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Microbial Analyses of Soil Samples in the Vicinity of Dye Pits in Zaria City, Northern Nigeria

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Authors' contributions

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

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ABSTRACT

This study was conducted to investigate the microbial enumeration and biomass of soil samples in the vicinity of dye pits in Zaria City, Northern Nigeria. A total of 90 soil samples were collected from five different dye sites with one location from non dye site. The pH of the non dye area was slightly acidic and ranged from 4.70- 5.40 both in $CaCl_2$ and H_2O while the contaminated samples ranged from 8.59-9.98 which indicate alkalinity. Microbial biomass carbon and nitrogen ranged from 170.82 - 334.44 mg/kg, and 16.89 - 37.28 mg/kg respectively. The soil microbial biomass, carbon and nitrogen including total bacterial and fungal counts of the soil samples were estimated using standard technique. Microbial enumeration showed the abundance of bacterial and fungal counts. The dominant genera of bacterial and fungal counts were *Bacillus* and *Aspergillus*. The highest bacterial count was observed in Kofar Gayan, while the lowest value was in Mabuga so also for fungal counts. Similar bacterial and fungal species were encountered in the different sampling locations in the course of this study, but their occurrences and levels of predominance were different. *Bacillus* spp dominated the bacterial isolates while *Aspergillus* spp was the most dominant

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fungus across the different sampling locations. Bacterial and fungal abundance were typical of an environment with high species richness and functional diversity.

Keywords: Culturable bacteria; fungi; dye effluent; contaminated soil.

1. INTRODUCTION

Soil is a vital part of the natural environment. It is just as important as plants, animals, rocks, landforms, and rivers. It influences the distribution of plant species and provides a habitat for a wide range of organisms [1]. Soil is a complex ecosystem where living organisms play a key role in the maintenance of its properties. Soil biota comprises a huge diversity of organisms belonging to different taxonomic and physiologic groups, which interact at different levels within the community [2].

Soil contamination is a condition that occur when soil loses its biophysical and biochemical properties due to anthropogenic and or natural influence in the soil environment. Anthropogenic influence includes chemical contaminants which adversely affect the soil by altering its physicochemical properties thereby making it imperfect for crop bearing. The excessive use of chemicals also adversely affects soil microbial diversity [3]. Contaminants in soil can easily be taken up by plants along with nutrients contained in the soil which, when consume by man, may pose a risk hazard [4]. Increasing industrialization and urbanization leads to risk of environmental pollution. The discharge of toxic effluents from various industries adversely affects water resources, soil fertility, aquatic organisms and ecosystem integrity. Activities by humans are greatly altering the ecosystem worldwide at unprecedented rates and leading to accelerated loss of biodiversity and environmental pollution [5].

The problem of environmental pollution is increasing day by day due to the release of xenobiotic substances into water, soil and air. These substances include organic compounds (pesticides, dyes, polymers etc.) and heavy metal ions. The damage caused by these pollutants to plants, animals and humans cannot be neglected and hence strategies must be developed to solve the problem of environmental pollution on the priority basis. Removal of dyes from the effluents or their degradation before discharge is a great environmental challenge for the industries [6]. The soil microbial biomass is the active component of the soil organic pool, which is responsible for organic matter decomposition affecting soil nutrient content and, consequently, primary productivity in most biogeochemical processes in terrestrial ecosystems [7,8]. Therefore, measuring microbial enumeration and microbial biomass is a valuable tool for understanding and predicting long-term effects on changes in land use and associated soil conditions.

2. MATERIALS AND METHODS

2.1 Study Area

Zaria lies between latitude 11º 03' 0" and 11º 05' 0" N and longitude 7º 41'0" and 7º 42' 0" E and located on a plateau at altitude of about 2200 feet above sea level in center of Northern Nigeria and more than 400 miles (634.71 km) away from the sea [9]. It has a tropical continental climate with the tropical climate more pronounced during the dry season, especially in December and January. The city lies within a region which has a tropical savanna climate with distinct wet and dry seasons [10]. The mean annual rainfall is 1060 mm [11].

A total of five locations were selected amongst the dyeing centers with one location selected from a non-dyeing centre which served as a control. The locations selected were Kofar Kuyanbana, Kofar Jatau, Kusfa, Mabuga, Angwan Iya and Kofar Gayan (the control). These locations represented the major dyeing centers in Zaria city.

2.2 Soil Sampling and Preparation

Fresh soil samples were collected in the immediate vicinity of dye pits and dump sites of the dye waste. Composite samples were collected at three depths (0-15 cm, 15-30 cm and 30-45 cm). The samples were collected from the dye pit; immediately within the pit.

2.2.1 Analytical procedures

Soil pH was determined using a glass electrode pH meter. The soil microbial biomass C and N was estimated by the fumigation-extraction method [12,13], using freshly collected moist soil samples. Ten grams of fresh soil sample was

extracted immediately after collection, with 50 ml of 0.5M K_2SO_4. A second subsample (10 g) was extracted and immediately fumigated in an incubator using reagent-grade ethanol-free chloroform. Ethanol was removed from the chloroform by washing the chloroform with about 5% by volume concentrated sulphuric acid (H_2SO_4) by shaking in a separating funnel and after separating the acid, the chloroform was washed with 10 rinses of distilled water. Water-saturated filter paper was placed in the incubator to keep the samples moist. After 5 days, the incubator was opened and after allowing the chloroform to dissipate, the soil was removed and immediately extracted with 0.5M K_2SO_4 as done for the unfumigated sample.

The extractable C and N in both fumigated and unfumigated samples were determined. Microbial biomass C was estimated by multiplying the difference in extractable C between fumigated and unfumigated samples by a conversion factor of 2.64 [14]. Microbial biomass N was calculated by multiplying the difference in extractable N between fumigated and unfumigated sample by a conversion factor of 1.46 [12].

$$\text{Microbial biomass carbon} = (\text{ExtractedCt1} - \text{ExtractedCt0}) \times 2.64$$

$$\text{Microbial biomass nitrogen} = (\text{Extracted Nt1} - \text{ExtractedNt0}) \times 1.46$$

2.2.2 Microbial enumeration (bacteria and fungi)

The procedure of Prescott et al. [15] was used as follows:

2.2.2.1 Preparation of media

For bacteria 22.5 g of plate count agar was weighed and dissolved in one litre of distilled water, heated to dissolution and sterilized by autoclaving at 121ºC for 15 minutes. For fungi 39 grams of saborand dextrose agar was done in the same manner.

2.2.2.2 Preparation of serial dilution for isolation of microorganism

One gram soil sample was weighed into universal bottle containing 9 ml of sterile distilled water (stock solution) shaken thoroughly to mix. 1 ml was taken into another 9 ml of sterile distilled water in the universal bottle this gave 10^1 dilutions, this process continued until 10^4 dilutions were obtained. 1 ml of 10^4 dilutions was transfer into sterile Petri dish. A 15 ml of molten agar cooled to 45ºC was added and swirled to mix completely. The plate was then allowed to solidify before incubation at 37ºC for bacteria and 25ºC for fungi. After the incubation period the isolated colonies were counted using a counter and result was expressed as the colony forming unit per gram ($CFUg^{-1}$). Bacteria incubation was done for three days while for fungi was for seven days. The result was expressed using the following relation.

$$\frac{Total\ no\ of\ colony\ counted \times dilution\ factor}{Volume\ of\ inoculum}$$

2.2.2.3 Preparation of smear

A smear was prepared in a clean glass slide. A drop of water was placed on a clean glass slide with the aid of a sterile wire loop; a bacterial colony was picked from the petri dish (cultured plate) and emulsified with the wire on the slide to obtain a thin film. A smear was allowed to air dried and fixed by heating over Bunsen burner flame by passing the slide trice with the smear facing upward.

2.2.2.4 Gram staining of fixed smear

The smear was stained with crystal violet and allowed to stand for 1 minute, and later washed with water and further flooded with grams iodine for 1 minute and again washed with water. It was decolorized with acetone and immediately washed with water and counter stained with safranine and allowed to stand for 1 minute, washed again and finally air dried. It was then examined under the microscope using × 100 objectives to view the organisms present.

2.3 Data Analysis

Statistical analyses were performed using SAS statistical package version 9.0 (SAS, 2002). Analysis of variance (ANOVA) was carried out to compare the difference of means from various sampling sites, followed by multiple comparisons using Duncan Multiple Range Test (DMRT) to separate the means.

3. RESULTS AND DISCUSSION

3.1 pH of Sampling Location

The pH of the- non dye area was slightly acidic and ranged from 4.70 - 5.40 both in $CaCl_2$ and H_2O while the contaminated samples ranged from 8.59-9.98 which indicate alkalinity. Mabuga

had the highest pH in both H_2O (9.98) and $CaCl_2$ (9.61) while Kofar Gayan had the lowest values for pH (H_2O) and pH ($CaCl_2$) which were 5.42 and 4.70 respectively. The control site, Kofar Gayan where dye activities did not take place had the lowest pH value. The high pH in Mabuga may be due to frequent discharge of dye chemicals in the area. The increase in the soil pH in the dye areas may be attributed to accumulation of various soluble salts. This is in consonant with the report of [16] on the influence of dye effluent in the soil of India. Soil pH affects the soil's physical, chemical, and biological properties and processes, as well as plant growth (Table 1).

Table 1. pH of the sampling locations

Location	pH (H_2O)	pH ($CaCl_2$)
KG	5.42±0.31^{c}	4.70±0.35^{d}
Kusfa	9.44±0.72^{b}	8.80±0.57^{c}
KJ	9.64±0.68^{a}	9.04±0.40^{b}
Mabuga	9.98±0.21^{a}	9.61±0.30^{a}
AI	9.78±0.32^{a}	8.63±0.45^{c}
KK	9.35±0.58^{b}	8.59±0.42^{c}

Note: Mean with the same letters along a column is not different at P=.05
KG= Kofar Gayan, KJ= Kofar Jatau, AI= Angwan Iya and KK= Kofar Kuyanbana

3.2 Soil Microbial Properties

Soil microbial biomass carbon and nitrogen showed significant differences in soil samples of dye and the non-dye areas (Table 2). However, microbial biomass C ranged from 170.82 mg/kg to 334.44 mg/kg, while microbial biomass N ranged from 16.89 mg/kg to 37.28 mg/kg. The highest was observed at Kofar Gayan for both MBC and MBN, while the lowest value was observed at Kusfa and Mabuga for both MBC and MBN respectively. The total bacterial counts (TBC) of the soil sample ranged from 3.60×10^{4} to 1.29×10^{7} $CFUg^{-1}$. The total fungi counts (TFC) of the soil ranged from 1.46 to $8.74\times10^{6}CFUg^{-1}$ (Table 2).

Even though there were differences in the total bacterial and fungal counts of the different sampling locations, these differences were not significant (*P=.05*). Kofar Gayan showed highest count for bacterial and fungal while, Mabuga showed the lowest (Table 2). Although down the soil profile, a significant variation (*P=.05*) was observed. The concentration of microbial organisms was highest at surface soil depth in most of the locations under study (Tables 3-8).

3.3 Variations in Bacteria Types in Sampling Locations

Throughout the sampling locations, a total of six (6) distinct genera of bacteria was observed, namely *Bacillus, Lactobacillus, Escherichia, Pseudomonas, Salmonella* and *Staphylococcus* (Table 9). Kusfa had all the six (6) genera with *Bacillus* being the most dominant. The fewer genera were *Lactobacillus* and *Escherichia.* In Kofar Jatau, six (6) genera were also observed, *Bacillus, Pseudomonas* and *Escherichia* genera were the most dominant while *Lactobacillus* and *Salmonella* were less dominant. Likewise in Mabuga six (6) genera were also recorded and the most dominant genera were *Bacillus* and the least is *Escherichia*. In Angwan Iya four (4) genera were recorded and *Bacillus* was the most dominant and *Escherichia* was the least. At Kofar Kuyanbana, five (5) genera were recorded and *Escherichia* was the most dominant, while *Pseudomonas* was the least; and finally six (6) genera were recorded in Kofar Gayan which is the control, *Lactobacillus* was dominant, while *Escherichia* and *Salmonella* were the less dominant genera as shown in Table 9.

Table 2. Microbial properties of soils under the influence of dye effluents in Zaria city

Location	MBC (mg/kg)	MBN (mg/kg)	BC ($CFUg^{-1}$)	FC ($CFUg^{-1}$)
KG	334.44^{a}	37.28^{a}	1.29×10^{7a}	8.74×10^{6a}
Kusfa	170.82^{d}	22.89^{b}	2.23×10^{5a}	2.53×10^{4a}
KJ	259.48^{b}	28.67^{b}	4.83×10^{5a}	4.70×10^{4a}
Mabuga	195.94^{d}	16.89^{c}	3.60×10^{4a}	1.46×10^{4a}
AI	215.28^{c}	23.78^{b}	4.17×10^{4a}.	8.06×10^{4a}
KK	203.32^{cd}	19.66^{b}	1.40×10^{5a}	5.53×10^{4a}

Note: Means with the same letters along the column are not significantly different at P=.05
KG = Control, MBC = Microbial biomass carbon, MBN = Microbial biomass nitrogen, BC = Bacterial carbon, FC= Fungal count

Table 3. Effect of soil depth on microbial biomass, bacterial and fungal count of soil under the influence of dye effluents in Kofar Gayan, Zaria city

Auger depth (cm)	MBC (mg/kg)	MBN (mg/kg)	BC ($CFUg^{-1}$)	FC ($CFUg^{-1}$)
0-15	405.60^{a}	45.10^{a}	7.60×10^{4a}	8.40×10^{4a}
15-30	370.50^{a}	41.00^{a}	1.06×10^{5a}	4.20×10^{4a}
30-45	233.22^{b}	25.65^{a}	2.40×10^{5a}	4.00×10^{4a}

Note: Means with the same letters along the column are not significantly different at P=0.05

Table 4. Effect of soil depth on microbial biomass, bacterial and fungal count of soil under the influence of dye effluents in Kusfa, Zaria city

Auger depth (cm)	MBC (mg/kg)	MBN (mg/kg)	BC ($CFUg^{-1}$)	FC ($CFUg^{-1}$)
0-15	216.12^{a}	24.12^{a}	3.26×10^{5a}	8.20×10^{4a}
15-30	120.84^{b}	13.00^{b}	3.02×10^{5a}	1.60×10^{4b}
30-45	175.50^{a}	19.02^{a}	1.42×10^{5a}	2.00×10^{3b}

Note: Means with the same letters along the column are not significantly different at P=.05

Table 5. Effect of soil depth on microbial biomass, bacterial and fungal count of soil under the influence of dye effluents in Kofar Jatau, Zaria city

Auger depth (cm)	MBC (mg/kg)	MBN (mg/kg)	BC ($CFUg^{-1}$)	FC ($CFUg^{-1}$)
0-15	284.92^{a}	37.12^{a}	8.40×10^{5a}	8.00×10^{5a}
15-30	253.50^{a}	28.61^{a}	3.57×10^{5a}	2.60×10^{4a}
30-45	280.02^{a}	31.00^{a}	2.25×10^{5a}	1.60×10^{3a}

Note: Means with the same letters along the column are not significantly different at P=.05

Table 6. Effect of soil depth on microbial biomass, bacterial and fungal count of soil under the influence of dye effluents in Mabuga, Zaria city

Auger depth (cm)	MBC (mg/kg)	MBN (mg/kg)	BC ($CFUg^{-1}$)	FC (CFUg-1)
0-15	225.40^{a}	25.00^{a}	9.28×10^{5a}	2.04×10^{5a}
15-30	213.72^{ab}	22.11^{ab}	1.76×10^{5a}	1.20×10^{4b}
30-45	170.82^{b}	18.65^{b}	1.48×10^{5a}	1.50×10^{4b}

Note: Means with the same letters along the column are not significantly different at P =.05

Table 7. Effect of soil depth on microbial biomass, bacterial and fungal count of soil under the influence of dye effluents in Angwan Iya, Zaria city

Auger depth (cm)	MBC (mg/kg)	MBN (mg/kg)	BC ($CFUg^{-1}$)	FC ($CFUg^{-1}$)
0-15	220.60^{a}	25.00^{a}	4.8×10^{4a}	8.21×10^{3a}
15-30	216.86^{a}	24.07^{a}	3.20×10^{5a}	8.00×10^{3a}
30-45	218.40^{a}	24.22^{a}	2.80×10^{4a}	8.20×10^{3a}

Note: Means with the same letters along the column are not significantly different at P =.05

3.4 Variations in Fungi Genera in Sampling Locations

A total of nineteen [17] distinct genera of fungi were recorded throughout the sampling locations, (Table 10). In Kusfa eight (8) genera were recorded with *Aspergillus* being the most dominant while the less dominant genera were *Rhizopus, Trichoderma* and *Sclerotium.* Similarly in Kofar Jatau twelve (12) genera were recorded, *Aspergillus* genera was the most dominant, while *Candida* and *Fusarium* were less dominant. At Mabuga eight (8) genera were also recorded and the dominant genera were *Aspergillus,*

Genicularia and *Fusarium,* while the less dominant genera were *Vulvularia* and *Gleosporium.* As observed in Angwan Iya, seven (7) genera were recorded and *Aspergillus* being the most dominant, while *Vulvularia, Sclerotium* and *Gleosporium* were the less dominant. At Kofar Kuyanbana, ten (10) genera were recorded and *Aspergillus* was the dominant, while *Alternaria* and *Rhizoctonia* were the less dominant and finally fourteen (14) genera were recorded in Kofar Gayan which is the control with *Fusarium* as the dominant genera. *Candida* and *Penicillium* were the less dominant as shown in Table 10.

Table 8. Effect of soil depth on microbial biomass, bacterial and fungal count of soil under the influence of dye effluents in Zaria, Kofar Kuyanbana, Zaria city

Auger depth (cm)	MBC (mg/kg)	MBN (mg/kg)	BC ($CFUg^{-1}$)	FC ($CFUg^{-1}$)
0-15	188.14^{a}	16.79^{a}	4.82×10^{5a}	6.80×10^{4a}
15-30	122.26^{a}	10.80^{a}	2.22×10^{5a}	6.00×10^{4a}
30-45	108.42^{a}	12.07^{a}	4.74×10^{5a}	2.61×10^{5a}

Note: Means with the same letters along the column are not significantly different at P =.05

Table 9. Occurrence of some genera of aerobic heterotrophic bacteria within the vicinity of dye pits in Zaria city

Bacteria	Kusfa	KJ	Mabuga	AI	KK	KG
Bacillus	+	+	+	+	+	+
Lactobacillus	+	+	+	+	+	+
Staphylococcus	+	+	+	-	-	+
Salmonella	+	+	+	-	+	+
Pseudomonas	+	+	+	+	+	+
Escherichia	+	+	+	+	+	+

KK and KG; Kofar Kuyanbana and Kofar Gayan respectively, + indicates present, - indicates absent

Table 10. Occurrence of some aerobic heterotrophic fungi within the vicinity of dye pits in Zaria city

Fungi	Kusfa	KJ	Mabuga	AI	KK	KG
Aspergillus	+	+	+	+	+	+
Rhizoctonia	+	+	+	-	+	+
Neurospora	+	+	+	-	-	-
Rhizopus	+	-	-	-	-	+
Fusarium	+	+	+	+	+	+
Cephallospora	+	+	+	-	-	-
Trichoderma	+	-	-	-	+	+
Alternaria	-	+	-	-	+	-
Cladosporium	-	+	-	-	-	+
Phoma	-	-	-	+	+	+
Genicularia	-	-	+	+	-	+
Vulvularia	-	-	-	+	+	+
Monilla	-	-	+	-	+	+
Cheatomilla	-	+	+	-	-	-
Candida	-	+	-	-	+	+
Canoplea	-	+	-	-	-	-
Sclerotium	+	+	-	+	+	+
Penicillium	-	-	-	-	-	+
Gleosporium	-	+	-	+	-	+

+ indicates present, - indicates absent

Bacterial and fungal populations were relatively greater in soil samples collected from non-dye areas when compared to soil samples from dye areas. Differences in bacterial and fungal counts between the different samples were not significant in all the location, although bacteria count was more than that of fungi. This may be due to the fact that most soil bacteria prefer a slightly alkaline medium while fungi grow in acidic medium. This finding corroborates with that of Ogunmwonyi et al. [18] on the microbial analyses of different top soil samples. Soil reactions also influence the type of the bacteria and fungi present in soil. Even though more strains of fungi were observed than that of bacteria, total bacterial counts were generally higher than that of fungi, irrespective of sampling locations. The predominance of bacteria over fungi observed throughout the sampling time has been reported by other workers [18]. *Bacillus* and *Aspergillus* were the dominant bacteria and fungi genera found in the soil samples from all the locations.

Relative microbial biomass decreased exponentially with depth. More bacterial and fungal counts were recorded most especially at 0-15 cm. It is widely presumed that the number of heterotrophic bacteria changes with depth. Fierer et al. [19] and Hartmann et al. [17] also reported that microbial biomass often exhibits exponential decreases with depth. However, even the deepest soils had microbial biomass levels that were measurable. This may be due to bacterial and fungal species having the ability to absorb and/or degrade textile dyes. It could also be attributed to spatial and resource factors such as nutrients that can influence the microbial diversity of the soil [20]. The control had the highest counts and highest microbial biomass C and N. This indicates that soils with the highest salinity level showed the lowest soil microbial biomass and activities while soils with low salinity levels showed no effect on soil microbial indices.

Even at the dye areas, there were measurable numbers of bacteria and fungi. This is because under osmotic stress, microorganisms enhance their survival by channeling the consumed C and N into biomass production or cell proliferation, which naturally results in a decline in the rate of C and N mineralization. Low average soil MBN content was also reported by Muhammad et al. [21].

4. CONCLUSION

Dye waste has significant effect on the physicochemical properties of the soil. Dye waste affects the microbial enumeration and biomass of the soil. Though the volume of effluents from individual small-scale dyers may be small, the concentration of pollutants is generally high. The impact is significant where several producers are located at one place and discharge effluents into the soil. It is concluded that soil from the dye areas in Zaria were highly coloured, foul smelling, alkaline in nature. The effluent from the textile areas were the major source of pollution impacting on the soil properties in such environment. Thus, there is need for proper treatment before disposal on land as it is not safe for final release which is also harmful to man and other living organisms which consequently decrease soil fertility and damage food quality.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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