The order of concentration of heavy metals in the effluents is Fe>Zn>Mn>Ni>Pb>Cd. The total bacterial counts (TBC) were generally higher than that of fungal counterparts (TFC) in the soil samples of study locations. The range for TBC was from 1.45 x 10^6 to 7.20 x 10^6 cfu/ml, while that for TFC was from 1.34 x 10^4 to 5.31 x 10^4 cfu/ml. Theidentified bacterial isolates were *Klebsiella aerogenes, Esherichia coli, Staphylococcus aureus, Pseudomonas* spp., *Proteus* spp., *Streptococcus* sp. and *Micrococcus* sp. and identified fungal isolates were *Mucor, Aspergillus, Penicillium*, *and Neurospora* spp., from the sampling locations. Findings from this study showed that all the chemicals analysed were withinthe standards set by WHO and FEPA, except for Zn and Ni, which were respectively higher one and three of the dye effluent sampling locations. The bacterial and fungal isolates could be consideredas biological pre- and post-treatment agents for cost-effective and eco-friendly remediation of textile effluents to minimize water and soil pollution, thereby, ensuringsustainable environmental and economic development.

Keywords: Textile dye effluents; *Pseudomonas* spp.; biochemical oxygen demand; zinc.

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1. INTRODUCTION

Globally, textile industry has been recognized as one of the major wastewater generating industries because they make use of large volumes of water during textile dyeing. The wastewater contains a mixture of different dyes and additives such as dispersants, levelling agents, acids and alkali that are released into water bodies by textile industries without any adequate effective treatment, causing serious environmental concerns [1,2]. Over the years, over 100,000 different synthetic dyes have been reported to be commercially available with an annual production of over 700,000 metric tons [3,4]. After analysing the composition of chemicals in textile dye effluents, it was reported that about 50% of the applied dye was present in the effluent [5]. The high concentration of dyes in textile industrial wastewater has been reported to be associated with the low absorption properties of fibres to it [6,7]. Synthetic dyes are made of complex compounds that are generally composed of azo, triphenylmethane or heterocyclic/polymeric structures, and based on their stable nature, can last long untransformed or discoloured in the natural ecosystem [3,2]. Hence, wastewater from textile industry could have adverse effect on the receiving water bodies or soil.

In water bodies, the effluent increases the chemical oxygen demand (COD) and biochemical oxygen demand (BOD), which alter the pH and gives the water bodies extreme colourations [1]. Andleeb et al*.* [7] identified coloured wastewater from textile industries as one of the most polluted effluents in almost all industrial sectors. The presence of a little quantity of dyes in water is significantly visible and can impact the physicochemical and photosynthetic activities of aquatic plants [8]. Also, in the water bodies, the dyes may be transformed into hazardous compounds or molecules. For instance, the azo group $(N = N)$ in dyes are converted to aromatic amines which are possible carcinogenic and mutagenic products [9,10]. Some of these chemicals could bio-accumulate and magnify along the food chain with the ultimate effects on humans.

To prevent the environmental effects of dye and other chemicals in the waste water, industries apply physical and chemical techniques such as membrane filtration, coagulation, precipitation, flotation, adsorption, ion exchange, chemical reduction, ultrasonic mineralization, electrolysis and advanced chemical oxidation to remove the dyes [11]. However, physicochemical techniques are met with series of draw backs, such as they are infrastructural intensive, costly and relatively ineffective, generation of toxic metabolites thereby creating disposal problems [3,2]. Currently, attention has turned to the biological option because they are cost-effective and environmentally friendly [11].

According to previous reports, most microorganisms have been found to decolorize and detoxify dyes and other additives in textile wastewater. The role of some bacteria, fungi and algal species for the decolourization and degradation of textile dyes has also been reported $[7,1,3,4,12,10,2]$. Hence, the need to explore more efficient microorganisms with potentials to degrade and decolourize dyes especially in developing countries where there is little or no efficient treatment for the large volume of wastewaters generated on daily production processes. This can be achieved by isolating efficient aerobic degraders for use in decolourization and degradation of textile dye waste effluents. Therefore, the aim of the present study isto evaluate the impact of textile dye effluents on the physicochemical and microbiological quality of the surrounding soil environment at Oshogbo, Osun State.

2. MATERIALS AND METHODS

2.1 Study Location

The study was carried out within Oshogbo, the Capital City of Osun State, South-Western Nigeria. Geographically, Osogbo metropolis lies on latitude $7^{\circ}49'$ N and a longitude $4^{\circ}37'$ E in South western Nigeria (Fig. 1). It covers an area of approximately 14,875 square kilometers with a total population of approximately 3,416, 959 [13].

2.2 Sample Collection

Textile dye effluents were sampled from the main effluent discharge sites within Oshogbo metropolis. The effluent samples were collected at four (A, B, C and D) different discharge pipe with three replicates. The sample containers (500ml sterile reagent bottles) were transported immediately to the laboratory in icepacked bags. Uncontaminated and contaminated soil samples in the vicinity of the dye dumpsites were also collected at two different locations from depths of 0- 15 cm using soil auger. The samples were subjected to immediate physicochemical and microbiological analysis.

2.3 Media Preparation and Sterilization

The major media used included nutrient agar (Oxoid), potato dextrose agar (Oxoid) and mineral salt medium [composition (g/L): $Na₂HPO₄, 3.6$; $KH₂PO₄, 1.0$; $(NH_4)_2SO_4$, 1.0; MgSO₄.7H₂O, 1.0; CaCl₂ .2H₂O, 0.1; Fe (NH_4) and citrate, 0.01]. The media were prepared according to specifications, and sterilized using the autoclave at 121° C for 15 minutes.

2.4 Physicochemical Analysis of Samples

The textile dye solution was analysed for biochemical oxygen demand (BOD), chemical oxygen demand (COD), total dissolved solids (TDS), Total Suspended Solids (TSS), Chlorides and Sulphates, and heavy metals according to the procedure reported earlier [14,15]. The contaminated soil samples were also analysed for heavy metal contents.

2.5 Determination of BOD

Textile dye effluent was taken in two different BOD bottles. One of the bottles was incubated for 3 days in a BOD incubator. To another bottle, 2ml of Manganese sulphate and 2ml of alkaline iodide – azide solution were added for the formation of precipitate. The precipitate was then dissolved with concentrated sulphuric acid. Twenty millilitres of the obtained solution was transferred into a clean conical flask and immediately titrated against 0.0125M sodium thiosulfate solution till the conversion of brown colour to pale straw colour. Starch was added as indicator for the formation of blue colour and titration was continued till the disappearance of formed blue colour. The amount of sodium thiosulfate consumed is noted as dissolved oxygen content of day 0 sample. The same procedure was followed for another bottle after incubation of 3 days. BOD was calculated with the dissolved oxygen of day 0 and day 3 [15].

2.6 Determination of COD

About 50 ml of textile dye effluent was dispensed into 500ml refluxing flask. To this 1g of mercuric sulphate and 5.0 ml of sulphuric acid reagent were added followed by 25 ml of 0.0417M potassium dichromate solution. The flask was attached to a condenser and cooling water was turned on. 70 ml of sulphuric acid reagent was added and the condenser was covered and refluxed for 2 hours. After 2 hours the condenser was cooled and washed. The contents of the flask was diluted to about twice its volume with distilled water and cooled to room temperature. Thereafter the contents were titrated against potassium dichromate with ferrous ammonium sulphate using ferroin as indicator. The end of the titration is the sharp colour change from blue green to reddish brown. A blank containing distilled water instead of the textile dye solution with all other reagents was also titrated against potassium dichromate using ferroin as indicator [15].

2.7 Determination of TSS

The disk with wrinkled side up was fitted into filtration apparatus. The vacuum was applied and the set up was washed with three successive volumes of water. The textile dye effluent was stirred and a measured volume was added to the filter paper placed on glass-fibre filter disk and suction was continued for about 3 minutes. The filter paper was weighed before and after filtration [15].

Fig. 1. Map of Nigeria showing Oshogbo, Osun State

2.8 Determination of TDS

The disk with wrinkled side up was fitted into filtration apparatus. An empty evaporating dish was weighed. The test solution was stirred with magnetic stirrer and a measured volume of test solution was added to the filtration apparatus and suction was applied for about 3 minutes. The filtrate was transferred to pre-weighed evaporating dish and evaporated to dryness on a steam bath. The dish was cooled and weighed [15].

2.9 Determination of Chlorides

To 20 ml of textile dye solution, 1.0 ml of potassium chromate was added and titrated against standard silver nitrate (0.0417M) till the appearance of pinkish yellow colour. A blank was also titrated with standard sodium chloride solution (0.0141M) [15].

2.10 Determination of Sulphates

To 25 ml of textile dye effluent, 20 ml of buffer solution was added and mixed thoroughly. To this, barium chloride crystal was added for the formation of turbidity. The turbidity was measured at 420 nm in a Spectrophotometer. The standard sulphate solution was also added with buffer and barium chloride crystals and turbidity was measured. Turbidity values of standard and textile effluent were compared and the amount of sulphates was calculated [15].

2.11 Determination of Heavy Metals

Heavy metal content of the textile dyes solution was determined by Atomic Absorption Spectrophotometer (AAS) [15].

2.12 Enumeration and Isolation of Total Aerobic Bacteria and Fungi

The collected samples were analysed for the load of bacteria and fungi present in them as described by Aneyo et al. [16]. First, 1 ml of the textile effluent sample was transferred into 9 ml of sterile saline solution in a test tube and shaken vigorously. The solution was serially diluted up to 10^{-6} . Thereafter, 10^{-7} 2 to 10^{-4} dilutions were taken and plated aseptically using the pour plate technique on sterile Petri dishes. An aliquot of the diluted effluent was inoculated on nutrient agar and incubated at 37 ºC for 24 hours. For the fungal count, it was cultured on potato dextrose agar with 10% tartaric acid (antibacterial agent) using the spread plate method as previously described. Microbial count of the effluent samples were reported

as colony forming units per millilitre (cfu/ml).The colonies were purified on fresh nutrient medium by sub-culturing until a pure culture was obtained. Bacterial identification was based on basic Gram staining reactions and other biochemical tests as described previously (Chowdhury, 2015). Identification of the bacteria was based on their cultural, morphological and biochemical reactions with reference to Bergeys Manual of Determinative Bacteriology.Identification of fungi was done according to the method described by Aneyo et al*.* [16]. This was based on the macroscopic colonial appearances of fungi observed in the plates, such as colony texture, size, pigmentation, time of growth, colour on the reverse side of the plate and colony margin. The microscopic appearance was studied using lactophenol cotton blue stain. To achieve this, a drop of lactophenol cotton blue was placed on a grease-free, scratch-free glass slide. A small portion of the fungal growth was picked with a wire loop and teased out using a mounting needle. The preparation was covered with a cover slip.The slide was observed under 10x and 40x objective lenses. Observed characteristics were recorded and compared with the established identification keys as described by Aneyo et al*.* [16].

2.13 Statistical Analysis

All the data generated were analysed using Microsoft Excel Windows 7 using descriptive statistical tools. The means were compared using ANOVA and significance was set at P<0.05.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Physicochemical properties of samples

The mean results of the physicochemical properties of effluents collected from the textile/dye industry discharge sites are given in Table 1. The mean and standard deviation of physicochemical parameters, such as pH, total dissolved solids (TDS), total suspended solids (TSS), biochemical oxygen demand (BOD) and chemical oxygen demand (COD), Chlorides and Sulphates were investigated. The pH of the collected textile effluent samples from selected sites was analysed and the mean was found to be 8.4. The mean levels of the other parameters investigated were 96.50 ± 0.02 mg/l, 10.08 ± 0.05 mg/l, 3.65±0.21mg/l, and 6.35±0.21 for TDS, TSS, BOD and COD respectively. The level of Chloride $(204.20\pm0.14 \text{ mg/l})$ was significantly high when compared with Sulphate that had 7.21±0.08 mg/l.

The results of the heavy metal content of the contaminated soil and dye effluent samples are presented in Table 2. The heavy metals investigated were Cadmium (Cd), Nickel (Ni), Lead (Pb), Zinc (Zn), Manganese (Mn) and Iron (Fe). The order of concentration of heavy metals in the effluents was: Fe>Zn>Mn>Ni>Pb>Cd. The level of concentrations of heavy metals in contaminated soil samples varied differently with respect to the sampling locations. However, similar patterns to the heavy metal contents of the dye effluents samples were observed. Zinc $(114.20 \pm 0.00 - 160.37 \pm 0.09 \text{mg/l})$ was generally the highest, while Cadmium $(0.15 \pm 0.00 - 0.32 \pm 0.01)$ was the least in all locations studied.

3.1.2 Enumeration of total aerobic bacterial and fungal counts

The total microbial counts obtained from analysis of the effluent are shown in Table 3. From the observations, total bacterial counts (TBC) were generally higher than their fungal counterparts (TFC) in all the locations samples. The range for TBC was from 1.45 x 10^6 to 7.20 x 10^6 cfu/ml, while that for TFC was from 1.34 x 10^4 to 5.31 x 10^4 cfu/ml.

3.1.3 Identification of bacterial and fungal isolates

A total of 24 bacterial isolates was recovered from the bacteriological analysis carried out. They included *Klebsiella aerogenes* (n=8), *Esherichia coli* (n=5), *Staphylococcus aureus* (n=3), *Pseudomonas* spp. (n=2), *Proteus* spp. (n=2), *Streptococcus* sp. (n=1), *Micrococcus* sp. (n=1) (Fig. 2). Tables 4 and 5 show the morphological characteristics of fungal isolates from the soil contaminated with textile dye locations. The main identified fungal isolates were *Mucor, Aspergillus, Penicillium* and *Neurospora* species, depending on the sampling location.

Table 1. Physicochemical composition of effluent samples

WHO-World health Organization, STD-Standard; SD-Standard Deviation

Key: Location A = Contaminated Soil; Location B = Contaminated Soil; Location C = Uncontaminated Soil (Control), Location D = Dye Effluent; FEPA-Federal Environmental Protection Agency

Fig. 2. Frequency of bacterial isolates

Table 5. Morphological characteristics of fungi isolates from contaminated soil samples with textile dye location 2

3.2 Discussion

This study investigated some samples of textile dye effluents from major factories in Osogbo, Osun State, Nigeria to isolate and identify their associated bacteria and fungi. The physicochemical properties of the effluents and contaminated soils were also determined. It was found that the mean pH of the textile effluent was 8.4. A comparison with the WHO standard showed that it was within the 6.5–8.5 recommended limits. This study has revealed that the pH of effluent samples from the study location tended towards alkalinity. A recent study by Younas et al*.* [17], reported that high pH values in wastewater effluents have the potential to increase the chances of solubilization of essential elements if released into natural water bodies, thereby affecting the aquatic life; Paul et al*.* [18] reported similar result as this study, he observed that the impacts of effluents of some selected dye mills were slightly alkaline in nature due to the presence of scouring and bleaching agents as well as the chemicals, sodium hypochlorite and sodium hydroxide, and various surfactants and sodium phosphate used in mercerizing the fabric, though higher level of pH value has been recorded by Desai and Kore [19] and Tiwari and Chauhan [20]. In addition to pH; other parametersvalues investigated were 96.50±0.02 mg/l, 10.08±0.05 mg/l, 3.65±0.21mg/l, and 6.35±0.21 forTDS, TSS, BOD and COD accordingly. All the parameters studied were all found to be significantly below the WHO textile effluents limitations standard.

According to Lokhande et al*.* (2011),TDS is the representation of the total inorganic contents of $Ca^{2+}Mg^{2+}$, K⁺, Na⁺, HCO₃⁻, Cl⁻, SO₄²⁻ and organic substance present in the wastewater. TDS in effluent isnot limited to the salts but also proportionally related to the salinity and conductivity of water.Low value TDS is an indication of low rate of mineralization in water. Generally, the WHO standard limit for TDS is 500 mg/l while the maximum is 2000 mg/l in waste water [21]. Bilotta and Brazier (2008) in their work reported a very high level of TSS in water which impacted significant on the physical, chemical and biological properties of the receiving environmental bodies especially in soil and water. The low levels of TSS, BOD, and COD in this study is a suggestion of decreased effect of the textileeffluents on the quality of the receivingbodiesi.e. soil and water. High level of chemical oxygen is an indication of a noxious conditions (BOD is very low)and presence of resistant organic impurities as reported by Bhatia et al*.* [22]. The level of Cl was significantly high when compared with SO_4^2 and this contributed to the relatively higher content of TDS observed in this

study. This finding is in agreement with the work of Paul et al. [18], who reported that higher values of chloride in wastewater were responsible for increase in total dissolved solids. Moreover, sulphate is the major anion occurring in natural water and has been reported to be generally non-harmful substance especially at low levels, but may exert laxative effect at high concentrations [23].

The heavy metals investigated were cadmium (Cd), nickel (Ni), lead (Pb), zinc (Zn), manganese (Mn) and iron (Fe). The levels of heavy metals in contaminated soil samples varied tremendously in accordance with the sampling locations, with Fe being generally the highest and Cd the least in all locations studied. Some of these heavy metals at concentrations above the standard limits could be dangerous to humans and the ecosystem. Studies have shown that the dye effluents of textile industry contain mostly heavy metals like Cu, Zn, Cr, Cd and Fe, thus the dyeing process usually contributes Cr, Pb, Zn and Cu to wastewater [24]. Some of the reported problems associated with pollutions of heavy metals include edema of eyelids, tumor, congestion of nasal mucous membranes and pharynx, stuffiness of the head and gastrointestinal, muscular, reproductive, neurological and genetic malfunctions [24]. In this study, most of the heavy metals were within the permissible limits according to the Federal Environmental Protection Agency in Nigeria [25], except for Ni and Zn which were prominently higher in oneand three of the locations respectively. Zinc is widely used in industries such as galvanization, paint, batteries, smelting, fertilizers and pesticides, fossil fuel combustion, pigment, polymer stabilizers, etc, and the wastewater from these industries is polluted with zinc, due to its presence in large quantities as electroplating agents. Although several metals can be used for electroplating, nickel, copper, zinc and chromium are the most commonly used metals, the choice depending upon the specific requirement of the articles. During washing of the electroplating tanks, considerable amounts of the metal ions find their way into the effluent and when it is present in less quantity in human body, it affects considerably human health. Although humans can handle high levels of zinc, too much of it can still cause health problems [26]. Similarly, Ni is generally considered to be one of the most toxic metals found in the environment; once it enters the food chain, progressively larger accumulation of nickel compounds take place in humans and animals.Nickel(II) is present in the effluents of silver refineries, electroplating, zinc base casting and storage as well as battery industries; at higher concentrations it can lead to cancer of lungs, nose and bone, headache, dizziness, nausea and vomiting, chest pain, tightness of the chest, dry cough and shortness

of breath, rapid respiration, cyanosis and extreme weakness [26]. Monitoring their levels is therefore vital in the effluents before discharge into the environments.

The total microbial counts obtained from analysis of the effluent revealed that total bacterial counts (TBC) were generally higher than their fungal counterparts (TFC) in all the locations samples. This finding suggests that bacteria are well adapted to the utilization of textile effluents. Previous studies have shown that bacteria demonstrated maximum degradation of dye effluents [27].

The predominant bacterial isolates from this study included *Klebsiella aerogenes, Esherichia coli, Staphylococcus aureus, Pseudomonas* spp., *Proteus* spp., *Streptococcus* sp. and *Micrococcus* sp., while the fungal isolates were *Mucor, Aspergillus, Penicillium*, *Rhizopus, Neurospora* and *Fusarium* species, from the sampling location. The presence of these organisms suggests that dye effluents could be utilized by the microorganisms as sources of nutrients. The ability of bacteria and fungi to decolorize dyes has been reported by past researchers. Some of the bacteria reported to decolourize dye effluents include *[Bacillus subtilis,](http://www.scialert.net/asci/result.php?searchin=Keywords&cat=&ascicat=ALL&Submit=Search&keyword=Bacillus+subtilis) [Aeromonas hydrophila,](http://www.scialert.net/asci/result.php?searchin=Keywords&cat=&ascicat=ALL&Submit=Search&keyword=Aeromonas+hydrophila) [Bacillus](http://www.scialert.net/asci/result.php?searchin=Keywords&cat=&ascicat=ALL&Submit=Search&keyword=Bacillus+cereus) [cereus](http://www.scialert.net/asci/result.php?searchin=Keywords&cat=&ascicat=ALL&Submit=Search&keyword=Bacillus+cereus)*, *Pseudomonas* species, *Rhodococcus species*, *Klebsiella pneumoniae* RS-13 and Acetobacter liquefaciens S-1 and *Pseudomonas desmolyticum.* For the fungi, some species have been reported to remove textile effluent dyes, among them are *Rhizopus arrhizus* and different species of *Phlebia*, *Penicillium, Fuasrium*, white rot and brown-rot fungi and mushrooms [28]. This is important because these microorganisms could be encouraged as native, readily available biological treatment agents for effluents by textile industries.

4. CONCLUSION

The effluents from textile dye industries within Oshogbo metropolis was investigated microbiologically and chemically to determine their quality and impact on the receiving environments. All the physical and chemical parameters investigated such as pH, BOD, COD, TSS, TDS, Chloride and Sulphate of the effluents and receiving soil were found to be significantly lower than the maximum permissible limits prescribed by WHO. In this study Ni and Zn were found to be above theFederal Environmental Protection Agency (FEPA) limitations guidelines in some of the locations. Therefore, there is a need to develop simple, environmentally friendly and low cost treatment systems for the treatment of textile effluents to minimize water and soil pollution. This study recommends the intentional treatment of

textile effluents before releasing it into receiving soil and water bodies.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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