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Survey of Microbial Diversity in Dye-Contaminated Soil of Kofar Na'isa Dyeing Pit, Kano, Nigeria

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ABSTRACT

Background and Objective: A wide variety of microorganisms have been reported to survive in dye-contaminated soils due to their ability to metabolise synthetic dyes. This study was carried out in one of the major dyeing sites in Kano: Kofar Na'isa dyeing pit and was aimed at detecting soil microbes (bacteria, fungi and microalgae) from the dye-contaminated soil. **Materials and Methods:** The microorganisms were isolated using serial dilution, pour plate, streak culture and direct isolation techniques. Identification of microbial isolates was based on identification guides and DNA analysis. **Results:** A total of fifteen microbial species were identified, which include two bacterial species (*Bacillus megaterium* and *B. velezensis*) and thirteen fungal species (*Aspergillus flavus, A. fumigatus, A. niger, A. ochraceous, A. parasiticus, A. striatus, A. terreus, Candida tetrigidarum, Fusarium equiseti, F. oxysporum, Penicillium chrysogenum, P. digitatum and Rhizopus microsporus*). There were no microalgal species isolated from the dye pit's soil. **Conclusion:** It was concluded that fungal species are more abundant in the dye-contaminated soil of the Kofar Na'isa dyeing pit.

KEYWORDS

Bacteria, DNA analysis, dye-contaminated soil, fungi, microalgae, microbial diversity, microorganisms

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INTRODUCTION

Soil is a natural medium containing organic and inorganic compounds that support the survival of microbes and other living systems¹. It houses a wide variety of microbes that are associated with its clay-organic matter complexes². Some of the soil microbes include species of algae, bacteria, fungi, protists and viruses³. Contaminated soils are extreme environments that have adverse conditions making survival difficult for inhabiting living organisms⁴. Jorquera *et al.*⁵ declared that species present in extreme environments modify their distribution and abundance, as a result, creating a biotechnological tool with the capacity to resolve environmental pollution.

Soil microbes play an important role in the maintenance of soil function in both natural and contaminated soils due to their involvement in key ecological processes like soil structure formation and dynamics, decomposition of organic matter/contaminants, cycling of major elements, regulation of plant



communities and suppression of soil-borne diseases⁶. They colonise contaminated environments by adjusting their metabolic activities, thus, increasing the chances of colonisation by other species acting as biological stabilizers⁷.

Dell' Anno *et al.*⁸ reported that microbes belonging to different taxa occur naturally in contaminated soil, surviving by metabolizing toxic contaminants in the soil⁹. The most common microbes dwelling in contaminated soil are genera from bacteria, fungi and algae. Bacteria have been known for their ability to degrade organic contaminants through enzyme activity and biosorption. Some of the bacterial species inhabiting contaminated soils belong to the genera *Alcanivorax*, *Thallassolitus*, *Cycloclasticus*, *Oleispira*, *Marinobacter*, *Alcaligens*, *Achromobacter*, *Acinetobacter*, *Alteromonas*, *Arthrobacter*, *Burkholderia*, *Bacillus*, *Enterobacter*, *Flavobacterium* and *Pseudomonas*^{10,11}.

The survival of fungi in contaminated soils is due to the secretion of enzymes (catalases, peroxidases and laccases, etc.) enabling them to degrade organic toxins and immobilise inorganic contaminants¹². Members of the genera *Aspergillus*, *Curoularia*, *Drechslera*, *Fusarium*, *Lasiodiplodia*, *Mucor*, *Penicillium*, *Rhizopus*, *Trichoderma*, *Candida* and *Cryptococcus* are the common soil-dwelling fungi¹³.

Microalgae of the genera *Selenastrum*, *Scenedemus*, *Chlorella*, *Spirulina*, *Spirogyra*, *Oscillatoria*, *Chlorococcum*, *Synechocystis*, *Nannochloropsis* and *Chlamydomonas* have also been reported to survive in contaminated soils remediating organic pollutants^{11,14}.

Dye pits in Kano dispose of their wastes into the environment which can lead to loss of topsoil and microbial biodiversity¹⁵. Dye-contaminated environments are unsuitable for the survival of many ecologically important organisms due to their toxicity. However, few organisms may try to adapt to the dye-contaminated environments. The effect of dye waste on soil microbial diversity is minimally studied in Kano's Kofar Na'isa dyeing pit.

This study was therefore, aimed at investigating the bacterial, fungal and microalgal flora of dye-contaminated soil at the Kofar Na'isa dyeing pit.

MATERIALS AND METHODS

Sampling site: The soil sample used in this study was collected from the Kofar Na'isa dyeing pit. The site is an ancient fabric re-dyeing site, situated in the ancient city of Kano, Nigeria. The specific geographical co-ordinates of the samplings sites are 11°58'56.442"N and 08°30'53.724"E. The study was carried out at Research Laboratory, Biological Sciences Department, Bayero University, Kano, Nigeria from July, 2017 to November, 2018.

Sample collection: A soil auger was used to collect the dye-contaminated soil sample at 10 cm depth from the sampling site as described by Shafi *et al.*¹⁶. The collected sample was placed into a sterilised, well-labelled sampling vial and transported to the laboratory for analyses.

Isolation of microorganisms

Sample processing and dilution: One gram of the dye-contaminated soil sample was placed in a separate, labelled sterilised test tube, to which 10 mL of sterile distilled water was added (stock solution). The solution was mixed thoroughly and allowed to sediment for 15 min. Another set of five sterilised test tubes labelled 10^{-1} - 10^{-5} were arranged accordingly with each containing 9 mL of distilled water. Using a sterilised syringe, 1 mL of the stock solution was transferred into the test tube labelled 10^{-1} and mixed carefully. Using another sterilised syringe, 1 mL from the 10^{-1} test tube was transferred to the second test tube labelled 10^{-2} . The dilution subsequently continued to the fifth test tube, giving dilutions of 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} and 10^{-5} , respectively.

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Media preparation and isolation of microorganisms: Culture media for the cultivation of algae, (bold basal medium) bacteria (nutrient agar) and fungi (potato dextrose agar) were prepared according to the manufacturer's instructions and were subsequently, poured into labelled Petri-dishes. One millilitre of each serially diluted sample was cultured on a respective medium and incubated at 30°C for 14 days (microalgae) and 37°C for 24 hrs and 5 days for bacteria and fungi, respectively. After incubation, microbial colonies were checked macroscopically and suspected colonies were individually sub-cultured on culture media to obtain pure cultures of the isolates for identification¹⁷.

Identification of microorganisms: The biochemical identification of the bacterial isolates was carried out according to the flow chart in Bergey's manual¹⁸, which identifies bacteria based on the following, gram staining, morphology, spore formation, starch hydrolysis, Voges-Proskauer Test (VP) and cell diameter. Fungal isolates were identified based on hyphae structure and mycelial appearance as described by Alsohaili and Bani-Hasan¹⁹. The DNA of the isolates were extracted and amplified through PCR and gel electrophoresis using bacterial primer: 16S rRNA Bact1442-F (5'-AGAGTTGATCCTGGCTCAG) and 16S rRNA Bact1492-R (3'-GGTTACCTTGTTACGACTT), with base pair and annealing temperature of 1,500 and 60°C, respectively²⁰. Fungal primer: 18S rRNA fung ITS-F (5'-ATATGCTTAAGTTCAGCGGGT) and 18S rRNA fung-ITS-R (3'-GTTCCGTAGGTGAACCTGC), with base pair and annealing temperature of 550-600 and 47°C, respectively²¹. The extracted DNAs were further sent for sequencing at Inqaba Biotech (South Africa) using the Sanger sequencing method. The DNA sequences were inputted into National Centre for Biotechnology Information (NCBI) for Identification based on the Basic Local Alignment Search Tool (BLAST). Sequences were aligned and compared with sequences available at the BLAST-n site in the GeneBank database²².

RESULTS

The results for microbial diversity of dye-contaminated soil of the K/Na'isa dyeing pit are presented in Table 1 and Fig. 1. Table 1 shows the morphological features and BLAST results for the fifteen species isolated from the dye-contaminated soil, while Fig. 1 shows their macroscopic and microscopic view. The species belonged to six genera (*Bacillus, Aspergillus, Candida, Fusarium, Penicillium* and *Rhizopus*). The data in Fig. 1a-b are showing the morphological features of the bacterial isolates (*Bacillus* species). The data in Fig. 1c-i are showing the *Aspergillus* species, Fig. 1j presents the *Candida* species, Fig. 1k-l is the *Fusarium* species, Fig. 1m-n show the *Penicillium* species and Fig. 1o presents the *Rhizopus* species.

Isolates	Morphology/biochemical tests	BLAST results		
		 Per. Ident. (%)	E value	Accession No.
Bacillus megaterium	Gram-positive, rod-shaped bacteria with	100	0.0	MG561346.1
	sub-terminal spore, ability to hydrolyse			
	starch and positive to Voges-Proskauer Test			
Bacillus velezensis	Gram-positive, rod-shaped bacteria with	100	0.0	OK625530.1
	terminal spore, ability to hydrolyse starch			
	and positive to Voges-Proskauer Test			
Aspergillus flavus	Yellowish-green and downy	100	0.0	MG991646.1
Aspergillus fumigatus	Bluish-green and downy	99	0.0	JX501388.1
Aspergillus niger	Black and powdery	97	0.0	EU440768.1
Aspergillus ochraceus	Golden brown and powdery	92	0.0	AF128851.1
Aspergillus parasiticus	Green and powdery	83	0.0	AY371490.1
Aspergillus striatus	White and downy	97	0.0	MT322248.1
Aspergillus terreus	Brown, fluffy and downy	91	0.0	MK039873.1
Candida tetrigidarum	Dirty-white and woolly	100	0.0	NG063272.1
Fusarium equiseti	Tan, fluffy and downy	99	0.0	MH542620.1
Fusarium oxysporum	Whitish and woolly	87	8.00E-147	KT357578.1
Penicillium chrysogenum	Grey and downy	93	0.0	MK267448.1
Penicillium digitatum	Blue and downy	87	0.0	CBS130527.1
Rhizopus microsporus	Grey, fluffy and downy	97	0.0	KJ935021.1

Table 1: Morphological features, percentage identity and accession number of first hit of NCBI BLAST of isolated species

Aspergillus genus had the highest number of species (seven), while Candida and Rhizopus had the least (one)

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Fig. 1(a-o): (a-o) Colony and (a'-o') Microscopic view of microorganisms isolated from dye-contaminated soil of K/Na'isa dyeing pit (Magnification, ×1/20 and ×40, respectively), (a) *B. megaterium*, (b) *B. velezensis*, (c) *A. flavus*, (d) *A. fumigatus*, (e) *A. niger*, (f) *A. ochraceous*, (g) *A. parasiticus*, (h) *A. striatus*, (i) *A. terreus*, (j) *C. tetrigidarum*, (k) *F. equiseti*, (l) *F. oxysporum*, (m) *P. chrysogenum*, (n) *P. digitatum* and (o) *R. microsporus* Letters with prime sign (') are microscopic view of organisms

DISCUSSION

The results of the study (Table 1 and Fig. 1) present some of the major microbes inhabiting dye-contaminated soil of the K/Na'isa dyeing pit to be species from the genera *Bacillus, Aspergillus, Candida, Fusarium, Penicillium* and *Rhizopus*. Fungal species were observed to be more abundant and there were no algal species in the soil.

Several reports have proven the presence of different microorganisms in dye-contaminated soils, acting as pollutant removal tools^{4,23-25}.

Vani *et al.*²⁶ revealed the presence of *Bacillus* species in soils of local dyeing sites, which is due to their capability to metabolise soil contaminants²⁷. *Bacillus cereus, B. flexus, B. firmus* and *A. niger* were isolated from dye spilled soil and used to degrade synthetic dyes and in the treatment of industrial effluents^{28,29}.

Šimonovicová *et al.*²⁵ reported that fungi thriving in harsh environments display high effectiveness in remediating toxic substances due to the production of unusual chemical structures. They also possess hyphae that enable them to penetrate deeply contaminated soil surfaces to degrade contaminants (such as hydrocarbons, pesticides and dyes, etc.) by enzyme activity³⁰.

Aspergillus species are cosmopolitan micro-filamentous fungi that exist in almost all soil types³¹. They have been reported to be isolated from different types of contaminated soils with pH ranging from 3.5-9.0, varying degrees of anthropic pollution, toxic elements exceeding permissible limits that may affect their growth as well as causing genome alteration resulting in physiological changes and production of different secondary metabolites such as mycotoxins²⁵. They are very important in the degradation of complex materials like plant polymers, toxic organic and inorganic compounds and as well human tissues and antique parchments³². Many types of research have proven the existence of a wide variety of *Aspergillus* species in dye-contaminated soils. Some of these include, *A. flavus, A. niger, A. fumigatus* and *A. terreus*^{24,33-36}. The presence of these species is a result of their potential in degrading dyes and other contaminants in the soils²⁴.

Candida species occur naturally on animal skin, mouth, vaginal mucous membranes, stools, plant leaves, flowers and soils associated with domestic and industrial wastes and aquatic environments, as such, are much more resistant to environmental influences³⁷. To date, *Candida* species have been reported to be isolated from oil-contaminated soils, with no records of isolation from dye-contaminated soils, which may be due to its high toxicity³⁰. Some species like *C. albicans* and *C. tropicalis* isolated from oil-sludge soil have been used in the remediation of azo dyes and other toxins^{30,38}.

One of the major inhabitants of soil are members of the genus *Fusarium* existing in both pathogenic and non-pathogenic forms. Many are soil-borne pathogens and/or facultative parasites associated with plant debris and roots that can survive in extremely contaminated soils³⁹. *Fusarium moniliforme, F. oxysporum, F. poae* and *F. solani* are some of the species isolated from dye-contaminated soils and as well used in the decolourisation of textile dyes and treatment of textile effluents^{40,41}.

Penicillium chrysogenum, P. frequentans, P. lanosum and *P. notatum* were reported to inhabit dye-contaminated soils, metabolising dyes and other elements^{36,42}.

Pele *et al.*⁴³ used *Rhizopus* strains (*R. arrhizus* and *R. microsporus*) isolated from contaminated soils of Caatinga to produce biosurfactants that play an important role in environmental protection, oil spill management, degradation and detoxification of oil-containing industrial effluents and soils. In another study, *Rhizopus* species isolated from dye-contaminated soil was used to decolourise azo dyes used in textile industries⁴⁴. *Rhizopus oryzae* and *R. stotonifer* isolated from the agricultural waste dump and oil spill sites respectively were subsequently used in degrading environmental pollutants⁴⁰.

In this study, there were no microalgal species isolated from the dye-contaminated soil which may be due to its toxicity, as most algae survive in less polluted environments. They are also termed bioindicators of pollution due to their high sensitivity to pollutants⁴⁵. Several types of research have revealed that some indigenous and tolerant microalgal species are introduced to and subsequently survive in polluted environments due to their ability to remediate toxic substances, thus, resulting in gradual clear-up of contaminants⁴⁶.

The presence of synthetic dyes in the environment is undesirable, as it causes serious environmental pollution due to their colour and toxicity. The species in this study can be used in the remediation of such dyes due to their tolerance and survival in the contaminated soil. Also, previous research has shown that most of the organisms surviving in contaminated environments can remediate dyes and other pollutants through enzymatic action and biosorption. There is a need to cultivate high biomass of such organisms which could be used to reduce environmental pollution.

CONCLUSION

A total of fifteen species were isolated from the dye-contaminated soil of the K/Na'isa dye pit. Two were bacterial species from the genus *Bacillus*, while the remaining thirteen were fungal species from different genera (seven from *Aspergillus*, one from *Candida*, two from *Fusarium*, two from *Penicillium* and one from *Rhizopus*) making them the most abundant. There were no algal species isolated from the soil.

SIGNIFICANCE STATEMENT

In Kano, wastewater from the re-dyeing process is usually discharged to open spaces as well as surrounding soil and drains that subsequently flow to ditches, burrow pits, ponds and other habitats. This makes the receiving soils poor in physicochemical properties, thus, increasing susceptibility to erosion, decreasing productivity and sustainability and as well affecting food chain quality and microbial biodiversity. However, few organisms may try to adapt to the dye-contaminated environments.

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