Survey of Microorganisms (Fungi) Associated with Dumpsites in Rumuolumeni, Port Harcourt, Rivers State

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Abstract

This study was conducted to survey and identify fungi associated with Eagle cement dumpsite, Rumuolumeni, Port Harcourt. Soil samples were taken from the two waste-dump sites at different points. Soil samples were taken into different clean nylon bags and taken for culture on sterile and freshly prepared Plate Count Agar (PCA). All techniques were carried-out under manufacturers' instructions standard laboratory conditions. The inoculation was done in triplicate and recorded as average total viable fungi in the soil sample. Four (4) Fungi were isolated, characterised, and identified from Eagle cement dumpsites. The fungi identified were Aspergillus flavus, A. niger, Penicillium spp., and Fusarium spp of all the organisms isolated during the period of survey. Aspergillus flavus fungi species has the highest occurrence of 36.3% with Penicillium spp. and Fusarium spp. having the least (18.2%). A total of fungi counts of 9.89 × 10^7 was recorded with an average mean of 3.29 × 10^7.

INTRODUCTION

Soil is a critically important component of the earth's biosphere, functioning not only in the production of food and fiber but also in the maintenance of local, regional and global environmental quality (Glanz, 2015). Increasing human populations, decreasing resources, social instability, and environmental degradation pose serious threats to the natural processes that sustain the global ecosphere and life on earth (Constanza, 2012). Of the many problems associated with urbanization especially in sub-Sahara Africa, waste management crisis has assumed an important position. Today, waste disposal has become an acute problem in numerous urban centers across Africa (Pasquini and Harris, 2005), with formal waste collection ranging from 11 to 44 % for households in cities such as Accra, kinshasha, Lagos, Kaduna and Enugu (Pasquini and Harris, 2010). Nigeria has an enormous waste management problem, all over the country; there are examples of unsanitary open dumps and industrial contaminations which are continuously discharged on land and into streams and rivers without treatment (Agunwamba, 2008).

Dumpsite is a traditional way of waste disposal same with landfill method for managing waste. A landfill site or dump-site is a site for the disposal of waste materials by incineration or burial and it is regarded as the oldest form of waste treatment (Babayemi, 2009). Many things are discarded everyday ranging from ordinary rubbish to old newspapers, packaging, cleaning materials, and many different kinds of junk. Methods of waste disposal pose threats to both man, animals and soil. Poisonous plants, insects, animals and other pathogens are biological hazards that may be encountered at the dumpsite (Achudume, 2007). Dumpsites are mostly established in disused mining quarries or excavated pits far away from residential settlement. Designated agency, corporate bodies and individuals routinely collect waste into these dumpsites (AbdulsSalam, 2009). Dumping waste into uncontrolled open landfills has been the predominant way of waste depositing for a long time (Reddy, 2011) and still is on a global scale. As some components of municipal solid waste and subsequently the leachate and gas emissions cause essential health and environmental problems (Reddy, 2011), developed countries became aware of the importance of reducing the amount of MSW that is landfilled and emphasised on certain political and legislative actions that would lead to the desired decline (Blumenthal, 2011). In Nigeria, modern landfill facilities are not easily found, therefore waste sorting-out into degradable, non-degradable and recyclable may not be achieved (Osazee, 2013). Poor waste dumpsites management could create adverse environmental impacts such as wind-blown litter, attraction of rodent and pollutant for example leachates that can contaminate underground soil bed or aquifer (Abduls-Salam, 2009).

Microorganisms present in the refuse use the refuse as a food source. The microorganisms found in waste dumpsites obtain their nutritional requirements from the waste constituents, thus aiding the detoxification of complex organic molecules (Osazee, 2013). However, some solid waste can also contain large amount of organic pollutants that can persist in the environment (Williams and Hakem, 2016). In a study carried out by Simon, (2018) on the identification of Fungi on two dumpsites in Akure. The following fungi species were identified; *Aspergilus Flavus*, *M. Mucedo*, *Aspergilus niger*, *Candidia Albicans*, and *Neurospora spp*. From the experiment, *Neurospora sp* showed the highest occurrence of 5.50 sfu which was significantly different ($p < 0.05$) from those of other fungi except *A. flavus* (5.25 sfu). *M. mucedo* showed the least occurrence of 2.00 sfu which was significantly lower than that of *A. flavus, A. niger, C. albicans* and *Neurospora spp*. In Fiwasaye dumpsite, the occurrence of *A. niger* was significantly higher ($p<0.05$) than those of *A. flavus, F. moniliforme, M. mucedo, C* *albican* and *Neurospora spp.* Similarly, *C. albican* and *A. fumigatus* showed the least occurrence in Fiwasaye dumpsite. In the month of August in Igbatoro dumpsite, *A. flavus* and *A. niger* showed the highest occurrence of 4.00 cfu which was significantly higher than that of *F. moniliforme* and *M. mucedo.*

Pathogenic microorganisms and harmful chemicals in solid waste can be introduced into the environment when the waste is not properly managed (Wai-Ogosu, 2004; Ogbonna et al., 2006). Waste can contaminate surface water, ground water, soil and air which poses more problems for humans, other species, and ecosystems (Obire et al., 2002). Thus, the objective of the study was to identify the microorganisms associated with some waste dump sites around Port Harcourt metropolis.

Clinical and public health microbiologist recognition that microbial biofilms are ubiquitous in nature has resulted in the study of a number of infectious disease processes from a biofilm perspective. Cystic fibrosis, native valve endocarditis, otitis media, periodontitis, and chronic prostatitis all appear to be caused by biofilm-associated microorganisms. A spectrum of indwelling medical devices or other devices used in the healthcare environment have been shown to harbor biofilms, resulting in measurable rates of device-associated infections (Donlan, 2002). Biofilms of potable water distribution systems have the potential to harbour enteric pathogens, *L. pneumophila*, nontuberculous *mycobacteria*, and possibly Helicobacter pylori. What is less clear is an understanding of how interaction and growth of pathogenic organisms in a biofilm result in an infectious disease process.

Microbial degradation of solid waste implies the breaking down of organic components of waste to inorganic form by the microorganisms, which can readily serve as nutrient for a variety of other organisms. When wastes are dumped on land, soil microorganism such as bacteria, fungi and worms (helminthes) readily colonize the waste carrying out degradation or transformation of degradable (organic) materials in the waste. Similarly, mesophilie, thermophilic and their thermo-tolerant bacteria in the waste use the constitutuents as nutrient, thus detoxifying the materials as their digestive process, breakdown complex organic molecules into simpler fewer toxic molecules (Boulter, 2002). Bacteria are the dominant population in the entire waste degradation process (Rebollido, 2008). A variety of such waste microbes are widespread in the environment. They are found in abundance in the soil habitat (108 -109 g -1 of soil) (Van Elsas, 2011). Of the waste degrading microorganisms, the aerobic populations are preferred because of their efficiency in degradation process. During the process, microorganisms use the crude organic matter as source of food thereby producing heat, carbon dioxide, water vapor and humus (Tiquia, 2005). Most of these microbes are potential human pathogens (such as *Salmonella spp, Escherichia coli*, Fungi and Protozoan) and may cause severe health hazards (Obire, 2002).

The number of pathogenic organisms in MSW in developing and non-developed countries is found to be higher due to improper waste management and lack of public conveniences in most Urban Centres (Obire, 2002). Pathogenic bacteria that may be associated with faecal contamination include pathogenic strains of *Escherichia coli, Salmonella species, Shigella species* and *Vibrio cholerae* (Achudume and Olawale, 2007; WHO, 2013).These are pathogens and, hence capable of causing infection and pose great risk to human health and animals when increased in population (Onweremadu, 2009). Many countries in Africa do not have efficient waste collection and disposal services, which often results in both environmental and health problems for the people. Because these wastes are not properly disposed off, they constitute serious health problems, such as dissemination of infectious diseases to man and animals living within the vicinity (Lorentz, 2000). The use of waste dump as a preferred method of solid disposal is unscientific and constitutes a nuisance to the public; hence, the resultant effect is pollution. When waste is dumped on land, microorganisms such as bacteria and fungi proliferate. Most of these microorganisms are potential human pathogens and may cause severe health hazards (Wachukwu, 2010). Bartram et al. (2003) and Onweremadu et al. (2009) discovered that bacterial pathogens in wastes that lack leachaete collection system are source of pathogens to the soil. These pathogens when increased in population, pose great risk to human health (Achudume and Olawale, 2007). The microbial community of soil may be expected to vary considerably, depending on the type of waste and other factors. Mbata (2008); Oviasogie et al. (2010); Wachukwu et al. (2010) Osunwoke and Kuforiji (2012) identified many bacteria general including *Arthrobacter spp.*, *Bacillus spp. Micrococcus spp.*, *Poteus spp.*, *Pseudomonas spp.*, *serratia spp.*, *staphylococcus aureus*, *staphylococcus spp.*, *Streptococcus*, *Esterichia coli*, *Klebsiella spp.*, *streptococcus spp.*, *Salmonella spp.*, *Vibrio spp.*, in waste. Staphylococcus is an opportunistic pathogen in that it causes infections most commonly at sites of lowered host resistance, for example, damaged skin or mucous membranes (Arora, 2004). *Staphylococcus aureus* has been associated with several diseases, especially superficial infections, osteomyelitis, septicaemia and otitis media. Klebsiellae in general, are more frequently involved in hospital-associated urinary tract infections, wound and burn infections and as secondary invaders in other respiratory infections. In fact, they are the most frequently encountered gram-negative pathogen causing nosocomial infections of the lower respiratory tract and are second only to *Escherichia coli* as a cause of primary bacteraemia by gram-negative organisms. They may also cause meningitis and diarrhoea (Harris and Paxman, 1982). *Bacillus anthracis* and *Bacillus cereus* have been incriminated in human and animal disease. *Bacillus anthracis* is one of the highly pathogenic microorganisms known to mankind. Infections by other members of the genus are rare; however, they occasionally cause gastrointestinal disease in the immunocompromised and acute purulent meningitis. Bennett (2011) reported the isolation of viable aerobic bacteria from hospital and municipal solid waste dumpsites in Benin City, Nigeria. *Aerobic bacteria Escherichia coli*, *Staphyloccusaureus*, *Klebsiella spp.*were recorded indecreasing order of prevalence from municipalsolid while *Pseudomonas aeruginosa*, *Klebsiellasp*, *Bacillus substilis*, *Serratia species*,*Staphylococcus aureus*, *and Escherichia coliwere isolated from the hospital waste.* Also, Berkeley and Campbelt (2012) recorded microbial pathogens of public health significance found in waste and common sites, collected from four different dumping sites and assessed for pathogenic agents in Ede Southwest of Nigeria. The results revealed the presence of bacterial species including *Pseudomonas, Mirococcus,Actinomyces, Neisseria, Bacillus* and *Klebsiella*.

The main aim of this study was to survey the microorganisms (fungi) associated with Eagle cement waste dump site of Rumuolumeni, Port Harcourt, Rivers State. The following are the objectives of the study;

- To isolate fungi in the dump site within the study area.
- To identify and characterize the fungi found in the dump site within the study area.
- To determine the prevalence fungi species found in the dumpsite within the study area.
- To determine the physicochemical parameters at the study area

MATERIALS AND METHODS

The study was conducted in Port Harcourt, Rivers State, Nigeria. Port Harcourt lies between latitude 4°46'38.71" N and longitude 7°00'48.24" E. It is located in the tropical rainforest in Nigeria. Port Harcourt is a metropolitan city with over a million people; it is made up of people from different ethnic groups. Rivers State is rich in oil and gas, and as a result, a significant number of foreign nationals work in the oil and gas/allied industries, most of which were resident in Port Harcourt. The population of people in the city has increased leading to increased industrial and domestic solid waste, which are dumped indiscriminately on open lands. The physical parameters observed on the waste dump sites include temperature with the use of a simple calibrated thermometer.

Sample Collection

Soil samples were taken from the Eagle cement waste dump sites at three different points (P1, P2 and P3) established at the front, middle and end of the dumpsite for isolation and identification of fungi species present. A random sample of the soils were taken into different clean nylon bags and taken for culture on sterile and freshly prepared Plate Count Agar (PCA). All techniques were carried-out under manufacturers' instructions standard laboratory conditions. The inoculation was done in triplicate and recorded as average total viable fungi in the soil sample (Ewekeye, 2012).

Preparation of Culture

The culture media used was Plate Count Agar (PCA). PCA prepared by dissolving 3.9 g to make up 100 ml of distilled water in a conical flask. One percent streptomycin was added to molten PCA in order to suppress bacteria growth. They were sterilized along with the agar in the conical flasks in an autoclave (GallenKamp) at 121°C for 15 minutes (Ewekeye, 2012) and Chloramphenicol an antibiotic was added after autoclaving to inhibit the growth of bacteria in the medium.

This involved transferring a small piece of mycelium free of medium using a sterile inoculation loop unto a glass slide containing a drop of cotton blue-in-lacto phenol and the mycelium was spread properly. The preparation was covered with a covered slip and observed under medium power (x100) and later at high power (x400) magnifications. The observations made were used in identifying the fungi organisms. Using visible observation (macroscopic examination) and microscope at low power magnification (x40), parameters such as colony characteristics of the submerged hyphae whether rhizoid, spiral or regular and characteristic shape of mature fruiting bodies were all observed.

Isolation of Fungi

Microbial (fungal) isolation was carried out on the soil samples using the standard method of serial diluted and pour plate technique. Preliminary investigations showed dilutions of 10^{-5} for fungal growth. One-milliliter of each dilution was plated in triplicate on PCA to which 0.1% Chloramphenicol has been incorporated to inhibit bacterial growth. The plates were incubated at 28±2°C for 3-5 days. The pure cultures of fungal isolates were identified based on cultural and morphological characteristics according to Barnet and Hunter (Faith, 2010).

Statistical Analysis

All data obtained were subjected to analysis of variance (ANOVA) at $p < 0.05$ and where significant, means were separated using Tukey's test.

RESULTS

Fungi associated with Eagle cement waste dumpsite.

Table 1 shows the total fugal count of 9.89×10^7 was recorded with an average mean of 3.29×10^7. Three points were established on the dumpsite. Point 2 which happens to be the middle of the dumpsite shows higher figure of 4.85×10^5 compared to other established points on the dumpsite.

Table 1: Total fungal count at P1, P2 and P3 of eagle cement waste dumpsite, Port Harcourt.

Sample site	Total Fungal count (cfu/g)
Point 1	**2.53×10^7**
Point 2	**4.85×10^7**
Point 3	**2.51×10^7**
Total Mean Value	**$9.89 \times 10^7 \pm 3.29 \times 10^7$**

Point 2 which happens to be the middle of the dumpsite shows higher figure of 4.85×10^5 compared to other established points on the dumpsite.

Table 2 result revealed that Fungi identified were *Aspergillus flavus, A. niger*, *Penicillium spp*., and *Fusarium spp* the average temperature obtained from the dumpsite was 27^0C and the average pH obtained was 7.7.

Table 2: Frequency Occurrence of Fungi species isolated from eagle cement dumpsite.

Fungi	**Fungi Isolates**	**Frequency Occurrence %**
Aspergilus flavus	4	**36.3%**
Aspergilus niger	3	**27.3%**
Penicillium spp.	2	**18.2%**
Fusarium spp.	2	**18.2%**
Total	**11**	**100**

Aspergillus flavus fungi species has the highest occurrence of 36.3% with *Penicillium spp.* and *Fusarium spp.* having the least (18.2%).

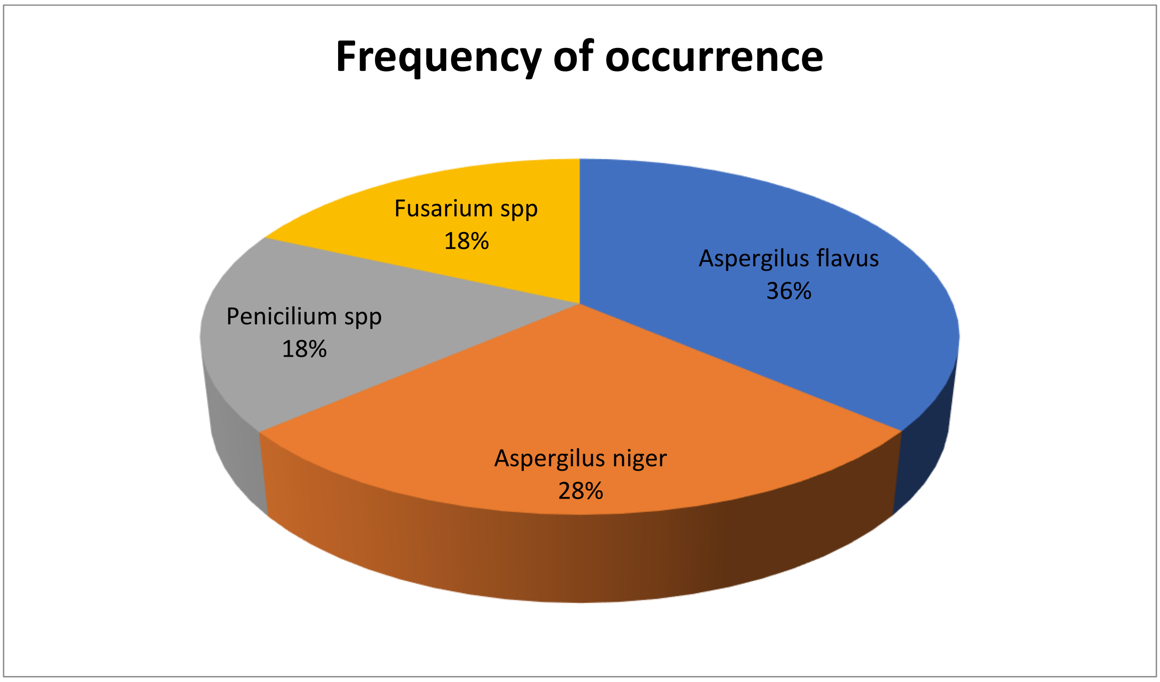

Fig 1: A pie chart showing the frequency occurrence of fungal species at Eagle cement dumpsite, Port Harcourt.

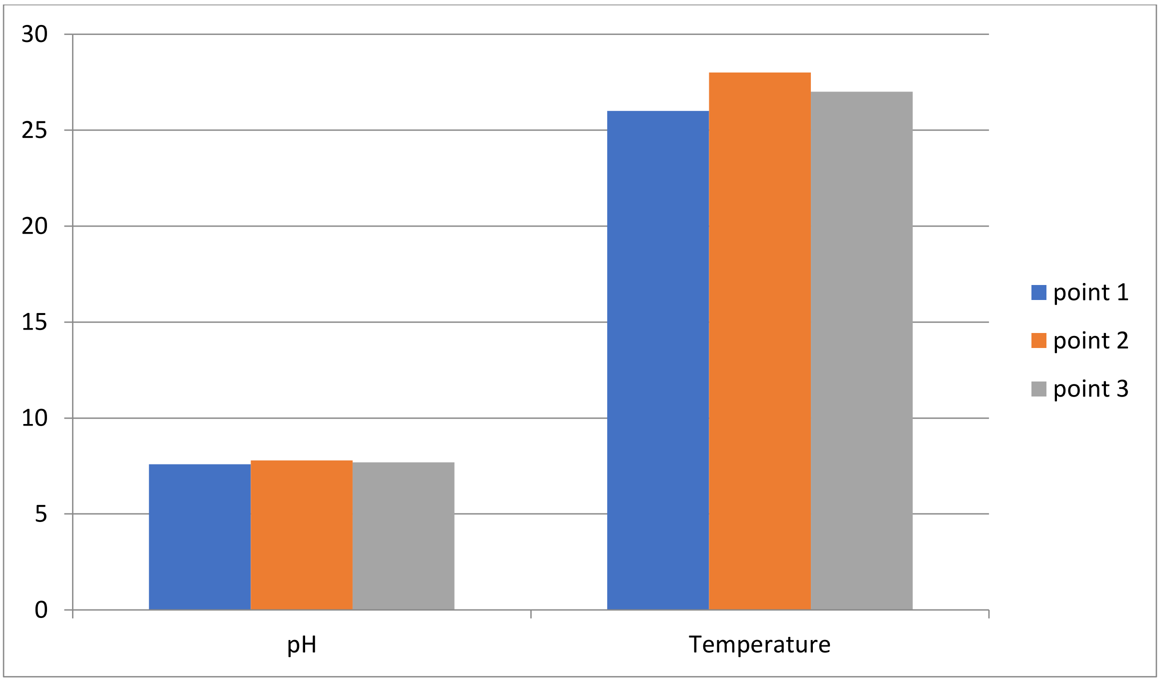

Fig 2: A chart showing temperature and pH of the dumpsite.

DISCUSSION

The total fungal count on the dumpsites (P1, P2 and P3) is shown in table 1. All samples were taken 10 feet away from each other. The species of fungi isolated in this study is also reported in table 2. Fungi species isolated were Aspergillus flavus, Aspergillus niger, Fusarium spp and Penicillium spp. Fungi, especially Aspergillus spp secrete mycotoxins that are poisonous to health when contacted (Onuegbu, 2002). The high occurrence of Aspergillus spp in the dumpsites may be attributed to its high ubiquitous nature. They are widely distributed and have been observed in a broad range of habitats because they can colonize a wide variety of substrates. Aspergillus spp is commonly found as a saprophyte growing on dead leaves, stored grain, compost piles, and other decaying vegetation (Velsivasakthivel, 2014). Also, the temperature observed at the Eagle Cement waste dumpsites ranged between 27 and 28°C. These values fell within the mesophilic range of temperatures for most pathogenic fungi whose optimum temperature for growth is 37 ˚C, with upper and lower temperature limits of 40-50 and 15-20 °C respectively (Arora, 2004). Obire et al. (2002) reported that during initial compositing development, the mesophilic flora predominates, and are responsible for most of the metabolic activities that occur. This increased microbial activity elevates the temperature of the compost with the subsequent replacement of the mesophilic population by thermophilic flora such as Aspergillus spp found in this study. However Liu et al., (2020) reported that in the first 2 to 3 days of composting, the pH drops to 5.0 or less, then begins to rise to about 8.5 for the remaining aerobic process. The degree of acidity (pH), reported in this study for all the waste dumpsites ranged from pH 6.5 and 7.8, which is been supported by previous findings which reported a pH range of 5.4 to 7.9 and temperature of 27-28 °C respectively (Obire et al., 2002; Ogunwande et al. 2020; Lee et al., 2019 and Al-khatib et al., 2019).

The isolated fungi species in the present study are responsible for causing many diseases and risks to the community, such as skin infection and pulmonary disease. A study by Oyedepo et al., (2019) reported that dumpsites were a breeding ground for pathogenic fungi, which could lead to respiratory problems and other issues. Many fungi are opportunistic organisms and cause infections during extended antibiotic treatment and severe immunosuppression such as Aspergillus spp (WHO, 2002). The use of phytoremediation techniques, such as phytoextraction and Phyto-stabilization has been shown to be effective in reducing fungal growth and contamination in dumpsites. A study by Singh et al. (2020) found that phytoextraction can significantly reduce the growth of fungal species in dumpsites, with Phyto-stabilization showing effectiveness in reducing the risk of fungal contamination in soil and water resources (Kumar et al., 2020). Interestingly, investigation of fungal communities revealed that diversity in the most contaminated soil was significantly higher at 1.0 m compared to the other soil samples; no other significant trends were observed within the community dynamics. However, the most contaminated soil (TP30) had a higher diversity and the highest capacity to support a broader diversity of species (carrying capacity) compared to the other soil samples.

Studies have consistently shown that dumpsites provide a conducive environment for the growth of microorganisms like fungi (Onuegbu, 2002; Velsivasaktivel, 2014, Kumar et al., 2019, Rajendran et al., 2019). The high occurrence of Aspergillus spp in this study agrees with previous findings that these species are widely distributed and can colonize various substrates (Singh et al., 2020, Zhang et al., 2018). The mesophilic temperature range observed in this study (27-28°C) is also consistent with the optimal growth temperature for most pathogenic fungi (Arora, 2004). The use of microbial fuel cells has been shown to be effective in reducing fungal growth and contamination in dumpsites. Zhang et al. (2020) previously reported that microbial fuel cells can significantly reduce the growth of fungal species in dumpsites, as well as the risk of fungal contamination in soil and water resources (Li et al., 2020).

In conclusion, dumpsite creates a good nutritional ground for the growth of microorganisms such as fungi. The movement of these organisms down the aquifers due to rainfall can lead to contamination and when used by human for drinking, cooking and other domestic activities can get the human body infected leading to possible disease. This study highlight the importance of a multi-faceted approach to reducing fungal growth and contamination in dumpsites, including the use of natural amendments, microbial amendments, physical barriers, chemical treatments, biological treatments, integrated approaches, monitoring and maintenance strategies, advanced technologies, policy and regulatory frameworks, public education and awareness campaigns, alternative waste management technologies, nanotechnology, bioremediation techniques, phytoremediation techniques, and microbial fuel cells. Therefore, the recommendation for effective waste management systems to prevent open dumping and reduce environmental and health risks is supported by the findings of this study and previous research. Due to the findings obtained from this research, it is therefore recommended that government should employ a good waste management system as to help curtail open dumping which can lead to soil and water contamination and possibly affecting human health.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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