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Physicochemical and Microbiological Evaluation of Soil and Effluent Contaminated with Azo-Dye in Itoku, Abeoukuta Ogun State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The global industrialization of chemical dyes used for household products has resulted in various dyes leaching into the soil. Azo dye textile contamination could lead to infertile soil. This study investigates the ecological effect of azo textile dye contamination on bacterial biomass, selective pressure, and associated physicochemical changes in soil samples contaminated with textile azo

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dye in Itoku, Abeokuta, Nigeria. Microbiological and physicochemical changes of the soil and effluent samples collected were determined using standard procedures. Results revealed that the average count of total viable bacteria in the contaminated sampling sites ranged from 0.82±021 x 10^6 cfu/g to 1.65±0.02 x 10^6 cfu/g which was significantly lower than the control bacterial counts. The dye contaminated and the control soil samples each contained a heterogenous population of bacteria which included *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 the azo dye contaminated soil (10.4–11.1) and electrical conductivity was also highest in the effluent (1250 to 2943 units) compared to the control (430 to 480). Importantly, the control samples were higher in all of the other tested soil parameters including cation exchange, the mean value of the organic matter, water holding capacity, particle density, moisture content, and total porosity. Conclusion- Azo dye contamination caused decreases in bacterial density and many other soil parameters along with higher PH and electrical conductivity which suggests that azo textile dye contaminated soil is unfavorable for agricultural purposes and potentially toxic to the ecosystem.

Keywords: Bacteria; contamination; degradation; heterogeneous; physicochemical.

1. INTRODUCTION

Environmental pollution of the ecosystem is becoming worrisome due to the increased industrialization of environmentally unfriendly chemical dyes that are 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 is an artificial dye, used in various aspects of human endeavors with the primary aim of adding color to materials [2]. This is mostly employed in tie dye of clothing, which is common in some parts of the country. Over the years, the use of azo dye has been increasing, which 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 via its mutagenic and carcinogenic potential that can affect 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, however, 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 an environment impregnated with dye, and some are even adapted to assist with the degradation of the dye [9]. However, 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, electrochemical 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 the 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 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 the structural state of the soil will affect the range of useful bacteria present. Dye has been linked to increased alkalinity and loss of fertility of the soil that could result in long-term consequences such as loss of substantial hectares of land for agricultural purposes and 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 a high turnover of mutation and the development of new and resistant species [19]. There is limited literature on the characterization and identification of bacteria biomass in a dye contaminated ecosystem. Thus, there is a need to isolate, characterize, and identify bacteria present in dye contaminated soil as well as the physicochemical properties of the soil which will secondarily affect the bacterial population.

2. EXPERIMENTAL DETAILS

2.1 Study Area

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

2.2 Sample Collection

Soil samples of 200g were 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 at ambient temperature and transported to the laboratory for analyses.

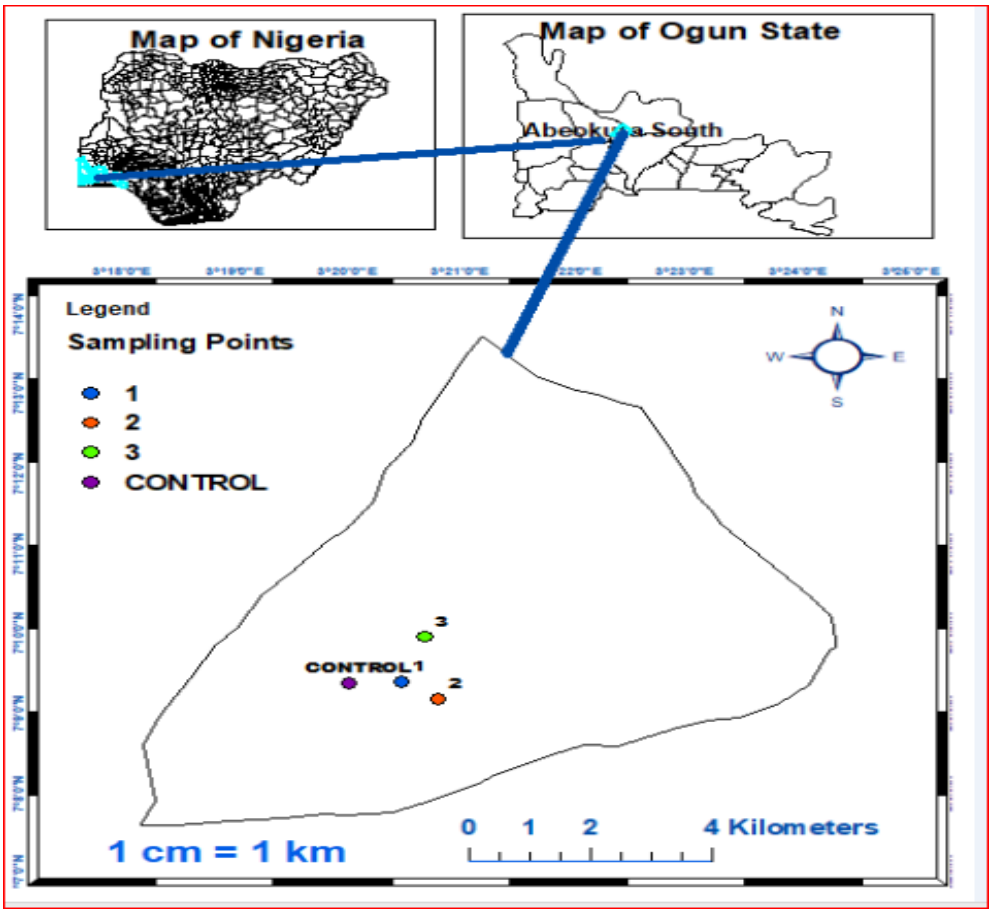

Fig. 1. Map showing study area & sample locations

2.3 Physical Parameters of Soil Samples and Chemical Parameters of Soil Samples

The parameters analyzed included pH by potentiometric meter, moisture content, bulk density and porosity. The chemical parameters included 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 is shaken in a polyethylene bottle of about 250 cm capacity on a 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].

2.4 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].

2.5 Biochemical Identification of Isolates

Phenotypic and biochemical identification of bacterial isolates will be conducted with the following tests Gram staining, 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.

2.6 Statistical Analysis

The data obtained in this study were evaluated using descriptive statistics through simple graphs, tables & charts 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 $\alpha_{0.05}$.

3. RESULTS AND DISCUSSION

Table 1A and 1B show the results of the bacterial isolation enumeration. The bacteria had the highest count in control soil ($4.06\pm0.09x10^6$ cfu/g) compared to the textile dye-polluted soil ($1.23\text{-}1.65\pm0.02x10^6$ cfu/g), while that of the effluent ranged from ($0.61\text{-}0.98\pm0.02x10^6$ 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 the soil may contain some compounds that prevent the high survival of heterotrophic bacteria. The study by Sirajo et al showed the total viable counts of bacteria from textile dye-contaminated soils were found to be comparable [23].

Table 1A. Colony count from soil Dye samples

Samples	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

Samples	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. Frequencies and percentages were used to identify the isolated bacteria using advanced biochemical procedures, *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 shown 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

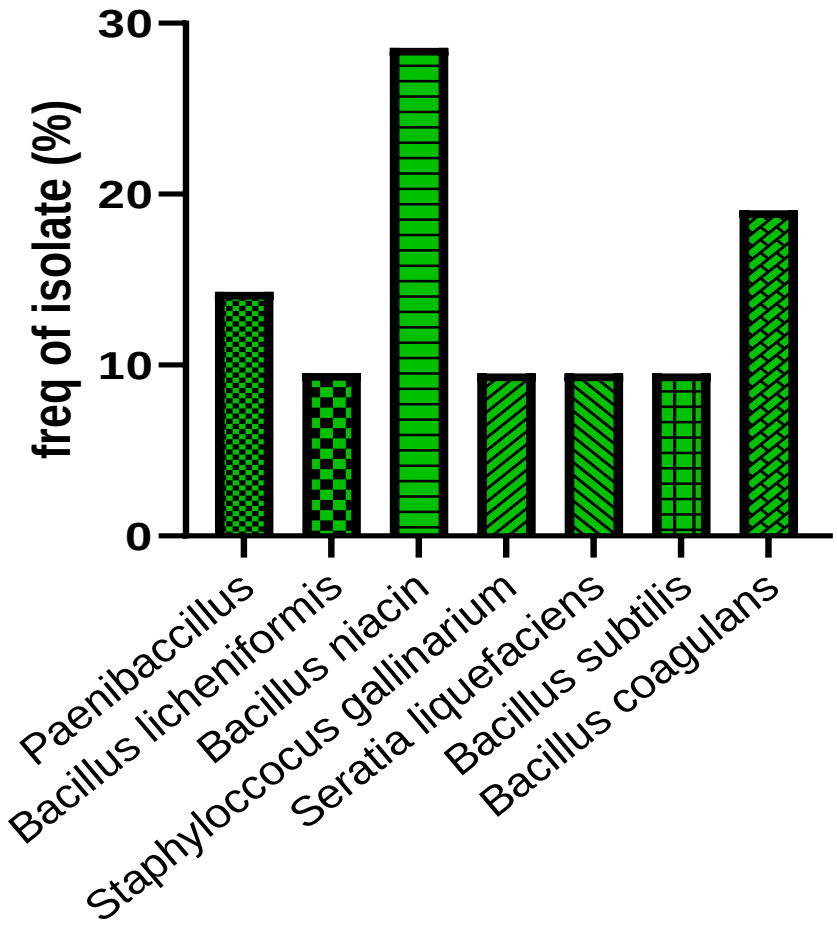

Fig. 2. Frequency and percentage occurrence of bacteria species

Table 4. Physicochemical parameters of the dye contaminated soil

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
CS	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), control soil (DCS)

Table 5A. Chemical parameters of the dye contaminated soil

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
CS	8.62± 0.02	500±0.02	0.45±0.03	1.64±0.02

Dye soil (DS 1-3), Control Soil (CS)

Table 5B. Physicochemical parameters of the dye contaminated effluents

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)

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).

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. The 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 onto 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 is 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.

4. CONCLUSION

There is biodiversity in the bacterial population and a high count in the normal soil sample compared to that of the dye contaminated soil. Most of the isolated bacteria are bacillus which has the potential to biodegrade azo dye. This could have been a result of environmental selective pressure or a mutation that allows for survival. Thus, there may be a need to genetically screen the isolated bacteria to determine if it is a new strain of bacillus. The physicochemical properties of the contaminated soil were significantly affected by the dye resulting in poor soil quality, which could lead to loss of soil fertility.

SIGNIFICANCE OF THE STUDY

This research is significant as pollution of our ecosystem is increasing. Due to the continuous disposal of effluents to the surrounding soil in Nigeria industries. The aim of this study was to investigate the ecological effect of azo textile dye contamination in the soil on bacterial biomass

and its selective pressure and to determine the associated physicochemical changes in Itoku, Abeokuta. The current study found that AZO dye contaminated soil altered the PH of the soil, significantly reduced bacterial abundance in the soil, and lowered many soil parameters. This data suggests that AZO dye in the soil results in adverse effects to the soil that could eventually lead to infertile soil.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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