

# Impact of scrap dumpsite leachates on African land snails: insights into toxicity, biochemical responses, and reproductive implications

Jimoh Olatunji Abubakar <sup>1,\*</sup>, Oluyemi Ayoola Abidemi <sup>2</sup>,  
Ayodele Simeon Olugbenga <sup>1</sup>, Osayande Unity Daniel <sup>3</sup> and  
Olarotimi Olumuyiwa Joseph <sup>4</sup>

\*corresponding author: abubakarjimoh2011@gmail.com

<sup>1</sup> Agricultural Technology Department, The Federal Polytechnic Ado Ekiti, Ekiti State, Nigeria.

<sup>2</sup> Bogoro Research Centre, Afe Babalola University Ado Ekiti, Ekiti State

<sup>3</sup> Department of Agricultural Technology, Edo State college of Agriculture and Natural Resources, Iguoriakhi, Edo State

<sup>4</sup>Department of Animal Science, Adekunle Ajasin University Akungba Akoko, Ondo State

## ABSTRACT

This research delved into the intricate physiological responses of African land snails exposed to leachates from metal scrap dumpsites in Ado Ekiti metropolis. Raw leachates were collected from different leachate wells at the two dumpsites, these were used to form concentrations (v/v; leachate: dechlorinated tap water) and offered as drinking water throughout the study. A total of 80 points of lay snails (*Archachatina marginata*) 160.25 ± 5.84g and 7–8 months were used as test organism to assess the effect of the leachate. The snails were randomly allotted into four treatments, with four replicates and five snails per replicate representing the different leachate sample concentrations i.e T1-0%, T2-33.3%, T3- 66.67% and T4-100%. Results obtained indicated that the sodium, calcium, potassium, chromium, manganese and magnesium values of the leachates were higher than safety limits. The final weight of snails in T3 was significantly (P<0.05) higher than other treatment. The gonadosomatic index of snails in T1 was similar to T3 and T4 but significantly (p<0.05) higher than those on T2. Snails exposed to metal leachates have lower antioxidant activities compared with those on T1. In conclusion, the exposure of snails to higher concentrations of the leachates indicates potential toxicity and a tendency for impairment in reproductive capacity.

**Keywords:** Environmental contamination, Leachate, heavy metal, bioaccumulation, land snails

## INTRODUCTION

The escalating challenges of environmental pollution have far-reaching consequences on diverse ecosystems, affecting various flora and fauna. Terrestrial mollusks such as African land snails have emerged as critical bioindicators of environmental stress due to their sensitivity to environmental alterations and their ability to bio-accumulate compounds (Ebenso et al., 2004a; Ebenso et al., 2005; Anim et al., 2011). These land snails are delicacy in diets of rural communities (Ebenso et al., 2004b).

One predominantly source of pollution with high concern is scrap dumpsites, notorious for releasing leachates that encompass array of contaminants into the surrounding environment. Scrap dumpsites serve as reservoirs for an amalgamation of discarded materials, including heavy metals, organic pollutants and other hazardous substances (Adetoro et al., 2018). These materials when exposed to environmental factors can generate leachates that percolate into the soil, potentially affecting the resident fauna (Ghosh et al., 2017; Gupta et al., 2019). African land snails, a slow-moving and soil-dwelling organisms become susceptible to the adverse effects of such leachates due to their tendency of prolonged exposure (Vega et al., 2012).

The growth pattern of African land snails in response to scrap dumpsite leachates represent a crucial aspect of their adaptive strategies. Previous study has highlighted the influence of environmental contaminants on the growth rates of terrestrial mollusks (Sharaf et al. 2015). Understanding these growth dynamics provides insights into the overall well-being of the snail population in contaminated environments. The gonadosomatic index (GSI), a metric reflecting the proportion of gonad weight to total body weight serves as a valuable indicator of reproductive health in snails (Barber and Blake, 2006; Jimoh and Akinola, 2020). Alterations in GSI of African land snails exposed to scrap dumpsite leachates may cause disruptions in their reproductive physiology. Studies on fish have demonstrated changes in GSI as a response to environmental stressors (Ahammad et al., 2021; Jamalzadeh et al., 2013). Also, biochemical analysis encompassing enzyme activities and metabolic parameters offer a comprehensive view of the physiological status of African Land snails under leachate exposure (Radwan et al., 2010; Ugokwe et al., 2020). Reduction or rise in enzyme activities or metabolic profiles can indicate stress-induced responses as observed in related research (Ugokwe et al., 2020; Ndebele, 2023). Therefore, this research was conducted to investigate the responses of African land snails exposed to leachates from scrap dumpsites and to contribute to the scientific understanding of the ecological consequences of scrap dumpsite leachate on African land snails.

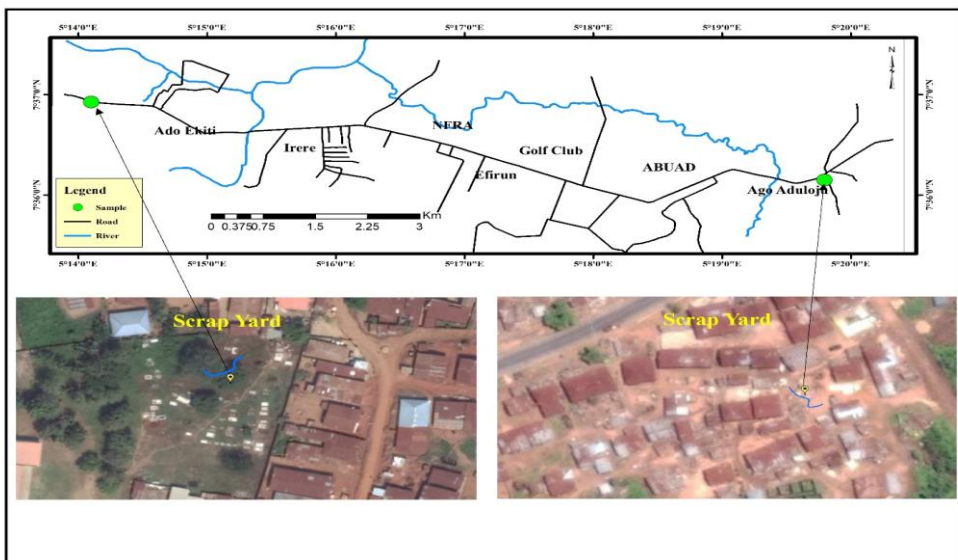
## MATERIALS AND METHODS

The institutional research and ethics committee at Federal Polytechnic Ado Ekiti, Nigeria is approved for animal research and experimental methods based on animal studies procedures. Approval for the conduct of this research was obtained from the Institutional Ethics Committee with IACUC approval number: - FPA/EC/22/0023, was performed in conformity with the ARRIVE 2.0 and NRC guidelines and appropriate measures were taken to minimize pain or discomfort to the animals. The authors confirm that the ethical policies of the journal were adhered to as noted on the journal authors' guideline page. The authors confirm that the standards for the protection of animals used for experimental/scientific purposes were followed.

### *Leachate collection*

Two metal scrap dumpsites situated in Ado Ekiti (Figure 1), Ekiti State, was chosen as the collection site for leachate sample. These sites are the primary metal scrap dumping site of Ado Ekiti and dumping of scraps at the sites is unrestricted and contains mixtures motor parts, electronics and machinery including industrial, agricultural, domestic and medical equipment. Raw leachates were collected from different leachate wells in the dumpsite, thoroughly mixed to provide a representative leachate sample. The leachate was taken to the laboratory in pre-washed 5-liter plastic container and stored at 4°C until use.

**Figure 1.** Map of Ado Ekiti showing sites of leachate collection



### *Heavy metal analysis of Leachate*

Leachate analysis was done by measuring the chemical components of the leachate. The concentrations of heavy metals; Sodium, Calcium, Magnesium, Potassium, Lead, Cadmium, Nickel, Chromium, Arsenic, Copper, Manganese were detected by heavy metal kits (Merck) and atomic absorption spectrophotometry (APHA, 1998), carried out using Buck Scientific Atomic absorption spectrophotometer 211VGP, Norwalk USA, Lamps and standards used are supplied by Buck scientific Inc. Norwalk, USA.

### *Biotoxicity Test*

A total of 80 points of lay snails (*Archachatina marginata*) with  $160.25 \pm 5.84$ g average body weight and age range 7–8 months were purchased as test organism to assess the toxicity of the collected waste leachate. The snails were purchased from the Institute for Agricultural Research and Training, Ibadan. They were kept at the Snail Research unit of Teaching and Research Farm, Department of Agricultural Technology, Federal Polytechnic, Ado Ekiti, Nigeria. Snails were acclimatized for seven days fed *ad libitum* with fresh unripe pawpaw (*Carica papaya*) and watermelon peel. Feed and water were provided in feeding and water trough. This research was undertaken with approval from the institutional ethics committee of the Department of Agricultural Technology, Federal Polytechnic, Ado-Ekiti. The institutional and national standards for the care and use of animals for research were followed, and appropriate measures were taken to minimize pain or discomfort on the animals.

The treatment groups consist of four different concentrations of the leachate sample (v/v; leachate: dechlorinated tap water) designated as T1 (0%), T2 (33.3%), T3 (66.67%) and T4 (100%) and offered as drinking water throughout the study. Sterile loamy soil was placed at base of each cage up to 2cm deep and moistened with the different treatment solutions respectively. This acted as both substrate and source of exposure. 80 snails were randomly allotted to the four treatment groups. These were allotted to four replicates with five snails per replicate in wooden cages with the dimension 30 cm × 40 cm × 24 cm. Snails were fed and watered twice a week, during a 28-day exposure period. At the end of the study, five (5) snails from each group were randomly selected weighed and euthanized and a small portion of the snails' shell situated above the heart was removed and a capillary tube was inserted into the heart to collect hemolymph out for evaluation of biochemicals and oxidative stress markers. The weights of visceral and various organs were obtained as well as the gonadosomatic index.

### *Biochemical assay*

Total protein, albumin, alanine amino transferase, aspartate amino transferase, alkaline phosphatase was carried out using fortress diagnostics commercial assay kits and its procedures. Determination of oxidative stress markers; total antioxidant capacity activities, superoxide dismutase, glutathione peroxidase, catalase activities and lipid peroxidation were assay as outlined in Jimoh et al., (2019).

### *Determination of serum total antioxidant capacity activities*

TA capacity assay was carried out according to Koracevic et al., (2001), reactive mixture containing 0.5 mL of a (10 mmol/L) Na-Benzoate, 0.2mL of H<sub>2</sub>O<sub>2</sub> (10 mmol/L), 0.49 ml of phosphate buffer (100 mmol/L, pH = 7.4) (prepared by mixing 19.5 ml of KH<sub>2</sub>PO<sub>4</sub> (100 mmol/L) with 80.5 ml of Na<sub>2</sub>HPO<sub>4</sub> (100 mmol/L), then adjusted the pH to 7.4 and 0.2 ml of Fe-EDTA complex (2 mmol/L) (prepared freshly by mixing equal volumes of EDTA (2 mmol/L), and ferrous ammonium sulfate (2 mmol/L), then left at 25 °C for 60 min. Ten microliters of the blood serum were added to the latter reactive mixture and were incubated at 37 °C for 60 min. Finally, 1 ml glacial acetic acid (20 mmol/L) and 1 ml thiobarbituric acid (0.8% w/v in 100 ml of 50 mmol/L NaOH) were added, and the absorbance at 532 nm was measured spectrophotometrically after incubation at 100 °C for 10 min. Total antioxidant capacity was calculated according to the following formula:

$$T A \text{ capacity (mmol/L)} = (CUA) (K - A) / (K - UA)$$

CUA (mmol/L); concentration of uric acid, K: absorbance of the control (K<sub>1</sub> - K<sub>0</sub>), A: absorbance of the sample (A<sub>1</sub> - A<sub>0</sub>), UA: absorbance of uric acid solution (UA<sub>1</sub> - UA<sub>0</sub>)

### *The assay for lipid peroxidation*

The reaction mixture in a total volume of 3.0 ml contained 1.0 ml serum and 1.0 ml of TCA (0.67%). All the test tubes were placed in a boiling water bath for a period of 45 min. The tubes were shifted to the ice bath and then centrifuged at 2500 rpm for 10 min. The amount of malondialdehyde (MDA) formed in each of the samples was assessed by measuring the optical density of the supernatant at 532Nm.

### *Superoxide dismutase (SOD)*

The reaction mixture includes 2.1 ml of 50 mM buffer, 0.02 ml of enzyme source, and 0.86 ml of distilled water. The reaction was initiated with 0.02 ml of 10 mM pyrogallol, and change in absorbance monitored at 420 nm. One unit of SOD is defined as the amount of enzyme required to inhibit to auto-oxidation of pyrogallol by 50% in the standard assay system of 3 ml. The specific activity is expressed as unit/min/mg protein.

*Glutathione peroxidase activity*

0.5 ml of 0.4 M buffer, pH 7.0, 0.2 ml enzyme source, 0.2 ml of 2 mM GSH, and 0.1 ml of 0.2 mM H<sub>2</sub>O<sub>2</sub> were added and incubated at room temperature for 10 min along with the control tube containing all reagents except enzyme source. The reaction arrested by adding 0.5 ml of 10 % TCA, centrifuged at 4000 rpm for 5 min, and the glutathione (GSH) content in 0.5 ml of supernatant was estimated. The activity expressed as microgram of GSH consumed/min/mg protein.

*Catalase activity*

The assay system contains 1.9 ml of 0.05 M buffer, pH 7.0, and 1.0 ml of 0.059 M H<sub>2</sub>O<sub>2</sub>. The reaction is initiated by addition of 0.1 ml enzyme source. The decrease in absorbance is monitored at 1 min interval for 5 min at 240 nm, and activity is expressed as nanomoles of H<sub>2</sub>O<sub>2</sub> decomposed/min/mg protein.

*Statistical analysis*

Data obtained from the study was tested using generalized linear model procedure of one-way Analysis of variance according to statistical analysis software IBM SPSS 20.

## RESULTS AND DISCUSSION

The mean concentration of heavy metals in the sampling points is shown in Table 1. The sodium calcium, potassium, chromium, manganese and magnesium values were higher than NESREA limit. However, the calcium, magnesium and manganese values from sampling point B are higher than sampling point A.

**Table 1.** Heavy metal analysis of water leachate samples

	Sampling Point A	Sampling Point B	*NESREA
Sodium (ppm)	41.45	39.65	0.50
Calcium (ppm)	95.15	103.45	50
Magnesium (ppm)	3.25	4.19	-
Potassium (ppm)	21.35	20.10	100
Lead (ppm)	0.02	0.02	0.05
Cadmium (ppm)	0.01	0.01	0.2
Nickel (ppm)	-	-	-
Chromium (ppm)	0.41	0.38	0.05
Arsenic (ppm)	0.02	0.02	-
Copper (ppm)	0.52	0.47	0.5
Manganese (ppm)	0.38	0.41	-
Zinc (ppm)	0.51	0.48	6.0 - 9.0

Key: NESREA=National Environmental Standards and Regulations Enforcement Agency

\*Adapted from Ugokwe *et al.*, (2020)

The result of the performance of African land snails exposed to metal leachate is presented on Table 2. The final weight of snails on T3 (123.61g) were significantly ( $p < 0.05$ ) higher than those from T1 (110.84g) and T2 (111.45g). The weight gain of snails on T3 and T4 were significantly ( $p < 0.05$ ) higher than those on T1 and T2.

**Table 2.** Performance of African land snails exposed to metal leachate

	T1	T2	T3	T4	SEM
Initial weight(g)	110.18	108.45	118.09	113.27	1.53
Final weight (g)	110.84 <sup>b</sup>	111.45 <sup>b</sup>	123.61 <sup>a</sup>	117.59 <sup>ab</sup>	1.48
Weight gain (g)	0.66 <sup>c</sup>	2.99 <sup>b</sup>	5.52 <sup>a</sup>	4.32 <sup>a</sup>	1.84

<sup>ab</sup> Means with the same superscript on the same row do not differ significantly ( $p > 0.05$ ), SEM: standard error of means

The result of the carcass and gonadosomatic index of African land snails exposed to metal leachate is presented on Table 3. There was no significant difference across the treatments for the carcass weight, visceral weight, and shell weight. The live weight of snails on T3 (137.34) was significantly ( $p < 0.05$ ) higher than those from T4 (126.54). The foot weight of snails in T2 (50.82) was significantly ( $p < 0.05$ ) higher than those from T3 (41.70). Also, the gonadosomatic index of snails in T1 (60.24) was similar to T3 (57.70) and T4 (50.21) but significantly ( $p < 0.05$ ) higher than those from T2 (42.65).

**Table 3.** Carcass and gonadosomatic index of African land snails exposed to metal leachate

	T1	T2	T3	T4	SEM
Live weight (g)	128.66 <sup>ab</sup>	129.64 <sup>ab</sup>	137.34 <sup>a</sup>	126.54 <sup>b</sup>	1.62
Carcass weight (g)	69.36	70.96	65.26	71.94	1.30
Visceral weight (g)	26.06	21.16	22.92	24.16	0.87
Shell weight (g)	32.64	34.06	38.10	32.66	1.27
Foot weight (g)	43.44 <sup>ab</sup>	50.82 <sup>a</sup>	41.70 <sup>b</sup>	48.08 <sup>ab</sup>	1.43
Gonadosomatic index	60.24 <sup>a</sup>	42.65 <sup>b</sup>	57.70 <sup>ab</sup>	50.21 <sup>ab</sup>	2.94

<sup>ab</sup> Means with the same superscript on the same row do not differ significantly ( $P > 0.05$ ), SEM: standard error of means

The haemolymph biochemicals of African land snails exposed to metal leachate is shown in Table 4. The total protein and albumin in hemolymph from snails in T2 (30.96g/dl and 24.00g/l respectively) were significantly ( $p < 0.05$ ) higher than other treatments. Alkaline phosphatase and alanine amino transferase of snails on T1 (43.06 U/I) was significantly ( $p < 0.05$ )

higher than those from T2, T3 and T4. Aspartate amino transferase of snails on T1 were significantly ( $p < 0.05$ ) higher than those on T4.

**Table 4.** Hemolymph biochemicals of African land snails exposed to metal leachate

	T1	T2	T3	T4	SEM
Total Protein (g/dl)	19.11 <sup>b</sup>	30.96 <sup>a</sup>	18.25 <sup>b</sup>	20.36 <sup>b</sup>	2.76
Albumin (g/l)	11.00 <sup>b</sup>	24.00 <sup>a</sup>	14.00 <sup>b</sup>	15.00 <sup>b</sup>	5.91
Alkaline phosphatase (U/I)	43.06 <sup>a</sup>	3.31 <sup>b</sup>	6.62 <sup>b</sup>	7.18 <sup>b</sup>	6.15
Alanine amino transferase (U/I)	50.33 <sup>a</sup>	26.48 <sup>b</sup>	10.94 <sup>b</sup>	27.91 <sup>b</sup>	4.76
Aspartate amino transferase (U/I)	20.98 <sup>a</sup>	17.86 <sup>ab</sup>	17.58 <sup>ab</sup>	14.04 <sup>b</sup>	3.27

<sup>ab</sup> Means with the same superscript on the same row do not differ significantly ( $P > 0.05$ ), SEM: standard error of means

The oxidative stress markers of African land snails exposed to metal leachate is shown in Table 5. The total antioxidant activity (TAC) snails on T1 and T2 were significantly ( $p < 0.05$ ) higher than those on T3 and T4. Superoxide dismutase (SOD) of snails on T4 and T1 were statistically similar and were significantly ( $p < 0.05$ ) higher than those on T2 and T3. Lipid peroxidation and catalase of snails on T1 was significantly ( $p < 0.05$ ) higher than those on other treatment. The glutathione peroxidase of snails on T1, T2 and T4 were similar and significantly ( $p < 0.05$ ) lower than those on T3.

**Table 5.** Oxidative stress markers of African land snails exposed to metal leachate

	T1	T2	T3	T4	SEM
Total Antioxidant activity (mmol/L)	0.84 <sup>a</sup>	0.93 <sup>a</sup>	0.50 <sup>b</sup>	0.63 <sup>b</sup>	0.20
Lipid peroxidation ( $\times 10^{-5}$ MDA/mg protein)	3.61 <sup>a</sup>	1.63 <sup>b</sup>	1.98 <sup>b</sup>	1.97 <sup>b</sup>	0.45
Superoxide Dismutase (U/min/mg protein)	8.93 <sup>a</sup>	0.54 <sup>b</sup>	10.74 <sup>a</sup>	1.92 <sup>b</sup>	2.30
Catalase (nm H <sub>2</sub> O <sub>2</sub> /min/mg protein)	20.33 <sup>a</sup>	7.00 <sup>b</sup>	4.89 <sup>b</sup>	5.21 <sup>b</sup>	3.21
Glutathione Peroxidase (mg GSH/min/mg protein)	1.65 <sup>b</sup>	1.00 <sup>b</sup>	3.03 <sup>a</sup>	1.73 <sup>b</sup>	0.41

<sup>ab</sup> Means with the same superscript on the same row do not differ significantly ( $P > 0.05$ ), SEM: standard error of means

The study contributes to the understanding of the ecological consequences of scrap dumpsite leachates on African land snails. It highlights the vulnerability of these organisms to environmental contaminants and the importance of monitoring and mitigating the impacts of waste disposal on



ecosystems. Environmental pollution is of great concern since several ecosystems are being increasingly affected by anthropogenic activities. The leachates from the scrap dumpsites contained high concentrations of heavy metals such as sodium, calcium, potassium, chromium, manganese, and magnesium, which exceeded the limits set by the National Environmental Standards and Regulations Enforcement Agency (NESREA). This suggests that these dumpsites are sources of heavy metal contamination, which can have detrimental effects on the environment and organisms living in proximity to the dumpsites. Waste leachates constitute a major source of environmental pollution and contain hazardous compounds such as heavy metals. However, how these heavy metals are bioaccumulated in tissues of living organisms differs significantly (Iwegbue et al., 2009) and their concentration depends on the form in which the metal is bound (Mariam et al., 2004). The quantity of metals in the soil, the pH of the soil, and the physiological traits of the species, such as their ability to absorb and excrete, are other factors that affect the build-up of heavy metals (Purchart and Kula, 2007). The application of ecologically relevant species like land snails offers a chance to perform early tests on ecosystem health in relation to exposure to these contaminants (Jha, 2004). Considering that the giant African land snails are omnivores and consume soil detritus that may include organic pollutants and heavy metal, they have the potential to accumulate these contaminants to dangerously high levels.

For heavy metal analysis of water leachate at the two sampling points for this study, sodium (Na), calcium (Ca) and chromium (Cr) measured was higher than the maximum tolerable limit set by National Environmental Standards and Regulations Enforcement Agency (NESREA), while potassium (K), lead (Pb), cadmium (Cd) and zinc (Zn) were below the maximum limit. NESREA is an environmental agency of the Nigerian Federal Government tasked with maintaining and enhancing the country's natural resources sustainably, protecting biodiversity, and protecting the environment. Certain metals are required for the maintenance of specific physiological and biochemical processes in animals and other species (Singh et al., 2011) in traces, sometimes referred to as microelements, these include Cr, copper (Cu), manganese (Mn), iron (Fe) and Zn, however, continuous exposure to these compounds at elevated quantities has been linked to systemic or cellular issues and might potentially contribute to pollution (Pandey et al., 2014). On the other hand, as a result of their lack of established biological roles, other metals such as Arsenic (As), Pb and Cd are considered contaminants and undesirable compounds for animals to consume (Aliasgharpour and Rahnamaye, 2013). Their ability to harm organs even at low exposure levels further indicates their severe toxicity.

The final weight and weight gain of snails offered 66.6% of leachate concentration in this study suggested that higher the concentration of leachate

might increase the weight gain as also observed with snails exposed to 100% of leachate concentration. However, this contradicts the report of Lopotych et al., 2020 on rats, where increase in the concentration of heavy metals decreases the body and organ weight. There are still scanty scientific reports about the influence of heavy metals on feed intake and weight gain.

The results of carcass characteristics of the snails in this study did not follow a particular trend, higher live weight did not correspond to higher carcass and foot weight indicating the influence of the exposure on weight is not sequential. The reproductive ability of snails is predicted by the gonadosomatic index. The result from this study shows that the gonadosomatic index of snails not exposed to metal leachate had significantly higher value when compared to those exposed to metal leachate. This could be as a result of the negative effect of the metal leachate on the reproductive efficiency of the snails exposed to metal leachate. This is in line with the findings of Ahmed et al. (2018) who reported that heavy metals in fish gonads negatively affected their gonadosomatic index. The variation in GSI across treatment groups suggests that exposure to scrap dumpsite leachates may impact the reproductive health and physiology of the snails. Changes in GSI can indicate alterations in reproductive activity and potential disruptions in the reproductive cycle. Changes in GSI can have indirect effects on population dynamics. A decrease in reproductive output due to leachate exposure could lead to reduced population growth and potentially impact the overall population size of the snails in the ecosystem. A higher GSI indicates a larger investment in reproductive organs, which is essential for successful reproduction. The lower GSI observed in some treatment groups may suggest a potential impairment in reproductive capacity, possibly due to the toxic effects of the leachates.

The biotoxicity test showed that exposure to the leachates had significant effects on the African land snails (*Archachatina marginata*). Snails exposed to higher concentrations of the leachate (T3 and T4) showed higher weight gain compared to those in lower concentrations (T1 and T2). However, there were no significant differences in carcass weight, visceral weight, and shell weight across the treatments. This indicates that while the leachate may stimulate growth in some aspects, it does not affect overall body composition significantly.

The analysis of haemolymph biochemicals showed that exposure to the leachate affected the levels of total protein, albumin, alkaline phosphatase, alanine amino transferase, and aspartate amino transferase in the snails. Snails in different treatment groups showed varying levels of these biochemicals, indicating potential effects on metabolic processes and organ function. All protein metabolisms in the body are susceptible to heavy metals, which can also disrupt the majority of animal processes that depend on proteins (Raghavendra et al., 2017). The total protein contents of the

haemolymph in this study was highest in snails with access to 33.3% concentration (T2) of leachate and the least in T3 (66.6%). The total protein content was lower than 35.23 and 52.03g/dl reported by Osunkeye et al. (2021) for haemolymph from snails obtained from Lagos and Osun State respectively. Snails in group T2 and T4 had total protein higher than the control, indicating that the level of heavy metals exposed to was not capable of depleting the amino acids in the body of the snails. Snails are known to be a good source of protein which can be consumed to ameliorate the problem of protein deficiency in diets (Akinnusi, 2002; Amusan and Omidiji, 2008) and the haemolymph also proof the presence of appreciable amount of protein than can be used as a regulator in human physiological processes especially, growth and repaired of worn-out tissue.

Albumin is part of protein responsible for maintenance of proper osmotic pressure, it binds and transports different molecules and the elimination of free radicals. Albumin is the most abundant protein in the body fluids. Its main function is to maintain osmotic pressure, the mechanism that prevents fluids from leaking out of the blood vessels and into surrounding tissues. It is an important transporter in the body fluids; albumin also binds fats and helps body with fat metabolism (Finlayson, 1975). The albumin contents of the haemolymph obtained in snails in this study have higher value with snails in T2 (66.6%) which differs significantly compared with other treatments, this confirms that protein was not depleted in a sequential manner as the concentration of heavy metal leachate. Alkaline phosphate (ALP), alanine amino transaminase (ALT) and aspartate amino transaminase (AST) are types of liver enzymes. The highest of in all these enzymes when measured in this study was in T1 (0.0%), in ALP and ALT this was significantly higher than the other treatments while in AST was similar to T2 and T3. Exposure to Arsenic (As) had been implicated in decreased ATP production, leading to cell damage (Shen et al., 2013). Likewise, concentration of Lead (Pb) at high level through drinking water for rats elevated the value of ALP and AST (Jadhav et al., 2007). The value of Pb at both sampling points for leachate collection was below the NESREA limit.

The fundamental cause of oxidative stress is an imbalance between the body's capacity to neutralize or detoxify free radicals by neutralizing their detrimental effects through the action of antioxidants. According to El-Demerdash (2007), oxidative damage is often indicated by extreme cell structural damage brought on by elevated ROS production leading to the damage of proteins, lipids, nucleic acids, and carbohydrates. The main ways that pollutants affect biological systems are through high ROS generation and direct interactions with biological tissues (Ma et al., 2013). The values obtained for the antioxidant enzymes observed in the present study did not follow a particular trend, although there was significant difference in all parameters measured i.e lipid peroxidation (MDA), superoxide Dismutase

(SOD), catalase (CAT) and glutathione peroxidase (GPx). The primary indication of lipid peroxidation is malondialdehyde (MDA) and measuring it can directly reveal oxidative stress. MDA levels have been shown to rise as a result of free radicals produced by contaminants in the biological system, elevation of MDA is well recognized as an indicator of oxidative stress. (Belhaouchet et al., 2012). The findings of this study reveal that MDA level was reduced in groups receiving different concentration of leachate compared to the control (0%). This indicated that lipid peroxidation was not aggravated in this study. Therefore, there is no indication of oxidative stress with increased concentration of leachate in this study.

The primary defensive mechanisms in antioxidant systems are CAT and SOD because of their important role in combating oxidative stress. In this study, the CAT level in T1 (20.33 nm H<sub>2</sub>O<sub>2</sub>/min/mg protein); receiving 0% of the leachate concentration was significantly higher the other treatments while the highest value for SOD was 10.74 (U/min/mg protein) in T3 (66.6%) but significantly similar to T1 (8.93 U/min/mg protein). This contradicts the report of Ugokwe et al (2020) that the level of antioxidant enzymes such as CAT and SOD were increased due to exposure to pollutants because of their ability to mitigate the effects of reactive oxygen species (ROS). Various pro-oxidant or antioxidant responses have also been said to be induced by toxicants based on their ROS-producing capacity (Barata et al., 2005). Considered as the most important peroxidase, glutathione peroxidase (GPx) detoxifies hydroperoxides and peroxides to hydroxyl and water molecules, respectively. This is an antioxidant enzyme that has the ability to scavenge free radical, therefore lowering intracellular oxidative stress. The GPx in this study indicated that snails in T3 (66.6%) had elevated GPx values which were significantly different from others that were similar, point to the fact that there is likelihood of increased ROS production in this group. The study also found that exposure to the leachate affected the oxidative stress markers in the snails. Total antioxidant activity (TAC) was higher in snails in lower concentrations of the leachate (T1 and T2), while superoxide dismutase (SOD) was higher in snails in higher concentrations (T3 and T4). This suggests that exposure to the leachate induces oxidative stress in the snails, which can have negative effects on their health and longevity.

The results of the study suggest that leachates from scrap dumpsites have significant toxic effects on African land snails, affecting their growth, biochemical profiles, and oxidative stress responses. This highlights the importance of proper waste management practices to prevent environmental contamination and protect the health of organisms living in proximity to dumpsites.

## CONCLUSION

Leachates from the scrap dumpsites contained high concentrations of heavy metals, exceeding regulatory limits. This indicates a serious environmental concern, and the need for better waste management practices to prevent contamination. The exposure of snails to the leachates resulted in significant effects on their weight gain and biochemical profiles. Higher concentrations of leachate stimulated weight gain, they also induced changes in haemolymph biochemicals and oxidative stress markers, indicating potential toxicity. This study emphasizes the need for stricter environmental regulations and effective waste management strategies to mitigate the adverse effects of scrap dumpsite leachates on ecosystems and wildlife. The findings of this study carry significant importance from a public health perspective, raising concerns about the safety of consuming these snails. If these contaminated snails enter the food chain, they could pose serious health risks to consumers due to the bioaccumulation of heavy metals and other toxic substances. This underscores the critical need for not only stricter environmental regulations but also vigilant monitoring of food sources, especially in areas near scrap dumpsites, to safeguard public health.

## ACKNOWLEDGEMENTS

The authors are grateful to the management and staff of the Animal Science Unit, Teaching and Research Farm, Agricultural Technology Department, Federal Polytechnic Ado Ekiti, for their assistance in providing housing for this work.

## REFERENCES

- Ademolu, K. O., Idowu, A. B., Jayeola, O. A. 2009. Changes in haemolymph biochemical values during different growth phases in African giant land snail, *Archachatina marginata* (Swainson). *Nigerian Journal of Animal Production*, 36(1): 161-166.
- Adetoro, F. A., Ikuabe, B. O., Lawal, R. A. 2018. Toxicological Response of *Poecilia reticulata*, *Hyla* species and *Culex* species to Leachates from Olusosun Landfill, Lagos State, Nigeria. *J. Appl. Sci. Environ. Manag.*, 22(5): 817-823.
- Ahammad, A. K. S., Hasan, N. A., Haque, M. M., Abul Bashar, M. D., Ahmed, B. U., Alam, M. A., Asaduzzaman, M. D., Mahmud, Y. 2021. Environmental factors and genetic diversity as drivers of early gonadal maturation: A gonadosomatic index based investigation on Indian Shad, *Tenulosa ilisha* population of Bangladesh. *Front. Mar. Sci.*, 8:758868
- Ahmed, E. H., Heba, A. M. E., Asmaa, M. R. G. 2018. Assessment of Water Quality and Heavy metals in Water, Sediments and Some Organs of African

- Catfish (*Clarias gariepinus*) in El-Serw drain, Nile Delta, Egypt. *International Journal of Environment*, 7(4): 124-141
- Akinnusi, O. 2002. Introduction to snail and snail farming. Triolas Exquisite Ventures. Abeokuta. 25 :1-6
- Ali, D., Alarifi, S., Kumar, S., Ahamed, M., Siddiqui, M. 2012. Oxidative stress and genotoxic effect of zinc oxide nanoparticles in freshwater snail *Lymnaea luteola*L. *Aquat. Toxicol.*, 124: 83–90.
- Aliasgharpour M, Rahnamaye F. M. 2013. Trace elements in human nutrition: A review. *International journal of medical investigation*. 2013;2(3)
- Amusan, J. A., Omidiji, M. O. 2008. Edible Land Snails. A Technical Guide to Snail Farming In the Tropic". Versity Press Ibadan 19-22.
- Anim, A. K., Ackah., M., Fianko, J. R., Kpattah, L., Osei, J., Serfor-Armah, Y., Gyamfi, E. T. 2011. Trace elements composition of *Achatina achatina* Samples from the Madina market in Accra, Ghana. *Research Journal of Environmental and Earth Sciences*, 3(5): 564-570.
- Barata, C., Varo, I., Navarro, J. C., Arun, S., Porte, C. 2005. Antioxidant enzyme activities and lipid peroxidation in the freshwater cladoceran *Daphnia magna* exposed to redox cycling compounds. *Toxicol. Comp. Biochem. Physiol.C.*, 140: 175–186.
- Barber, B.J., Blake N.J. 2006. Reproductive Physiology. In *Scallops: Biology, ecology and aquaculture*, second Edition, S. E Shumway and G.J. parsons, eds. Elsevier science publisher.
- Belhaouchet N, Djebar MR, Meksem L, Grara N, Zeriri I, Berrebbah H. 2012. Evaluation of the biomarkers of the oxidative stress induced by a biopesticide. The Spinosad on an alternate model: *Helix aspersa*. *J. Appl. Sci. Res.* 8: 4199-4206.
- Ebenso, I. E. 2004a. Egg-water relations of incubated eggs of African giant land snail in wetland ecosystem within Cross River basin of guinean forest of Niger Delta, Nigeria. *Bulletin of Pure and Applied Sciences (India)*, 23A : 65-69.
- Ebenso, I. E. 2004b. Molluscicidal effect of neem *Azadirachta indica* extracts on edible tropical land snails. *Pest Management Science (England)*, 60 : 178 – 182.
- Ebenso, I. E., Ita, B., Umoren, E. P., Ita, M., Binang, W., Edet, G., Izah, M., Udo, I. O., Ibanga, G., Ukpong, E. E. 2005. Effect of carbamate molluscicide on African giant land snail *Limicolaria aurora*. *J. Appl. Sci. Environ. Mgt.*, 9(1): 99-102
- El-Demerdash, F. M. 2007. Lambda-cyhalothrininduced changes in oxidative stress biomarkers in rabbit erythrocytes and alleviation effect of some antioxidant. *Toxicology in Vitro*. 21: 392-397.
- Finlayson J.S. 1975. Physical and biochemical properties of human albumin. In: *Proceedings of the workshop on albumin*, Sgouris J.T Rene A, eds, 31-56.

- Ghosh, P., Thakur, I. S., Kaushik, A. 2017. Bioassays for toxicological risk assessment of landfill leachate: A review. *Ecotoxicol. Environ. Saf.*, 141: 259-270.
- Gupta, P., Arpit, B., Roshani, K., Lalit, L., Rajnarayan, T., Pushpendra, K. G., Neha, B., Ravindra, S., Pradyumna, K. M. 2019. Impairment of Mitochondrial-Nuclear Cross Talk in Lymphocytes Exposed to Landfill Leachate. *Environ. H Insi.*, 13: 1-11.
- Iwegbue, C. M., Arimoro, F. O., Nwajei, G. E., Eguavoen, O. 2009. Heavy metal content in the African giant snail *Archachatina marginata* (Swainson, 1821) (Gastropoda: Pulmonata: Achatinidae) in southern Nigeria. *Folia Malacologica*, 16 (1): 31-34
- Jamalzadeh, H. R., Akhundian, M., Kabir, M., Khara, H., Elham, M., Hajirezaee, S., Golpour, A. 2013. Changes of gonadosomatic index and plasma levels of cortisol in the male and female captive Caspian brown trout, *Salmo Trutta Caspius* (Kessler, 1877) during the reproductive cycle. *J. Appl. Ani. Res.*, 41(2): 133-136.
- Jadhav, S. H., Sarkar, S. N., Patil, R. D., Tripathi, H. C. 2007. Effects of subchronic exposure via drinking water to a mixture of eight water-contaminating metals: a biochemical and histopathological study in male rats. *Arch. Environ. Contam. Toxicol.* 53 (4), 667–677. doi:10.1007/s00244-007-0031-0
- Jha, A. N. 2004. Genotoxicological studies in aquatic organisms: an overview. *Mutat Res.*, 552: 1–17.
- Jimoh, O.A., Akinola, M. O. 2020. Reproductive performance of laying snails (*Archachatina marginata*) fed on roughages and different concentrate mix. *Bulletin of the National Research Centre*, 44: 118
- Lopotych N., Panas N., Datsko T., Slobodian S. 2020. Influence of heavy metals on hematologic parameters, body weight gain and organ weight in rats. *Ukrainian Journal of Ecology*, 2020, 10(1), 175-179, doi: 10.15421/2020\_28
- Ma H., Diamond S. A. 2013. Phototoxicity of TiO<sub>2</sub> nanoparticles to zebrafish (*Danio rerio*) is dependent on life stage. *Environ. Toxicol. Chem.*, 32: 2139–2143.
- Mariam I., Iqbal S., Nagra S. A. 2004. Distribution of some trace and macrominerals in beef, mutton and poultry. *International Journal of Agriculture and Biology*, 6: 816-820
- Ndebele D. 2023. Trace metal Levels and Oxidative Stress Biomarkers in Land Snails, *Achatina Fulica*, Exposed to Soils from a Coal Mining Area in Zimbabwe. *Biomark. J.*, 9: 035
- Ojha, A., Yaduvanshi, S. K., Sivastava, N. 2011. Effect of combined exposure of commonly used organophosphate pesticide on lipid peroxidation and antioxidant enzymes in rat tissues. *Pesticide Biochem Physiol.*, 99: 148-156.

- Osunkeye, O. J., Abiona, J. A., Onagbesan, O. M and Omole, J. A. (2021). Haemolymph biochemical and mineral properties with morphomeric parameters of reproductive organs of *Archachatina marginata* as affected by humid agro-ecological zones in Nigeria. *Nigerian Journal of Animal Science*, 23 (1), 53 – 60.
- Pandey G., Madhuri S. 2014. Heavy metals causing toxicity in animals and fishes. *Research Journal of Animal, Veterinary and Fishery Sciences*.2(2):17-23
- Purchart, L., Kula, L. 2007. Content of heavy metals in bodies of field ground beetle (Coleoptera: Carabidae) with respect to selected ecological factors. *Polish Journal of Ecology*, 35: 305-314.
- Radwan, M. A., El-Gendy K. S., Gad, A. F. 2010. Biomarkers of oxidative stress in the land snail, *Theba pisana* for assessing ecotoxicological effects of urban metal pollution. *Chemosphere*, 79: 40-46
- Raghavendra Rao M. V., Yogesh Acharya, Jitendra Kumar Nayak, Sireesha Bala, Simi Paramban, Anusha C. Pawar 2017. Study of the effects of heavy metals on the biochemical constituents of proteins metabolism and total ninhydrin positive substances using alternate animal model: *Heterometrus fulvipes*. *International Education & Research Journal [IERJ]*. E-ISSN No: 2454-991: 3(5).
- Sharaf, H. M., Salama, M. A., Abd El-Att, M. S. 2015. Biochemical and Histological alterations in the digestive gland of the land snail *Helicella vestalis* (Locard, 1882) exposed to Methiocarb and Chlorpyrifos in the Laboratory. *J. Cytol. Histol.*, 6: 327
- Shen, S., Li, X.-F., Cullen, W. R., Weinfeld, M., Le, X. C. 2013. Arsenic binding to proteins. *Chem. Rev.* 113 (10), 7769–7792. doi:10.1021/cr300015c
- Singh R, Gautam N, Mishra A, Gupta R. 2011. Heavy metals and living systems: An overview. *Indian Journal of Pharmacology*. 43(3):246
- Ugokwe, C. U., Okafor, F. C., Okeke, P. C., Ezewudo, B. I., Olagunju, T. E. 2020. Induction of genetic alterations and oxidative stress in giant African land snail (*Limicolaria aurora*) exposed to municipal waste leachate. *Rev Toxicol.*, 19–25.
- Vega, I. A., Arribére, M. A., Almonacid, A. V., Ribeiro, G. S., Castro-Vazquez, A. 2012. Apple snails and their endosymbionts bioconcentrate heavy metals and uranium from contaminated drinking water. *Environ. Sci. Poll. Res. Int.*, 19: 3307.
- Wu, G., Yi, Y. 2015. Effects of dietary heavy metals on the immune and antioxidant systems of *Galleria mellonella* larvae. *Comp. Biochem. Physio., Part C*. 167: 131-139.