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IMPACT OF DUMPSITE SOIL SIMULATED LEACHATE ON HAEMATOLOGY AND HISTOPATHOLOGY OF SWISS ALBINO MICE (*MUS MUSCULUS*)

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Abstract

Solid waste dumpsites/landfills are significant sources of emerging contaminants to terrestrial ecosystems. This study assessed the toxicological effect of exposure to leachate on mice body weight changes, relative organ weight, haematological parameters and histological biomarkers effects after oral administration with diluted Ajaokuta soil simulated leachate (15%–75%) for 28 days. Mice exposed exhibited significant decreases in body weight, percentage weight lost and significant decreases in relative organ weight in a dose dependent manner compared to controls, suggesting possible chemically induced metabolic disruptions. Haematological analysis showed significant alterations in red blood cell indices, haemoglobin concentration, and total leukocyte counts, reflecting impaired haematopoiesis and systemic toxicity. The pathological lesions also showed indirect tissue damage due to reactive oxygen species elicited by the landfill chemicals. This study shows that exposure to pollutants poses substantial risks to blood cell integrity, it also suggest potential health risk to residents within the vicinity of

landfills. Hence the need for remediation measures to reduce health risks associated with pollutants from waste decomposition.

Keywords: Dumpsites; pollutants; haematology, histopathology, health risk

Introduction and literature review

Pollution of aquatic and terrestrial ecosystems has reached unprecedented levels globally, primarily due to the discharge of wastewater and solid wastes from anthropogenic activities. Landfill/dumpsite has emerged as a significant contributor to environmental pollution, with the production of leachate discharge into the environment (Vasilyeva *et al.*, 2019). Although, landfills waste management systems serve as final repositories for a wide array of discarded materials. The most significant environmental concerns associated with landfilling is the generation of leachate, a complex and potentially hazardous liquid produced when water infiltrates the waste matrix, dissolving and mobilizing various contaminants (Nanda and Berruti, 2021). Leachate is also an heterogeneous and complex type of wastewater characterized by significant highly variable organic matter content, reported as bio and chemical oxygen demand (BOD, COD) and dissolved organic matter (DOM), ammonia nitrogen and salts (Lebron *et al.*, 2021; Nanda and Berruti, 2021). Pollutants found in leachate include heavy metals and persistent organic compounds categorized to have detrimental effects on both human health and ecological systems (Ma *et al.*, 2022; Abdel-Shafy *et al.*, 2024; Sulistyanning *et al.*, 2022). In Nigeria, the situation is worsen due to inadequate waste management practices, particularly in urban areas where landfill can receive up to 10,000 tons of waste daily, including electronic and hazardous materials (Oshode *et al.*, 2008). Leachate from such dumpsites can contaminate groundwater and surface water, posing serious health risks to nearby communities (Adeyemi *et al.*, 2024). These pollutants can disrupt ecosystems, harm biodiversity, and pose risks to human health through the consumption of contaminated water and aquatic organisms (Alabi *et al.*, 2017). Exposure to landfill leachates has been associated with a wide range of adverse public health effect, including developmental abnormalities, reproductive impairments, and genotoxic or genomic instability effects (Alimba and Bakare, 2016; Ogunlaja *et al.*, 2019; Ademola *et al.*, 2020; Escalante-Mane *et al.*, 2022; Torres-Gonzalez *et al.*, 2021). Decades ago, the incidents of cancer, liver problems and low birth weights were reported among Niagara, United States residents exposed to chemicals discharged from Love Canal landfills (Gill and Mix, 2020), who encountered hazardous chemicals in landfill leachate. The prevalence of diseases such as immune and respiratory system disorders, degenerative neurologic and endocrine disorders, cancer, stroke, diabetes, and liver dysfunctions was also reported among individuals residing in close proximity to landfills (Afolabi *et al.*, 2022; Saha *et al.*, 2023). These reports on the toxic effects of landfill leachates have raised awareness

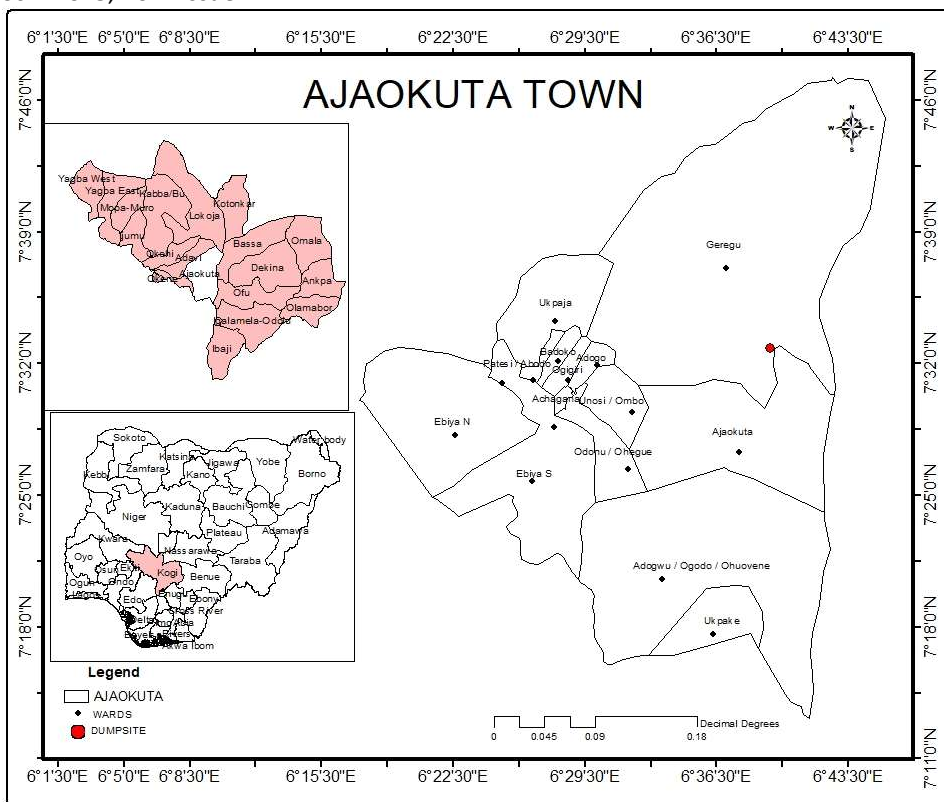
about potential adverse health effects associated with continuous exposure to landfill chemicals, and thus prompted landfill toxicity studies, with the identification of specific constituents of these chemicals being the most commonly used approach to demonstrate the hazardous nature of chemicals in landfill wastes (Ogunlaja *et al.*, 2019).

Although extensive literature exists on the toxicological consequences of landfill leachates and their potential risks to environmental and public health, there remains a paucity of such information from Kogi State. In the present study, we aimed to document the presence of metals and other contaminants of emerging concern in leachates from Ajaokuta landfill, Kogi State as well as the systemic changes in Swiss albino mice (*Mus musculus*) exposed to varying leachate concentrations under laboratory conditions as a baseline toxicity test report required for proper management. Animal models have been pivotal in elucidating the toxicological mechanisms of leachate exposure, providing valuable insight into systemic and cellular responses. Among these, mice are particularly useful model organisms due to their physiological, biochemical, and genetic similarities to humans (Rydell-Törmänen and Johnson, 2019). Systemic tissues and organs in the mammalian body are sensitive to chemical induced toxicity due to their involvement in various metabolic activities like detoxification, regeneration, excretion, secretion and storage of chemicals (Alimba *et al.*, 2021). The liver and kidneys are highly sensitive indicators of chemically induced toxicity due to their central roles in the metabolism, detoxification, storage, and excretion of xenobiotics and their metabolites. Consequently, these organs are primary targets for xenobiotic-induced damage.

Experimental Methods

Study area

The Landfill used for the study is located in Ajaokuta Steel Company Limited in Kogi State, Nigeria. The study area lies on the coordinates of 7°33'22''N 6°39'18''E. Ajaokuta occupies a land area of 1,362 square kilometers, which is about 5% of the total land of Kogi State, Nigeria.



Dumpsite Soil Collection, Leachate Simulation and Preparation of Test Samples

The composite soil samples were collected at a depth of 0-10cm labeled in such a way that represented the entire dumpsite using cleaned trowel. The samples were collected in 10 randomly selected points within the site. The samples were placed in a sterilized transparent bottle and transported to Animal and Environmental Biology Laboratory. The sample was properly air dried at room temperature void of cross contamination for two weeks and then sieved with 0.5mm size sieve to remove unwanted particles, the sieved soil was grounded into fine powder.

Leachate simulation from the soil was carried out according to the American Society for Testing and Materials category A, extraction procedure as modified by Bakare *et al.*, (2007). Briefly, 2000g of the soil samples were soaked in 4000mL of distilled water for a period of 72 hours, filtered using a 15cm Whatman filter paper and sieved to remove debris. From the resultant stock solution (simulated leachate) of the landfill waste, different concentrations of 15, 25, 50, and 75% (v/v, simulant/distilled water) solutions were prepared and stored at 4⁰C until use based on standard procedure. Distilled water was used as a negative control to compare the analysis of the parameters.

Landfill Soil Simulated Leachate Pollution Index (LPI) and Physico-chemical Assessment

The leachate pollution index (LPI) was calculated according to the method of Kumar and Alappat (2005) and Fagbenro *et al.* (2024) using the following modified formula:

$$LPI = \frac{\sum_{i=1}^n w_i \times p_i}{\sum_{i=1}^n w_i}$$

Equation (1)

where: W_i = pollutant variable weight, P_i = pollutant variable sub-index value, n = number of factors for leachate pollution used in the LPI computation. Pollutant weight (W_i) and sub-index value (P_i) values for all the analyzed parameters are presented according to Kumar and Alappat (2005) specific sub-index curves.

Standard physical and chemical parameters of the leachate including chemical oxygen demand (COD), biochemical oxygen demand (BOD), total dissolved solid (TDS), Calcium (Ca), magnesium (Mg), phosphorus (P), sulphur (S), Chloride (Cl^-), alkalinity and turbidity were measured following standard methods for water/wastewater analysis according to APHA (2005). Nine (9) heavy metals including Manganese (Mn), iron (Fe), zinc (Zn), copper (Cu), lead (Pb), chromium (Cr), nickel (Ni), arsenate (As) and barium (Ba) were analyzed and quantified using inductively coupled plasma-mass spectrometry (ICP-MS).

Animal Collection and Housing

Thirty-six (36) swiss albino mice aged six-eight weeks, weighing 25g-35g were purchased from an inbred colony, Department of Animal and Environmental Biology, Anyigba and used for the study. The animals were housed in a neat and well ventilated plastic cage. The animals were allowed to acclimatize for a period of seven (7) days. The animals were kept in group of six (6) for experimental and control groups respectively in a suspended cage placed in a well-ventilated and hygienic animal house under suitable conditions of temperature and humidity. Their bedding which was of saw dust was regularly changed and they were supplied with commercially pellet Feed and drinking water *ad libitum*. The animals were subjected to natural photoperiod of 12 hrs light and 12 hrs dark cycles. The animals were handled humanely during the experimental period of twenty-eight (28) days in compliance with international accepted principles for laboratory animal use. The experimental protocols were approved by the College of Health Science Research Ethical Committee (CHSREC), Prince Abubakar Audu University, Kogi State, Nigeria (PAAU/CHS/PRV/CHSREC/Vol 01/ 035).

Experimental design (leachate administration and Exposure)

The animals were divided into five groups consisting of six mice each per group;

Negative control (NC): Animals received 0.5ml of distilled water every 24 hours for a period of twenty-eight days.

Group I: Animals were treated through oral gavage for a period of twenty-eight days with the 15% (v/v, simulant/distilled water) solutions 0.5ml once every 24 hrs.

Group II: Animals were treated through oral gavage for a period of twenty-eight days with the 25% (v/v, simulant/distilled water) solutions 0.5ml once every 24 hrs

Group III: Animals were treated through oral gavage for a period of twenty-eight days with the 50% (v/v, simulant/distilled water) solutions 0.5ml once every 24 hrs.

Group IV: Animals were treated through oral gavage for a period of twenty-eight days with the 75% (v/v, simulant/distilled water) solutions 0.5ml once every 24 hrs

Body weight change

The body weights of the mice were recorded every week, before and at the termination of the experiment using electronic analytical weighing balance. Percentage body weight gain was determined using equation (1).

$$\frac{\text{finalbodyweight} - \text{initialbodyweight}}{\text{initial body weight}} \times 100$$

Blood Collection and Haematological analysis

Upon the completion of the 28 days period of experimentation, blood was withdrawn from the heart of all the mice in each respective group by cardiac puncture and stored in an EDTA container and used for haematology. The haematology parameters were analyzed using SYSMEX automated haematological analyzer. The parameters measured were White Blood Corpuscles($\times 10^3/\mu\text{l}$) (WBC), Red Blood Corpuscles ($\times 10^6/\mu\text{l}$)(RBC), Pack Cell Volume % (PCV),Granulocyte ($\times 10^3/\mu\text{l}$) (GRAN), Plateletcrit%(PCT), Monocytes ($\times 10^3/\mu\text{l}$) (MID), Mean Corpuscular Volume (FL)(MCV) haemoglobin (HBG) and lymphocytes (LYM).

Relative organ weight (ROW)

The organs (liver and kidneys) of the animals were removed using a pair of scissors and forceps, rinsed to remove excess blood, blotted dry with whatman filter paper and weighed using electronic analytical weighing balance in grams to determine the absolute weight of the organs.

The relative organ weight (ROW) of each organ was calculated using the equation.

$$\frac{\text{absolute organ weight (g)}}{\text{final body weight}} \times 100$$

Histopathology Analysis

The animals were thereafter sacrificed by cervical dislocation and dissected to excise the organs (liver and kidneys) which were fixed in a 10% formalin solution and stored in a sterile universal container. The organs were cut into small pieces of not more than 4mm thick into pre-labelled cassettes. These were further immersed in 10% formol saline for 24 hours to fix. The tissues specimen from the organ

were processed with an automated tissue processor (leica tp1020) programmed to run for 12 hours. Subsequent dehydration by allowing to pass through various reagents including; station one containing 10% formol saline, station 3 to station 7; alcohol (70%, 80%, 90%, 95%, absolute 1 and absolute 11), the tissues continued to pass through station 8 and station 9 containing two changes of xylene for the purpose of clearing and finally transferred into three wax baths for infiltration/impregnation, and embedding, serial sections of 4 μ m thickness were obtained on glass slides using rotatory microtome, deparaffinised and stained using haematoxylin and eosin (H and E) and examined microscopically.

Data Analysis

The result of the study was subjected to the mean and standard error of means analysed using the SPSS software program. ($P < 0.05$) level of significance was considered using the Duncan Multiple Range Test.

Results and Discussions

Physico-chemical profiles and Landfill Leachate Pollution Index (LPI) Assessment

Table 1 presents the physical and chemical properties and pollution load of Ajaokuta soil simulated leachate using the Leachate Pollution Index (LPI) as an integrative tool for overall leachate quality. All evaluated physicochemical parameters were significantly higher than their respective regulatory standards, indicating severe contamination and potential environmental and public health risks associated with the leachate. The analyzed leachate sample exhibited a significantly high chloride concentration (1075.00mg L⁻¹), alkalinity (2755.00mg L⁻¹), COD, BOD and TDS values were also high (192.70, 118.22 and 251.43 mg L⁻¹ respectively), indicating active leachate generation within the sampled site and may exert deleterious effects on exposed organisms within the vicinity (Escalante-Mañe et al., 2022). Similarly, most of the metals concentrations exceeded both national and international permissible limits. High concentrations of heavy metals in landfill leachate pose significant risks to human health through bioaccumulation and biomagnification in the food chain. Chronic exposure to metals such as Pb, Cd, As, and Ni can lead to hematological, renal, hepatic, neurological, and reproductive disorders (Bakare *et al.*, 2013; Alimba *et al.*, 2015; Alimba and Bakare, 2016; Olawale *et al.*, 2019; Ogunyemi et al., 2022). These metals induce oxidative stress, DNA damage, and enzyme inhibition, resulting in anemia, organ dysfunction, and increased cancer risk (Aromolaran et al., 2019). Contaminated groundwater and crops near landfill sites serve as major exposure pathways, particularly in developing countries like Nigeria, where poor waste management worsen leachate contamination (Adeniyi et al., 2019; Olawale et al., 2019). The calculated LPI value was 69.58. According to Kumar and Alappat's (2005) classification, LPI values above **30** indicate severe leachate contamination,

suggesting that the site pose serious pollution risks. The LPI values obtained in this study is higher than those previously reported in other regions of Nigeria. Fagbenro *et al.* (2024) reported LPI values of 8.55 at Aba-Eku landfill, Adegbola *et al.* (2023) reported LPI values of 17.55 at Awotan and 15.67 at Ajakanga dumpsites in Ibadan, Oyo State. Salami *et al.*, (2014) reported values ranging from 16.87 to 23.54 across four major dumpsites in Lagos, Ibezute and Erhunmwunse, (2018) recorded a mean LPI of 22.31 and 37.93 in leachates from Benin City, Edo State. The LPI values in this study suggests that landfill and dumpsite management in the studied areas is associated with more acute pollution risks potentially linked to waste composition, higher organic fractions, and lack of leachate containment systems.

Table 1: Leachate Physico-Chemical Characteristics and Leachate Pollution Index (LPI)

S/N	Parameters	WHO ^a / NESREA ^b LIMIT	Significance	Pollution weight (Wi)	Pollution concentration (mg/L)	Sub-index value (Pi %)	Overall pollution rating (Wi×Pi)
1	pH	6.0-9.0 ^b	3.509	0.055	7.40	0.00	0.0000
2	TDS*	500 ^b	3.196	0.050	251.43	50.29	2.5143
3	BOD*	50 ^b	3.902	0.061	118.22	100.0	6.1000
4	COD*	90 ^b	3.963	0.062	192.70	100.0	6.2000
5	Turbidity	5.0 ^b	-	0.049	32.40	100.0	4.9000
6	Alkalinity	150 ^b	-	0.047	2755.00	100.0	4.7000
7	Chloride	250 ^b	3.078	0.048	1075.00	100.0	4.8000
8	Calcium	75 ^a	-	0.044	4.87	6.49	0.2857
9	Magnesium	50 ^a	-	0.044	1.36	2.72	0.1197
10	Phosphate	2.0 ^b	-	0.049	4.94	100.0	4.9000
11	Sulphate	250 ^b	-	0.046	1.66	0.66	0.0305
12	Mn	0.4 ^b	-	0.045	120.182	100.0	4.5000
13	Fe	0.3 ^a	2.830	0.045	124.773	100.0	4.5000
14	Zn	3.0 ^{a,b}	3.585	0.056	186.538	100.0	5.6000
15	Cu	2.0 ^a	3.170	0.050	2.001	100.0	5.0000
16	Pb	0.01 ^a	4.019	0.063	0.063	100.0	6.3000
17	Cr	0.05 ^a	4.057	0.064	0.207	100.0	6.4000
18	Ni	0.02 ^a	3.321	0.052	0.005	25.0	1.3000
19	As	0.05 ^{a,b}	3.885	0.061	0.011	22.0	1.3420
20	Ba	0.05 ^a	-	0.043	0.001	2.00	0.0860
	LPI						69.58

Legends: All values for pollutant concentrations are expressed as mg/L except pH and Colour. The significance, Pollutant weight (*Wi*) and Sub-index value (*Pi*) were adapted from Kumar and Alappat (2005).

NESREA- National Environmental Standards and Regulations Enforcement Agency in Nigeria maximum permissible limits for effluent from wastewater; **WHO-** World Health Organization leachate; **TDS-** Total dissolved solids; **BOD-**Biochemical oxygen demand; **COD-**Chemical oxygen demand.

1. **Body Weight Change**

The effects of Ajaokuta soil simulated leachate on the body weight of mice over 28 days are presented in Table 2. The negative control (NC, 0%) group showed a gradual and consistent increase in body weight (26.33 ± 1.91 g on day 0 to 31.13 ± 1.87 g on day 28). Mice exposed to 15% and 50% leachate concentrations exhibited fluctuating weight patterns, the 15% group showed a significant weight drop to 25.77 ± 8.02 g by day 28, while the 50% group decreased to 22.52 ± 7.37 g. Mice exposed to 25% and 75% leachate showed significant weight increases. The 25% group maintained elevated weights from day 7 (37.97 ± 2.52 g) to day 28 (38.34 ± 3.23 g). Similarly, the 75% group showed significantly higher weights from day 0 (40.54 ± 1.21 g) through day 14 (39.10 ± 1.15 g), before declining by day 28 (29.06 ± 9.71 g). The study observed that prolong exposure to varying concentrations of leachate lead to increased and decreased body weight in mice. This pattern suggests a biphasic response with early stage stimulation followed by toxicity induced weight loss. This observation aligns with findings from the study of Bakare *et al.* (2003) who reported that exposure to simulated leachates from various dumpsites, including Abadina, Orita-Aperin, and Oworonsoki, led to weight loss, sluggishness, hair loss, and reduced food intake in mice. The severity of these effects varied among the leachates, with Oworonsoki raw leachate being the most toxic. Alabi *et al.* (2017) observed that exposure to leachates from petrol, diesel, and kerosene dispensing sites resulted in reduced body weight gain in mice. The study also noted reproductive and hematological toxicity, indicating systemic effects beyond weight alterations. These studies suggest that leachates from various sources in Nigeria can have significant toxic effects on mice, including alterations in body weight. The biphasic weight response observed in this study may reflect a complex interaction between leachate concentration, exposure duration and the specific chemical composition of the leachate.

Table 2: Percentage weight Alterations in mice exposed to Ajaokuta soil simulated leachate (Values are mean \pm SEM; n=6)

GROUP	DAYS				
	0	7	14	21	28
NC (0%)	26.33 ± 1.91	30.14 ± 1.94	30.95 ± 1.50	30.85 ± 1.49	31.13 ± 1.87
15%	32.14 ± 3.04	35.85 ± 2.12	32.01 ± 2.91	32.82 ± 2.62	25.77 ± 8.02
25%	31.95 ± 2.71	$37.97 \pm 2.52^*$	$38.50 \pm 3.04^*$	$38.52 \pm 3.12^*$	$38.34 \pm 3.23^*$
50%	32.23 ± 3.91	34.20 ± 2.67	35.52 ± 1.58	31.69 ± 1.45	22.52 ± 7.37
75%	$40.54 \pm 1.21^*$	$40.78 \pm 2.37^*$	$39.10 \pm 1.15^*$	37.06 ± 3.29	29.06 ± 9.71

NB: *p < 0.05 statistically significant differences compared to NC.

Haematological Changes in Mice Exposed to Ajaokuta Soil Simulated Leachate

Table 3 presents the effects of Ajaokuta soil simulated leachate on haematological parameters in mice. The negative control (NC, 0%) group displayed baseline values consistent with normal murine haematology, including WBC ($5.48 \pm 1.62 \times 10^3/\mu\text{L}$), RBC ($3.94 \pm 0.93 \times 10^6/\mu\text{L}$), HBG (6.55 ± 1.39 g/dL), and PLT ($493.5 \pm 204.5 \times 10^3/\mu\text{L}$). Exposure to 15% leachate led to a significant increase in WBC ($11.83 \pm 2.49 \times 10^3/\mu\text{L}$, $p < 0.05$) and a decrease in MCV (49.0 ± 0.64 fL, $p < 0.05$) compared to the NC, suggesting an early immune or inflammatory response. At 25% concentration, most parameters remained close to control values, indicating minimal haematological disturbance at this moderate exposure level. The 50% leachate group exhibited alterations with significant elevations in WBC ($16.42 \pm 3.09 \times 10^3/\mu\text{L}$), RBC ($6.98 \pm 0.11 \times 10^6/\mu\text{L}$), HBG (11.25 ± 0.35 g/dL), GRAN ($8.55 \pm 2.15\%$), LYM ($77.75 \pm 7.65\%$), and PCV ($36.40 \pm 2.50\%$), all statistically significant ($p < 0.05$). This pattern indicates leukocytosis, erythrocytosis and potential stress induced haemoconcentration, reflecting systemic toxicity. The 75% leachate group showed less consistent changes, with some parameters elevated (PLT = $690.5 \pm 457.5 \times 10^3/\mu\text{L}$) but others reduced comparable to control values, possibly due to severe toxicity or homeostatic dysregulation at higher leachate concentrations. The haematological data indicate a concentration dependent effect of Ajaokuta soil leachate on mice, with moderate to high exposure (50%) producing significant immunological and erythropoietic alterations, suggesting systemic stress and potential toxicity. The study observed significant haematological changes in mice exposed to varying concentrations of Ajaokuta soil simulated leachate. The high leachate concentration led to increases in white blood cell count (WBC) and granulocyte percentages, reduced red blood cell count (RBC), hemoglobin (HGB), and packed cell volume (PCV). These alterations suggest a concentration dependent haematotoxic effect, indicative of systemic toxicity. These findings support with the study of Alabi *et al.* (2017) reported that exposure to simulated leachates from petrol, diesel, and kerosene dispensing sites induced significant increases in WBC, lymphocytes, neutrophils, monocytes, eosinophils, and mean corpuscular volume (MCV), while decreasing basophils, hemoglobin, MCH, PCV, and MCHC in mice. The study attributed these effects to the presence of toxicants such as lead, copper, mercury, polycyclic aromatic hydrocarbons, and benzene in the leachates. Alabi (2021) investigated the haematotoxic potential of cocoa industry wastewater in mice. The study found significant increases in WBC and MCV, alongside decreases in MCHC and MCH, suggesting macrocytic anemia. These alterations were linked to the presence of heavy metals like lead and chromium in the wastewater. Adeyemi *et al.* (2024) examined the effects of leachate from the Uyo village road dumpsite on haematological parameters in rats. The study observed significant reductions in RBC, HGB, and WBC levels, indicating potential anaemia and compromised immune function. These

studies revealed the haematotoxic potential of leachates from various sources in Nigeria. The observed haematological alterations, including changes in WBC, RBC, HGB, PCV, and MCV, are consistent across different leachate types and concentrations. Epidemiological evidence further suggests that continuous human exposure to these hazardous substances may contribute to elevated body burdens of toxicants and increased incidences of disease conditions, particularly among populations residing or working near landfill environments (Adewuyi *et al.*, 2011; Alimba and Bakare, 2016).

Table 3: Haematological changes in mice exposed to Ajaokuta soil simulated leachate (Values are mean \pm SEMn=6)

Grp	Parameters								
	WBC	RBC	HBG	PLT	MCV	GRAN	LYM	MID	PCV
NC (0%)	5.48 \pm 1.62	3.94 \pm 0.93	6.55 \pm 1.39	493.5 \pm 204.5	55.9 \pm 0.35	3.0 \pm 0.35	93.7 \pm 0.80	3.35 \pm 0.45	22.15 \pm 4.15
15%	11.83 \pm 2.49*	5.60 \pm 0.27	7.80 \pm 0.31	576.0 \pm 224.9	49.0 \pm 0.64*	2.6 \pm 1.01	92.0 \pm 2.44	5.40 \pm 2.13	27.50 \pm 1.72
25%	4.98 \pm 1.48	4.94 \pm 0.40	6.90 \pm 0.25	264.0 \pm 20.6	52.9 \pm 1.56	4.6 \pm 2.05	83.1 \pm 7.26	8.35 \pm 3.90	24.65 \pm 5.22
50%	16.42 \pm 3.09*	6.98 \pm 0.11*	11.25 \pm 0.35*	256.5 \pm 96.5	52.1 \pm 2.80	8.55 \pm 2.15*	77.75 \pm 7.65*	12.70 \pm 4.50*	36.40 \pm 2.50*
75%	7.02 \pm 2.07	4.01 \pm 1.02	5.90 \pm 2.90	690.5 \pm 457.5	54.2 \pm 0.85	4.4 \pm 1.50	89.1 \pm 0.50	6.50 \pm 1.00	21.85 \pm 5.00

NB: *p < 0.05 statistically significant differences compared to NC.

Legend: Negative control (NC) group, White blood cell (WBC), Red blood cell (RBC), haemoglobin (HBG), platelet count (PLT), mean corpuscular volume (MCV), granulocytes (GRAN), lymphocytes (LYM), monocytes (MID), and packed cell volume (PCV)

Relative Organ Weight (ROW) Changes in Mice Exposed to Ajaokuta Soil Simulated Leachate

Table 4 shows the effects of oral exposure to Ajaokuta soil simulated leachate on the relative weights of liver, kidney, and spleen in mice. The negative control (NC, 0%) group displayed baseline organ weights of 4.06 ± 0.22 g (liver) and 1.27 ± 0.06 g (kidney). Mice exposed to 15% leachate exhibited increases in all organs, with the liver (5.04 ± 0.31 g) and kidney (1.47 ± 0.05 g) showing the highest relative weights among all treatment groups. This suggests potential organ hypertrophy, possibly due to early stage adaptive or inflammatory responses. At 25% and 50% concentrations, organ weights were similar to or slightly below control values, indicating minimal effects at these exposure levels. The liver weights decreased to 4.29 ± 0.41 g at 25%, and 3.92 ± 0.19 g at 50%, suggesting a potential onset of toxicity or organ tissue stress. The 75% leachate group showed increases in organ weights compared to 25% and 50% groups (liver = 4.09 ± 0.20 g, kidney = 1.36 ± 0.07 g), indicating a possible adaptive response at higher concentrations or variability in individual responses. The data indicate that exposure to Ajaokuta soil simulated leachate can induce concentration dependent alterations in relative organ weights, with 15% exposure producing hypertrophy, while moderate-to-high exposures (25–50%) may cause slight organ weight reductions, reflecting potential systemic toxicity.

Table 4: Effect of oral exposure of Ajaokuta dumpsite soil simulated leachate on Relative Organ Weights (ROW) of mice (Values are mean \pm SEM n=6)

Group	Organ (g)	
	Liver	Kidney
NC (0%)	4.06 ± 0.22	1.27 ± 0.06
15%	5.04 ± 0.31	1.47 ± 0.05
25%	4.29 ± 0.41	1.19 ± 0.11
50%	3.92 ± 0.19	1.24 ± 0.10
75%	4.09 ± 0.20	1.36 ± 0.07

Histopathological Analysis

Photomicrograph of selected organs (liver and kidney) of mice section stained by HandE exposed to the Ajaokuta soil simulated leachate produced dose-related alterations or lesions in organ morphology compared to the control (Table 5 and Figure 2). The liver showed some hepatocellular hypertrophy, sinusoidal dilatation, Kupffer cell hyperplasia, focal vacuolar degeneration, mild portal inflammation, hepatocyte shrinkage, focal necrosis, mild hepatocellular hypertrophy, scattered inflammatory foci. The kidney showed lesion such as glomerular hypertrophy, tubular epithelial proliferation, early tubular atrophy, interstitial fibrosis, glomerular collapse, tubular epithelial loss, focal tubular regeneration, vascular congestion. These lesions suggest an active immune

response to xenobiotic insult. The present study also demonstrated that exposure to leachate produced concentration dependent alterations in the relative weights of the organs in mice. Mice exposed to lower concentrations of leachate exhibited significant increases in organ weights, indicative of potential organ hypertrophy that may reflect early adaptive responses, inflammation or mild stress induced by leachate constituents, including heavy metals and other toxicants. Moderate to high concentrations suggests onset of organ tissue stress or mild toxicity, which could be attributed to individual variability or compensatory hypertrophy in response to higher toxicant loads. Our study gets support with the study of Bakare *et al.* (2003) observed significant hypertrophy of liver and spleen in mice exposed to municipal solid waste leachates from dumpsites, suggesting systemic toxicity and inflammatory responses. Alabi *et al.* (2017) reported increases in liver and kidney weights in mice exposed to petroleum contaminated site leachates, attributed to hepatotoxic and nephrotoxic effects of heavy metals such as lead, chromium, and nickel. Adeyemi *et al.* (2024) also documented relative organ weight alterations in rats following exposure to dumpsite leachates, highlighting dose-dependent effects of leachate on organ morphology and function. These studies indicate that leachate exposure in Nigeria can induce significant organ specific toxicity, with liver and kidney being particularly sensitive. The observed hypertrophy at lower concentrations may represent an initial adaptive response, while reductions or variable weights at higher concentrations reflect progressive organ stress and toxicity.

Table 5: Severity of Histological Lesions in the Liver and Kidney of mice Exposed to Dumpsite soil simulated leachate

Organ	Histological Changes	NC (0%)	15%	25%	50%	75%
Liver	hepatocellular hypertrophy	–	+	+	++	+++
	vacuolar degeneration	–	–	++	–	++
	Kupffer cell hyperplasia	–	–	+	++	++
	Multifocal inflammation	–	+	+	–	+++
	Congestion of hepatic blood vessels	–	–	–	++	+++
	Necrosis	–	+	++	++	++
Kidney	Congestion of renal blood vessels	–	+	+	+	+++
	glomerular hypertrophy	–	–	–	–	++
	interstitial fibrosis	–	–	+	++	++
	focal tubular regeneration	–	+	+	+	+++

Legend: NC = Negative Control (distilled water);

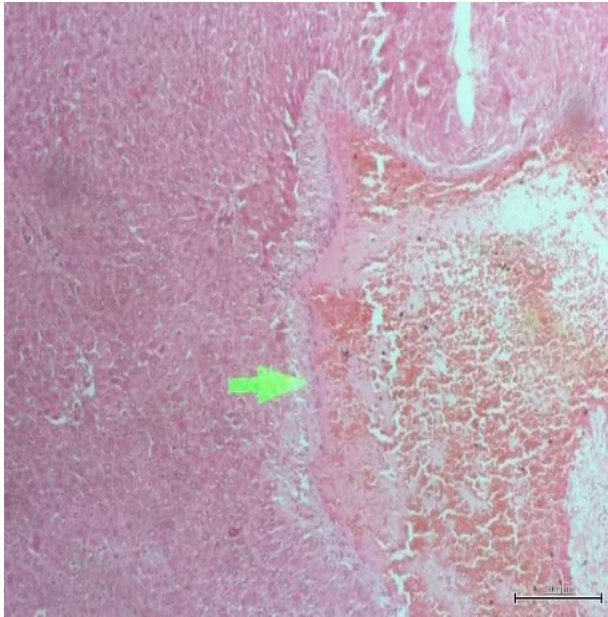
Severity of the liver and kidney histological changes were assessed using the following scale:

– change that was either absent or sporadic in all the group

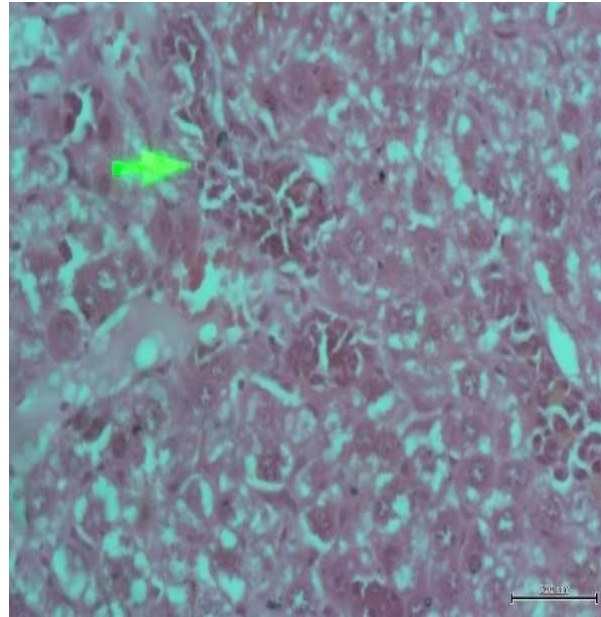
+ changes observed in a few animals of the group

++ changes relatively common in the group

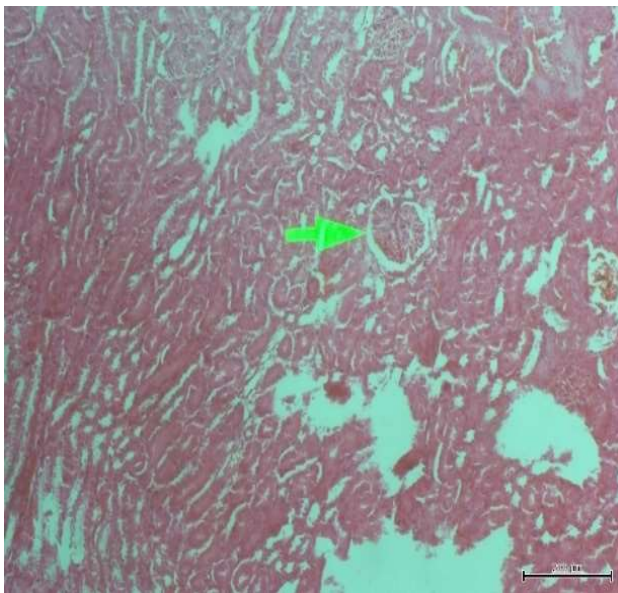
+++ changes very frequently observed in the group



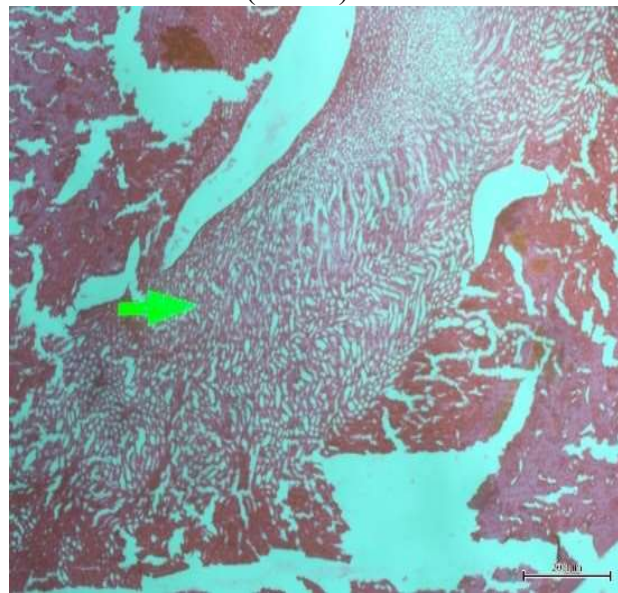
Section of the liver show periportal fibrosis



Section of the liver show injured hepatocytes with mild steatosis(Arrow)



Section show normal glomerulus(arrow) and tubules with infiltration of interstitium by lymphocytes.



Section of the kidney focal area of necrosis (Arrow).

Figure 2: Photomicrographs of liver and kidney tissue section of mice exposed to different concentrations of leachate stained by haematoxylin and eosin (X 400 magnifications)

Conclusions

The findings from this study provide strong evidence that chronic exposure to contaminants from the environment exerts multifaceted toxic effects on mammalian physiology. Significant decreases in body weight gain in exposed animals suggest possible metabolic disruptions resulting from the bioaccumulation of environmental contaminants. Haematological evaluations revealed reductions in red blood cell (RBC) counts, haemoglobin concentration (Hb), and packed cell volume (PCV), indicative of anaemia likely caused by contaminants induced suppression of erythropoiesis or disrupted erythropoietic balance, interference with normal haematopoiesis and oxidative damage to erythrocyte membranes. These disruptions are indicative of anaemia, immunotoxicity and possible bone marrow suppression which signify the systemic toxicity of leachate exposure. Increases in white blood cell (WBC) counts point to an inflammatory or immune activation response, while platelet fluctuations suggest possible clotting disturbances, with the most adverse effects observed at high leachate concentration. Thus, continuous exposure to leachate contaminated groundwater or soil from dumpsites could play significant roles in the development of haematological disorders and related systemic dysfunctions. These findings underscore the importance of monitoring environmental leachate exposure, given its potential systemic health impacts, the need for stringent environmental monitoring and regulation to mitigate the adverse health impacts of leachate exposure. The study therefore highlight the needs for further research on elucidating the molecular and cellular mechanisms underlying leachate induced haematotoxicity, including oxidative stress pathways, DNA damage and apoptosis in blood forming organs.

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Supplementary Material

Not Applicable

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