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Review

Genotoxicity and mutagenicity of solid waste leachates: A review

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Solid waste production is inevitable and its unsanitary disposal in the environment is of public and environmental health concern. Leachate, generated due to the infiltration of water/precipitation through the waste mass and the wastes biodegradation, is a mixture of dissolved organic matter, inorganic macro-components, heavy metals, xenobiotic organic compounds and microorganisms. Several studies have reported the acute toxicity of leachate using different end points, while evidences are accumulating on their potentials to induce genetic damage. In this wise, different short-term *in vivo* and *in vitro* bioassays are being utilized in the evaluations of genotoxicity and mutagenicity of leachates; and the possible mechanisms of genetic damage. This paper reviews reports on leachate-induced genetic damage. There is need for a shift from waste disposal to sustainable waste management. Awareness on possible health impacts or consequences of exposure to solid waste should also be created through health education.

Key words: Solid waste leachate, genotoxicity, mutagenicity, environmental pollution.

INTRODUCTION

Solid wastes are mostly undesirable by-products of economic development and technological advancement. With the effort to increase standard of living by most countries, increase in solid waste production became inevitable and will continue due largely to accelerated industrialization, urbanization, and population growth. The waste types (including metal scraps, chips and grits from iron and steel industries, paper and pulp industries, tyres, spent oil from machines, synthetic textile dye and tans from textile and tannery industries, radioactive substances, pharmaceutical and personal care products, carcasses and other animal wastes, electrical and electronic wastes, among others) may possess flammable, irritable, toxic, carcinogenic and or corrosive properties. This suggests that solid wastes may be dangerous to life if not properly managed; and have elicited strong international concerns about the possible environmental and health effects of living in the vicinity of the wastes.

In an attempt to guide against the release of hazardous chemicals capable of polluting the environment and harming biotic communities, different methods of waste management (incineration, landfilling, recycling, composting, disposal into sea and water ways, burning along major roads and surface dumping) are utilized worldwide. Among these, landfilling is the most common, accounting for the disposal of about 95% of the total solid waste collection worldwide (Kurniawan et al., 2006). Very few of the landfills and incinerators are designated for either domestic or industrial solid waste disposal while majorities are for co-disposal of wastes. Contrary to situations in some developed nations, landfills in most

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developing and some developed nations are unsanitary without liner, covers and leachate collecting systems. They are located in public places surrounded by residential quarters and in wetland or other places with seasonally high water tables. Sometimes wastes are also disposed into water bodies and around river banks, in gullies excavated by erosions and human activities, in gutters and channels constructed for flooding and burning on major roads. These disposal methods are capable of releasing hazardous substances into the environment which can endanger the survival of living organisms including human (Alimba, 2013).

During disposal and processing of solid wastes in landfills and dump sites, they undergo a combination of physical, chemical and microbial processes (Christensen et al., 2001). These processes transform the solid wastes into different water-soluble compounds and particulate matter which can be transferred from the wastes to the percolating water (Bjerg et al., 2003). The contaminant-rich aqueous solution of pollutants formed is termed "leachate". The sources of the percolating water may include precipitation, irrigation, surface runoff, groundwater intrusion and the initial moisture content present within the wastes (El-Fadel et al., 1997).

WASTES LEACHATE COMPOSITION

Leachates could be natural or synthetic. Natural leachate is the liquid originating from full-scale existing landfill operations. They fall into three classes; raw, diluted and treated (Cameron and Koch, 1980). Raw leachate is derived directly from solid wastes filled areas either from wells driven into the landfills or leachate springs. Diluted natural leachate is formed when it flows through surface waters like creeks and its concentrations diluted by the water body and/or attenuated during its passage through the landfill and soil layers. Treated leachate receives varying degrees of physical and/or chemical treatment to attenuate its concentrations. Synthetic or simulated leachates are produced from test lysimeters containing composite solid wastes operated under a carefully controlled set of temperature and precipitation rate that mimics the actual landfill conditions (Cameron and Koch, 1980). Numerous studies have classified leachate generated from solid wastes into a complex mixture of chemicals and microorganisms. Christensen et al. (1994) describe landfill leachate as a mixture of four major groups of pollutants: dissolved organic matter, inorganic macro-components, heavy metals and xenobiotic organic compounds. Other compounds that may be present, but in minute amounts are: boron, arsenic, selenium, lithium, mercury and cobalt (Christensen et al., 2001). These compounds which are of secondary importance vary in their compositions depending on the type of solid waste and age, landfill technology, degree of compaction, the hydrology of the site and climatic change (Bjerg et al., 2003; Fan et al., 2006). Macro-components which are the major inorganic constituents of leachates [calcium (Ca), magnesium (Mg), sodium (Na), potassium (K), ammonium (NH^{4+}), iron (Fe), manganese (Mn), chloride (Cl^-), sulphates (SO_4^{2-}) and bicarbonates (HCO_3^-)] and their concentrations depend on the stabilisation processes in the landfills or dumpsites (Kjeldsen et al., 2002).

Dissolved organic matter describes the soluble organic component of the solid waste leachates and is usually expressed as chemical oxygen demand (COD), total organic carbon (TOC) or biochemical oxygen demand (BOD). At early stages of solid wastes in landfills, leachates usually have high BOD (9500 mg/L) value and even higher COD (14 000 mg/L) content (Kjeldsen et al., 2002). Dissolved organic matter is a bulk parameter covering a wide range of organic degradation products including methane (CH_4), volatile fatty acids and some refractory compounds such as fulvic and humic-like compounds. The decomposition of organic matter present in solid wastes gives leachates their colours: yellow, brown, dark or black (Aziz et al., 2007) and also determine to a greater extent the volume and diversities of microorganisms present in such leachates (Donnelly et al., 1988). More than 200 organic compounds have been identified in solid waste leachates (Paxeus, 2000; Schwarzbauer et al., 2002), with about 35 of these having the potential to cause harm to the environment and human health (Paxeus, 2000). Some of the identified organic compounds in leachates obtained from municipal landfills and incineration residues from Japan include persistent organic pollutants (POPs) such as non-ortho and mono-ortho substituted chlorobiphebyls (dioxin-like PCBs), polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDDs/DFs) (Ham et al., 2008). Similarly, low concentrations of polycyclic aromatic hydro-carbons (PAHs) and polychlorinated biphenyls (PCBs) were reported in fly and bottom ashes of a medical waste incineration facility from Greece (Valavanidis et al., 2008). Matejczyk et al. (2011) also detected low concentrations of hexachlorobutadiene, pentachloro-benzene and PAHs in the leachates from 22 municipal solid waste (MSW) landfill sites in Southern Poland. Brown and Donnelly (1988) concluded from their findings that the numerous organic compounds reported in 58 hazardous and municipal waste landfill leachates may exhibit similar toxic and carcinogenic effects in biologic systems. The reports of Christensen et al. (2001), Kjeldsen et al. (2002) and Cuadra et al. (2006) showed that during exposure, the organic compounds in leachates are capable of bioaccumulating in fish and human tissues.

The compounds widely described as xenobiotic organic compounds (XOCs) are chiefly associated with industrial or conventional hazardous solid wastes, but a large number are also present in municipal or household solid wastes (Schwarzbauer et al., 2002; Slack et al., 2007). Also, paint, garden chemicals, household cleaning agents, human and veterinary medicines, motor vehicle

products, waste electrical and electronic equipment and batteries, are all potential sources of XOCs (Slack et al., 2005, 2007). Hao et al. (1995) reported that significant quantities of XOCs are present in municipal landfill leachates from Taiyuan, China, and those detected were chlorinated and non chlorinated hydrocarbons, carbon tetrachloride, chlorobenzene, toluene, chloromethane, chloroethylene, xylene, phenols, phthalate, camphor, decanoic and nonanoic and cresol. The concentrations of XOCs are higher during the active stage of decomposition and gradually decrease as the landfill stabilizes (Christensen et al., 2001) but they can continue leaching from the wastes for decades. The types and concentrations of XOCs frequently identified in solid waste leachates differ from one leachate to another and the difference is a reflection of the age of the landfill, waste composition and landfill management processes occurring at the site (Oman and Rosqvist, 1999; Christensen et al., 2001). Benzene, toluene, ethyl-benzene and xylenes (BTEX compounds) are XOCs commonly found in high concentrations in most solid waste leachates, due to their common usage as solvents in large range of products and waste generating pro-cesses (Kjeldsen et al., 2002). Next to BTEX are the halogenated hydrocarbons; tetrachloroethylene, trichloro-ethylene and dichloroethanes (Krug and Ham, 1991; Kjeldsen et al., 2002). These compounds are almost universal in their occurrence in leachates. They are among the commonly analyzed XOCs in leachate samples in most developed countries due to their being designated by USEPA as priority pollutants due to their aquatic polluting capability (Kjeldsen et al., 2002).

Plasticisers are another group of XOCs which have been widely reported in landfill leachates. The principal members of XOC in this group of compounds are phthalates, of which di-(2-ethylhexyl) phthalate (DEHP), diethyl phthalate (DEP), diisononyl phthalate (DINP) and dibutyl phthalate (DBP) have been extensively used in the production of consumer goods (Jonsson et al., 2003; Marttinen et al., 2003). Public health concern for this group of compounds has increased since they were being classified as endocrine disruptors and listed as priority pollutants by the USEPA (Schwarzbauer et al., 2002). The tendency to persist in the environment and bioaccumulate in organisms are the main contributory factors for their priority status (Schwarzbauer et al., 2002). These xenoestrogens are metabolites of various widely used substances that are preferentially adsorbed onto sewage sludge (Marcomini et al., 1989), suggesting a possible entry route into landfills.

Heavy metals are of particular interest due to their hazardous nature (European Commission, 2002). The commonly occurring heavy metals in landfill leachates include copper, cadmium, lead, nickel, chromium, arsenic, manganese, zinc and mercury (Reinhart, 1993) and they can form metal colloids or complexes, particularly with organic matter (Jensen and Christensen, 1999). As such, the heavy metal content of leachates can be significantly high. Generally, heavy metals with high level of sorption and precipitation do not constitute a groundwater pollution threat due to poor migration into the leachate plume and low initial concentrations leaching from the solid waste. However, they can reach problematic levels (despite their low concentrations in the leachate) due to their ability to bioaccumulate in tissues of living organisms (Slack et al., 2005; Cuadra et al., 2006; Sanchez-Chardi et al., 2007). Particulate matters contaminated with heavy metals are also known primary sources of heavy metal emissions from solid wastes (Parker et al., 2002). Concentrations of heavy metals in landfill leachates are sometimes low and may not pose a great deal to groundwater pollution (Bjerg et al., 2003). However, when they are present in high concentrations, heavy metals can cause a little volume of leachate to be highly toxic (Sawaittayothin and Polprasert, 2007). Table 1 presents range of concentrations of some physico-chemical properties and heavy metals that are commonly analysed in solid waste leachates.

Pathogenic and opportunistic microorganisms have also been isolated from solid waste leachates. Donnelly et al. (1988) observed different species of Gram-positive and Gram-negative bacteria from different bacterial genera in hazardous solid waste and simulated leachates from experimental landfills. The Gram-positive species include the genus *Clostridium* while the Gram-negative species are mainly rod shaped genera *Citrobacter*, *Enterobacter*, *Klebsiella* and *Pseudomonas*. A total of 112 bacteria belonging to about 17 genera were isolated from Aba Eku landfill leachate from Nigeria (Oshode et al., 2008). These bacterial species including *Bacillus aureus*, *Escherichia coli*, *Staphylococcus aureus*, *Clostridium sordelli*, *Clostridium perfinges* and *Salmonella arizonae* are of public health concern. Some of the isolates investigated for virulence factors and antibiotic resistance produced enterotoxins (Efuntoye et al., 2011). Some genera of fungi group have also been identified in solid waste leachates. These are *Fusarium*, *Monosporium*, *Penicillium*, *Phoma* and *Pleospora* (Donnelly et al., 1988), *Aspergillus niger*, A*. flavus*, A*. terreus*, *Fusaruim oxysporium*, *penicillium spp* and *Rhizopus spp* (Oshode et al., 2008). These are potential pathogenic and toxin–producing species which can be transported along with leachates into ground or surface water sources and contaminating potable water (Daskalopoulos et al., 1998). Similarly, Matejczyk et al. (2011) characterized (by evaluation of chemical, microbiological and ecotoxicological parameters) the leachates from 22 municipal solid waste (MSW) landfill sites in Southern Poland. The leachates were conta-minated with bacteria, including aerobic, psychrophilic and mesophilic bacteria, coliform and fecal coliforms, and spore-forming-bacteria, including *Clostridium perfringens*, and with filamentous fungi. From their analysis of specific micro-organism groups, it can be concluded that the landfill

Table 1. Concentration range of some physico -chemical components and heavy metals analyzed in solid wastes leachates

Parameters*	CRL	TCR	NESREA[a]	USEPA[b]
Colour	Light-Dark brown	-	-	-
pH	4.76 – 8.10	5.0-7.5	6.0 – 9.0	6.5 – 8.5
Nitrate	0.12 - 98.65	0.1-10	10	10
Ammonia	1.41 - 126.81	100 - 400	10	0.02
BOD	306 - 601	1000 - 30000	50	-
COD	120.0 – 2480	1000 - 50000	90	-
Phosphate	1.04 - 122.02	0.5 - 50	2.0	-
Chloride	27.53 - 2470	100 - 2000	250	250
Sulphate	24.66 - 387.61	10 - 1000	250	250
Hardness	793 - 805	500 - 10000	150	0 - 75
Alkalinity	62 - 502	500 - 10000	-	20
TS	318.04 - 3116.67	3000 - 50000	-	-
Copper	0.08 – 22.31	0.02 – 1.00	0.5	1.3
Iron	0.48 - 8.65	-	-	0.3
Lead	0.011 – 130.89	0.1 – 1.0	0.05	0.015
Cadmium	0.008 – 8.04	0.001 – 0.10	0.2	0.05
Manganese	0.61 - 5.26	-	0.2	0.05
Arsenic	0.004 - 1.50	-	-	0.01
Nickels	1.88 – 2.41	-	0.05	-
Chromium	0.02 – 8.00	0.05 – 1.0	0.05	0.1

*All values are in mg/L except pH. BOD, biochemical oxygen demand; TS, total solid; COD, chemical oxygen demand ; [a]National Environmental Standards and Regulation Enforcement Agency (2009) (Nigeria) maximum permissible limits for wastewater. [b]United State Environmental Protection Agency (2006) (www.epa.gov/safewater/mcl.html); CRL, Concentration range for leachates from municipal landfill wastes (Bakare et al., 2005; Oni et al., 2011; Alimba and Bakare, 2012), hospital waste incinerator bottom ash (Akinbola et al., 2011), Polyfiber factory, aeronautical industry plants and municipal sludge (Bakare et al., 2007) and electronic wastes (Alabi and Bakare, 2011) ; TCR, typical concentration range of landfill leachate (Lee and Jones-Lee, 1994).

leachates showed sanitary and epidemiological hazard (Matejczyk et al., 2011).

The incidence of cancers (Janerich et al., 1981), low birth weight (Vianna and Polan, 1984) and increasing frequencies of chromosomal changes (Heath et al., 1984) among residents exposed to hazardous chemicals leaching from landfills increased the awareness on the adverse human health effects that might be associated with landfills. These reports drew the attention of researchers to solid waste toxicity studies, with the identification of some physical and chemical constituents of leachates as most commonly used approach to show the hazardousness of chemicals in waste leachates (Ikem et al., 2002; Oyeku and Eludoyin, 2010; Laniyan et al., 2011). This method has a major limitation in its inability to provide information about all the toxic chemicals present in the waste mixture and the potential synergistic and antagonistic interactions of these chemicals in living organisms. Since human health effects commonly associated with exposures to genotoxic compounds include cancer, birth defects and heart diseases (Houk, 1992), the need to evaluate the mutagenic and genotoxic effects of solid waste leachates in living organisms became necessary.

INDUCTION OF GENETIC DAMAGE BY SOLID WASTE LEACHATES

Genotoxicity and mutagenicity bioassays provide means for assessing the DNA and chromosome damaging abilities of a complex nature of xenobiotics without the need for their standard chemical characterizations (Claxton et al., 1998). Genotoxicity and mutagenicity tests are *in vitro*, *in vivo* and or *in situ* experiments designed to assess substances capable of inducing genetic damage through different mechanisms. Substances that prove positive to genetic tests are potential human carcinogens and/or mutagens. The genotoxicity of an unknown mixture is usually evaluated by exposing the samples to biologic substances and then examined for genetic damage. A number of bioassays using prokaryotic and eukaryotic organisms have been used to assess the potentials of solid waste leachates to induce genetic

damage. These studies have shown that leachates and leachate contaminated water bodies induced genotoxic and mutagenic effects in microbial, plant and animal systems. Different short-term tests have also been developed and utilized in the last three decades to aid in the identifications of genetic damage in biologic systems within the vicinities of solid waste facilities (Heath et al., 1984; Vrijheid et al., 2002). This is necessary since many congenital and non congenital diseases are often been linked to DNA damage.

MICROBIAL BIOASSAYS

Microbial bioassays such as Ames mutagenicity assay are commonly used worldwide and for a long time to evaluate agents that are capable of inducing genetic mutations (Ames *et al*., 1973; Maron and Ames, 1983; Claxton et al., 2010; Wolz et al., 2011). The utilization of these bioassays with bacteria has proven to be very effective for monitoring the mutagenicity of surface water due to their sensitivity, reliablity, and can be performed in a short period of time with relatively low cost (Ohe et al., 2004). These assays basically employ strains of the autotrophic bacteria, *S. typhimurium*. Some other microbial systems that have also been used are *E. coli* (WP2) bacterial assay (Green and Muriel, 1976), fungal; *Aspergillus nidulans* and yeast (*Saccharomyces cerevisiae*) (Scott et al., 1982). Among these test systems, the Salmonella mutagenicity test has been the most widely used for detecting mutagenicity/genotoxicity in leachate samples. The different responses of the Salmonella strains can provide information on the classes of mutagens present in leachate samples. This test developed by Ames et al. (1973) and Maron and Ames (1983) is based on the detection of histidine-independent revertants in selected Salmonella strains after exposure to mutagens with or without additional activating enzymes. The dose response can be quantified by varying sample concentration and counting revertant colonies per plate at each concentration and samples to be tested must be filter sterilized under normal conditions (Junk et al., 1974).

USEPA (1980) evaluated the mutagenicity of leachates simulated from solid wastes collected from different sources and their XAD-2 concentrates (XAD resins is the most commonly applied method for concentrating organic substances from different kinds of surface waters) using three different microbial bioassays with and without metabolic activation in two microorganisms. These are: (1) *Salmonella/*microsome assay, (2) *Saccharomyces* $can^1/hist^+$ dual assay and (3) *Salmonella* UvrB repair assay. The results show that except for the arsenic-contaminated groundwater, none of the leachates from wastes or their XAD-2 concentrates showed positive mutagenic activity in the first assay. In the second assay, only the XAD-2 concentrate of the arsenic-contaminated sample showed positive mutagenic effect in the test system without metabolic activation in a 24 h exposure. None of the leachates showed positive mutagenic activity in the third assay, either with or without metabolic activation. Similarly, Omura et al. (1991) reported that leachates from solid wastes in domestic landfills in Japan induced mutagenic effects in the *S. typhimurium*/mammalian-microsome assay. Their study also showed that the acidification of the leachate before XAD treatment effectively increased the mutagenicity of the leachate samples. In an attempt to further ascertain the mutagenic agents in the solid waste leachates, Omura et al. (1992) recovered the organic concentrates from the leachates using XAD-2/8 resin adsorption and their mutagenic activities were tested for 8 months using the Ames *Salmonella* /microsome assay. Highly polluted leachates (COD and BOD > or = 40 mg/l) generally had equal or higher mutagenic activities than lightly polluted leachates (COD and BOD < 40 mg/l). But there was no clear difference in the mutagenicity per amount of concentrate between the two leachates. These results suggest that the mutagenic activity of landfill leachate is determined to some degree by the organic concentrates in the leachate. Kamiya *et al*. (1989) studied the mutagenicity of refuse leachate from municipal incinerator using liquid rec-assay and Ames assay. The volatile components of the leachate did not induce mutagenic effects while the non volatile components induced mutagenicity in the Ames assay. Also, the leachate induced DNA damage in the liquid rec-assay with S-9 mix. The PAHs extracted from tobacco ash and carbonyl compounds obtained by the putrefaction of foods were confirmed to be main contributors to the DNA damaging capacity and mutagenicity of the refuse leachate (Kamiya et al., 1989).

Bessi et al. (1992) evaluated 14 series of toxic wastes from different origins (metallurgy, chemical industries, incinerators, industrial treatment plants, and others) for their mutagenic properties using the *Salmonella* reversion assay. To consider realism of environmental impact, a strategy was proposed to assess mutagenicity of the water-soluble fraction of the wastes. Water-extractable micro pollutants were further concentrated by liquid–liquid extraction or lyophilisation prior to mutagenicity testing. None of the 14 crude aqueous fractions was shown to be mutagenic. On the contrary, positive responses were recorded on the concentrated phases of two solid wastes derived from one chemical industry and from the treatment of organophosphorus wastewaters. Chemical analysis did not reveal the presence of known mutagens in the extracts with mutagenic effects. The authors hypothesized that genotoxicity was due to interacting effects between micro pollutants. McDaniels et al. (1993) compared ten soil samples from a hazardous waste site for their mutagenic activities by the Ames test and a modified SOS colorimetric test. Organic extracts were prepared from the soil sample by soxhlet extraction.

Analysis of variance indicated that with S-9 mix, Ames and SOS results were similar for the same soils and solvent extractions. However, without S-9 mix, the SOS test was significantly more sensitive than the Ames test to the mutagens extracted from the soils. Both the Ames and SOS test detected lower concentrations of mutagens in methylene chloride than in cyclohexane extracts of the soil samples (McDaniels et al., 1993).

Schrab et al. (1993) assessed the genetic and acute toxicities of four municipal solid waste (MSW) landfill leachates and groundwater samples using the *Salmonella*/microsome (Ames test) bioassay, the *B. subtilis* (DNA repair) bioassay, the diploid *A. nidulans* (chromosome damage) bioassay and the Microtox test. All the tested leachate samples were acutely toxic, while three of the four leachate samples were genetically toxic. The authors concluded from their results that two of the leachates had mean estimated cumulative cancer risks with similar order of magnitude (10^{-4}) as leachates from co-disposal and hazardous waste landfills. In a comparative study to evaluate the performance of four bacterial short-term genotoxicity assays (*Salmonella*/microsome assay, SOS chromotest, Microscreen phage-induction assay, differential DNA repair test) that are widely used and/or have a promising potential for the genotoxicity testing of water samples, landfill leachate was found to display higher genotoxic activity than other samples tested (Helma et al., 1996). The tested landfill leachate samples induced genotoxic effects in all the assays with the exception of the Microscreen assay, which failed to detect genotoxicity due to viral toxicity.

Koikawa-Mutoh et al. (1992) studied the mutagenicity of leachates from a MSW landfill using the Ames assay with five tester strains of *S. typhimurium*. Of the 36 leachate samples studied, 12 showed significant mutagenicity for both tester strains TA98 and TA100, and 3 for TA98 but not for TA100. The mutagenicity of these leachate samples was observed mostly without metabolic activation by liver homogenates from rats (S9). Further examinations with selected samples showed mutagenicity for TA1537, a frameshift mutant, but not for TA1535, a base-pair mutant, nor TA1977, a frameshift mutant which had an excision repair system. The increase in a concentration of S9 up to 150 µg/plate caused the decrease in mutagenicity for both TA98 and TA100 down to 40 to 70%. The addition of a low concentration of S9 (20 µg/plate), however, decreased markedly bactericidal effects observed under higher concentrations of extracts than 250 µg/plate. The authors concluded that the active ingredients of the leachate extracts consisted mainly of direct mutagens which induced frameshift mutation by reacting with *Salmonella* DNA in a covalent bonding.

Singh et al. (2007) analysed leachates derived from dry wastes of metal, tannery, and dye industries from the State of Uttar Pradesh (India) for their mutagenic potential using *Salmonella* reverse mutation assay. Both the spot and plate incorporation assays were conducted with four tester strains of *S. typhimurium* (TA97a, TA98, TA100, and TA102). Their result suggests that leachates derived from metal and tannery wastes possess mutagenic properties.

Kwasniewska et al. (2012) used the Ames test and the *umu*-test to assess the genotoxicity of aqueous leachates from municipal solid waste landfill sites in Southern Poland. None of the tested 22 leachate samples revealed genotoxic activities in the *umu*-test, while two samples were described as genotoxic in the Ames test. Also, Ribe *et al.* (2012) evaluated the genotoxic potentials of landfill leachates treated with pine bark biosorbent and untreated leachates using bacterial *umu*-test. No genotoxic effects were detected in any of the tested leachate samples (treated and untreated) with the *umu*-C genotoxicity test using the *S. typhimurium*. These authors concluded that *umu*-test appeared to be insensitive enough in the mutagenicity evaluation of leachates.

Although, these microbial bioassays are useful in the detection of mutagens, studies have however shown that they have low sensitivity for heavy metals (Gatehouse et al., 1994). These may suggest why in some studies, negative mutagenic effects of solid waste leachate using the Ames test was reported (Radetski et al., 2004). Also, one major deficiency of using microbial assays in testing for the mutagenic effects of agents is that bacteria lack many of the metabolic enzymes present in animals. Some of these enzymes activate chemicals into electrophilic compounds capable of binding covalently to DNA (Houk, 1992). This may suggest why most recent studies use battery of assays including plant and animals to assess genetic effects of solid waste leachates (Radetski et al., 2004; Lah et al., 2008; Chakraborty and Mukherjee, 2009).

PLANT BIOASSAYS

Plant bioassays, which are sensitive and simple in comparison with animal bioassays, have been validated in international collaborative studies, and prove to be efficient tests for genotoxic monitoring of environmental pollutants (Turkoglu, 2009).

The use of meristematic cells in these plants makes it possible to determine different cytogenetic end points: cytotoxicity, genotoxicity and mutagenicity, when treated with test agents. The analysis of the cytotoxicity was done by evaluating the mitotic index and cell death. The induction of aberrant metaphase, anaphase and telophase, such as bridge, loss and chromosome stickiness, polyploidy, irregular nuclei and nuclear buds are parameters for the genotoxicity, while the micronuclei and chromosome breaks are used to assess the mutagenicity effects (Fernandes et al., 2007; Leme and Marin-Morales, 2009; Souza et al., 2009).

Plant materials have been successfully utilized in the assessment of the genotoxicity and mutagenicity of solid waste leachates. Sang et al. (2006) investigated the genotoxicity of a municipal landfill leachate with the *Hordeum vulgare* root-tip cytogenetic bioassay, using chemical oxygen demand (CODCr) as a measure of leachate concentration. Leachate decreased the mitotic index but caused significant increase of micronucleus frequencies in a concentration-dependent and a time-dependent manner. In addition, pycnotic cells (PNC) occurred in root tips at the tested leachate concentrations, and PNC frequencies had a positive relationship with the treatment concentrations and exposure time.

The results suggest that components of leachates may be genotoxic in plant cells and imply that long term exposure to leachate at low concentrations in both aquatic and terrestrial environments may pose a potential genotoxic risk to organisms. Helma et al. (1994) used the *Tradescantia* micronucleus (Trad-MCN) assay to determine the clastogenic effects of contaminated groundwater collected near a hazardous waste landfill. The water samples gave positive dose-dependent effects before filtration and irradiation.

Li et al. (2008) investigated the physiological and genetic toxicity of leachate, generated from Xingou Municipal Landfill in China with *T. aestivum* (wheat) bioassay. The results show that lower leachate concentrations stimulated the germination, growth and cell division, and did not induce obvious increase in micronucleus (MN) frequency in root tips; while higher concentrations inhibited the processes, and significantly induced MN frequency in a concentration and time dependent manner. In addition, pycnotic cells and sister chromatid exchange (SCE) occurred in root tips at all leachate treatment concentrations, and the frequencies had positive relation with the treatment concentration and time. The results suggest that the wheat bioassay is efficient, simple and reproducible in monitoring genotoxicity of solid waste leachates.

Simulated leachates from MSW incineration bottom ash (MSWIBA), within 3.4 and 100% concentrations, induced significant increase in micronucleated cells of treated *Vicia faba* root tip (Radetski et al., 2004). Landfill leachates collected during different seasons caused decrease in the mitotic index and significantly caused increase in micronucleated (MN) and anaphase aberration (AA) frequencies of treated *V. faba* root tip in a concentration dependent manner (Sang and Li, 2004). Tannery solid waste leachates significantly inhibited the mitotic index and induced chromosomal and mitotic aberrations in a dose dependent manner in the somatic cells of treated *V. faba* (Chandra et al., 2004). Radiowo municipal landfill leachate induced a concentration dependent increase in anaphase aberration of *V. faba* root tip (Obidoska and Jasinska, 2008). Feng et al. (2007) characterized the heavy metal composition of a MSWIBA leachate from Macao and the genotoxicity of the leachates was evaluated using micronucleus assay in the root tip cells of treated *V. faba*. The results showed that aluminum, manganese, cobalt, cadmium, mercury, iron, copper, molybdenum, chromium, zinc, selenium, strontium, barium and caesium and lead were detected at higher concentrations than standard permissible limits. Compared with the negative group, a significant increase of MN frequencies was observed in the leachate exposed groups ($p<0.05$). With increase in heavy metals in the leachates, the toxic effects on the *V. faba* root tip cells also increased, implying that heavy metals were the main agents causing the genotoxic effects. Manier et al. (2012) evaluated landfill leachate contaminated soil in a plant species, *Trifolium repens*, using the alkaline comet assay. They observed increase in the percentage Olive tail movement of the aerial part of the plant compared to the control.

Among the plants used for cytogenetic test systems, chromosomal aberration in *A. cepa* (2n = 16) assay was the first of nine plant systems to be accepted in the genotoxic programme of the USEPA and is widely used for monitoring water contamination (Fontanetti et al., 2011). The sensitivity of the *A. cepa* chromosome aberration assay was attributed to the large size of the chromosomes, which are also metacentric (Ma et al., 1995). It has been widely used to evaluate the cytogenotoxic and mutagenic activities of leachates from municipal landfill, rural refuse, industrial solid waste, hospital waste incinerated bottom ash and electronic wastes. Results from these studies showed that solid waste leachates inhibited the mitotic index of the dividing cells and induced different types of chromatid and chromosome aberrations, as well as micronuclei in the meristematic cells of *A. cepa* (Cabrera and Rodriguez, 1999; Bakare et al., 2000, 2012a; Bakare, 2001; Bakare and Wale-Adeyemo, 2004; Chandra et al., 2005; Iwegbue et al., 2007; Obidoska and Jasinka, 2008; Akinbola et al., 2011; Kwasniewska et al., 2012). These reports show the efficiencies and reproducibility of plant bioassays in the genotoxic and mutagenic evaluations of solid waste leachates.

ANIMAL BIOASSAYS

The use of animals in solid waste leachates safety and toxicological evaluation has become a well-established and essential practice. They are useful as predictive model for leachate actions and transformation in humans. In addition to the information generated from leachate induced genotoxicity and mutagenicity in plants, experiments using animals have provided the necessary building blocks that have permitted the explosive growth of knowledge in the field of solid waste leachate genotoxicity, mutagenicity and public health in recent years. Animal test systems are often generally accepted than microbial and plant test systems in predicting toxicity and

genotoxicity in humans. This is because animals are similar in the way they enzymatically metabolize many carcinogens (Stegeman and Lech, 1991), and respond to oxidative enzyme damage (Washburn and Di Giulio, 1989). In animal experiments, chemical exposure and environmental conditions can be precisely controlled, and virtually all types of toxic effects can be evaluated along with their various mechanisms of occurrences.

Different animal models with varying cytogenetic biomarkers have been utilized in evaluating the genotoxic and mutagenic effects of solid waste leachates. Bakare et al. (2005) reported that raw and simulated solid waste leachates from three dumpsites in Nigeria induced dose-dependent increase in the frequency of sperms with abnormal morphology in mice. There were similar observations in mice treated with leachates from electronic wastes and from hospital waste incinerated bottom ash (Alabi and Bakare, 2011; Akinbola et al., 2011). During spermiogenesis; the process of sperm elongation, the male germ cells undergo dramatic morphological changes whereby the spermatozoon gains its specific shape, the nucleus condenses and the flagellum extends (Henkel, 2011). During this process, xenobiotics may cause damage to the DNA of the developing spermatozoa. It therefore suggests that the interactions of the leachate constituents with the genetic materials of the differentiating cells during spermiogenesis may be responsible for the observed sperm shape abnormalities in the treated mice. Abnormal sperm morphology had been correlated with a range of adverse clinical outcomes including impaired fertilisation, disrupted preimplantation embryonic development, increased rates of miscarriage and an enhanced risk of disease in the progeny (Aitken et al., 2009; Aitken and de Iuliis, 2010). Treated and untreated domestic and industrial solid waste leachates from Japan induced increase in frequencies of micronuclei in gill cells of goldfish (*Carassius auratus*) (Deguchi et al., 2007). Likewise, raw leachate from Abule Egba municipal landfill, Nigeria significantly increased the frequencies of micronuclei and nuclear abnormalities in peripheral erythrocytes of African catfish, *Clarias gariepinus* (Alimba et al., 2011). Municipal landfill leachate from China significantly induced micronucleated polychromatic erythrocytes (MNPCE) in bone marrow of male and female mice orally treated with different concentrations of the leachate solutions for 7 days (Li *et al*., 2004). Similarly, municipal landfill leachates induced MNPCE in peripheral blood cells of mice, micronucleated and nuclear abnormalities in peripheral erythrocytes of Japanese quail, and micronucleus in polychromatic erythrocytes of bone marrow cells of mice and Japanese quail (*Coturnix japonica*) (Alimba, 2013). Municipal waste leachate significantly induced increase MNPCE frequencies in bone marrow (Tewari et al., 2005), dose-dependent inhibition in the mitotic index and increased the frequency of chromosome aberrations in the bone marrow of mice (Sang and Li, 2005; Tewari et al., 2005). Leachate from electronic wastes was also found to induce dose-dependent increase in the frequencies of micronucleated PCE in bone marrow of mice and also induced chromosome aberrations in both bone marrow and spermatogonia of treated mice (Alabi and Bakare, 2011). The erythroblasts of the bone marrow cells of albino rat exposed to municipal landfill leachates examined at post-treatment showed dose dependent increase in structural chromosomal abnormalities such as breaks, gaps, rings and acentrics (Alimba et al., 2006). In an *in vitro* study, landfill leachates from Settat town in Morocco induced micronucleus and also affected proliferation kinetics of human peripheral blood lymphocytes (Amahdar et al., 2009). Manier et al. (2012) evaluated the mutagenicity of soil contaminated with landfill leachate in a non clitellated species of adult earthworm, *Eisenia fetida*, using real-time quantitative polymerase chain reactions, (PR-PCR), with *Cd-mt* expression primers. The landfill leachate contaminated soil induced increase in Cd-Metallothionein-coding mRNA quantity in *E. fetida*.

The single-cell gel electrophoresis technique or Comet assay has also been utilised to evaluate the DNA damaging potentials of solid waste leachates. This assay, which is a rapid and highly sensitive fluorescent microscopic test, is capable of detecting low levels of DNA damage (one break per 1010 Da of DNA; Gedik et al., 1992) and repair in cell, tissues, organs and organismal level in both plants and animals. Other advantages in using Comet assay includes low cost, ease of application, short time needed to complete a study, the flexibility to use proliferating as well as non proliferating cells, and it can be conducted on cells that are the first site of contact with mutagenic/carcinogenic substances (example, oral and nasal mucosal cells) (Dhawan et al., 2009).

This assay, along with *in vitro* cytogenetic studies with cell lines grown in culture media, has been well used in the mutagenic assessment of solid waste leachates. In an *in vitro* study using Comet assay, leachates from solid wastes obtained from a polyfiber factory, an aeronautical plant, a municipal sludge and electronic-wastes induced significant concentration-dependent increase DNA damage in human peripheral blood lymphocytes (Bakare et al., 2007; Alabi et al., 2012). The municipal sludge leachate also induced DNA damage (as assessed by the comet assay) and oxidative stress in somatic tissues and organs of the mouse (Bakare et al., 2012b). Widziewicz et al. (2012) assessed the genotoxic effects of landfill leachates before and after biological treatment was conducted with two human cell lines (Me45 and NHDF) and *Daphnia magna* somatic cells using the alkali version of the comet assay. They observed significant differences between DNA strand breaks in cells incubated with leachate before and after treatment in a time-reliable and concentration dependent manner. Fly ash leachate induced significant concentration-dependent increase in DNA damage in treated human peripheral blood

lymphocytes by increasing comet parameters via percentage tail DNA, tail length and olive tail movement (Chakraborty and Mukherjee, 2009). *In vitro* genotoxic effects of leachates from electric arc furnace dust evaluated on human peripheral lymphocytes using the alkaline comet assay showed significant increase in the mean values of the tail lengths in treated lymphocytes than the control (Garaj-Vrhovac et al., 2009). Gajski et al. (2012) assessed solid waste leachates collected from a Rovinj town sanitary landfill, Croatia in human peripheral lymphocytes using the alkaline comet assay and cytokinesis-block micronucleus (CBMN) test. The results showed that the two leachate samples induced increase in tail length and micronucleus test parameters (micronucleus, nucleoplasmic bridges, nuclear buds and lymphocyte proliferation).

In vivo, leachates from tannery wastes, metal-based wastes, dye and pigment wastes and municipal sludge induced systemic genotoxicity by increasing percentage tail DNA, tail length and olive tail movement in tissues and organs of the treated mice compared to the control (Chandra et al., 2006; Tewari et al., 2006). Untreated and treated landfill leachates from Brazil induced genotoxic effects in exposed *A. cepa* and *Geophagus brasiliensis* than the control as determined by the comet assay (Bortolotto et al., 2009). Using same assay, industrial solid waste leachates from flashlight battery, pigment and tannery wastes induced dose-dependent increase in DNA damage in the midgut cells and brain ganglia of treated *Drosophila melanogaster* mutant deficient in DNA repair proteins (Siddique et al., 2005, 2008a) and in the gill epithelial cells of goldfish (*Carassius auratus*) (Deguchi et al., 2007). Landfill leachates from Tunisia induced cytotoxic effects by inducing necrosis in MCF-7 cells. It also enhanced the expression of Heat shock protein (Hsp) and various stress related proteins (heterogeneous nuclear ribonucleoprotein E1, phosphoglycerate mutase and nuclear matrix protein 200) in the MCF-7 cells (Talorete et al., 2008). Similarly, tannery waste leachates caused a concentration- and time-dependent significant increase in Hsp70 expression, ROS generation, antioxidant enzyme activities and MDA content of treated larvae of transgenic *D. melanogaster* (Siddique et al., 2007, 2008b).

The induction of these genetic anomalies suggests that xenobiotics in solid waste leachates are capable of inducing DNA damage in somatic and germ cells in animal systems. These findings also suggest that solid waste leachates are capable of contaminating different components of the ecosystems (aquatic, terrestrial and arboreal) and induced genotoxic and mutagenic effects in the resident animals. In this review, 34.92% of the studies utilized plant bioassays to evaluate the genotoxic and mutagenic effects of solid waste leachates, 28.57% used animal test system while microbial and *in vitro* (lymphocytes and cell lines) test systems formed the least (Figure 1) . The probable reason for the high use of plant bioassay may be due to their higher sensitivity, hence providing information about a wide range of genetic damage; gene mutation and chromosome aberrations (Dhawan et al., 2009), like animal bioassays. Also, plant test systems are readily available, relatively cost effective with cheap techniques compared to other test systems. The use of both multiple bioassays with different model organisms along with chemical analysis of the leachates allows important hazardous properties to be addressed (Deprez *et al*. 2012) and may be very useful in determining the components of the leachates responsible for the genotoxicity and mutagenicity.

POSSIBLE MECHANISMS OF LEACHATE-INDUCED GENOTOXICITY AND MUTAGENICITY

It is now well known from *in vivo* and *in vitro* studies that solid waste leachates are capable of inducing genetic damage in both eukaryotic and prokaryotic systems. Due to the complex nature of leachate composition, different mechanisms of genotoxicity and mutagenicity have been suggested. Reports showed that individual leachate constituent might have acted singly, or interacted synergistically, and or antagonistically to induce genotoxic and mutagenic effects in living systems. Most reports on genotoxicity and mutagenicity studies have been implicating the metallic contents of leachates as the main agent to which we can ascribe DNA damage. Heavy metals such as Cd, Pb, Fe, Zn, As, Cu, Mn, Hg and Ni, have been found in leachate samples at concentrations higher than the limits set by international regulatory authorities. These metals have the potential to induce toxicity, mutations and cancer in living cells. Epidemiological studies and studies in experimental animals indicated that Pb is both genotoxic (Shaik et al., 2006) and carcinogenic (Fowler et al., 1994), and that Ni (Haugen et al., 1994) and Cd (Elinder and Jarup, 1996) are carcinogenic. Hexavalent Cr was reported to induce chromosomal aberrations, micronuclei, and single strand DNA breaks in mammalian cells (Wise et al., 2002), and gene mutation in bacteria (De Flora et al., 1990). Trivalent Fe also was reported to induce a high level of micronuclei in newt larvae at concentrations of 12.5 and 25 mg/l (Godet et al., 1996). Mice exposed to Zn salt showed increased single-strand DNA breaks as measured by the Comet assay (Banu et al., 2001) and chromosomal aberrations (Gupta et al., 1991). Welders exposed to both Ni and Cr exhibited sister chromatid exchange (SCE) frequencies that represent an additive response for these two genotoxins (IARC, 1990). The combined effects of Fe and Cr produced an elevated percentage of micronucleated red blood cells and other genotoxic effects in assays conducted with newt larvae (Godet et al., 1996). Cu produces free radicals, and when present in an unbound form, it produces reactive oxygen species (ROS) that cause DNA, protein, and lipid damage

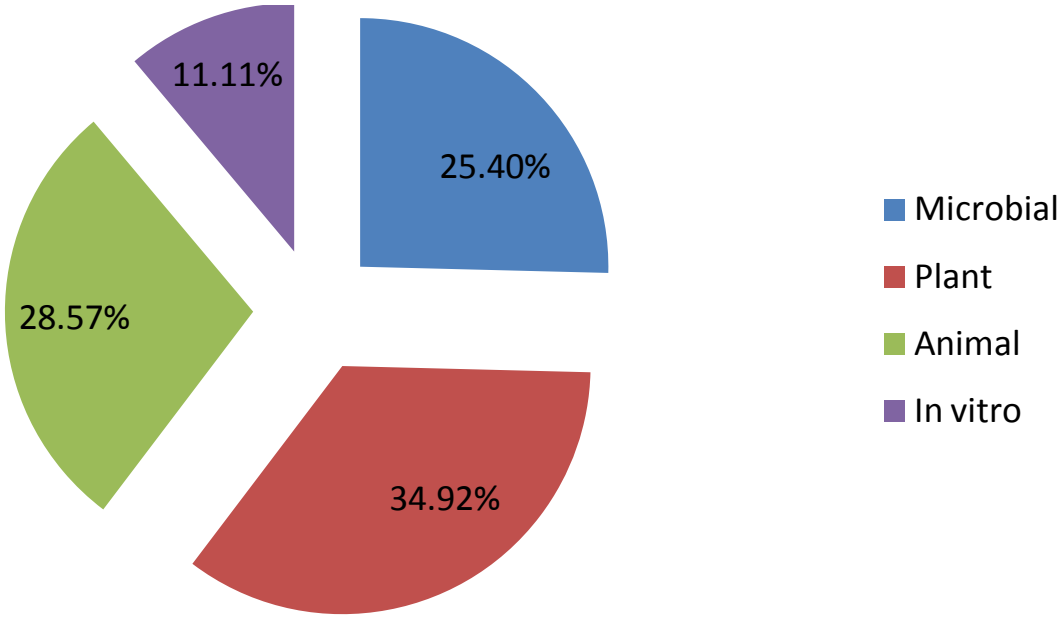

Figure 1. Frequencies of organisms utilized in the bioassays for the assessment of the genotoxic and mutagenic effects of solid waste leachates.

(Galaris and Evangelou, 2002). The genotoxic activities of heavy metals were reported to be the result of formation of DNA-DNA and DNA-protein cross-links (De Flora et al., 1990; Costa, 1991; Costa et al., 1994).

The proposed mechanism of induction of DNA damage by leachate in living cells may be due to the cross-linking of some of the metals with DNA and/or proteins. It could be via inhibition of DNA repair processes in the cells, and metals have been reported to exert unique mechanism(s) of repair inhibition (Hartwig and Schwerdtle, 2002). It also may be due to generation of free radicals, either via auto-oxidation or by enzyme-catalyzed oxidation of organic compounds in the leachate. These free radicals can induce a broad spectrum of genetic mutations including DNA single-strand and double strand breaks, apurinic/apyrimidinic (AP) sites base modification and bulky adducts (Halliwell and Aruoma, 1991; Sang and Li, 2004). In this regard, it would be noteworthy to state that Cd, Cu, and Fe have been reported to induce reactive oxygen species in eukaryotic systems (Ghio et al., 2002; Radetski et al., 2004). Evidence in support of leachate – induced genotoxicity in eukaryotes via free radical generation in the exposed cells has been provided (Ferrarri et al., 1999; Ali et al., 2004; Radetski et al., 2004; Li et al., 2006a; 2006b; Bakare et al., 2012; Alimba et al., 2012).

Weinberger et al. (2002) reported that increase in urinary levels of thiobarbituric-acid-reacting substances (TBARS), which are proportional to lipid peroxidation and oxidation stress, correlated with elevated serum transaminases levels in preterm infants. Sanchez-Chardi et al. (2007) also reported that the bioaccumulation of Pb, Hg, Cd, Fe, Mg, Zn, Cu, Mn, Mo and Cr in the tissues of wood mice (*Apodemus sylvaticus*) inhabiting Garraf landfill, correlated with elevation of serum aminotransferases. It has long been known that marked release of aspartate amino transferase (AST) and alanine amino transferase (ALT) into the circulatory system is an indication of severe damage to tissue membranes (Molander et al., 1955; Zimmerman, 1999). Recently, Alimba et al. (2012) showed that increase in serum transaminases along with histopathological lesions in the liver and kidney tissues of landfill leachate treated Wistar rats suggested that leachate constituents provoked loss of functional integrity of the cellular membranes of the rats through free radical formation. This deduction corroborated the study of Talorete et al. (2008), wherein they exposed human breast cancer MCF-7 cells to different concentrations of leachates from two landfills in Tunisia using modified E-screen assay; and they concluded that leachate-induced cytotoxicity was through damage to cell membranes by necrosis. It is possible that the histopathological findings in the liver and kidney of leachate treated rats (Alimba et al., 2012) may also suggest a possible pathway of leachate induced genotoxicity. The observed inflammatory cells were elicited in response to tissue damage and their presence shows the generation of reactive oxygen species by endogenous substances. This may account for the observed necrosis and apoptotic cell damage in these tissues (Alimba et al., 2012). This assertion corroborated the findings of Siddique et al. (2008b) wherein the generation of reactive oxygen specie (ROS) by tannery leachates positively correlated with the induction of apoptotic cell death in treated *D. melanogaster*.

The propensity for cells to undergo necrosis and or apoptosis depends on the intracellular oxidant status, the level of ATP in the cell and the extent of induced membrane damage and this may determine the extent of observed DNA damage in the cell (Fenech et al., 1999). The observed histological lesions with inflammatory cells production in leachate treated rats, may suggest that these cells were elicited in response to the presence of invading microorganisms (Oshode et al., 2008; Efuntoye et al., 2011), heavy metals and other chemical sub-stances (though not analysed) present in solid waste leachates. These cells are capable of destroying and removing apoptotic and necrotic cells (cellular debris) through phagocytosis (Jaeschke, 2000; Ramaiah and Jaeschke, 2007). These phagocytic cells do not attack healthy cells but respond to distressed or dying cells (Jaeschke, 2006). These may be supported by the report that apoptotic and necrotic cells trigger neutrophilic inflammatory process (Lawson et al., 1998; Faouzi et al., 2001). During phagocytosis, mediators of neutrophilic inflammation generated by Kupffer cells including tumour necrosis factor α (TNF- α), interleukins (IL-1 and IL-6), chemokines and ROS (Sweet and Hume, 1996) activate neutrophil induced tissue damage. The presence of neutrophil in cells triggers a long-lasting oxidative stress through Nicotinamide adenosine dinucleotide hydrogen (NADH) oxidase. This enzyme generates superoxides from oxygen and hydrogen peroxides (ROS), which is highly diffusible (Jaeschke, 2003; Gujral et al., 2004). These intracellular ROS formed through autoxidation (endogenous source) (Adamson and Billings, 1992; Jaeschke, 2000, 2003; Gujral et al., 2004), have been associated with genotoxic damage (Chellman et al., 1986) and cytotoxicity (Schacter et al., 1988). Also, ROS, whether exogenously or endogenously generated have been associated with lipid peroxidation, protein and DNA damage, aging and carcinogenicity (Frenkel et al., 1986; Nag, 2009). ROS can cause damage to any base or sugar moiety in DNA leading to strand breaks (Cadet et al., 2003). It is plausible that leachate constituents gene-rated ROS through multiple exogenous and endoge-nous sources or acted directly in the test systems to cause the observed genotoxic and mutagenic alterations reported by the various authors.

CONCLUSION

Solid wastes leachates are complex mixtures of chemical substances and particulate matters, including microorganisms. The constituents are capable of causing DNA damage in biological systems; and are an important prerequisite for exogenously induced mutations. Bio-assays for identifying mutagenic/genotoxic sub-stances in leachates are necessary in order to minimise the risk of exposure to xenobiotics with suspected carcinogenic properties. In an ecological context, mutagens/genotoxins might induce substantial reproductive loss in exposed populations and could further influence individual fitness by a toxicity-related phenomenon described as genotoxic disease syndrome (Kurelec, 1993). Additional investigation is needed to explore the potential genotoxic and mutagenic effects and systemic toxicity of leachates on the major organs and tissues in biological systems. This is important for further understanding of the mechanisms and pathway of leachate genotoxicity and mutagenicity. There is need for a shift from wastes disposal to sustainable waste management including wastes reduction, minimization, recovery, recycling and reuse.

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