The thought-provoking research papers presented at the Humboldt Kolleg-Osogbo 2017, compiled in this book give insight into evidence-based quality improvement research in developing countries. The book is divided into three chapters covering subject areas that includes Microbiology and Parasitology, Biochemistry, Physics, Agricultural Science, Statistics, Engineering, Food science and Technology among others. The need for rational use as well as the development of new antibiotics and the usefulness of plant extracts for treatment are presented in the first chapter under the title Health, Treatment and Infection. The second chapter titled Agriculture, Food and Nutrition highlights the importance of biotechnology and effective storage management as a means of improving and increasing agricultural yield. The third chapter is dedicated to the important topics on Waste and Environmental Pollution which still remain a major challenge that requires urgent attention in developing countries. This book will therefore be valuable for students, academicians, researchers and policy makers who are interested in research that focuses on improving the quality of life.

Olusola Ojurongbe is a Professor of Medical Parasitology and the Head of Tropical Disease Research group, Ladoke Akintola University of Technology, Nigeria. He has many publications focusing on tropical parasites and has served as a consultant to the World Health Organization. He is a fellow of the Alexander von Humboldt foundation, Germany.

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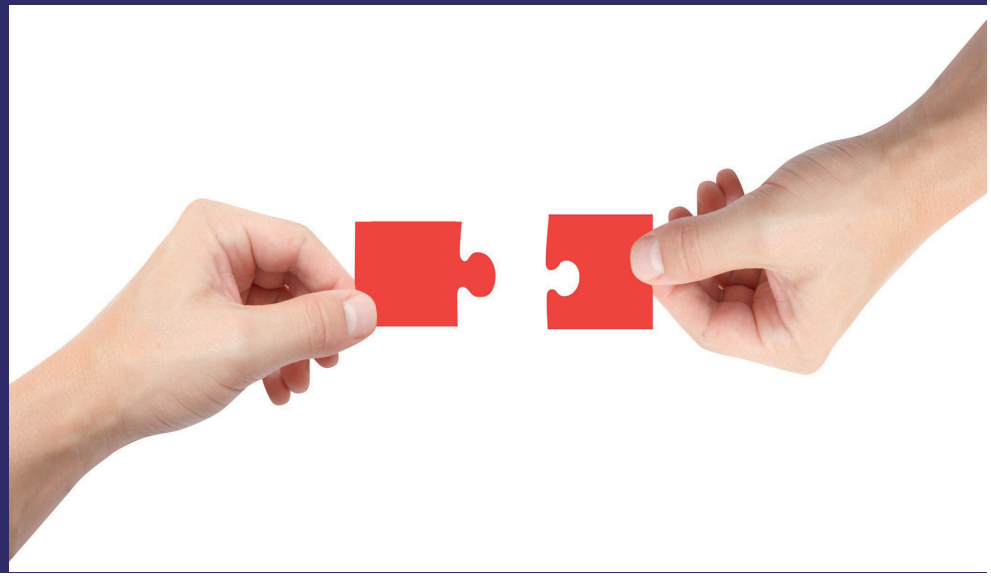

Olusola Ojurongbe

# Translating Research Findings into Policy in Developing Countries

## Contributions from Humboldt Kolleg Osogbo-2017

Ojurongbe

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# Contents

# Simulated landfill soil leachates induced cytotoxicity and DNA damage in human cells in vitro

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**ABSTRACT**
Inappropriate solid wastes disposal into unsanitary dumpsites has emerged a major source of underground and surface water, and soil contamination. This may increase human and wildlife exposure to mixture of emerging carcinogens, teratogens and mutagens. The study herein characterized chemical constituents of landfill soil leachates from Nigeria and India and investigated their DNA damaging potentials and cytotoxicity in lymphoma (Jurkat), osteosarcoma (HOS) and hepatocarcinoma (HepG2) cells. Cells were incubated with 0, 6.25, 12.5, 25, 50, 75 and 100% of Aba Eku (AEL) and Nagpur (NPL) leachates for 24h and assessed for morphological alterations and cell viability using MTT assay. Cells were exposed to sub-lethal concentrations of the leachates for 24h and analyzed for DNA damage using Comet assay. Metals and organic compounds were characterized in the leachates using inductively coupled plasma-mass spectrometry (ICP-MS) and gas chromatography-mass spectroscopy (GC-MS) respectively. The soil leachates significantly induced concentration dependent cytotoxicity in the cells with evidence of apoptosis, shrunken morphologies, detachment from the substratum and cytoplasmic vacuolations. There was significant DNA damage induced in the cells, with concentration dependent increase in Olive tail moment. Jurkat was the most sensitive (Jurkat>HepG2>HOS) to the cytotoxic and genotoxic effects of the leachates. All the analyzed metals were lower than standard allowable limits except Cd, Zn, Fe, Al and Mn. 32 and 23 different PAHs and PCBs were detected in AEL and NPL respectively, at varying retention peak times. Solid waste soil leachates contain toxic metals and organic constituents which induced cytotoxic and genotoxic effects in human cells.
**Keywords:** Comet assay, cytotoxicity, DNA damage, heavy metals, human cells, landfill soil leachates, PCBs and PAHs, MTT assay.

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## INTRODUCTION

Indiscriminate disposal of solid wastes into unsanitary landfills and open dumps in cities and around residential quarters are common methods of solid waste management in Nigeria and India [1,2].These landfills release significant amount of xenobiotics via leachates into the environment and this constitutes risk to human health and wildlife conservation [3]. Many of these xenobiotics are highly mutagenic, estrogenic, teratogenic and carcinogenic even at trace concentrations [4, 5]. Heap of soils are produced during solid waste decomposition in landfills and this serves as ultimate sinks for xenobiotics (metals, organo-metallic, water soluble inorganic and hydrophobic organic compounds) present in the wastes [6]. The mixture of xenobiotics strongly bound to the soil particles [7], hence, constituting issues of great concern to wildlife and human via environmental contamination [8]. 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 the vegetables/plants [9], and can biomagnify in humans and wildlife through the food chains. Also landfill soil increases human exposure to xenobiotics via consumption of contaminated underground and surface water and accidental ingestions of soil particles [8].

Studies have characterized chemical and microbial components of leachates with the aim of understanding the health risk associated with the leachate constituents [3]. However, this is not adequate to increase the understanding of the hazardousness of the complex mixture of xenobiotics in leachates, due to their synergistic, additive and antagonistic activities in biological systems. Studies have assessed the systemic toxicity, genotoxicity and mutagenicity of leachates in microbial, plant and animal models in order to understand the possible toxicological profile of leachates as complex mixture of xenobiotics [10, 11]. However, insufficient information exist on the chemical characterization of landfill soil leachates with cytotoxicity and DNA damage *in vitro* using human cells, and none exist that examined the toxic effects of leachate constituents on morphological alterations of human cell line. The use of cell line 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 cytogenotoxicity screening of xenobiotics [12, 13]. In *in vivo* mammalian system, liver and bone marrow cells are readily prone to leachate toxicity due to the detoxification of chemical substances in the liver and increase cell proliferation in both liver and bone marrow [14, 15]. In this study, three widely used human cell lines; hepatocarcinoma (HepG2), Osteosarcoma (HOS) and lymphoma (Jurkat) are selected as model cells to represent three different potential targeted tissues and organs of the mammalian body; liver, bone marrow and lymphocytes respectively, during leachate toxicity. These cells have been extensively used and approved for their sensitivity in toxicological screening of different toxicants.

The study herein utilized MTT and alkaline comet assays to assess the cytotoxicity and DNA damage induction by two simulated landfill soil leachates from Nigeria and

India on HepG2, HOS and Jurkat. Heavy metal and organic chemical constituents of the leachate samples were also analyzed.

## MATERIALS AND METHODS

### Landfill soil collection and leachate preparation

The study sites; Aba-Eku landfill in Nigeria and Nagpur landfill in India which are currently in use, were selected based on previous studies reporting their pollution status and public health impacts [1, 16]. Soils were randomly collected from 20 different spots at a depth not less than 15cm on each landfill in accordance with Baderna et al. [8]. 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-mm (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) [17]. This procedure which was undertaken to maximize the detectability of organic and inorganic constituents of the soil samples from the landfills was well described by Alimba et al., [18]. The simulated landfill soil leachate samples were designated as Aba-Eku leachate (AEL) and Nagpur simulated leachate (NPL) and stored at 4ºC until used.

### Heavy metal and volatile organic characterization of leachate samples

Fourteen heavy metals were analyzed in the leachate samples using inductively coupled plasma-mass spectrometry (ICP-MS). Cadmium (Cd), zinc (Zn), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), manganese (Mn), nickel (Ni), lead (Pb), selenium (Se), aluminum (Al), arsenate (As), beryllium (Be) and vanadium (V) concentrations in the leachate samples were quantified. Pre-acidified leachate samples were digested using standard analytical methods with protocol code 3030F (Nitric acid – Hydrochloric acid digestion) [19], and quantified using ICP-MS; Perkin Elmer: Optima 4100 DV (Labtam Plasmalab-8440, Mordialloc, Victoria, Australia), with concentrations of the metals determined against the standards.

Polycyclic aromatic hydrocarbons (PAHs), polycyclic brominated diphenyl ethers (PBDEs) and polycyclic chlorinated biphenyls (PCBs) in the simulated soil samples were

analyzed using gas chromatography-mass spectroscopy (GC-MS) (Perkin Elmer, Clarus 680C GC and Perkin Elmer, Clarus 600C MS) and detailed in Alimba et al., [18]. The detection of compounds was carried out based on relative retention times/peak area using NIST MS search program and TurboMass GC/MS Software (PerkinElmer, USA).

**Cytotoxicity testing of landfill leachates**

HepG2, Jurkat and HOS cells, obtained from National Cell Centre for Cell Science (NCCS, Pune, India), were maintained on DMEM, RPMI and MEM respectively, and supplemented with 10% FBS and 1% penicillin/streptomycin. The cells were grown in humidified 5% $CO_2$ incubator at 37ºC. HepG2 and HOS cells were seeded at a density of 2 x $10^5$ viable cells / mL per well, while Jurkat was seeded at a density of 4 x $10^5$ viable cells / mL per well at 37$^o$C in 5% $CO_2$. HepG2 and HOS cells were allowed to attach for at least 18–20 h, prior to leachate treatments. Using 92 well culture plates, 190 µL of medium was added to each well and 10 µL of each concentrations (6.25, 12.5, 25, 50, 75 and 100 % leachate/medium, v/v) of the leachate solutions, medium (0.00 µL; negative control, NC) and hydrogen peroxide (50 µM; positive control, PC), was also added to achieve a 2 x $10^5$ viable cells / mL in 200 µL per well for HepG2 and HOS, and 4 x $10^5$ viable cells / mL in 200 µL per well for Jurkat. The experiment was set up in three replicates for each concentration of the leachate samples and incubated for 24h. At post-incubation, cell morphological changes were observed using optical microscope (Catcom 130 Image analysis software, Catalyst Biotech, India). Cell viability was assessed by the MTT assay, following the manufacturer's protocol [18]. Cell inhibition was calculated from the Optimum Density (OD) as follows: (OD of negative control group - OD of experimental group)/(OD of negative control group - OD of positive control) x 100. Percentage (%) viability of the negative control cells was arbitrarily set at 100%, while the lethal concentration ($LC_{50}$) of the leachate samples that resulted into 50% cell death during leachate treatments was determined for each cell line from the dose-response curve analysis.

**Comet assay in the analysis of DNA damage in cells exposed to landfill leachates**

HepG2 and HOS cells were seeded at a density of 3 x $10^5$ viable cells / mL per well, while Jurkat was seeded at a density of 4 x $10^5$ viable cells / mL per well in 6 well flasks at 37ºC in 5% $CO_2$. Cells were treated with $LC_{50}$, 0.5x $LC_{50}$ and 0.2x $LC_{50}$ values obtained for each leachate samples per cell line for 24h at 37ºC to evaluate DNA damage using alkaline comet assay [18]. Slides were scored under blind code, using a fluorescence microscope (Olympus BX51 U-LH100HG, Tokyo Japan) equipped with excitation filter (N2.1, Ex. 515–560 nm, band pass; Em. 590 nm, long pass), with final magnification of 4003. 100 cells/slide were measured for Olive tail moment (OTM) using Komet 5.1 image-analysis software (Liverpool, UK).

**Statistical analysis**

Data are presented as means ± SD of triplicate (n=3) per concentrations performed for each leachate sample. Correlation coefficients were calculated to analyze concentration response relationships. The cytotoxic and genotoxic effects of leachates on the human cells and control were analyzed using one-way analysis of variance (ANOVA) and the difference between treated and control determined with Dunnett posthoc multiple comparison test ($p<0.05$) using Graphpad prism 5.0® software.

**RESULTS**

**Heavy metal and volatile organic characterization of leachate samples**

Heavy metal concentrations in the tested leachates are presented in Table 1. Cd, Fe, Mn and Al concentrations in the leachate samples were higher than national and international allowable limits. Pb and Be concentrations were below detectable limits in the leachate samples, Co and V were detected in the leachate samples. Zn was very high (17.35 mg/L) in AEL, but lower than permissible limits in NPL samples.

The GC-MS screening of the leachate samples produced different profiles of organic components at their retention times (Tables 2, 3). 30 organic pollutants were recorded in AEL (Table 2) and 23 organic pollutants in NPL (Table 3). The full scan analyses of the organic compounds revealed that they are mainly different types of polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs).

**Cytotoxicity evaluation of the leachates in Jurkat, HepG2 and HOS cell lines**

The effect of leachate treatment on cell viability as assessed using MTT assay showed that the tested leachates induced concentration dependent significant increase cytotoxicity in Jurkat, HepG2 and HOS cells as shown by decreased cell viability of the treated cells compared to the control (Figure 1). The $LC_{50}$ values derived from the dose response curves (Table 4) present different degrees of cytotoxicity induced on the cells by the leachates. Jurkat was the most sensitive cell to the leachate cytotoxicity among the cells while HOS was the least sensitive cell; Jurkat > HepG2 > HOS. The degree of cytotoxicity induced by the constituents of the leachate samples on the cells showed positive correlation: Jurkat cell {AEL ($p<0.0001$; $r=0.8599$) and NPL ($p<0.0001$; $r=0.8563$)}, HepG2 cell {AEL ($p<0.0001$; $r=0.8772$) and NPL ($p<0.0001$; $r=0.8407$)}, and HOS cell {AEL ($p<0.0001$; $r=0.9186$) and NPL ($p<0.0001$; $r=0.9203$)}.

**Morphological changes in Jurkat, HepG2 and HOS following leachate treatment**

Morphological alterations induced by the leachate samples on Jurkat, HOS and HepG2 cells are presented in figures 2–4. The tested leachates induced conspicuous cell death on all the cells and this mortality increased with leachate concentrations. Figure 2a presents the normal spherical Jurkat morphology from the negative control, while Figures 3b–d presents shrunken morphology of the treated Jurkat cells due to flaccidity induced by the leachates. They are smaller in size compared to the negative control and not in normal aggregation. Figures 3a and 4a present the regular dimensional shapes of HOS and HepG2 cells respectively. They grow attached to the substrata, while HOS divides faster than HepG2 and covering the substratum of the culture flask, HepG2 grows in discrete patches. Figures 3b–d and 4b–d showed the detachment of these cells from the substrate, cytoplasmic vacuolization and cells became shrunken in response to leachate treatment. Many of the cells showed decrease cell size compared to the negative control. The observed morphological alterations are related to cell death or apoptosis.

**Table 1.** Heavy metals detected in Aba Eku leachate (AEL) and Bhandewadi Nagpur leachate (NPL) samples and national and international permissible limits [18]

| Metals[a] | AEL | NPL | WHO[b] | USEPA[c] | BIS[d] | NESREA[e] |
|---|---|---|---|---|---|---|
| As | 0.0044 | 0.0018 | 0.05 | 0.01 | - | - |
| Cd | 0.0874 | 0.0740 | 0.003 | 0.005 | 0.01 | 0.01 |
| Co | 0.0006 | 0.0002 | 0.05 | - | - | - |
| Cr | 0.0006 | 0.0006 | 0.05 | 0.10 | 0.05 | 0.01 |
| Cu | 0.0006 | 0.0002 | 2.0 | 1.3 | 0.05 | 0.1 |
| Fe | 8.5132 | 8.9586 | 0.3 | 0.3 | 0.30 | 3.0 |
| Mn | 7.6302 | 5.6680 | 0.4 | 0.05 | 0.01 | 5.0 |
| Ni | 0.0008 | 0.0006 | 0.02 | - | - | - |
| Pb | BDL | BDL | 0.01 | 0.015 | 0.05 | 0.05 |
| Se | 0.0024 | 0.0128 | - | - | - | - |
| Zn | 17.3582 | 0.0210 | 3.0 | - | - | 3.0 |
| Al | 0.1778 | 0.2588 | - | - | - | - |
| Be | BDL | BDL | - | - | - | - |
| V | 0.0010 | 0.0004 | - | - | - | - |

*[a]All values are in mg/L.; BDL: Below detectable limit; [b]WHO guidelines for drinking water quality [39]; [c]United States Environmental Protection Agency. Maximum permissible limit for effluents from wastewater (www.epa.gov/safewater/mcl.html) [40]; [d]Bureau of Indian Standards [41] (IS 10500); [e]National Environmental Standards and Regulations Enforcement Agency in Nigeria [42].*

**DNA damage assessment in Jurkat, HepG2 and HOS cells exposed to the leachates**

Figures 5 presents the mean value (± SD) of the Olive tail moment measured in Jurkat, HepG2 and HOS cells treated for 24h with the sub-lethal concentrations of the leachates. There was concentration dependent significant increase in Olive tail moment of the leachate treated cells compared to the untreated cells; Jurkat cell ($p<0.0001$; $r=0.8941$), HepG2 cell ($p<0.0001$; $r=0.9583$), and HOS cell ($p=0.0031$; $r=0.9031$). Jurkat was the most sensitive to leachate induced DNA damage than HepG2 and HOS. $H_2O_2$ (positive control; 50 µM), a known reactive oxygen species, similarly induced DNA migration and breakage on all the cells.

**Table 2.** Identified organic compounds at their various peak retention times in Aba Eku landfill leachate (AEL) sample [18]

| Peak retention time (min) | Identified organic compounds |
|---|---|
| 2.03 | Methylene chloride |
| 2.80 | [1,4] Dioxino [2,3-B]-1,4-hexahydro-2,3-dimethyl dioxin |
| 3.82 | 1-isopropoxy-2,2,3-trimethylaziridine(sin) |
| 5.57 | N-(2,2-dichloro-1-hydroxy-ethyl)-2,2-dimethyl-propionamide |
| 6.17 | 1,6-anhydro-2,4-dideoxy-beta-D-ribo-hexopyranose |
| 6.73 | 2-methyl-3-propyloxaziridine |
| 7.13 | 2-trifluoroacetylaminopropenoic acid |
| 8.94 | 4-Ethylamino-N-Butylamine |
| 9.92 | 1-[N-Dodecyl] Aziridine |
| 10.64 | 2,3,4,5,6,7-hexahydro-3,6-dihexyl-10,11-diphenyl-bis[1,3]oxazino[6,5F] |
| 11.85 | 1,2-N-(2-Aminoethyl)ethanediamine |
| 12.90 | 2,3-Epoxyhexanol |
| 13.32 | 1,6-anhydro-3,4-dideoxy-beta-D-manno-hexa-pyranose |
| 15.08 | 1,2-cyclopentanediol (trans) |
| 15.37 | Azacyclodecan-5-ol |
| 15.61 | 1,3-Bis-T-Butylperoxy-phthalate |
| 16.02 | 2-T-butylperoxy-2-ethylbutan-1-ol (Butyrate ester) |
| 16.99 | Gamma-guanidinobutyricacid |
| 17.86 | 2,2,4-trimethyl-1,3-pentanediol |
| 18.59 | O-methyloximedecanol |
| 18.98 | 1-Chloroacetylpiperidine, |
| 19.56 | 2-Carbamyl-9-[Beta-D-Ribofuranosyl] Hypoxanthine |
| 20.00 | N-(2-Aminoethyl) 1,2-Ethanediamine |
| 20.27 | 4-Biguanidoantipyrine |
| 21.39 | Ala-Gly,Trimethylsilyl Ester |
| 22.47 | Propanamide |
| 23.96 | 2-Nonenoicacid |
| 24.90 | 2-cyanoAcetamide |
| 25.71 | 1,3-bis-t-butylperoxy-phthalan |
| 27.15 | Emylcamate |

**Table 3.** Identified organic compounds at their various peak retention times in Bhandewadi Nagpur landfill leachate (NPL) sample [18].

| Peak retention time (min) | Identified organic compounds |
|---|---|
| 2.03 | Methylene chloride |
| 3.68 | N-butyl-N-Nitro-1-Butanamine |
| 4.49 | 2-methyl-N-(2-methylpropyl)-N-nitroso-1-propanamine |
| 9.29 | 3,3'-Iminobispropylamine |
| 12.00 | 1,3-bis-t-butylperoxy-phthalate |
| 12.41 | 1,6-anhydro-2,4-dideoxy-beta-D-ribo-hexopyranose |
| 13.31 | Cyanoacetylurea |
| 14.67 | 1,3-methylene-D-arabitol |
| 15.07 | Octanal |
| 15.44 | Tert-butylmethylcarbonate |
| 15.74 | 2-cyanoacetamide |
| 16.03 | 2-TrifluoroAcetoxydodecane |
| 16.50 | 1-Pentyn-3-Ol,3-Ethyl |
| 17.02 | Phenol,3,5-Bis(1,1-dimethylethyl |
| 19.61 | Propanamide |
| 20.17 | Guanidine,Methyl |
| 20.50 | Tetracetyl_D-XylonicNitrile |
| 20.99 | 2-Formyl-9-[Beta-D-Ribofuranosyl]Hypoxanthine |
| 23.06 | Propanamide,N-(Aminocarbonyl) |
| 23.96 | 1,3-bis-t-butylperoxy-phthalan |
| 24.49 | Cyclopropenecarboxylamide |
| 25.53 | Butylaldoxime,2-methyl- (Syn) |
| 25.79 | Butylaldoxime,2-methyl- (Anti) |

**Table 4.** Regression analysis from the dose-response curve of AEL and NPL cytotoxic effects on Jurkat, HepG2 and HOS cells [18].

| Cells | | AEL | NPL |
|---|---|---|---|
| **Jurkat** | **$LC_{50}$** | 26.15% | 24.89% |
| | **$R^2$** | 0.7394 | 0.7333 |
| | **RE** | Y= -0.72x + 68.77 | Y= -0.72x + 67.85 |
| **HepG2** | **$LC_{50}$** | 30.58% | 27.45% |
| | **$R^2$** | 0.7695 | 0.7068 |
| | **RE** | Y= -0.74x + 72.63 | Y= -0.68x + 68.67 |
| **HOS** | **$LC_{50}$** | 54.87% | 30.33% |
| | **$R^2$** | 0.8438 | 0.8467 |
| | **RE** | Y= -0.60x + 82.79 | Y= -0.78x + 81.11 |

*RE (Regression equation); $LC_{50}$ (Leachate lethal concentration that produced 50% cell death), $R^2$ (Regression coefficient); AEL (Aba Eku landfill leachates); NPL (Bhandewadi Nagpur landfill leachate).*

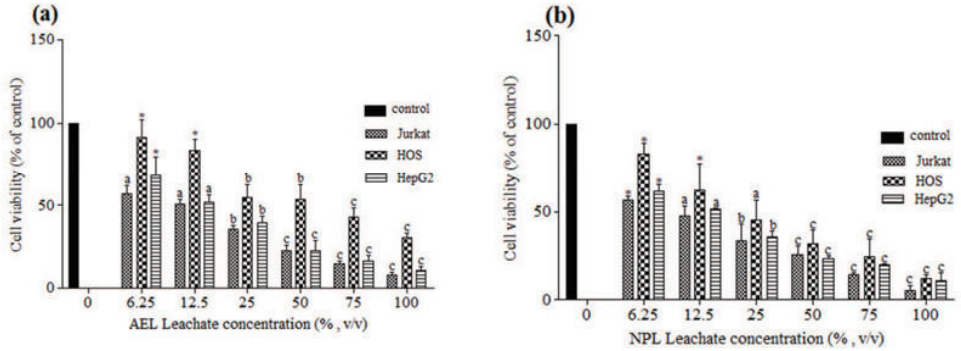


**Figure 1**. Percentage cell viability from (a) Aba Eku leachate and (b) Bhandewadi Nagpur leachate treated Jurkat, HepG2 and HOS cells. Results are expressed as mean ± SD (n=3). Values are significantly different *p>0.05, $^{a}$p<0.05,$^{b}$p<0.01 and $^{c}$p<0.001 compared to negative control [18].

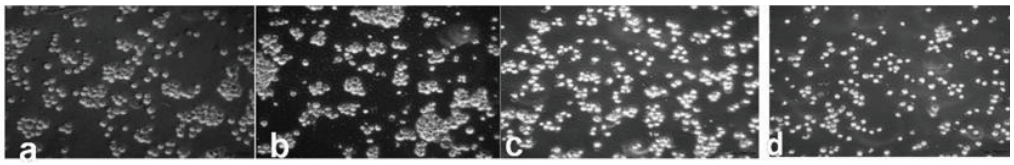


**Figure 2**. Alterations in Jurkat morphology induced by landfill leachates: a) Normal spherical Jurkat morphology in the negative control cells. b) Cells with abnormal cell morphologies. c) Shrunken cells with decreased cell sizes. d) Shrunken cells with reduced cell sizes and not in aggregations [18].

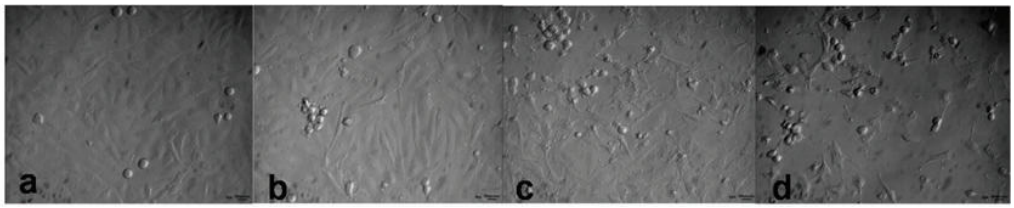


**Figure 3**. Alterations in HOS morphology induced by landfill leachates: a) Regular dimensional HOS morphology of the negative control cells. b) Shrunken cells that detached from the substrate. c) Cells with shrunken shape and smaller in sizes compared to control. d) Cells having star-like morphological shape with vacuolations [18].

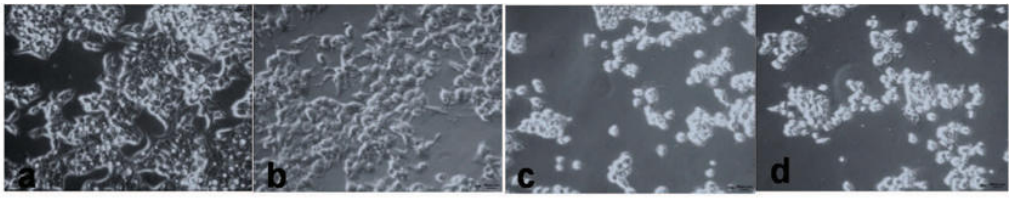


**Figure 4**. Alterations in HepG2 morphology induced by landfill leachates: a) Regular dimensional HepG2 morphology of the negative control cells. b) Cells detached from the substrate. c-d) Cells with shrunken shape, small sizes compared to control, discrete with cytoplasmic vacuolization [18].

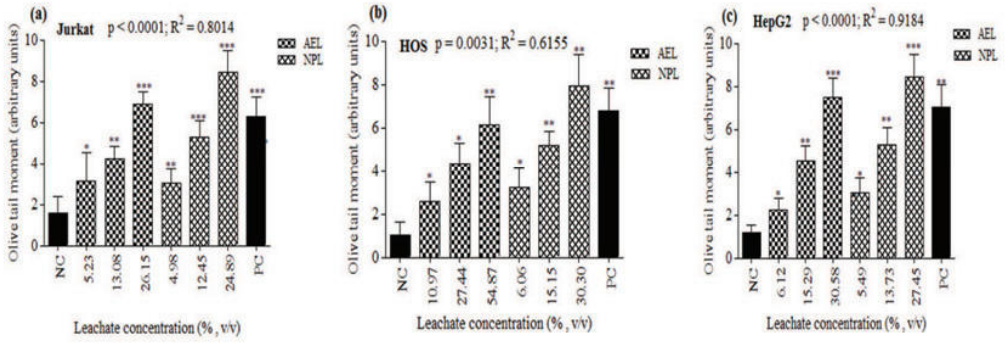


**Figure 5**. DNA damage, Olive tail moment (arbitrary units), in (a) Jurkat, (b) HOS and (c) HepG2 cells exposed to the simulated landfill soil leachates. Results are expressed as mean ± SD (n=50 cells). Values are significantly different *p<0.05, **p<0.01 and ***p<0.001 compared to the negative control [18].

## DISCUSSION

The use of MTT assay in *in vitro* cytotoxicity studies for assessing cell viability during exposure to toxic substance is reliable and sensitive, since it links possible cause of cell death to mitochondrial damage or alterations in mitochondrial functions [20]. The

concentration dependent significant increase in percentage cell viability induced by landfill leachates in the treated cells herein showed that chemical constituents of the leachates induced pathological lesions on the cells. This deduction is supported by the morphological alterations induced by the leachates on the cells, which were signs of apoptotic and or necrotic cell death [21]. Furthermore, physiologically, when cells detached from the base of the culture plate as observed with HepG2 and HOS, it reveals an interruption of the extracellular matrix and inhibition of cell-cell contact, and when it becomes flat and shrunken in appearance or reduction in size (Figures 2-4), it suggests apoptotic bodies.

It is well known that leachate constituents are capable of inducing reactive oxygen species (ROS) in biological systems [14, 15]. This may suggest that decrease cell viability and morphological alterations (cytotoxicity) induced by the constituents of the leachate samples may be attributed to ROS induced alterations in the cells' mitochondrial system which probably led to the induction of active oxygen related cell death. The reports that e-waste leachate decreased mitochondrial potentials (cytotoxicity) of NIH/3T3 mouse fibroblast cell via oxidative stress induced apoptosis [22], and landfill leachate induced cytotoxicity on human breast cancer (MCF-7) cell via oxidative stress associated necrosis [23] are in support of the oxidative stress related cell death assertion. Furthermore, reports from *in vivo* studies that landfill leachates altered erythrocyte morphology in Wistar rats [24] and sperm morphologies in mice [25] are in consistence with the morphological alterations observed in the treated cells. Similarly, reduction in cell proliferation and viability induced by industrial solid waste leachates on human peripheral lymphocytes [26] and e-waste leachate on NIH/3T3 mouse fibroblast cell [22] are in consistence with decrease cell viability induced by AEL and NPL on the cells.

The genotoxic effects induced by the sub-lethal concentrations of the leachates showed that the leachate constitutes increase genome instability in mammalian system. The DNA damage detected in the leachate treated cells may have originated from DNA single and double strands breaks, DNA adducts formations, DNA-DNA and DNA-protein cross-links, alkali-labile sites and incomplete excision repair [27]. Many of the metals analyzed in the leachates, which are classified as mutagens and carcinogens, or probable mutagens and carcinogens [28], have been implicated with cytotoxic effects and DNA

damage in cells. For instance, Cd, a known carcinogen, exerted cytotoxic effect on two hepatoma cells (HTC and HepG2) [29] and genotoxic effect on white blood cells and fibroblast cells [30] via oxidative stress induced apoptosis. Al elicited cytotoxic and genotoxic effects on different phases of human lymphocyte cycle by producing free radicals [31]. Also Al and Fe are known to synergistically interact and induced DNA damage in human neural (HN) cells via free radical production [32]. Therefore, the leachate constitutes possibly acted singly and/or interacted among one another to induce the observed decreased cell viability and DNA damage on the treated cells.

Organic compounds present in landfill leachates have been described as emergent chemicals which are capable of contaminating both aquatic and terrestrial environments, posing treat to human health and wildlife diversities [6]. Many of these compounds in the tested leachates belong to the groups of polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) are described as carcinogens and mutagens. Even at trace concentrations, they are harmful to cells hence are on the priority lists of hazardous substances [33, 34]. For instance, nitrobenzenes are known to elicit free radicals which induced DNA damage on human lymphocytes [35]. Dibutylphthalate and diisobutylphthalate (phthalate esters) detected in the leachate samples (Tables 2-3) induced DNA single-strand breaks in nasal mucosa and oropharyngeal epithelia cells [36]. PAHs induced their deleterious effects via adduct formation with many proteins and nucleic acids, while PCBs produce intermediates which act as alkylating agents and form nucleic acid adducts [37].

Heavy metals, polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons detected in the leachate samples are stable pollutants that are present in almost all compartments of the aquatic and terrestrial ecosystems. Being lipophilic compounds, they possess the ability to bioaccumulate in lower forms and plants, and biomagnify through food webs to accumulate in the fat-rich tissues of higher trophic animals including humans. Studies have shown that human population working in and living around landfill facilities harboured higher concentrations of toxic metals and organic pollutants in their blood and breast milk than control population [38]. This suggests the possibilities of human exposure to chemical mixtures in leachates via soil, water and food. The presence of toxic metals and organic pollutants in blood and human

breast milk is of public health concern as breast milk serves as source of nutrient to infants, considering that these compounds are endocrine disrupting chemicals capable of impairing reproduction and developmental processes and may lead to carcinogenesis [5].

In conclusion, municipal landfill leachates from Nigeria and India caused decrease cell viability and abnormal cell morphology in human cells *in vitro*. They also increased DNA damage in Jurkat, HepG2 and HOS suggesting their ability to induce cytotoxic and genotoxic effects in human cells *in vivo*. Chemical analysis of the leachates revealed the presence of toxic metals and numerous organic compounds (classified as PCBs and PAHs) which possibly acted singly or synergistically to induce the cytotoxic and genotoxic effects on the cells. Indiscriminate co-disposal of industrial, agricultural, medical, institutional and municipal solid wastes into unsanitary landfills is capable of increasing aquatic and terrestrial pollution hence posing treat to human health, plant and animal diversities.

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