Antifungal Susceptibility and Evaluation of Risks Associated with Heavy Metals Distributed in Selected Dumpsite Soils of Osogbo Metropolis, Southwest Nigeria

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Abstract: The study assessed the drug susceptibility, ecological and human health risks associated with toxic metal concentrations of five dumpsite soils in Osogbo metropolis, Southwest Nigeria. Fungal distribution and susceptibility, heavy metal (Cd, Cu, Fe, Pb, and Zn) levels of Egbedi, Gbonmi, Ilesa garage, Omobolanle, and Oke-Baale dumpsite soils were determined following standard protocol. Pollution, ecological, and human health risk indices were also estimated. A total of 17 fungal isolates were obtained, with *Aspergillus* and *Penicillium* genera being prevalent. All the isolates (17) were susceptible to voriconazole while 10 and 16 were resistant to amphotericin B and fluconazole, respectively. The level of toxic metals in the soils was in the descending order: Cu > Fe > Pb > Cd > Zn except in Egbedi whose Zn (64.05±0.03 mg/kg) was higher than Cd (48.45 ± 0.02 mg/kg). In this investigation, Omobolanle and Egbedi dumpsite soils showed high-level pollution. Cadmium was the major contaminant and contributes majorly to the high ecological risk in the areas. For both children and adults, the level of metals in the soils do not suggest a significant cancer threat. Similarly, Cd through inhalation signals extremely high non-carcinogenic risk. There is a need for effective monitoring of pollution in the dumpsites to safeguard environmental and human health.

Key word: Dumpsite soils, fungi, heavy metals, pollution, risk assessment

INTRODUCTION

Anthropogenic activities including agriculture, and industrialization, coupled with the increasing urbanization, generate wastes that have become an emerging public health concern in many nations of the world (Abdus-Salam, 2009; Abdus-Salam *et al*., 2011). Generally, municipal solid wastes (MSW) are classified as domestic, commercial, and industrial, and comprise paper, broken bottles, batteries, food wastes, glassware, metal scrapes, textiles, wood, leaves, ceramics, leather, rubber, plastics, concretes, ashes, amongst others (Obire *et al*., 2002; Obasi *et al*., 2017).

Diverse microorganisms are associated with MSW (Oshoma *et al*., 2017). Typically, dumpsites harbour bacteria and fungi capable of degrading complex organic materials while obtaining nutrients for growth and metabolism from the waste constituents (Osazee *et al*., 2013).

Furthermore, pathogenic microbes are also encountered on trash sites (Obire *et al*., 2002; Williams and Hakam, 2016). Bacteria such as *Arthrobacter* sp., *Bacillus* sp., *Escherichia coli*, *Klebsiella* sp., *Micrococcus* sp., *Proteus* sp., *Pseudomonas* sp., *Serratia* sp., and *Streptococcus* sp.; moulds including *Aspergillus* sp., *Fusarium* sp., *Mucor* sp., *Penicillium* sp., *Trichoderma* sp., and *Rhizopus* sp.; and yeast *Saccharomyces* sp. have been previously associated with waste dumpsites microbial community (Obire *et al*., 2002; Omusi *et al*., 2017; Oshoma *et al*., 2017).

In many major cities of developing countries, MSW are disposed of and incinerated in undesignated open fields, even around residential areas (Abdus-Salam, 2009; Oshoma *et al*., 2017; Boateng *et al*., 2019), which in turns breeds pathogens and disease vectors, making it completely unsafe and unhealthy for the environment and human living. It can also be a source of irritating odour, smoke nuisance, fire hazard, and pollutants which can consequently affect underground soil beds, and/or aquifers (Obire *et al*., 2002; Abdus-Salam, 2009;

Abdus-Salam *et al*., 2011; Williams and Hakam, 2016; Boateng *et al*., 2019).

Heavy metals when ingested, contacted via the skin, and inhaled, pose detrimental health effects, especially at concentrations beyond the stipulated criteria (Chonokhuu *et al*., 2019). The heavy metals are characterized by various health-related challenges including cancer, liver disorder, ataxia, dermal allergy, renal, neurological, cardio-respiratory, gastrointestinal, reproductive, and psychological imbalances (Adewoyin *et al*., 2019; Boateng *et al*., 2019). Similarly, heavy metals with their characteristic toxicity, poor biodegradability, and bioaccumulation, concentrate in animals and humans via the food chain when absorbed by plants (Li *et al*., 2016a, b). Also, they possess the ability to form complexes with dust particles that settle on surfaces and when inhaled, endanger public health (Xiao *et al*., 2017).

More than a few studies on risk assessment of heavy metals have been conducted elsewhere including but not limited to waters (Titilawo *et al*., 2018; Adewoyin *et al*., 2019; Boateng *et al*., 2019; Ighariemu *et al*., 2019), soils (Abdelhafez *et al*., 2015; Xiao *et al*., 2017; Chonokhuu *et al*., 2019), surface dust (Ma and Singhirunnusornb, 2012), cultivated plants (Obasi *et al*., 2017), frozen fish (Ukoha *et al*., 2014), and MSW dumpsites (Obasi *et al*., 2012; Teka *et al*., 2018; Tang *et al*., 2019).

The evolution of antimicrobial resistance is an almost unavoidable universal process in the microbial world. Although, fungal resistance is not as popular as bacterial resistance, yet the economic loss associated with the former is indisputably on the rise (Srinivasan *et al*., 2014). Previous works have documented development of antibiotic resistance among isolates from heavy metal-polluted sites (Dickinson *et al*., 2019). Olayiwola and Onwordi (2015) reported only the environmental risk of a major heavy metal-contaminated MSW dumpsite in a neighbouring location in Osogbo, Southwest Nigeria. In this work, the study areas are majorly surrounded by residential buildings, farming activities, and groundwater observed close to some. Children and young adults are also often seen scavenging on the dumpsites. This poses a huge health risk to the population through ingestion, dermal contact, and inhalation of fungal spores and heavy metals from the soil. In the light of this background, the present study sought to profile firstly, the distribution and susceptibility of fungal isolates from heavy metal polluted dumpsites in and around Osogbo, Southwest Nigeria, and secondly, to assess associated ecological and human risks of the fungal agents and heavy metals.

MATERIALS AND METHODS

Description of study areas and collection of dumpsite soil sample: Composite topsoil samples (0 to 10 cm deep) from Gbonmi (7°45'53''N 4°33'32''E), Ilesa garage (7°44'54''N4°34'14''E), Omobolanle (7°47'25''N4°30'5''E), Onibu-Eja (7°38'34''N4°10'44''E) and Oke-Baale (7°46'11''N4°34'21''E) environs of Osogbo, Osun State, Nigeria were collected in May 2019 into clean ziplock bags. Samples were immediately conveyed to the laboratory on icepack for further processing. Debris at ten different locations on each site were cleared, and topsoil samples (0 to 10 cm deep) were collected into ziplock bags and immediately conveyed to the laboratory on icepack for further processing. The soils collected from each location were mixed to make a composite sample and used for analysis.

Isolation and presumptive identification of fungi: A composite soil sample (1 g) was weighed, and dispensed into a well-labelled glass test tube filled with 9 ml of sterile distilled water, and serial dilution was done to the appropriate factor. Fungi were isolated using standard spread plate technique on potato dextrose agar (PDA) medium (Oxoid, England, UK) in duplicates. An uninoculated sterile PDA plate served as a control. The Petri dishes were incubated at room temperature (28 ± 2°C) for 3 to 5 days, and distinctive colonies were sub-cultured on sterile PDA plates to obtain pure cultures.

Macroscopic identification of the isolates was done by observing the growth, colour, and texture of the colonies. For microscopic identification, pure cultures of 3 to 5 days old were stained in lactophenol blue on a clean slide and examined under a microscope (X40 magnification). Hyphae, conidiophore, and conidia appearance were observed and recorded. The fungal isolates were presumptively identified by comparing the colony and microscopic characteristics with Barnett and Hunter (1999).

Antifungal susceptibility testing of the fungal isolates: Disc-diffusion (Kirby-Bauer *et al*., 1966) was employed in antifungal susceptibility testing. Three antifungal discs impregnated with voriconazole (1 µg), fluconazole (10 µg), and amphotericin B (50 µg) (Oxoid, UK) were employed. Spore suspension of the fungal isolates was prepared and standardized to 10^6 spores/ml in sterile physiological saline. One hundred microlitre (100 µl) of the standardized spore suspension was swabbed on the entire agar surface and allowed to dry, the discs were placed gently and subsequently incubated at 28 ± 2°C for 48 h. The experiment was done in duplicate, and the diameters of zone of inhibition were measured in the nearest millilitre using a ruler.

Detection of heavy metal concentrations in the dumpsite soil samples: Soil samples collected at the different sites were analyzed to detect and quantify the levels of cadmium (Cd), copper (Cu), iron (Fe), lead (Pb), and zinc (Zn). Air-dried samples were sieved through a 500 µm pore size and 10 ml of aqua regia (25 % HNO_3; 75 % HCl) was added to 1 g of each soil sample. A blank, without a soil sample served as control. Digestion was done on a heating panel until thick fumes evolved. The crystal-clear solution obtained was passed through a 0.45 µm millipore filter (Millipore, Ireland) and reconstituted to 50 ml with distilled water. Atomic Absorption Spectrometry (AAS 6200, Shimadzu, Japan) was employed to measure the concentration of Cd, Cu, Fe, Pb, and Zn in the samples (Kojuncu *et al*., 2004).

Pollution and ecological risk assessment analysis

Contamination factor (CF_r):

Contamination factor is the ratio of heavy metal concentration in the soil to the baseline value. It is expressed as equation 1.

$$\mathrm{CFr} = \frac{\mathrm{Chm-sample}}{\mathrm{Chm-baseline}} \quad (1)$$

Where, ‘$C_{hm\text{-}sample}$’ and ‘$C_{hm\text{-}baseline}$’ is the ‘heavy metal in the soil sample’ and ‘the geochemical baseline concentration’ respectively. The baseline values for the test metals are as follows: Fe: 3800, Cd: 0.8, Cu: 36, Pb: 85, Zn: 140 (IRR, 2002). Four contamination categories have been interpreted as ‘$CF_r < 1$: low contamination’, ‘$1 \leq CF_r < 3$: moderate contamination’, ‘$3 \leq CF_r < 6$: high contamination’ and ‘$CF_r \geq 6$: severe contamination’ (Islam *et al*., 2015).

Potential ecological risk (RI):

Potential ecological risk measures the possible harmful effects of heavy metals in the environment. It comprehensively evaluates heavy metals concentration in the soil, the effects of their toxicity on the environment, and pollution in relation to the equivalent property index grading method (Soliman *et al*., 2015). The RI was computed from three components: contamination factor (CF_r), estimated toxic-response factor (TR), and potential ecological risk index (E_r) (equations 2 and 3).

$$E_r = TR \times CF_r \quad (2)$$

$$RI = \Sigma E_r \quad (3)$$

The following terms were recommended for E_r and RI values:

‘$E_r < 40$, low ecological risk’, ‘$40 < E_r \leq 80$, moderate ecological risk’, ‘$80 < E_r \leq 160$, appreciable ecological risk’, ‘$160 < E_r \leq 320$, high ecological risk’, and ‘>320, serious ecological risk’.

‘RI < 150, low ecological risk’, ‘150 < RI < 300, moderate ecological risk’, ‘300 < RI < 600, high ecological risk’, and ‘RI ≥ 600, significantly high ecological risk’ (Soliman *et al*., 2015).

In this study, potential ecological risk factor index was determined subject to the availability of TR. Except, Fe, TR for Cd, Cu, Pb, and Zn were defined as 30, 5, 5, and 1 respectively (Igwe *et al*., 2017).

Health risk assessment: Evaluation of the level of effects following human exposure to heavy metals is known as health risk assessment (Titilawo *et al*., 2018). The United States Environmental Protection Agency (USEPA) carcinogenic and non-carcinogenic models by have been universally adopted to quantify human health risks through ingestion, dermal contact, and inhalation (Liu *et al*., 2013). Usually, risk assessments involve hazard identification, exposure assessment, toxicity (dose-response) assessment and hazard characterization (USEPA, 2015; Kamunda *et al*., 2016).

In the present study, Cd, Cu, Fe, Pb, and Zn were identified as potentially hazardous agents in the dumpsite soils relevant to human health. Exposure assessment was estimated for adults and children using average daily intake (CDI) of heavy metals through ingestion, dermal contact, and inhalation (Wang *et al*., 2005). Dose-response assessment estimates toxicity from exposure to levels of chemicals using the indices; slope factor (CSF, a carcinogen potency factor), and reference dose (R*f*D, a non-carcinogenic threshold). The R*f*D is derived from animal experiments using the "no observable effect level" principle (Kamunda *et al*., 2016). However, in studies involving humans, the values are multiplied by 10 to account for uncertainties (Titilawo *et al*., 2018). Risk characterization envisages the possible cancerous and non-cancerous health risk of children and adults in the study sites by employing all necessary data to obtain quantitative values of cancer risk and hazard indices (USEPA, 2004; Kamunda *et al*., 2016).

Average intake of heavy metals from the soils:

The average daily intake of heavy metals from soil through ingestion, dermal contact, and inhalation was expressed in equations 4,5, and 6.

$$\mathrm{CDIing-soil} = \frac{\mathrm{Chm{-}sample\ x\ IRS\ x\ EFY\ x\ EDN}}{\mathrm{BWT\ x\ ATM}} \times CFR \qquad (4)$$

$$\mathrm{CDIing-soil} = \frac{\mathrm{Chm{-}sample\ x\ SSA\ x\ FEY\ x\ SAF\ x\ ABS\ x\ EFY\ x\ EDN}}{\mathrm{BWT\ x\ ATM}} \times CFR \qquad (5)$$

$$\mathrm{CDIing-soil} = \frac{\mathrm{Chm{-}sample\ x\ IHS\ x\ EFY\ x\ EDN}}{\mathrm{PEF\ x\ BWT\ x\ ATM}} \times CFR \qquad (6)$$

Where '$\mathrm{CDI}_{\mathrm{ing\text{-}soil}}$' is the average daily intake of heavy metals ingested from the soil in mg/kg/day, '$\mathrm{CDI}_{\mathrm{derm\text{-}soil}}$' is the exposure dose via dermal contact (mg/kg/day), '$\mathrm{CDI}_{\mathrm{inh\text{-}soil}}$' is the average daily intake of heavy metals inhaled from the soil in mg/kg/day. The parameters employed for the health risk assessment under standard exposure conditions through different pathways is in Table 1.

Carcinogenic risk assessment:

The increasing probability of a person developing cancer as a result exposure to potential carcinogenic compound over a time of life is defined as the carcinogenic risk and evaluated using equation 7.

$$\mathrm{CR = CDI\ x\ CSF} \qquad (7)$$

Where 'CR' is carcinogenic risk, a probability (unitless) of a person developing cancer over a lifespan, 'CDI' (mg/kg/day) and 'CSF' $(\mathrm{mg/kg/day})^{-1}$ are the average daily intake and the cancer slope factor, respectively for individual heavy metal. In this study, CR was calculated for Cd and Pb, where CSF is available (Table 2). The CSF converts the calculated day-to-day intake of the heavy metal averaged over a lifespan of exposure directly to the increasing risk of a person developing cancer (USEPA, 1989). If CR < 1×10^{-6}, the carcinogenic risk to human health is considered as negligible, however, the range 1×10^{-6} to 1×10^{-4} is regarded as posing an acceptable risk to humans. CR > 1×10^{-4} specifies a high risk for the development of cancer in humans (Liu *et al*., 2013; Diami *et al*., 2016).

An individual total lifespan cancer risk was estimated from the contribution of each heavy metals for all the pathways as expressed in equation 8.

$$\mathrm{LCR}_{(\mathrm{total})} = \mathrm{CR}_{(\mathrm{ing})} + \mathrm{CR}_{(\mathrm{dermal})} + \mathrm{CR}_{(\mathrm{inh})}$$

Where, '$\mathrm{LCR}_{(\mathrm{total})}$' is the total carcinogenic risk over a lifetime, '$\mathrm{CR}_{(\mathrm{ing})}$', '$\mathrm{CR}_{(\mathrm{dermal})}$', and '$\mathrm{CR}_{(\mathrm{inh})}$' are risk contributions through ingestion, dermal and inhalation pathways. In this study, LCR was calculated from

$CR_{(ing)}$ and $CR_{(inh)}$. The range of tolerable value LCR is from 1 x10^{-6} to 1 x 10^{-4} (Liu *et al*., 2013; Diami *et al*., 2016).

***Non-carcinogenic risk assessment*:**

Non-carcinogenic risk expressed as hazard index (HI), is the summation of hazard quotients for the different heavy metals. It was evaluated for children and adults using equations 9 and 10.

$$\mathrm{HQ} = \frac{\mathrm{CDI}}{\mathrm{R}f\mathrm{D}} \qquad (9)$$

$$\mathrm{HI} = \Sigma \mathrm{HQ} \qquad (10)$$

With the threshold of R*f*D value (Table 2), estimation of the prevailing adverse health effects to humans is possible. Where R*f*D value is higher than the CDI, there is no adverse health effect and vice versa (USEPA, 1993). Moreover, HQ < 1 signifies there are no adverse health effects and HQ > 1 suggests probable adverse health effects (USEPA, 1986).

Here, Cd and Pb are classified as metals with carcinogenic risk, and Fe, Zn, and Cu are grouped as non-carcinogenic (Weissmannová *et al*., 2019). If the HI is ≤ 1, the risk from non-carcinogenic effects is not possible, and if the HI is > 1, there is the possibility of adverse health effects, and the likelihood of the effect rises with the increasing value of HI (USEPA, 2001; Chonokhuu *et al*., 2019).

Table 1: Health risk assessment parameters via the three exposure pathways for soil

Parameters	Units	Children	Adult
Average time (ATM)			
For carcinogens	Days	365 x 70	365 x 70
For non-carcinogens		365 x EDN	365 x EDN
Body weight (BWT)	Kg	15	70
Conversion factor (CFR)	kg/mg	10^{-6}	10^{-6}
Dermal absorption factor (ABS)	None	0.1	0.1
Dermal exposure ratio (FEY)	None	0.61	0.61
Exposure frequency (EFY)	days/year	350	350
Exposure duration (EDN)	Years	6	30
Ingestion rate (IRS)	mg/day	200	100
Inhalation rate (IHS)	m^3/day	10	20
Particulate emission factor (PEF)	m^3/kg	1.3 x 10^{9}	1.3 x 10^{9}
Soil adherence factor (SAF)	mg/cm^2	0.2	0.07
Skin surface area (SSA)	cm^2	2100	5800

Table 2: Cancer slope factors (CSF) and reference doses (R*f*D) for the different heavy metals

		Cancer slope factor (CSF)	
Metal	Ingestion	Dermal	Inhalation
Cd	-	-	6.3 x 10^{0}
Pb	8.5 x 10^{-3}	-	4.2 x 10^{-2}
		Reference dose (R*f*D) (mg/kg-day)	
Metal	Ingestion	Dermal	Inhalation
Cd	5.0 x 10^{-4}	5.0 x 10^{-4}	5.7 x 10^{-5}
Cu	3.7 x 10^{-2}	2.4 x 10^{-2}	-
Pb	3.6 x 10^{-3}	-	-
Zn	3 x 10^{-1}	7.5 x 10^{-2}	-

Table 3: Fungal species in the dumpsite soil samples

Site	Fungal isolates	Frequency
Gbonmi	*Aspergillus niger*, *A. flavus*, *A. terreus*, *Chrysosporium* sp. and *Penicillium chrysogenum*	5
Ilesa garage	*Alternaria* sp., *Aspergillus niger* and *Penicillium* sp.	3
Omobolanle	*Aspergillus niger*, *Penicillium chrysogenum* and *Penicillium* sp.	3
Egbedi	*Aspergillus niger*, *Penicillium chrysogenum* and *P. purpurogenum*	3
Oke-Baale	*Aspergillus ochraceus*, *Penicillium chrysogenum* and *Penicillium* sp.	3

Table 4: Zones of inhibition (mm) of fungal isolates to selected antifungal agents

S/N	Location	Fungal species	Zone of inhibition (mm)		
			Voriconazole	Amphotericin B	Fluconazole
1	Gbonmi	*Aspergillus niger*	4.85 ± 0.25	1.65 ± 0.13	1.60 ± 0.06
2		*Aspergillus terreus*	4.40 ± 0.08	0.95 ± 0.05	0.00 ± 0.00
3		*Penicillium chrysogenum*	4.75 ± 0.13	0.00 ± 0.00	0.00 ± 0.00
4		*Aspergillus flavus*	4.85 ± 0.25	1.55 ± 0.05	0.00 ± 0.00
5		*Chrysosporium* species	5.35 ± 0.05	0.00 ± 0.00	0.00 ± 0.00
6	Ilesa garage	*Penicillium* species	4.95 ± 0.60	1.20 ± 0.08	0.00 ± 0.00
7		*Aspergillus niger*	4.25 ± 0.13	0.00 ± 0.00	0.00 ± 0.00
8		*Alternaria* species	3.85 ± 0.13	2.00 ± 0.08	0.00 ± 0.00
9	Omobolanle	*Penicillium purpurogenum*	3.85 ± 0.05	1.10 ± 0.02	0.00 ± 0.00
10		*Aspergillus niger*	5.55 ± 0.85	1.00 ± 0.00	0.00 ± 0.00
11		*Penicillium* species	4.75 ± 0.13	0.00 ± 0.00	0.00 ± 0.00
12	Egbedi	*Aspergillus niger*	3.65 ± 0.05	0.00 ± 0.00	0.00 ± 0.00
13		*Penicillium chrysogenum*	3.65 ± 0.05	0.00 ± 0.00	0.00 ± 0.00
14		*Penicillium purpurogenum*	4.60 ± 0.32	0.00 ± 0.00	0.00 ± 0.00
15	Oke-Baale	*Aspergillus ochraceus*	3.75 ± 1.13	0.00 ± 0.00	0.00 ± 0.00
16		*Penicillium chrysogenum*	4.80 ± 0.72	0.00 ± 0.00	0.00 ± 0.00
17		*Penicillium* species	5.15 ± 0.25	0.00 ± 0.00	0.00 ± 0.00

Table 5: Heavy metal concentrations (Cd, Cu, Fe, Pb, and Zn) in the dumpsite soil samples

Location	mg/kg				
	Cd	Cu	Fe	Pb	Zn
Gbonmi	31.60 ± 0.00	423.14 ± 0.01	291.25 ± 0.03	70.18 ± 0.01	20.51 ± 0.02
Ilesa garage	58.16 ± 0.01	258.52 ± 0.02	120.79 ± 0.02	87.45 ± 0.02	16.88 ± 0.01
Omobolanle	58.79 ± 0.00	414.71± 0.02	293.18 ± 0.01	120.15 ± 0.03	28.16 ± 0.00
Egbedi	48.45 ± 0.02	550.89 ± 0.01	121.99 ± 0.02	108.90 ± 0.00	64.05 ± 0.03
Oke-Baale	36.29 ± 0.01	353.32 ± 0.01	195.69 ± 0.00	71.69 ± 0.00	33.81 ± 0.02

Table 6: Contamination factor of the heavy metals in the dumpsite soils

Location	Cd	Cu	Fe	Pb	Zn
Gbonmi	39.50	11.75	0.01	0.83	0.15
Ilesa garage	72.70	7.18	0.00	1.03	0.12
Omobolanle	73.49	11.52	0.01	1.41	0.20
Egbedi	60.56	15.30	0.00	1.28	0.46
Oke-Baale	45.36	9.81	0.01	0.84	0.24

Table 7: Geo-accumulation index of heavy metals in the dumpsite soils

Location	Cd	Cu	Fe	Pb	Zn
Gbonmi	4.72	2.97	-7.61	-0.86	-3.36
Ilesa garage	5.60	2.26	-8.88	-0.54	-3.64
Omobolanle	5.61	2.94	-7.60	-0.09	-2.90
Egbedi	5.34	3.35	-8.87	-0.23	-1.71
Oke-Baale	4.92	2.71	-8.19	-0.83	-2.63

Table 8: Potential ecological risk factor and index of the heavy metal in dumpsite soils

	Potential ecological risk factor (E_r)				RI (% contribution of Cd)
Location	Cd	Cu	Pb	Zn	
Gbonmi	1185.00	58.77	4.13	0.15	1248.04 (94.95)
Ilesa garage	2181.00	35.91	5.14	0.12	2222.17 (98.15)
Omobolanle	2204.63	57.60	7.07	0.20	2269.49 (97.14)
Egbedi	1816.88	76.51	6.41	0.46	1900.25 (95.61)
Oke-Baale	1360.88	49.07	4.22	0.24	1414.41(96.22)

Table 9: Cancer risk associated with ingested or/and inhaled Cd and Pb

	Location	Children		Adult	
		Cd	Pb	Cd	Pb
Ingestion	Gbonmi	-	6.54E-07	-	3.50E-07
	Ilesa garage	-	8.15E-07	-	4.36E-07
	Omobolanle	-	1.12E-06	-	6.00E-07
	Egbedi	-	1.01E-06	-	5.43E-07
	Oke-Baale	-	6.68E-07	-	3.58E-07
Inhalation	Gbonmi	6.21E-05	8.37E-08	2.89E-05	4.29E-07
	Ilesa garage	7.73E-05	1.04E-07	5.33E-05	5.34E-07
	Omobolanle	1.06E-04	1.43E-07	5.39E-05	7.34E-07
	Egbedi	9.63E-05	1.30E-07	4.44E-05	6.65E-07
	Oke-Baale	6.34E-05	8.55E-08	3.32E-05	4.38E-07
LCR	Gbonmi	6.28E-05		2.97E-05	
	Ilesa garage	7.82E-05		5.43E-05	
	Omobolanle	1.07E-04		5.52E-05	
	Egbedi	9.74E-05		4.56E-05	
	Oke-Baale	6.42E-05		3.40E-05	

Table 10: Hazard quotients and index of heavy metals in the dumpsite samples for children and adults

Exposure pathway	Location	HQ				HI	HQ				HI
		Children					Adults				
		Cd	Cu	Pb	Zn		Cd	Cu	Pb	Zn	
Ingestion	Gbonmi	1.54E-02	1.46E-02	2.14E-03	8.74E-05	3.22E-02	3.71E-03	1.57E-03	1.14E-03	9.37E-06	6.43E-03
	Ilesa garage	1.92E-02	8.93E-03	2.66E-03	7.19E-05	3.08E-02	6.83E-03	9.57E-04	1.43E-03	7.71E-06	9.22E-03
	Omobolanle	2.63E-02	1.43E-02	3.66E-03	1.20E-04	4.44E-02	6.90E-03	1.54E-03	1.96E-03	1.29E-05	1.04E-02
	Egbedi	2.39E-02	1.90E-02	3.32E-03	2.73E-04	4.65E-02	5.69E-03	2.04E-03	1.78E-03	2.92E-05	9.53E-03
	Oke-Baale	1.57 E-02	1.22E-02	2.18E-02	1.44E-04	3.02E-02	4.26E-03	1.31E-03	1.17E-03	1.54E-05	6.75E-03
Dermal	Gbonmi	9.19E-04	5.98E-04	-	9.28E-06	9.28E-04	8.87E-04	4.11E-05	-	4.48E-05	1.52E-03
	Ilesa garage	1.69E-03	3.65E-04	-	7.64E-06	1.68E-03	1.63E-03	5.12E-05	-	3.67E-05	2.06E-03
	Omobolanle	1.71E-03	5.86E-04	-	1.27E-05	1.72E-03	1.65E-03	7.03E-05	-	6.15E-05	2.30E-03
	Egbedi	1.41E-03	7.79E-04	-	2.90E-05	1.42E-03	1.36E-03	6.37E-05	-	1.40E-04	2.19E-03
	Oke-Baale	1.06E-03	4.99E-04	-	1.53E-05	1.06E-03	1.02E-03	4.19E-05	-	7.38E-05	1.55E-03
Inhalation	Gbonmi	3.43E05	-	-	-	3.43E05	1.60E07	-	-	-	1.60E07
	Ilesa garage	6.32E05	-	-	-	6.32E05	2.95E07	-	-	-	2.95E07
	Omobolanle	6.39E05	-	-	-	6.39E05	2.98E07	-	-	-	2.98E07
	Egbedi	5.26E05	-	-	-	5.26E05	2.46E07	-	-	-	2.46E07
	Oke-Baale	3.94E05	-	-	-	3.94E05	1.84E07	-	-	-	1.84E07

Key: HQ = Hazard Quotient; HI = Hazard Index; Cd = Cadmium; Cu = Copper; Pb = Lead; Zn = Zinc; ‘-’= Not Determined

RESULTS AND DISCUSSION

Presumptive identification and distribution of fungal isolates in the dumpsite soils

In this study, a total of 17 fungal isolates belonging to 9 fungal species were presumptively identified. These included *Aspergillus flavus*, *A. niger*, *A. terreus*, *A. ochraceus*, *Alternaria* sp., *Chrysosporium* sp., *Penicillium chrysogenum*, *P. purpurogenum*, *Penicillium* sp. (Table 3). Previous studies reported that different species of *Aspergillus*, *Fusarium*, *Mucor*, *Penicillium*, *Saccharomyces*, *Trichoderma* and *Rhizopus* are predominantly found in MSW dumpsite soils (Oshoma *et al*., 2017; Williams and Hakam, 2016). Aside *Chrysosporium* species, other isolates obtained in our work had been reported elsewhere (Iram *et al*., 2013; Rasool and Irum, 2014).

Five different fungal species were observed at Gbonmi, whereas it was 3 each at Ilesa garage, Omobolanle, Egbedi and Oke-Baale (Table 3). Similar findings were obtained from major dumpsites in the South-South region of Nigeria including Port Harcourt (Obire *et al*., 2002; Williams and Hakam, 2016) and Benin (Oshoma *et al*., 2017). Except for the Oke-Baale dumpsite, *A. niger* was common in all the study sites.

Antifungal susceptibility testing (AST)

The MSW dumpsites provide a conducive environment for the proliferation of antimicrobial-resistant microorganisms (Waturu *et al*., 2017). In the current study, the fungal isolates from the 5 sites were sensitive to voriconazole with a diameter of inhibition ranging between 3.65 ± 0.05 and 5.55 ± 0.85 mm. Also, 7 isolates from Gbonmi, Ilesa garage, and Omobolanle were susceptible to amphotericin B, while all the isolates except *Aspergillus niger* from Gbonmi were resistant to fluconazole (Table 4). The findings of this study disagree with Osuntokun *et al*. (2018) whose fungal isolates from hospital dumpsite were susceptible to fluconazole. The resistance observed in the fungal isolates suggests contamination of the dumpsites with unused and expired amphotericin B and/or fluconazole, and drug-resistant fungi through household and illegal clinical wastes. This poses a serious threat to environmental and public health. Drug-resistant fungi can persist in dumpsite soils and leachate leading to groundwater contamination (Wang *et al*., 2015; Bartkowiak *et al*., 2016). Moreover, the fungal spores can be inhaled and have contact with the skin during anthropogenic activities and ingested through geophagy (Karimian *et al*., 2021). These possibly result in life-threatening medical conditions and increased economic burdens through extended hospital stays, unaffordable healthcare costs, and mortality (Anand *et al*., 2021).

Heavy metal concentration in the dumpsite soils

Overall, the concentration of heavy metals in the soils was in the decreasing order: Cu > Fe > Pb > Cd > Zn except in Egbedi whose Zn was higher than Cd (Cu > Fe > Pb > Zn > Cd). The heavy metals in the dumpsite soils varied across sampling locations with Cd, Cu, Fe, Pb and Zn ranging from 31.60 ± 0.00 to 58.79 ± 0.00; 258.52 ± 0.02 to 550.89 ± 0.03; 120.79 ± 0.02 to 293.18 ± 0.01; 70.18 ± 0.02 to 120.15 ± 0.03; and 16.88 ± 0.01 to 64.05 ± 0.03 mg/kg respectively (Table 5), with Cu and Zn being the most and least predominant metals detected in the investigated dumpsite soils respectively. These findings are higher than those previously reported on agricultural soils (Abdelhafez *et al*., 2015; Proshad *et al*., 2019), waters (Ighariemu *et al*., 2019;), sediment (Ighariemu *et al*., 2019), MSW (Obasi *et al*., 2017; Teka *et al*., 2018; Tang *et al*., 2019), plant (Obasi *et al*., 2017) and landfill leachate (Boateng *et al*., 2019). Varying concentrations of the heavy metals have equally been documented elsewhere (Ma and Singhirunnusornb, 2012; Olayiwola and Onwordi, 2015; Bongoua-Devisme *et al*., 2018; Chonokhuu *et al*., 2019). The disparity observed in the heavy metal concentrations could be attributable to the source and type of waste, differing from one place to the other and season to season (Titilawo *et al*., 2018).

Ecological risk assessment of the heavy metals concentration of the dumpsite soil samples

Contamination factor

The ratio of heavy metal to baseline value gives a clue into possible contamination of an area (Abdelhafez *et al*. 2015). Table 6 reveals that the five dumpsites were severely contaminated with Cd and Cu whereas, the contamination level of Zn and Fe was low. Conversely, Egbedi, Ilesa garage, and Omobolanle were moderately contaminated with Pb (Table 6). Previous studies also reported high-level contamination with Cd in soils (Abdelhafez *et al*. 2015; Proshad *et al*. 2019). The high-level contamination of the dumpsites with Cd and Cu is particularly worrisome because Cd is a potent toxin at relatively low concentrations (EC 2001), while Cu is poisonous at higher levels (Soliman *et al*. 2015).

Geo-accumulation index

The geo-accumulation index estimated for Cd, Cu, Fe, Pb, and Zn is presented in Table 9. Interestingly, I_{geo} for the metals followed the same order in all the locations i.e. Fe < Zn < Pb < Cu < Cd. The experimental data gathered shows that the dumpsite soils were practically uncontaminated with Zn, Fe, and Pb (values obtained were < 1). While Gbonmi and Oke-Baale were heavily to extremely contaminated with Cd (4.72 and 4.92 respectively), Egbedi, Ilesa garage, and Omobolanle were extremely contaminated with the same metal. Also, soils from Gbonmi, Ilesa garage, Omobolanle, and Oke-Baale were moderately to heavily contaminated with Cu while Egbedi was heavily contaminated with the same metal (Table 7). The I_{geo} values corroborate the high-level contamination of the soils with Cd and Cu as recorded for CF_r above.

Potential ecological risk index

The potential ecological risk factor (E_r) and risk index (RI) are presented in Table 10. The order of E_r in the five locations followed the same pattern, Zn < Pb < Cu < Cd. Generally, all the metals pose potential ecological risk, but at different magnitude. The degree of ecological risk contributed by Zn and Pb was low, moderate for Cu, and severe for Cd. Furthermore, the values obtained for RI, ranging between 1248.04 and 2269.49, show that all the sampling sites were at significantly high ecological risk with Cd contributing ≥ 94.95% (Table 8). This observation is however, not surprising since Cd was the major pollutant amongst the five heavy metals tested. Soliman *et al*. (2015) earlier noted low RI (mean value 29.85) in sediments from Egyptian Mediterranean Coast.

Health risk assessment

Toxic metals and exposure pathways of utmost concern are essential in evaluating human health risk (Saghatelyan *et al*., 2014). In the current study, CDI via ingestion, dermal, and inhalation contact were included in our calculations on the assumption that scavengers will ingest soil particles attached to waste, have skin contact with the soils, and dust generated especially during the dry season will be inhaled.

Cancer risk

The carcinogenic risks estimated for ingestion and inhalation exposure pathways are presented in Table 9. Generally, the level of carcinogenic risks posed by ingesting Pb was lower than the tolerable range (10^{-6} to 10^{-4}) in adults. Similar findings were obtained for children except in Omobolanle (1.12E-06) and Egbedi (1.01E-06) whose values were within the permissible limit. However, the risk calculated for inhaling Cd in both children and adults were within the acceptable range, the effect of breathing in Pb can be overlooked for both children and adults (Table 9). Additionally, LCR values for both children and adults were within the standard limit. Although the values recorded in this study do not pose any cancer risk, environmental and regulatory agencies are particularly advised to monitor the levels of these carcinogens in the soils to prevent future danger to human health.

Non-carcinogenic risk assessment

The data obtained showed that HQ for the five metals, which were estimated for ingestion and dermal exposure pathway, were below the standard value of 1 for both children and adults (Table 10). This suggests no significant non-carcinogenic effect. However, HQ > 1 may be observed if the average daily doses were in the multiples of 10 or higher and or in case of higher exposure frequency. Nevertheless, inhalation of Cd poses an extremely high non-carcinogenic risk to both children (range from 3.43E05 to 6.39E05) and adults (range from 1.60E07 to 2.98E07) (Table 10). Furthermore, HI values (ingestion exposure pathway) ranged from 4.65E-02 (Egbedi) to 3.02E-02 (Gbonmi), and 9.53E-03 (Egbedi) to 1.04E-02 (Omobolanle) in children and adults, respectively (Table 10). This indicates no adverse health effect for children and adults with direct contact with the soils for about 6 and 30 years respectively on an average of 350 days annually (assumptions for calculations are listed in Table 1) (USEPA, 2004; DEA, 2010; Kamunda *et al*., 2016). Similarly, for the dermal exposure pathway, there was no indication of non-carcinogenic risk as the HI values obtained in the five dumpsites were less than 1. On the other hand, HI from inhaling sand or dust particles from the sites poses a high adverse health risk with a range from 3.43E05 to 6.39E05 and 1.60E07 to 2.98E07 for children and adults respective (USEPA, 2001; Chonokhuu *et al*., 2019).

CONCLUSION

Fungal isolates were obtained in the five dumpsite soils, with the genera *Aspergillus* and *Penicillium* being the most prevalent. The resistance of the isolates to the antifungals and high concentration of the toxic metals in the soils poses a serious threat to environmental and public health through persistence in dumpsite soils and leachate leading to groundwater contamination. In addition, fungal spores can be inhaled and have contact with the skin leading to infections and life-threatening diseases. The heavy contamination of the sites with Cd poses a serious ecological risk and highly influences the risk of non-carcinogenic origin. Thus, proper monitoring and effective waste management strategies are essential to keep the metals, especially resistant fungi and Cd and other heavy metals below the safe level. Appropriate governmental policies and public education on MSW dumpsites associated risks are also advocated.

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