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# Chemical characterization of simulated landfill soil leachates from Nigeria and India and their cytotoxicity and DNA damage inductions on three human cell lines

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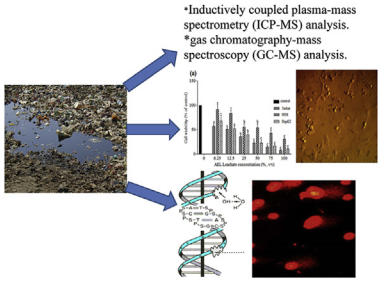
## HIGHLIGHTS

- Chemical analysis of simulated soil leachates from dumpsites revealed toxic metals and organic compounds (PAHs and PCBs).
- The simulated leachates induced morphological alterations in lymphoma, hepatocarcinoma and osteosarcoma.
- The leachates decreased cell viability of the exposed cell lines as assessed using MTT assay.
- The leachates increased DNA damage in the exposed cell lines.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Landfill soils are sources of emerging carcinogens, teratogens and mutagens in the environment. There is inadequate information on its possible health risk and cytogenotoxicity. This study evaluated chemical characterization of four simulated landfill leachates with their cytotoxicity and DNA damage in human cells. Hepatocarcinoma (HepG2), lymphoma (Jurkat) and osteosarcoma (HOS) cells, incubated with 6.25, 12.5, 25, 50, 75 and 100% of Aba Eku (AEL), Olusosun (OSL), Awotan (AWL) and Nagpur (NPL) simulated leachates for 24 h, were assessed for cell viability using MTT assay and morphological alterations. DNA damage was also assessed after 24 h treatment of cells with sub-lethal concentrations of the leachates using comet assay. Metals and organic compounds in the soil leachates were determined using inductively coupled plasma-mass spectrometry (ICP-MS) and gas chromatography-mass spectroscopy (GC-MS) respectively. The leachates induced significant cytotoxicity in the treated cells with evidence of apoptosis; shrunken morphologies, detachment from the substratum and cytoplasmic vacuolations. Similarly, there was significant DNA damage induced in the treated cells, with increased Olive tail moment, tail length and % tail DNA. Jurkat was the most sensitive (Jurkat > HepG2 > HOS) to the cytotoxic and genotoxic effects of the leachates. All the analyzed metals except Cd, Fe, Zn and Mn were found at levels lower than standard allowable limits. 32, 17, 23 and 23 different PAHs and PCBs were

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detected in AEL, AWL, OSL and NPL respectively, at varying retention peak times. These toxic constituents induced the observed cytogenotoxicity in the cells and may suggest possible public health risk.


## 1. Introduction

Nigeria with an estimated population size of 170 million and India with 1.21 billion (United Nation, 2013) are populous countries in Africa and Asia continents, respectively. They generate daily per capital solid waste of 0.44–0.66 kg (Ogwueleka, 2009) and 0.12–2.1 kg (Annepu, 2012), which amounts to approximately 25 million and 68.8 million tons per year, respectively. Indiscriminate disposal of these wastes into unsanitary landfills and open dumps usually located in cities and around residential quarters are common methods of solid waste management in these countries (Annepu, 2012; Alimba, 2013). Landfilling which accounts for the management of about 95% of the total solid waste collection worldwide (El-Fadel et al., 1997), is known to accumulate significant amount of xenobiotics in landfill soils. Heap of soils which are produced during solid waste decomposition in landfills are the ultimate sinks for xenobiotics (metals, organo-metallic, water soluble inorganic and hydrophobic organic compounds) present in the wastes. Many of these xenobiotics, classified as highly mutagenic, estrogenic, teratogenic and carcinogenic, are released into the environment in varying concentrations (Eggen et al., 2010; Vilavert et al., 2012). Generally, these complex mixture of chemicals strongly bound to the soil particles (Schuhmacher et al., 1998), and constituting issues of great concern to wildlife and human due to environmental contamination (Baderna et al., 2013). In most developing countries, landfill soils are used to fill excavated canals and as organic matter in the cultivation of vegetables and seed crops. Chemicals in landfill soils are usually transferred to vegetables/plants (Shagal et al., 2012), and can biomagnify in humans and wildlife along the food chains. Also landfill soil increases human exposure to xenobiotics via contaminated underground and surface water consumption and accidental ingestions of soil particles (Lah et al., 2008; Baderna et al., 2011).

Attempts to understand the possible health risk associated with exposure to xenobiotics in landfill soils spurred researchers into characterizing chemical and microbial constituents of landfill soils (Schuhmacher et al., 1998; Vilavert et al., 2012). Chemical and microbial analyses of leachates are not adequate to increase the understanding of the hazardousness of mixture of xenobiotics in landfill soils on biological systems. This is due to possible synergistic and antagonistic activities of the soil leachate constituents. Systemic toxicity, genotoxicity and mutagenicity assessments of leachates using microbial, plant and animal test models are necessary and relevant in presenting the possible toxicological profile of leachates as complex mixture of xenobiotics (Baderna et al., 2011; Salem et al., 2014; Alimba and Bakare, 2016). However, there is paucity of information on the chemical characterization of landfill soil leachates and their cytotoxicity and DNA damaging potentials using different human cell lines, as bioindicators. Similarly, no reports have examined the cytotoxic effects of simulated landfill soil leachates on the morphological characterization of human cell lines. This information is expected to increase knowledge on the sensitivity of human cell lines to the biological effects of mixture of xenobiotics in landfill soils. The use of cell lines in toxicological assessment of chemicals offers several benefits which include the ability to determine cell or organ specific mechanisms of toxicity and to minimize the need for animal use. Furthermore, cell lines in *in vitro* toxicological studies are sensitive to the cytotoxic and genotoxic screening of xenobiotics (Sahu et al., 2014). In this study, three widely used human cell lines; hepatocarcinoma (HepG2), osteosarcoma (HOS) and lymphoma (Jurkat) were selected as model cell lines to examine their sensitivities to possible cytotoxic effects and DNA damage as may be applicable to the three different potential targeted tissues and organs of the mammalian body; liver, bone marrow and lymphocytes respectively, during landfill leachate toxicity (Li et al., 2010; Bakare et al., 2012).

The study herein utilized MTT and alkaline comet assays to assess the cytotoxicity and DNA damage induction by four simulated landfill soil leachates from Nigeria and India on HepG2, HOS and Jurkat. Heavy metal and organic chemical constituents of the simulated soil leachate samples were also analyzed.

## 2. Materials and methods

### 2.1. Chemicals

Dulbecco's Modified Eagle's Medium (DMEM), Minimum Essential Medium (MEM), Roswell Park Memorial Institute 1640 medium (RPMI), fetal bovine serum (FBS), penicillin, streptomycin and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were obtained from GIBCO-Invitrogen (Life technology, Spain). Dimethyl sulfoxide (DMSO), hydrogen peroxide, normal melting agarose (NMA) and ethylenediaminetetraacetic acid ($Na_2EDTA$), low melting agarose (LMA), ethidium bromide (EtBr), triton X-100, were from Sigma Aldrich Chemicals Co. Ltd, USA. Sodium hydroxide (NaOH) and Tris buffer were obtained from Merck Pvt. Ltd, India. All other chemicals obtained locally were of analytical grade.

### 2.2. Landfill soil collection and simulated leachate preparation

The study sites; three landfills in Nigeria and one in Nagpur, India which are currently in use, were selected based on previous studies reporting their pollution status and public health impacts (Pujari et al., 2007; Alimba, 2013). Soils were randomly collected from 20 different spots at a depth not less than 15 cm on each landfill in accordance with Baderna et al. (2011). The soil samples from a site were mixed together to form a composite representation, air-dried, finely ground with a mortar and pestle, and sifted through a 63-μm (pore size) sieve to obtain a homogeneous mixture sample for each study site. Leachates were prepared from the homogenous soil mixture according to toxicity characteristic leaching procedure (TCLP) (USEPA, 1992). Briefly, 20 g of each soil was added to 400 mL of extraction fluid (w/v) (5.7 mL of glacial acetic acid was diluted to 1000 mL milli Q water). 64.3 mL of 1 N NaOH was added and the pH adjusted to $4.93 \pm 0.2$. The mixture was thoroughly mixed for 18 h on a rotary shaker at 30 ×g at room temperature. This procedure was undertaken to maximize the detectability of organic and inorganic constituents of the soil samples from the landfills. The samples were allowed to settle for 30 min to sediment visible particles, and then filtered with a 2.5 μm filter (Whatman® No. 42) to remove suspended particles. Finally, each sample was centrifuged at 600 ×g for 15 min at room temperature to collect the supernatant, and filter sterilized using 0.22 μm syringe filter. Samples were designated as Olusosun simulated landfill