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Isolation and characterization of some autochthonous microorganisms from dumpsite leachates in Ogun State, Nigeria

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Abstract

Municipal solid wastes are materials generated through man's daily needs and used for life sustainability that presumably regarded as unwanted to individuals. Soil microorganisms, fungi and bacteria are known to colonize the waste, the degradation and transformation of biodegradable materials in the waste contents. The physical and chemical assessment of the leachate samples parameters measured were the hydrogen ion concentration (pH), Total Dissolved Solids (TDS), and electrical conductivity (EC) parameters, and metals such as K+, Ca2+, Mg2+, Na+, Cl-, NO3-, SO42-, and PO43-. The Aliquots from each leachate-filled bottle were drawn with a sterile pipette and used for isolating the bacterial and fungal ecology aseptically. One-milliliter of leachate samples were poured into 9 ml of distilled water and mixed thoroughly. Serial dilutions were made at 10-1 to 10-5 for the bacteria. The same dilution levels for fungi, were cultured using the pour plate method on Potato Dextrose Agar (PDA) to determine heterotrophic fungi (HF) in the leachate. The physical and chemical parameters of the leachate are Temp (°C), 27.00; pH 8. 5; TDS (mg/L), 280; BOD (mg/L), 11; EC (µS/cm), 632; K+ (mg/L, 26; Ca2+ (mg/L), 170; Mg2+ (mg/L), 32; Na+ (mg/L), 12; Cl- (mg/L), 121; NO3–(mg/L), 27; SO42- (mg/L), 58; PO43- (mg/L), 132. And the autochthonous microbial contents from the soil and leachate from the dumpsite are similar; vis: *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger, Penicillin* sp., *Rhizopus* sp., *Saccharomyces* sp., *Fusarium* sp., *Bacillus* sp., *Micrococcus* sp., *Corynebacterium* Sp. Pseudomonas sp. and Escherichia coli.

Keywords: *Aspergillus flavus*; Degradation; Heterotrophic Fungi; Leachate; Municipal solid wastes and Parameters

1. Introduction

Human anthropogenic engagements in manufacturing products and processing varieties of goods for consumption have resulted in the proliferation of solid waste generation; landfilling and dumpsites are simple, cost-effective means of managing municipal solid wastes; interestingly, most dumpsites and landfills in third-world nations run less than the recommended limits [1], in these countries, dumpsites and waste landfills are openly-sited around public institutions, estates and sometimes close to streams and water servicing the community [2]. Municipal solid wastes are materials generated through man daily needs and used for life sustainability that presumably regarded as unwanted to individuals, Although man has control over their generation and disposal [3]; the challenge of waste management is increasingly becoming alarming due to some waste not being reused or recycled [4].

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Waste that is not treated and handled very well threatens human life in the short term and the environment in the long run [5]. Some waste disposal techniques in some countries, like waste dumps, landfills and incinerators, have proved inadequate [6]. And this has contributed to the pollution of the environment.

Microbial degradation involves chemical transformations mediated by soil microorganisms during which they satisfy their growth and energy requirements and detoxify their immediate environment in the process [7]. Soil microorganisms, fungi and bacteria are known to colonize the waste, carrying out the degradation and transformation of biodegradable (organic) materials in the waste contents [8].

Bioremediation is a strategy that utilizes biological agents to convert, extract or treat a polluted environment with their metabolic activities, use plants to remove harmful contaminants and pollutants and eventually renew the ecosystem to its status quo before it was polluted [9]. These strategies include natural attenuation, bio-augmentation, or bio-stimulation, involving microscopic organisms such as fungi, algae, and bacteria. Microorganisms are ubiquitous and found in any habitat. They thrive in soil, water, plants, animals, deep water, and ice. Biological mediation technology is widely used and growing exponentially [10].

Leachate is a liquid formed when water passes through the waste in a dumpsite [11]. Leachates have more pronounced characteristics than sewage. According to Englehardt et al. [12], a typical young leachate may have a chemical oxygen demand (COD) 36 times higher than raw sewage.

If the leachate discharge is not controlled and treated, it will cause serious environmental problems and threaten the surrounding environment, including soils, aquifers, and surface water [13]. Hence, this study aims to isolate and identify autochthonous microbial contents in the leachate from a dumpsite in Ikoto. Ogun, Nigeria.

2. Methods

2.1. Study area

The soils and leachates from a solid waste dumpsite, Ikoto, Odogbolu Local Government, Ogun State, were sampled.

2.2. Collection and processing of samples

2.2.1. Soil samples

Soil samples of 50 g each starting from the base of the dump site were taken randomly from 100 cm intervals upstream until five samples were collected, each sampled in separate sterile zip polyethene bags.

2.2.2. Leachate sampling

Ten milliliters of leachate samples were collected randomly at a distance of 100 cm from each other. Five samples were collected and transported in individual coloured sterile PET bottles and rapped with aluminum foil to the laboratory within 30 min.

2.2.3. The physical and chemical characterization of the leachate sample

The physical and chemical assessment of the leachate samples was carried out, following Solesi et al. [14], by measuring the hydrogen ion concentration (pH), Total Dissolved Solids (TDS), and electrical conductivity (EC) parameters, and metals such as K^+, Ca^{2+},Mg^{2+}, Na^+, Cl^-, NO^{3-}, SO_4^{2-}, and PO_4^{3-} elemental content. Hydrogen ion concentration (pH), Temperature (°C), Electrical Conductivity (EC), Total dissolved solids (TDS), Ca^{2+}, Mg^{2+}, Na^+, K^+, and Cl^-, NO_3^{2-}, SO_4^{2-}, and PO_4^{3-}, and biochemical oxygen demand (BOD), using HI98129 electrode calibrated with buffers of pH 4.0 and 9.0 to measure pH, Temperature, electrical conductivity, and total dissolved solids (TDS) on site. Cat-ions determination of Ca^{2+}, Mg^{2+}, Na^+, and K^+, using PinAAcle 500 Flame Atomic Absorption Spectrometer and Hach DR/2000 spectrophotometer for NO^{3-}, SO_4^{2-}, and PO_4^{3-}.

2.2.4. Isolation and identification of microbial ecology

The Aliquots from each leachate-filled bottle were drawn with a sterile pipette and use for isolating the bacterial and fungal ecology aseptically. One-milliliter of leachate samples were measured, poured into 9 ml of sterile distilled water and mixed thoroughly. Serial dilutions were made at 10^{-1} to 10^{-5} for the bacteria. The same dilution levels for fungi were cultured using the pour plate method on Potato Dextrose Agar (PDA) to determine heterotrophic fungi (HF) in the

leachate. All media and distilled water were autoclaved at 121°C for 15 minutes. Each distinct colony was picked with an inoculating loop for subculture to determine the pure cultures of the isolates.

Two grams of each soil sample were diluted with 10 ml of sterile distilled water in test- tubes and shaken vigorously. A drop of the aliquot from each samples-aseptically placed in the centre of a disposable petri- dish using the pour plate technique and incubated for five days for mycelium growth of the heterotrophic fungi species; the PDA was fortified with Streptomycin (0.03 g/l) and hydrogen peroxide (6%) drop-wisely for fungi to avoid bacteria contamination and 48 h in nutrient agar for bacterial species.

The identification of isolates was through their colonial cell morphology and biochemical tests. Microscopic identification of the isolated fungi was under-taken using methylene blue to stain the fungal slides under the light microscope. For bacterial identification, the data obtained were compared with standards obtainable in Bergeys manual of the determination bacteriology [15].

3. Results

3.1. Physical and chemical characterizations

Table 1 The parameters measured from the leachate samples

SN	Parameters	Values (mean(n=5))	WHO STD	FEPA STD
1	Temp (°C)	27.00	20 – 30	NA
2	pH	8. 56	6.5 - 8.50	6.00-9.00
3	TDS (mg/L)	280	500.00	NA
4	BOD (mg/L)	11	NS	30.000
5	EC (µS/cm)	632	100.00	125.000
6	K^+ (mg/L	26		100.00
7	Ca^{2+} (mg/L)	170	150.00	50.00
8	Mg^{2+} (mg/L)	32		125.00
9	Na^+ (mg/L)	12		0.5
10	Cl^- (mg/L)	121	250.00	100.000
11	NO^{3-} (mg/L)	27		20.00
12	SO_4^{2-} (mg/L)	58	100	100.000
13	PO_4^{3-} (mg/L)	132		50.00

3.2. Isolation and identification of isolates

The total number of isolates found in this study was 239. One hundred and forty-six were fungi, while the bacteria were 93 isolated from the dumpsite - Soil and leachate (Table 2). The fungi were identified following Ahmed et al. [16], using the mycology atlases and other studies based on colony morphologies and microscopic identification.

Table 2 Isolates identified in soil and leachates of the dumpsite

Name of isolates	Soil sample					No of species	Leachate sample					No of species	Total no. of isolates
	S1	S2	S3	S4	S5		L1	L2	L3	L4	L5		
Aspergillus flavus	5	7	3	4	6	25	1	0	1	1	3	6	31
Aspergillus fumigatus	8	2	0	4	0	14	0	2	1	1	0	4	18
Aspergillus niger	6	4	6	3	7	26	0	0	1	0	1	2	28
Penicillin sp.	2	1	0	2	3	08	1	2	3	1	0	7	15
Rhizopus sp.	3	5	7	1	0	15	0	0	0	0	0	0	16
Saccharomyces sp.	7	4	0	3	4	18	2	0	1	0	0	3	21
Fusarium sp.	2	0	4	2	6	14	1	0	0	1	1	3	17
Bacillus sp.	6	4	7	3	2	22	1	0	0	0	0	1	23
Micrococcus sp.	2	5	3	1	0	10	0	2	0	1	0	3	13
Corynebacterium sp.	4	6	2	1	1	14	0	0	0	0	0	0	14
Pseudomonas sp.	3	7	3	0	2	15	0	0	1	0	0	1	16
Escherichia coli sp.	6	7	2	2	3	22	1	2	1	0	2	6	27
Total						203						36	239

4. Discussion

The average values from several points of the leachate samples taken from the dumpsite were showed in Table 1. The temperature value is 27 °C. This value falls within the WHO and FEPA limit values. And TDS (mg/L), BOD (mg/L), K^+ (mg/L), Mg^{2+} (mg/L), and SO_4^{2-} (mg/L) were also within the FEPA and WHO guidelines.

Nitrate value is in excess could be attributed to bio-combustion in the dumpsite releasing nitrogen oxide, as reported by Ojoawo et al. [17] The result of leachate characteristics here agrees with those quoted by [18] in a study on Lagos dumpsites.

The values for Ca^{2+} (mg/L), Cl^- (mg/L) and PO_4^{3-} (mg/L) were more than the recommended values by FEPA at the study dumpsite because this site commonly other cities near it, with all kinds of waste ranging from paper waste, foodstuff waste from markets, metals processing waste etc.

The conductivity mean value of 632 g/cm was higher than the specified limit of 125 g/cm. Electrical Conductivity and Total Dissolved Solids are significant measures that pinpoint the degree of salinity and minerals of the leachate sample.

The pH value of the leachate sample was 8.56, indicating that the leachate sample of the study area was alkaline, reflecting the mature state of the dumpsite. This pH value was suitable for many alkalophilic fungi and suggested the kind of fungi found in the dumpsites, as reported by Naga et al. [19]. The genera of fungi isolated in the leachates are classified as filamentous fungi family and indigenous in soils [20]. The finding is in concordance with the pH values, as reported by Sebastião and Paulo [21]. The authors isolated soil fungi, such as *Penicillium* sp., *Fusarium* sp. and *Aspergillus* sp., which usually grow in alkaline pH. In this study, the fungal communities isolated from the dumpsite were *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Penicillium* Sp., *Rhizopus* Sp., *Saccharomyce*s Sp., and *Fusarium* sp., and are similar to the ones identified by Obire et al. [22] from the soils sampled from a similar dumpsite in Rivers State, Nigeria, This explains the fungi high dispersal potential; they propagate their spores by air dispersal and assist in ecosystem equilibrium by creating ecological niches, recycling nutrients, contaminants biodegradation and importantly in symbiotic associations [23, 24].

The species of *Aspergillus flavus*, *Aspergillus fumigatus*, and *Aspergillus niger* were in high proportion in the soil samples from the dump site. *A. flavus* and *A. fumigatus* are the two most risky strains of aspergillus that cause aspergillosis infections in humans. Typically, the spores of these strains can cause upper respiratory sensitivity, manifesting as

asthmatic spasms, fever, and malaise. Thus, this commonly occurs in tropical countries-of the world and withstanding the weather of these nations [25].

In this study, the bacterial species isolated were *Bacillus* sp., *Micrococcus* sp., *Corynebacterium* Sp., *Pseudomonas* Sp., and *Escherichia coli*. The frequency of Escherichia coli population is so high in the leachate compared to other bacteria species. Few of these gastrointestinal pathogens, such as Escherichia coli, have been identified in leachate samples from the San Nicolas landfill, Mexico [26]. The Municipal Solid Waste biodegradation potency of many microorganisms associated with waste is a factor in their generation time or ability to increase their populations in time. Some of these bacterial isolates with high potential to degrade municipal solid waste and use them as the sole source of carbon/energy were Pseudomonas spp. and Bacillus sp., while *Aspergillus* sp. *Mucor* sp. and *Saccharomyces* sp. are the fungal species that can use municipal solid waste as source of carbon/ energy [22]

5. Conclusion

Leachates from the dumpsites pose a high level of danger in environmental pollution. The results from this study showed that autochthonous fungi; *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Penicillium* Sp., *Rhizopus* Sp., *Saccharomyces* Sp., and *Fusarium* Sp were predominantly organisms in the leachate, though, they also contain a meaningful range of pathogenic indicating bacterial species.

Although microbial isolated may be used in the bioremediation of the leachate, leachate usually consists of a large quantity of organic materials resulting from biological degradation of the dumpsite.

Compliance with ethical standards

Disclosure of conflict of interest

All authors declare no conflict of interest.

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