# *Original Research Article*

**PHYSICOCHEMICAL AND MICROBIOLOGICAL EVALUATION OF SOIL AND EFFLUENT CONTAMINATED WITH AZO-DYE IN ITOKU, ABEOUKUTA OGUN STATE, NIGERIA**


## ABSTRACT

The study was aimed at investigating the ecological effect of azo textile dye on the bacterial biomass and its selective pressure and also to determine the associated physicochemical changes in the soil samples contaminated with textile azo dye in Itoku, Abeokuta. The microbiological and physicochemical changes of soil and effluent samples collected were determined using standard procedures. The results revealed the average count of total viable bacteria in sampling sites ranges from 0.82±021 x $10^6$ cfu/g to 1.65±0.02 x $10^6$ cfu/g. The results showed the azo dye contaminated and the control soil sample contained a heterogenous population of bacteria but with less density in the azo dye contaminated soil, the bacteria isolated include: *Paenibacillus validus, Bacillus licheniformis, Bacillus niacin, Seratia liquefaciens, Staphylococcus gallinarum, Bacillus subtilis, and Bacillus coagulans.* The physicochemical analysis of the soil revealed high levels of pH in azo dye contaminated soil (10.4–11.1). The electrical conductivity is highest in the effluent with a range of (1250 to 2943 units) and a control with a range of (430 to 480), which is the least. However, the cation exchange capacity in the control is significantly higher than other samples. The control also has the highest mean value of organic matter at 4.13%. The bulk density is almost the same across the groups with an average value ranging between 1.59-1.65 g/$cm^3$, while the other parameters like water holding capacity, particle density, moisture content, and total porosity have the highest value in the control sample. There is a decrease in bacteria count and the soil parameters of the azo textile contaminated soil, which thus makes it unfavorable for agricultural purposes and toxic to the ecosystem**.**



Keywords: Azo dye, Contamination, Degradation, Heterogeneous, Physicochemical


SIGNIFICANCE STATEMENT: The particular significance of study was aimed at investigating the ecological effect of azo textile dye on the bacterial biomass and its selective pressure and to determine the associated physicochemical changes in the soil samples contaminated with textile azo dye in Itoku, Abeokuta. The microbiological and physicochemical changes of soil and effluent samples collected were determined using standard procedures. The results showed the azo dye contaminated and the control soil sample contained a heterogenous population of bacteria but with less density in the azo dye contaminated soil, the bacteria isolated include: *Paenibacillus validus, Bacillus licheniformis, Bacillus niacin, Seratia liquefaciens, Staphylococcus gallinarum, Bacillus subtilis, and Bacillus coagulans. It was observed that the pollution of dye to the surrounding soil had deleterious effect on the physicochemical properties of the soil.*

## INTRODUCTION

Environmental pollution of the ecosystem is becoming worrisome owing to increased industrialization of the environmentally unfriendly chemical (dye) that is produced and consumed, affecting both living and nonliving components{1}. Synthetic dyes are of great importance in textile dyeing, paper printing, food, pharmaceuticals, cosmetics, photography, paint, petroleum products, and other industrial uses. Azo dye, being an artificial dye, has been used in various aspects of human endeavor with the primary aim of adding color to materials {2}. This is mostly employed in tie and dye, which is common in some parts of the country. Over the years, the use of azo dye has been increasing, and this constantly raises concern as it has been linked to many disease conditions {3}. Dyes and pigments are designed to resist degradation, such that they remain in the environment for a long period of time. For example, the half-life of the hydrolyzed dye Reactive Blue 19 is about 46 years at pH 7 and $25^0$C {4}.

Azo dye is well known to cause damage to living cells by its mutagenic and carcinogenic potential, by affecting the genome of living cells, it also affects the ecosystem and thus puts humanity at risk {5}. There is a continuous search for the best and most environmentally friendly method of cleaning, also partially degraded dyes are more toxic than the mother molecule{6,7}. This toxic potential of the dye creates a selective pressure on the bacteria population and causes adaptation for survival{8}. The bacterial population and type present in a soil determine the ecological state and, subsequently, the fertility of the soil. Some bacteria are well adapted to live in the environment impregnated with dye, while some are even adapted to degradation of the dye{9}. It has been reported that many of the azo dyes and aromatic amines produce potent toxins during their degradation and they contain heavy metals and carcinogens which may have a deleterious negative impact on bacterial cells and ecology {10}.

Conventional physical methods of treatment only transfer the pollutants from one form to another and produce secondary waste products. Examples include physical or chemical flocculation; membrane filtration; electro kinetic coagulation; electro chemical destruction; and precipitation{11}. These types of technologies are inefficient, expensive, and have low adaptability to diverse types of dye effluent. Dyes escape conventional wastewater treatment processes and persist in the environment as a result of their high stability to light, temperature, water, detergents, soap and other parameters{12}. The use of microorganisms for biodegradation of dye is convenient and versatile, with dynamic metabolisms and potential machinery of enzymes. Biodegradation is a nonhazardous, cost-efficient, environmentally friendly, and often more effective alternative to conventional methods of treatment of textile dye waste {13}.

The physico-chemical character of a soil contaminated with dye is affected. This ranges from the PH, conductivity, CEC, organic and non-organic matter, and soil structure{14}. Damage to soil property and structure can encourage leaching, thus loss of fertility. In another view, the survival of certain bacteria is dependent on the structural and chemical state of the soil, damage to structural state of soil will affect the range of useful bacteria present. Dye has being linked to increased alkalinity and loss of fertility of soil with long-term consequence of loss of substantial hectares of land, especially down the stream for agricultural purposes, and also damage to aquatic life. Research has shown that there is a decrease in the physicochemical properties of soil irrigated with industrial dye with a corresponding impact on crop yield{15,16}.

The search for a biological method of control of dye pollution is a necessity. This could be achieved through bioremediation or biodegradation as other methods of decontamination of dye are expensive and non-effective{17}. Bacteria that can survive in dye-contaminated soil may have the potential to degrade dye through mutation and selective pressure of the environmental toxicant{18}. The bacteria type and population are transitory as there is high turnover of mutation and development of new and resistant species{19}. There is limited literature on characterization and identification of bacteria biomass in dye contaminated ecosystem thus, there is a need to isolate, characterize and identify bacteria present in dye contaminated soil and the physicochemical property of the soil which will secondarily affect the bacteria population.

## EXPERIMENTAL DETAILS

### Study Area

The study was carried out in Olumo rock area, Itoku, Abeokuta, Ogun state.

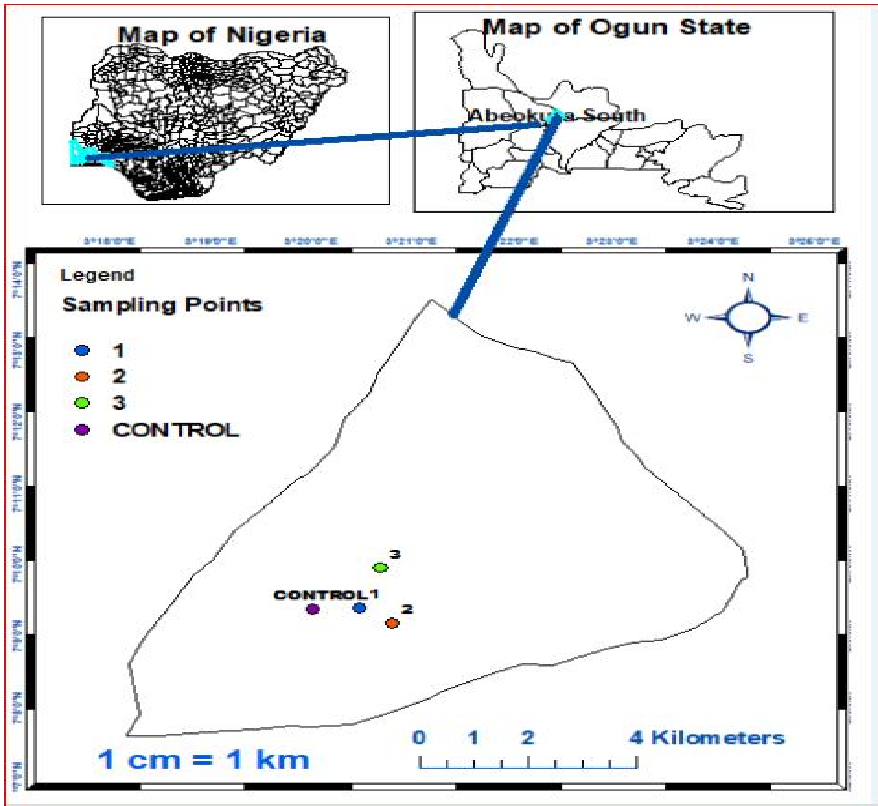


Figure 1:Map showing study area & sample locations

**Sample Collection**

Soil samples of 200g  was collected from three different dye contaminated sites in Itoku, Abeokuta, from the top soil layer profile of 0 - 20cm; in a sterile plastic bag and stored in ambient temperature and transported to the laboratory for analyses.

**Physical parameters of soil samples and Chemical parameters of soil samples**

The parameters analyzed include pH by potentiometric meter. Moisture content, bulk density and porosity. The chemical parameters include Electrical Conductivity (EC), Cation Exchange Capacity (CEC), a sample weight of 20 g was air dried and treated with 100 $cm^3$ of 0.1 mol $dm^{-3}$ Hydrochloric acid solution (in the ratio w: v of 1:5). The suspension will be shaken in polyethylene bottle of about 250 cm capacity on rotary shaker (40 rpm) at room temperature for 1hour, and then filtered. The filtrates will be titrated with 0.1 mol $dm^{-3}$ Sodium hydroxide solution in presence of phenolphthalein indicator and the sum of exchangeable alkaline cations will be calculated from the amount of sodium hydroxide solution used {20,21}.

**Isolation and Characterization of Isolates Microorganisms**

Isolation and enumeration of total viable bacteria will be carried out using Nutrient agar by pour plate methods. Colonies will be sub-cultured on freshly prepared Nutrient agar and the plates will be incubated at 37 $^{0}$C for 24 h {22}

**Biochemical identification of isolates**

Phenotypic and biochemical identification of bacterial isolates will be done by carrying out Gram staining reactions, catalase test, coagulase test, oxidase test, spore test, motility test, and growth on differential media, citrate utilization, Methyl Red, Voges Proskauer reactions, urease production, nitrate reduction, and sugar fermentation tests using Advance bacteria identification software 2021 version.

**Statistical analysis:** The data obtained in this study were evaluated using descriptive statistic through simple graphs, tables & chart and the one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test, using Graphpad prism version 8.0 software (La Jolla, California, USA). The level of significance was set at $\alpha_{0.05.}$

## RESULTS AND DISCUSSION

Tables 1A and 1B show the results of the bacterial isolation enumeration. The bacteria had the highest count in control soil (4.06±0.09 x $10^6$ cfu/g) compared to the textile dye-polluted soil (1.23-1.65±0.02 x $10^6$ cfu/g), while that of the effluent ranged from (0.61-0.98±0.02 x $10^6$ cfu/ml). These statistics were based on the average count of all viable bacteria and the standard deviation of colonies that were isolated. The low bacterial counts in the soil that was tainted with textile dye suggest that soil may contain some compounds that prevents high survival of heterotroprhic bacteria. The study by {23} showed the total viable counts of bacteria from textile dye-contaminated soils were found to be comparable{23}.

**Table 1 A: Colony count from soil Dye samples**

| Sample codes | **TVBC (x10$^6$ cfu/g)** |
|---|---|
| DS1 | 1.65± 0.02 |
| DS2 | 0.82±0.21 |
| DS3 | 1.23±0.11 |
| DCS | 4.06±0.09 |

All values in mean±SEM, Total Viable Bacterial Counts (TVBC),

Dye Soil (DS1-3), Dye Control Soil (SC)

**Table 1B: Colony count from Dye effluent samples**

| Sample codes | **TVBC (x10$^6$ cfu/ml)** |
|---|---|
| DE1 | 0.98±0.05 |
| DE2 | 0.63±0.04 |
| DE3 | 0.61±0.05 |

All values in mean±SEM, Total Viable Bacterial Counts (TVBC),

Dye Effluent (DE1-3)

A total of seven bacterial isolates were found in the results using advance bacteria identification software (2021) version. Frequencies and percentages were used to identify the isolated bacteria using advanced biochemical procedure, *Bacillus niacin* had the highest frequency and percentages of bacterial species in this study, with 6 (28.57%), followed by *Bacillus coagulans* 4 (19.05%), *Paenibacillus validus* 3 (14.29%) and *Bacillus licheniformis Seratia liquefaciens, Staphylococcus gallinarum,* and *Bacillus subtilis,* with 2 each (9.52%) It may not be surprising that there were many different species of *Bacillus* found in the polluted soil given that these organisms are native to soil environments and are known to persist there {24}. The isolated organisms from dye-contaminated soil are able to break down both the organic and inorganic dye constituents. Despite the dye's poisonous and resistant constituents, bacteria continue to exist by adapting to their surroundings. Similar research has suggested that members of the *Bacillus genus* are capable of decolorizing azo textile dye. Research has showed that *Bacillus subtilis*, and *B. cereus*, can decolorize azo dyes(25).



**Table 2: Cultural and biochemical characteristics of Bacterial isolated from Soil and Effluent samples**

| | ***Paenibacillus validus* similarity 96.5%** | ***Bacillus licheniformis* similarity 87.8%** | ***Bacillus niacin* similarity 90.8%** | ***Seratia liquefaciens* similarity 93.4%** | ***Staphylococcus gallinarum* similarity 80.2%** | ***Bacillus subtilis* similarity 91.2%** | ***Bacillus coagulans* similarity 95.5%** |
|---|---|---|---|---|---|---|---|
| Gram stain | + | + | + | - | + | + | + |
| Spore | + | + | + | - | - | + | + |
| Catalase | + | + | + | + | + | + | + |
| Glycerol | + | + | + | + | + | + | + |
| $H_2S$ | - | - | - | + | - | - | - |
| Lactose | - | - | - | - | - | - | - |
| Sucrose | + | + | + | + | - | - | + |
| Glucose | + | + | + | + | - | + | + |
| MR | + | + | - | + | + | + | - |
| V.P | _ | - | - | + | + | + | - |
| Starch | + | - | + | - | - | + | + |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| hydrolysis | | | | | | | |
| Casein hydrolysis | - | - | - | - | - | + | - |
| Lipase | - | + | + | + | + | + | + |
| Nitrate | + | + | + | + | + | + | + |
| Motility | + | + | - | + | + | + | + |
| Indole | - | - | _ | - | - | - | - |
| Citrate | - | + | + | + | - | - | - |
| Arabinose | - | + | - | + | + | + | + |
| Xylose | + | + | + | + | + | + | + |
| maltose | + | + | + | + | + | + | + |
| mannitol | + | + | - | + | + | + | _ |
| Erucrose | + | + | + | + | + | + | + |
| Raffinose | - | + | - | + | + | - | + |
| sorbitol | + | + | - | + | + | + | + |
| galactose | + | + | + | + | + | + | + |
| Ribose | + | + | + | + | + | + | + |
| rhamnose | - | + | + | - | + | - | + |

Table 3: Occurrence of bacterial isolates in the soil and effluent samples

| Samples | ***Paenibacillus validus*** | ***Bacillus licheniformis*** | ***Bacillus niacin*** | ***Seratia liquefaciens*** | ***Staphylococcus gallinarum*** | ***Bacillus subtilis*** | ***Bacillus coagulans*** |
|---|---|---|---|---|---|---|---|
| Dye Soil 1 | + | + | + | - | - | - | - |
| Dye Soil 2 | - | - | + | + | - | - | - |
| Dye Soil 3 | + | - | - | + | + | - | - |
| Control | - | - | - | + | + | - | - |
| Dye effluent 1 | - | - | + | - | - | + | + |
| Dye effluent 2 | - | + | + | - | - | + | - |
| Dye effluent 3 | + | - | + | - | + | - | + |

Keys: + = present - = absent

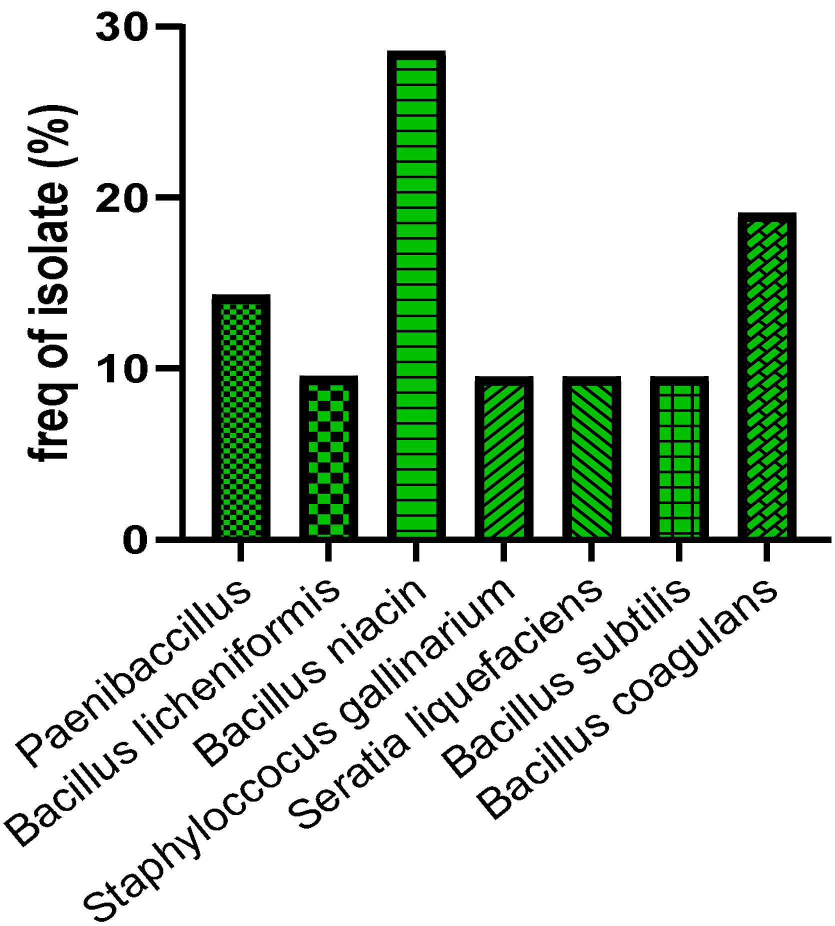


Fig. 2: Frequency and percentage occurrence of bacteria species isolated from soil contaminated with dye effluents

Alkalinity is a measurement of a soil's buffering capacity. It is a critical indicator of how effectively soil can scavenge acids from effluent wastes. At higher alkalinity values, bicarbonates and carbonates are more common in effluents. The azo textile dye-contaminated soil during this study exhibited higher pH levels (11.1–11.5) in every sampling site, exceeding the tolerated levels {23}. The findings showed that the soil moved toward an alkaline state and that it was over the allowable limits of (6-9). The pH in the effluent is moving toward a higher value, indicating alkalinity conditions, and this could negatively affect the permeability of the soil and the development of the soil microbial flora. Increased carbonates and bicarbonates from textile dye effluents may potentially be a contributing factor to high alkalinity. This is consistent with {23} findings, which revealed a high pH level (9.36- 9.44).

Table 4: Physicochemical parameters of the dye contaminated soil samples

| Samples | Water holding capacity (%) | Bulk density (g/cm) | Particle density (g/cm) | Moisture content (%) | Total porous (%) |
|---|---|---|---|---|---|
| DS 1 | 24.44±0.34 | 1.59±0.05 | 2.26±0.18 | 8.57±0.02 | 26.59±0.28 |
| DS 2 | 19.26±0.33 | 1.65±0.03 | 2.21±0.01 | 8.39±0.04 | 22.19±0.22 |
| DS 3 | 17.27±0.55 | 1.61±0.03 | 2.27±0.02 | 9.29±0.07 | 21.11±0.23 |
| DCS | 28.40±0.36 | 1.60±0.03 | 3.24±0.03 | 9.82±0.08 | 32.68±0.12 |

Dye soil (DS 1-3), Dye control soil (DCS)

Table 5A: Chemical parameters of the dye contaminated soil samples

| **Samples** | **Ph** | **Electrical conductivity (µS/cm)** | **CEC (Mol/Kg)** | **Organic matter (%)** |
|---|---|---|---|---|
| **DS 1** | 11.06±0.01 | 3273±0.03 | 0.25±0.00 | 1.31±0.02 |
| **DS 2** | 10.38±0.03 | 1250±0.03 | 0.25±0.01 | 2.44±0.05 |
| **DS 3** | 10.43±0.03 | 2943±0.02 | 0.14±0.02 | 1.89±-0.03 |

Dye soil (DS 1-3), Dye control soil (DCS)

Table 5B: Physicochemical parameters of the dye contaminated effluent samples

| **Samples** | **Ph** | **Electrical conductivity (μS/cm)** | **CEC (Mol/Kg)** | **Organic matter (%)** |
|---|---|---|---|---|
| **DE 1** | 12.90± 0.03 | 9267±0.03 | 0.16±0.01 | 3.10±0.02 |
| **DE 2** | 13.31±0.02 | 8769±0.03 | 0.17±0.01 | 2.86±0.02 |
| **DE 3** | 13.56±0.05 | 9511±0.03 | 0.05±0.042 | 2.58±0,03 |

Dye effluent (DE 1-3), Cation exchange capacity (CEC)

The ability of the soil to retain positively charged ions known as cations is known as its cation exchange capacity (CEC). Electrostatic forces cause the negatively charged clay and organic matter particles in the soil to hold onto these cations (negative soil particles attract the positive cations). According to the results above, the control soil sample's cation level was greatest, ranging between 0.4 and 0.5, while the levels in the soil polluted with textile dye and the textile dye effluents were between 0.1 and 0.2. Because they can be readily exchanged with other cations, the cations on the CEC of soil particles are available to plants. More clay or organic matter is present in the soil the greater the CEC. This often means that clay soils with high CEC have a higher water retention capacity than soils with low CEC (sandy soils). The organic matter norm ranges from 2.00 to 6.00. The organic matter content ranges between 1-3, which is extremely low for the survival of soil organisms and the maintenance of plants {26}

Cations are positively charged ions, the capacity of the soil to hold on to these cations is called the cation exchange capacity (CEC). These cations are held by the negatively charged clay and organic matter particles in the soil through electrostatic forces (negative soil particles attract the positive cations). From the result above result the cation from the control soil sample had the highest which ranged between 0.4-0.5 while the textile dye contaminated soil and the textile dye effluents ranged between 0.1-0.2. The cations on the CEC of the soil particles are easily exchangeable with other cations and as a result, they are plant available. The higher the CEC the more clay or organic matter present in the soil. This usually means that high CEC (clay) soils have a greater water holding capacity than low CEC (sandy) soils.

An organic matter concentration of more than 8.00 percent results in an excessive availability of nutrients as well as other issues like high pH, high soluble salts, etc. Additionally, in both uncontaminated and contaminated soil samples, soils with an organic matter content of less than 3.00 percent are stated to be less productive.

## CONCLUSION

There is biodiversity in the bacterial population and high count in the soil sample compared to the dye. Most of the isolated bacteria are bacillus which has potential to biodegrade azo dye. This could been a result of environmental selective pressure or mutation that allow survival. However there may be need to genetically screen the isolated bacterial if it of a new strain of bacillus. The physicochemical property of the soil is significantly affected by dye and thereby resulting to poor soil quality thus dye can cause loss of soil fertility.

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