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Impact of (ELIOZU) Dumpsite Leachates on Some Haematological Parameters in Wistar Rats

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Leachates contain deleterious constituents that may cause contamination of ground and surface water leading to serious risks to the ecosystem and human health. The exposure of the environment to dumpsite leachate may occur in different ways, such as uncontrolled overflow, rainfall and infiltration. This study therefore, attempts to determine the possible effects of (Eliozu) dumpsite leachates on some haematological parameters in Wistar rats. 25 Wistar rats weighing between 180g and 250g were divided into five groups of 5 animals each. Groups are as follows: Group 1: received 1ml of commercial bottle water, Group 2: received near-by borehole water, Group 3: received 10% of leachate concentrations, Groups 4 and 5: received 50% and 100% of leachate concentrations every 24 hours for 90 consecutive days. At the end of the gavaging of the leachates, blood samples were collected for heamatological parameters analysis using automated method. Results obtained shows a significant (p<0.001) reduction in some hematological parameters compared with control. Paradoxically, the mean corpuscular volume (MCV), red blood

cell distribution width-standard deviation and red blood cell distribution width-coefficient of variation show significant increase (p<0.001) compared with the control. There were also significant decreases in weights of the experimental animals while compared with the control group. Eliozu dumpsite leachates demonstrated some toxicological potentials on Red Blood Cells (RBCs) and RBC indices; White Blood Cells (WBCs) and WBC Differentials in Wistar rats. We recommend further studies in this regard.

Keywords: Leachates; Eliozu Dumpsite; Heamatological; Heamoglobin.

1. INTRODUCTION

Leachate is any liquid that in the course of passing through matter extracts soluble or suspended solids, or any other components of material through which it has passed [1]. When water infiltrates through waste, it promotes and helps the process of decomposition by bacteria and fungi [2]. This process releases by-products of decomposition and rapidly use up any available oxygen, buildina an anoxic environment. Eliozu dumpsite receives a mixture of municipal, commercial and mixed industrial waste but excludes significant amounts of concentrated waste and the leachate may be characterized as a water-based solution of groups of contaminants (Cheng et al., 2004). The exposure of the environment to leachate may occur in different ways, such as uncontrolled overflow, rainfall, subsidence and infiltration. A number of incidences have been reported previously where leachate had contaminated the surrounding soil and polluted the underground water acquifer or nearby surface water [3].

Eliozu dumpsite is one of the biggest dumpsite in Port Harcourt metropolis. It receives deposits of both domestic and industrial wastes from Port Harcourt, these wastes are dumped untreated, and thus may pose serious environmental risks to inhabitants in the area and entire Port Harcourt population directly or indirectly [4-6]. Despite its potential hazardous nature to Port Harcourt metropolis, no study has been done on the toxicological effect of Eliozu leachate on human population living around the vicinity. Residents near the dumpsite are oblivion of the possible contamination of water sources from leachate thus, the need for the present research [7-10]. This study therefore, attempts to evaluate the effects of Eliozu dumpsite leachate on some haematological parameters using experimental animals (Wistar rats) as models and is part of a more extensive assessment of the potential toxicological effects of this leachate apparently ignored by municipal authorities in Port Harcourt, Nigeria [11-13].

2. MATERIALS AND METHODS

2.1 Sampling Procedures and Analysis

Raw leachate was collected from leachate well on the dumpsite and sample was taken to laboratory in a clean and dry 5litres plastic containers. The obtained leachate was filtered using glass wool and Whatmann No.42 filter paper to remove suspended particles. The filtrate was centrifuged at 3000rpm for 10 minutes and the supernatant fluid obtained was considered as stock samples (100%) and labeled as Eliozu dumpsite leachate (EDL) and stored at 4^oC until ready for analysis and experimentation. Serial dilution of the leachate was subsequently prepared by diluting with distilled water. The different concentrations were thus determined, 10%, 50%, and 100% of leachate.

2.2 Acute Toxicity: Determination of LD₅₀

LD₅₀ of the supernatant fluid was determined using the Kerbars method in wistar rats. The value was estimated at 1.5mlper body weight.

2.3 Determination of the Physicochemical Properties of Leachate

Physicochemical properties of the leachate were analyzed according to (APHA standard method). Nitrate, ammonia, chloride, hardness, alkalinity, biochemical oxygen demand (BOD), chemical oxygen demand (COD) total suspended solid (TSS) and total dissolved solid (TDS) were determined. The concentration of the heavy metals was then estimated using PerkinElmer A3100 atomic absorption spectrophotometer.

2.4 Experimental Animals

25 Wistar rats weighing between 180g and 250g were obtained from the animal house unit of Faculty of Basic Medical Sciences, University of Port Harcourt, Nigeria. The animals were acclimatized for 14days before treatment and were maintained under standard laboratory conditions of 12 hours dark and light cycle with ready access to drinking water and standard rodent chow (*ad libitum*). The animals were treated according to the guide for the Care and use of laboratory animals published by United State for Laboratory and Animal Research 1996.

2.5 Experimental Design

The rats were divided into five groups of five animals each. Each experimental group was treated as follows,

Group 1: received 1ml of commercially bottled water

Group 2: received 1ml of water from borehole about 1Km from Eliozu dumpsite.

Group 3: received 1ml of 10% of leachate concentrations

Group 4: received 1ml of 50% of leachate concentrations

Group 5: received 1ml of 100% of leachate concentrations

All water and leachate were administered once daily using an oro-gastric cannula for 90 consecutive days. All animals had free access to rat feeds and water *ad libitum*

2.6 Determination of Haematological Parameters

On day 91, blood was collected by direct cardiac puncture after chloroform anaesthesia for determination of haematological parameters. About 4ml of blood was obtained from each animal. The following haematological parameters were determined, Red blood cell count, Heamoglobin concentration, Heamatocrit, Mean Corpuscula volume, Mean Corpuscular Heamoglobin concentration, Mean Corpuscular Haemoglobin, Red Blood Cell Distribution Width. The following leucocyte parameters were also determined, White Blood Cell count, percentage Lymphocytes, Neutrophil, Monocyte, Eosinophil counts. These analyses were done using an autoanalyzer machine, (autoanalyzer, Mayamed, England. 2018 model) and Total lymphocyte count was determined as a product of percentage lymphocyte and total white blood cell count as described by (Dapper et.al., 2008).

2.7 Body Weight Measurement

Rats in each of the groups were weighed at the beginning of the experiment using an electronic

analytical weighing balance. At the end of the treatments after 90 days, rats were fasted overnight and the final weight of the animals were taken and recorded before the animals were sacrificed.

2.8 Statistical Analysis

Data obtained were subjected to statistical analysis and the analysis of variance (ANOVA) was used to compare the various groups and the significant differences were set at a p level of 0.05. Results are as shown on Tables 2 and 3.

3. RESULTS

Table 1 shows the result of the physicochemical characteristics of Eliozu dumpsite leachate (EDL) thus, pH 7.8, with dark brown coloration, the Total Dissolved solvent (TDS), Biological oxygen Demand (BOD), Chemical Oxygen Demand (COD), are 7640mg/l, 8000mg/l and 43978mg/l respectively. The TSS and ammonia have values as 2020mg/l and 29mg/l.

Also in the leachate sample is high alkalinity, hardness, Chloride with values as 9000mg/l, 1001mg/l and 2000mg/l. The heavy metals are iron (Fe), lead (Pb), chromium (Cr), copper (Cu), Nickel (Ni), arsenic (As) with values as follows; 4.31mg/l, 1.95mg/l, 0.8mg/l, 1.45mg/l, 0.307mg/l and 0.2mg/l respectively. Cadmium (Cd) and manganese (Mn) have values as 0.2mg/l and 0.435mg/l.

The EDL also have a total hetrotrophic bacteria count (THBC) of 2.13×10^{6} (Cfu/100ml).

Table 2 shows the effect of administration of Eliozu dumpsite leachate on red blood cell count and other red blood cell indices investigated. Compared to control (group 1 commercial bottle water) rats, administration of Eliozu dumpsite leachate significantly reduced the red blood cell count in a dose dependent manner amongst Groups 3,4 and 5 rats (p<0.001). Similarly, haemoglobin concentration. values of heamatocrite, mean corpuscular haemoglobin count, mean corpuscular heamoglobin showed significant dose dependent decrease amongst Groups 3, 4 and 5 rats as compared to the control Group 1 rats (p<0.001). For instance, the value of mean corpuscular haemoglobin count reduced from 47.79±0.13(g/dl) amongst Group 3 rats to 45.78±0.50(g/dl) amongst Group 4 rats to the value of 43.94±0.40(g/dl) amongst Group 5 rats. These differences were found to be significant (p<0.001) and dose dependant.

However, values of mean corpuscular volume, red blood cell distribution width-standard deviation and red blood cell distribution widthcoefficient of variation showed dose dependent significant increase (p<0.001) amongst Group 3, 4 and 5 rats when compared to control Group 1. For instance, the value of mean corpuscular volume increased from 53.78 ± 0.20 (fl) amongst Group 3 rats to the value of 55.36 ± 0.40 (fl) amongst Group 4 to the value of 57.84 ± 0.70 (fl) amongst Group 5 rats. These differences were found to be significant (p<0.001) and dose dependant.

Table 3, shows the effects of the administration of Port Harcourt Eliozu landfill leachate on white blood cell count and some white blood cell indices. Compared to Group 1 (control), administration of Port Harcourt Eliozu landfill leachate significantly reduced the white blood cell count in dose dependent manner amongst Group 3, 4 and 5 rats (p<0.001). A similar dose dependent reduction in the value of Neutrophil count, Monocyte count, Eosinophil counts and Total lymphocyte counts were observed amongst Groups 3, 4 and 5 rats compared to control Group 1 rats (p<0.001). For instance the value of Neutrophil reduced from 35.30 ± 0.40 (%) to the value of 28.70 ± 0.24 (%) amongst Group 4 rats to the value of 21.82 ± 0.23 (%) in Group 5 rats. These differences were found to be significant (p<0.001) and dose dependent.

However, significant dose dependent increase was observed for only percentage lymphocyte amongst Group 3, 4 and 5 rats compared to Group 1 (control) rats (p<0.002).

Table 4 shows the result of change in weight (g) of animals, the results across groups are thus from group 1 to group 5, initial weight 194 ± 40 , 198 ± 66 , 198 ± 7.3 , 202 ± 3.7 and 202 ± 3.7 . The final weights across group 1 to group 5 are $233^{*}\pm3.0$, $175^{*}\pm2.2$, $192^{*}\pm5.8$, $179^{*}\pm2.4$ and $167^{*}\pm2.6$ respectively. The percentage changes are 20.1%, -11.6%, -3.0%, -11.6% and -17.3%.

4. DISCUSSION

Municipal solid waste disposal poses environmental challenge worldwide, [14]. In developing countries unavailability of suitable technological choices could be the reason why our solid wastes are dumped (Vacearo *et al.*, 2012, [15]. Exposure to leachate causes health risk [16].

Table1. Physicochemical para	ameters analyzed in Elioz	u dumpsite leachate
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Parameters	Pell	Nesrea
Colour	Dark brown	
pH	7.8	6.0-9.0
Ammonia (mg/l)	29.00	10
BOD (mg/l)	8,000	50
COD (mg/l)	43,978	90
Chloride (mg/l)	2000	250
Hardness (mg/l)	1001.1	
Alkalinity (mg/l)	9000	150
Cu (mg/l)	1.45	0.5
Fe (mg/l)	4.31	
Cd (mg/l)	0.2	0.2
Pb (mg/l)	1.95	0.05
Mn (mg/l)	0.458	0.2
As (mg/l)	0.2	
Ni (mg/l)	0.307	0.05
Cr (mg/l)	0.8	0.05
TSS (mg/l)	2020	
TDS (mg/l)	7640	
THBC	2.13×10 ⁶	

National Environmental Standards and Regulations Enforcement Agency (2009) maximum permissible limits for effleuent for waste water, www.epa.gov/safewater/mcl.html . THBC. Total hetrotrophic Bacteria count. TSS, Total solid suspension, (BOD) Biological oxygen demand, (COD) Chemical Oxygen Demand.

Parameters	Group1 (commercially obtained bottle water)	Group2 (Near-by borehole water)	Group3 (PELL 10%)	Group4 (PELL 50%)	Group5 (PELL 100%)
Red blood cell ($\times 10^{12}$)	6.31±0.18	6.5±0.10*	6.36±0.07*	6.18±0.20*	5.19±0.03*
, , , , , , , , , , , , , , , , , , ,	(5.9-6.9)	(6.2-9.8)	(6.2-6.6)	(5.5-6.6)	(5.1-5.3)
Haemoglobin	17.36±0.11	14.86±0.72*	16.46±0.40*	14.90±0.60*	14.30±0.30*
concentration (g/dl)	(16.9-17.9)	(12.3-16.4)	(15.7-17.8)	(12.6-15.8)	(13.4-14.8)
Haematocrit (%)	36.42±0.63	31.84±0.72*	34.08±0.41*	31.84±0.63*	27.42±1.40*
	(34.4-38.2)	(30.0-34.2)	(33.2-35.6)	(30.5-33.9)	(22.5-30.1)
Mean corpuscular volume	51.60±0.20	56.10±0.10*	53.78±0.20*	55.36±0.40*	57.84±0.70*
(fl)	(51.1-52.3)	(55.9-56.3)	(53.1-54.1)	(54.0-56.2)	(55.4-59.1)
Mean corpuscular	54.92±0.70	42.70±1.90*	47.68±0.13*	45.78±0.50*	43.94±0.40*
haemoglobin count (g/l)	(52.8-56.8)	(35.5-45.7)	(47.2-47.9)	(44.3-47.1)	(43.245.0)
Mean corpuscular	28.12±0.30	24.96±0.02*	25.98±0.23*	24.80±0.31*	24.36±0.10*
haemoglobin (pg)	(27.5-0.30)	(24.8-24.9)	(25.5-26.9)	(23.9-25.4)	(24.2-24.6)
Red blood cell	29.38±0.31	40.92±0.34*	35.36±0.04*	40.70±0.30*	44.38±0.30*
Distribution Width-	(28.5-30.3)	(40.2-42.1)	(35.3-35.5)	(39.5-41.2)	(43.4-44.9)
Standard Deviation (fl)					
Red blood cell	14.58±0.10	18.50±0.20*	16.44±0.05*	18.54±0.40*	21.02±0.50*
Distribution width coefficient of variation (%)	(14.3-14.9)	(18.1-19.1)	(16.3-16.6)	(17.8-19.8)	(19.4-21.9)

Table 2. Effects of Eliozu dumpsite leachate on red blood cell and red blood cell indices

*= Significant difference compared with Group 1 (p<0.05)

PARAMETERS	Group1 (commercially obtained bottle water)	Group2 (Near-by borehole water)	Group3 (10% PELL)	Group4 (50% PELL)	Group5 (100% PELL)
White blood cell	33.36±0.40	10.73±0.42*	11.76±0.22*	9.94±0.37*	7.60±0.11*
(cell/µl)	(32.5-34.4)	(9.6-11.9)	(11.1-12.5)	(9.0-10.9)	(7.2-7.8)
Lymphocyte (%)	54.60±1.40	60.56±3.50	63.00±0.40*	64.90±0.32*	68.56±0.20*
	(50.0-57.7)	(46.6-64.6)	(61.6-63.7)	(64.2-66.1)	(67.9-68.9)
Neutrophil (×10^9)	37.50±0.22	28.96±0.40*	35.30±0.40*	28.70±0.24*	21.82±0.23*
,	(36.9-38.1)	(27.9-30.1)	(34.9-35.7)	(27.8-29.1)	(21.3-22.4)
Monocyte (× 10^9)	15.84±0.50	7.54±0.53*	11.68±0.30*	7.70±0.30*	4.70±0.10*
	(14.6-16.9)	(6.1-9.2)	(10.7-12.3)	(6.7-8.4)	(4.5-4.9)
Eosinophil (×10^9)	7.20±1.30	3.18±0.20*	4.02±0.13*	3.44±0.10*	2.50±0.20*
,	(5.4-12.3)	(2.8-3.6)	(3.6-4.3)	(3.2-3.7)	(2.1-2.8)
Total Lymphocyte	1821.18±105.32	652.95±127.96*	740.95±35.07*	643.82±57.63*	520.89±18.35*
count (%cell/µl)	(1660.0-1943.6)	(433.4-762.8)	(701.5-791.3)	(581.4-720.5)	(492.5-537.4)

Table 3. Effects of Eliozu dumpsite leachate on white blood cell and white blood cell indices

*= significant difference compared with Group 1 (p<0.05)

S/N	PARAMETERS	INITIAL WEIGHT (g)	FINAL WEIGHT (g)	PERCENTAGE CHANGE (%)
1	Group 1	194 <u>+</u> 4.0	233*±3.0	20.1
2	Group 2	198 <u>+</u> 6.6	175* <u>+</u> 2.2	-11.6
3	Group 3	198±7.3	192* <u>+</u> 5.8	-3.0
4	Group 4	202 <u>+</u> 3.7	179* <u>+</u> 2.4	-11.6
5	Group 5	202 <u>+</u> 3.7	167* <u>+</u> 2.6	-17.3

Table 4 Changes in weight (g) of Animals compared in the various parameters

Physicochemical characterization is the common or only monitoring method employed in the majority of dumpsite [17]. However, it could not identify some compounds because of limitations of traditional chemical analysis [18].

The phytochemical properties of Eliozu Dumpsite leachate and nearby borehole water are comparatively higher than the required values of Environmental Standard National and Regulations Enforcement Agency (2009)maximum permissible limits for effluent from waste water. The physicochemical properties of Eliozu dumpsite leachate differs with that of leachate from dumpsite in Effurn, Delta State, work by [19], leachate from landfill in India also differs from the studied leachate [20] The differences could be as a result of the component of the waste, the industrial activity, geographical and weather condition.

The result shows significant decrease in red blood cell of animals treated with Eliozu dumpsite leachate, our result is supported by previous work by [21] Monsa and Khaltab 2003, Vosyliene and Kazlauskein, 2004).

There were significant decreases (p<0.01) in the concentrations of haemoglobin and haematocrit, this could be as a result of heavy metals like Cadmium that is capable of reducing attractive force towards oxygen binding capacity, making erythrocyte more permeable, friable, resulting to damage (Witiska and Koscink 2003).

Erythrocyte indices depend on blood capsule count, haemoglobin concentration and packed cell volume. Elevation in mean corpuscular volume, red blood cell distribution width-standard deviation, red blood cell distribution widthcoefficient of variance with concomitant depression in mean corpuscular haemoglobin count and mean corpuscular haemoglobin as compared with the control, suggest macrocytic and hypochromic anaemia in leachate treated 2003). lt rats. (Barger also suggests compensating activity of heamopoietic system as

a result of response to haemolytic action Eliozu dumpsite leachate and nearby borehole water constituents, which corroborates with [22] [23].

Significant dose dependent decreases in white blood cell, neutrophils, monocytes, eosinophils counts and concomitant increase in percentage lymphocytes were observed and this is similar and varies in some way with previous report by [24] on a work, the effect of municipal solid waste leachate on leucocytes and differentials in rats [25] On human exposed to marijuana smoke [26] rats exposed to single oral dose of 23-30mg of cannabinoid per kilogram body weight.

We further observed significant decrease in the Total lymphocyte counts; this is similar with a report by (Alimba *et al.*, 2008). It may be plausible that some of the leachate constituents are capable of suppressing the activity of lymphoid tissues in bone marrow of rats and may inhibit T and B lymphocytes production in rats.

Heamatopathology best describes the haematological changes and data from the toxicological studies in Wistar rats is a way of forecasting human risk assessment (Evans 2008).

The present study revealed dose depended decrease in body weights of EDL and near-by borehole water treated animals compared with control, our result is similar with (Farombi *et al.*,2011), but in variance with reports of [27-28], (Li *et al.*, 2006) who reported increase in body weight of mice treated with municipal landfill leachate. Difference in these results may be related to the season and composition of the landfills.

5. CONCLUSION

In conclusion, this study shows that Eliozu dumpsite leachate have high concentrations of both the physical and chemical properties when compared with the NESREA standard. The concentration dependent alterations in the studied haematological parameters and body weight loss are indications that Eliozu dumpsite is very toxic. Our study reports the potential toxicological effects of EDL and urges municipal authorities to intervene in public interest.

ETHICAL APPROVAL

Ethical approval for this study was sought and obtained from the University of Port Harcourt Research Ethics committee on 3rd July, 2018. The approval reference number is UPH/R&D/REC/04.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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