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BIOCHEMICAL AND HISTOPATHOLOGICAL PROFILES OF *Oreochromis niloticus* (*O. niloticus* - LINNAEUS, 1758) EXPOSED TO SOLID WASTE LEACHATE

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ABSTRACT

Landfill sites pose significant environmental and human health risks due to the production of leachate, which is heterogeneous and varies temporally and seasonally. This study evaluates the impact of leachate from the Olusosun landfill on 300 post-juvenile *Oreochromis niloticus* (*O. niloticus*), assessing biochemical and histopathological toxicity. Fish were exposed to acute concentrations of leachate for 96 hours, and the lethal concentration (LC_{50}) was determined using probit analysis. The percentage mortality was recorded at increasing leachate concentrations (580 mL, 600 mL, 620 mL, 640 mL, and 660 mL). Blood samples were collected for biochemical analysis, while organs (liver, kidney, gills, and muscles) were extracted for histopathological examination after 28 days. Statistical analysis was conducted using ANOVA. The 96-hour LC50 value of leachate was 39.05 mL/L, with mortality rates increasing from 0% to 100% as concentration levels rose. Fish exposed to higher concentrations exhibited abnormal swimming behaviors and progressive skin discoloration, with dead fish becoming noticeably lighter in color. Histopathological analysis revealed severe necrosis in the gills and sinusoidal congestion in the liver. Biochemical analysis showed fluctuating creatinine levels, indicating physiological stress. The findings suggest that lower leachate concentrations increase survival chances, while high concentrations pose severe toxic effects. Due to the toxic effects of landfill leachate on *O. niloticus*, it is recommended that landfills be located away from aquatic ecosystems or that alternative waste management strategies such as closed landfill systems be considered to prevent water contamination.

Keywords: Landfill leachate, Oreochromis niloticus, histopathology, biochemical toxicity, LC50, aquatic pollution

INTRODUCTION

Continuous increase in urban population leads to constant increase in the quantity of waste generated in such areas. Disposal of municipal solid wastes (MSW) are a challenge for many communities in the under-developed and developing countries due to its environmental contamination, social inclusion and economic sustainability (Ferronato and Torretta, 2019; Akmal and Jamil, 2021). In Nigeria, the annual production of MSW is almost unquantifiable and continues to increase at geometric rate due to industrialization, urbanization and simultaneous population explosion (Nnaji, 2015)

Wastes in developing and /or underdeveloped communities are commonly disposed in uncontrolled, unmonitored and unlined landfills and/or improperly sited open dumps located in public places and in wetland or other areas with seasonally high-water tables (Sangodoyin, 1993). The decomposition of these wastes (the biodegradable components) from landfills and the mixture of water (such as from rainwater) produces a foul-smelling, micro-organism-rich, dark-colored liquid known as leachate. The principal impact of the landfill leachate is the contamination of both the groundwater and surface water which has led to a number of studies over the years (Jaskelevicius and Lynikien, 2009). Leachate contain a range of chemical compounds, which may percolate to the groundwater and pose serious risks to ecosystems and human health if the chemicals migrate to surface waters or to drinking water sources (Olukanni *et al.*, 2017)

In Nigeria, Sea foods have been documented as one of the commonest sources of proteins, minerals, vitamins and other important elements for proper growth in humans and animals (Akinjogunla *et al*., 2017, Akinjogunla *et al*., 2021; Akinjogunla and Usman, 2023), thus contamination of water bodies where these sea foods are collected from deserves adequate monitoring of their chemical and biological content levels to ensure food safety (Plessl *et al.*, 2017; Akinjogunla and Lawal-Are, 2020, Akinjogunla *et al.*, 2023).

Fish has been recognized as bio-indicators for environmental conditions such as contamination of the aquatic habitat and also for providing an integrated insight into the status of their environment over longer period of time. The name tilapia actually refers to species of mostly freshwater and brackish fish from the Cichlidae family. Nile tilapia (*Oreochromis niloticus*) is widely distributed in tropical and sub-tropical Africa (Natarajan *et al.*, 2016, Ejikeme *et al.*, 2019).

A variety of biological monitoring approaches have been used to evaluate the effects of pollutants on the health of fish populations. Among those, necropsy, age and growth analysis, biometric analysis, and moreover, biochemical, immunological, hematological, physiological, and gross pathology, as well as microscopic and histopathological approaches, can be used to assess fish health (Van Dyk *et al*., 2009).

Biochemical evaluations are essential for assessing the toxicity of environmental contaminants in aquatic ecosystems. By analyzing specific biochemical biomarkers in fish, researchers can detect and quantify exposure to pollutants, providing insights into the health of aquatic organisms and the ecosystems they inhabit (Dalzochio and Gehlen, 2016).). According to Haluzová *et al*., (2011), Biochemical biomarkers are integral to environmental risk assessments and monitoring programs. They offer early warning signs of ecosystem health deterioration due to pollutant exposure.

Histopathology is the microscopic examination of human and animal tissues in order to study the manifestation of diseases that have advanced beyond physical observation (Slaoui and Fiette, 2011). Histopathological analysis is used in the evaluation of environmental contaminations through the examination of vital organs of fish such as the gills, livers, intestine, kidneys responsible for major body processes. One of the great advantages of using histopathological biomarkers in environmental monitoring is that this category of biomarkers allows examining specific target organs, including gills, kidney and liver, that are responsible for vital functions, such as respiration, excretion and the accumulation and biotransformation of xenobiotics in the fish (Gernhofer *et al.,* 2001). Furthermore, the alterations found in these organs are normally easier to identify than functional ones (Fanta *et al.*, 2003), and serve as warning signs of damage to animal health (Marina *et al*., 2007).

Literature survey has no documentation of the study of these leachates from the study site on the commercially, economically important finfish commonly consumed in various forms (roasted, boiled, fried, grilled and /or smoked) by the inhabitants of this community. This research was therefore, aimed to evaluate the harmful effects of short-term exposure of cultured species of Nile tilapia to solid waste leachate through recording of clinical abnormalities, histopathological changes and biochemical alterations associated with leachate poisoning.

Materials and methods

Study Area

This study was carried out in the aquaculture unit of the biological gardens of the Department of Marine Sciences, Faculty of Science, University of Lagos, Nigeria.

Experimental setup

Test solution: Leachate

The leachate use was collected from Olusosun landfill located around Ojota axis in Lagos State, Nigeria. using sealed 25 litres kegs with minimized exposure to oxygen. Olusosun occupies approximately a land mass of 42.7 hectares (Akinranti and Ajibola, 2017) and shares boundary with Ketu, Ojota and Oregun communities. Prior to testing, the leachates were kept at room temperature for a period of 2 weeks.

Sample collection and acclimatization of test species

Fish samples (300) of the Nile tilapia - *Oreochromis niloticus* (Figure 1) used in this study were obtained from a local breeder (Ayoola fish farm, Ipaja, Lagos) and transported to the aquaculture unit in aerated plastic containers. The fish were first transferred to water tanks stocked and maintained in aquaria under a natural photo-regimen and left to acclimatize to the new environment for 14 days, prior to the toxicity testing. The water temperature as at the time of transferring the fish to the tanks was at 25 ± 2°C. During acclimatization, the water was changed once in three days and the fish were fed on commercial ration of floating feeds, once a day first, then twice a day. The mean length (range) of the fish was 6.78 cm (5.0 - 8.5cm) and weight (range) was 5.73g (3.8 - 7.3g).

For each tested concentration of leachate, a total of 5 fish were kept in six different plastic containers with punctured covers (for aeration), this container contains 15 liters of filtered tap water with a pH value of 7.2, dissolved oxygen concentration value of 5.3 - 7.5 mg/L and mean temperature value of 29.9°C and leachate volume of 350ml, 4000ml, 4500ml, 500ml and 550ml in containers labeled A, B, C, D, E respectively and one control container with no leachate.

Figure 1: Nile Tilapia (*Oreochromis niloticus*) X15mg

Acute Toxicity Testing

The acute toxicity tests were carried out, observing the adverse effects of the leachate on the exposed fish using the standard 24hr interval period. Range finding tests were first carried out on the chosen fish species to determine the lethal concentration of the effluent in use (Leachate). Different volumes of raw landfill Leachate were added to 15L of water in plastic containers. Five acclimatized fishes were introduced in each concentration and based on the percentage of mortality the LC_{50} value of Leachate for 96hrs was assessed using Probit analysis (Promkaew *et al.*, 2010).

Sub-Lethal Assay

For biochemical and histopathological studies, 17 plastic containers (two control and five triplicates of experiment) having a capacity of 30L were taken and previously acclimatized. 10 fishes were transferred to each container (containing 15L of water and varying Leachate concentrations of below the previously calculated LC_{50}). Of the 17 containers, two were kept as control, without any toxicant and fifteen others were contaminated with five different concentrations below the 96hrs LC_{50} concentration of Leachate. The troughs were kept under continuous aeration and food. The experiment was continued for 30 days and dead fish were immediately removed.

Collection of blood samples for biochemical analysis

The blood samples were collected from the caudal peduncle (between the anal fin and caudal fin) of the fish with 2ml syringe. The collected blood samples were dropped in EDTA bottles and allowed to clot and then centrifuged at 3000 r. p. m for 10 minutes to obtain serum. Serum was obtained using micropipette model for biochemical studies. The serums total proteins, albumin, creatinine, Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) were determined calorimetrically using readily made kits. While, the concentration of serum urea was measured enzymatically using the methods / authors adapted by Mohammed *et al*., (2017). All of the biochemical and Immunological parameters were measured using specific reagent kits and the analysis was carried out at the APIN laboratory in Lagos University Teaching Hospital, Lagos Nigeria, using the Roche Hitachi Cobas C311 device.

Histopathological Analysis

One fish from each group was subjected to histological procedures, the abdominal cavities of the sacrificed fishes were opened and organs (kidney, muscles, liver and gills) were removed. The organs were fixed in 10 % buffered formalin before they were taken for analysis (Olujimi *et al.*, 2016). At the end of the exposure period, 5 fish samples were taken from each duplicate tank. The gill archways of the fish were cut out from both sides. Random Fish samples were collected from each tank and were dissected, the abdominal cavity was opened and the liver, muscles and kidney were excised quickly and fixed in 10 % buffered formalin at 4^0C, dehydrated in ascending grades of alcohol and cleared in xylene. The histopathological profile of the exposed fish samples was understudied at Molecular Pathology Department of the Lagos University Teaching Hospital, Lagos Nigeria where the samples were tainted/ stained with haematoxylin and eosin. Finally, the prepared sections were examined and photographically enlarged at 400mg using light microscope. Photomicrographs of the examined parts were taken using a microscope ((Hamilton compound photomicroscope) fitted with a camera unit (Kaoud and El – Dahshan, 2017).

Statistical Analysis

The basic statistics (means, standard deviations and standard errors) of the measured parameters were estimated. Data were analyzed by one-way analysis of variance (ANOVA) test. Probit analyses of the parameters and biochemical results were done using Microsoft office's Excel Worksheet. All statistical analyses were done, using the computer program of SPSS Inc. (version 17.0 for Windows) at the 0.05 level of significance. Histopathological tests are presented as photomicrographs.

RESULTS

Probit Mortality of the Nile Tilapia - Oreochromis niloticus

The mortality profile was observed when *Oreochromis niloticus* was exposed to lethal concentrations of raw landfill leachate in order to determine acute toxicity and the 96-hour LC_{50} (Figure 2). The 96hr LC_{50} is on the Log 2.785 at 500 ml. The sublethal concentrations were deduced from the 96hr acute toxicity test. The five different concentrations were 350ml, 400ml, 450ml, 500ml, 550ml of leachate.

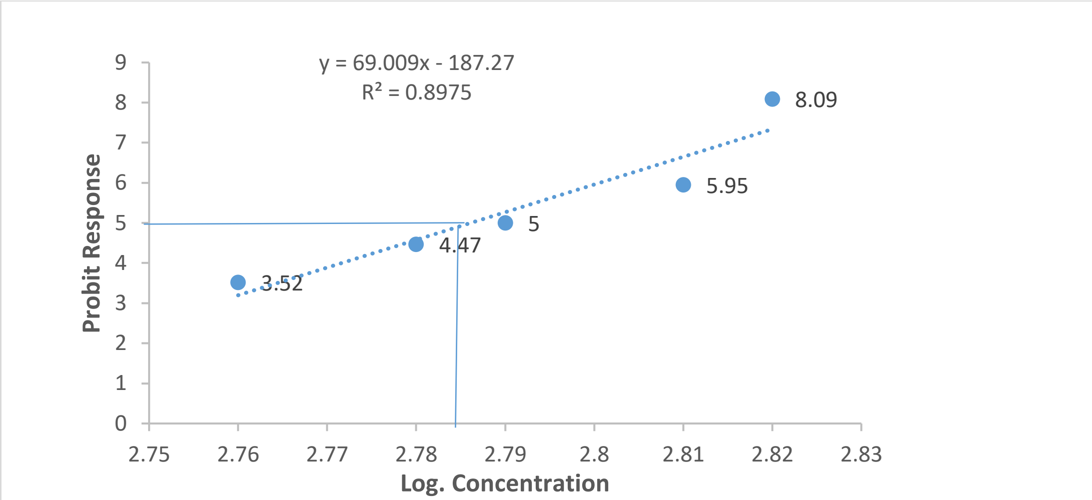

Figure 2: Probit Mortality against Log of concentration to determine 96hr LC_{50}

Biochemical Tests of the Nile Tilapia - Oreochromis niloticus

The results of the biochemical responses are presented in Table 1. The highest Aspartate Aminotransferase (AST) level was recorded as 18.6 IU/L in the fish taken from the tank containing leachate volume of 400ml. Creatinine level recorded highest in the effluent volume of 550ml at 112.2µmol/L. Alanine Aminotransferase (ALT) level shot up from leachate volume of 400ml to 450ml, with levels recorded at 2.2 IU/L and 5.4 IU/L respectively. The highest AST level was recorded 5.4IU/L in the fish taken from the tank containing leachate volume of 450ml. The urea level increased gradually as the effluent concentration increased. The highest urea level was recorded as 3.10 IU/L in the fish taken from the tank containing leachate volume of 550ml. The Albumin (ALB) level fluctuates, increasing and decreasing at almost an insignificant rate, when the effluent concentration was increased. The highest ALB level was recorded as 12.3g/L in the fish at 500ml leachate tank and lowest at 11.0g/L in 400ml leachate. The highest Total protein (TP) level was recorded as 38.10 mmol/L in the fish taken from the 350ml tank. The Triglycerides (TG) level fluctuated consistently at each effluent volume increase, with the levels decreasing after each increase as the effluent volume increases. The control experiment was marked as having the lowest TG level at 2.45mmol/L. The highest alkaline phosphatase (ALP) level was recorded as 29.0IU/L in the fish taken from the tank containing leachate volume of 450ml. The Calcium highest level was recorded as 2.10 mmol/L in the fish taken from the control tank. Sudden movement, grasping for air, change in fish colour and the fish drowning were observations recorded in the cause of the study.

Table 1: Summary of the biochemical profile of the blood samples of *Oreochromis niloticus* post juveniles subjected to different concentrations of raw leachate.

Solution	AST (iu/l)	CREAT (µMol/l)	ALT (iu/l)	UREA (mMol/l)	ALB (g/l)	TP (mMol/l)	TRIG (mMol/l)	ALP (iu/l)	CA (mMol/l)
Control	4.9±0.01	28.5±1.1	2.6±0.32	1.7±0.04	11.5±1.0	26.0±0.53	2.45±1.8	18.1±0.85	2.10±0.14
350ml	14.9 ±0.11	29.6±0.03	2.7±0.24	1.8±0.01	11.9±1.9	28.1±0.06	4.32±0.02	25.6±1.3	1.19±1.1
400ml	18.6±0.136	41.4±0.041	2.9±0.32	2.4±0.11	11.0±0.9	28.4±0.02	3.30±1.1	26.5±0.66	1.22±1.6
450ml	19.5±0.88	62.5±0.079	5.4±1.09	2.7±0.32	11.7±1.2	33.0±0.2	4.30±0.9	29.0±0.35	0.25±1.4
500ml	20.6±0.07	65.4±0.06	4.9±0.08	2.9±0.08	12.3±0.1	35.2±1.3	3.88±0.02	26.3±1.23	0.25±1.4
550ml	19.1±0.17	112.2±0.13	3.8±0.16	3.1±0.05	11.4±1.8	37.8±0.91	4.76±1.3	28.7±0.7	0.32±0.24

***Keys**: AST – Aspartate aminotransferase; CREAT – Creatinine; ALT – Alanine aminotransferase; UREA -Urease; ALB – Albumin; TP – Total Protein; TRIG – Triglycerides, ALP – Alkaline phosphatase; CA – Calcium*

Histopathological profile of the Nile Tilapia - Oreochromis niloticus

Table 2 shows the observation of the examined organs (Gills, liver, kidney and muscles) with respect to congestion and necrosis at all levels of concentration. Exposure to concentration at 350ml, 400ml and 450ml showed no reaction(s) on the organs examined.

Table 2: Summary of histopathological changes observed in the examined organs of *Oreochromis niloticus* post juveniles subjected to different concentrations of landfill leachate

Stock concentration	Organs	Congestion	Necrosis
Control	Gills	None	None
	Kidney	None	None
	Liver	None	None
	Muscle	None	None
350ml	Gills	None	None
	Kidney	None	None
	Liver	None	None
	Muscle	None	None
400ml	Gills	None	None
	Kidney	None	None
	Liver	None	None
	Muscle	None	None
450ml	Gills	None	None
	Kidney	None	None
	Liver	None	None
	Muscle	None	None
500ml	Gills	None	Mild
	Kidney	None	None
	Liver	None	None
	Muscle	None	None
550ml	Gills	None	Severe
	Kidney	None	None
	Liver	Sinusoidal	None
	Muscle	None	None

The gills were unaffected at the control up till it was exposed to 450ml of leachate (Plate 1a). At 500ml of leachate volume in water, the exposed fish gills showed shortening / blunting of secondary lamellae but preservation of the primary lamellae which indicates a mild lamellar necrosis (Plate 1b) while at 550ml, the exposed fish gills showed necrosis and destruction of both primary and secondary lamellae, indicating severe lamellar necrosis (Plate 1c).

Plate 1a: A histological profile of the gills showing the presence of primary and secondary lamellae from the control to the exposed gills at 450ml of leachate. X400mg

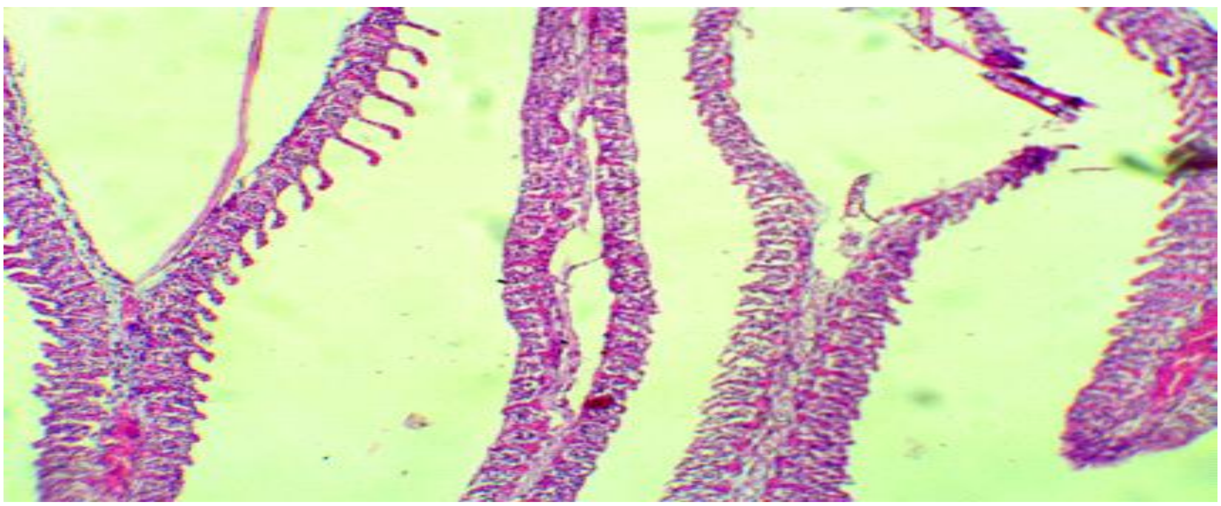

Plate 1b: At 500ml of leachate volume in water, the exposed fish gills showed shortening / blunting of secondary lamellae but preservation of the primary lamellae X400mg

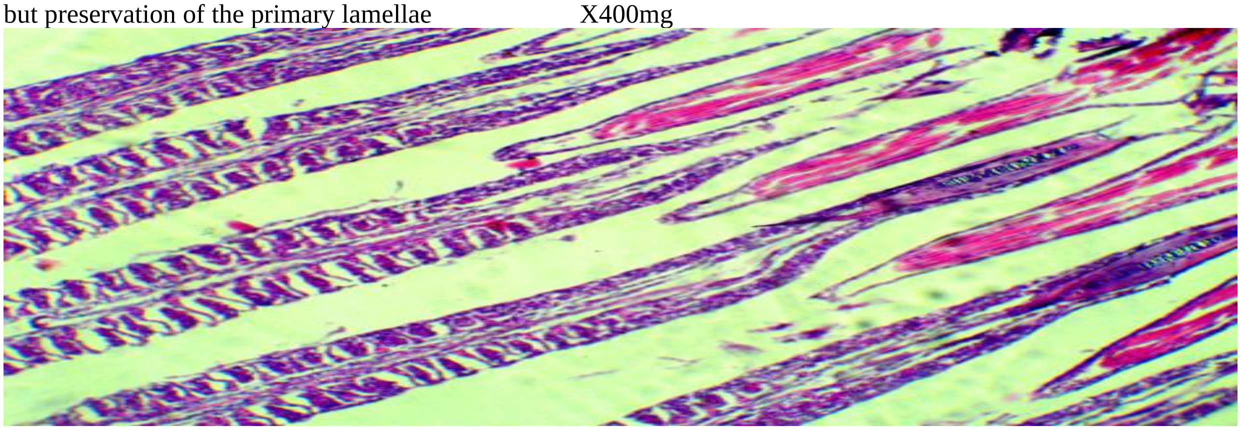

Plate 1c: A histological profile of the exposed gills at 550ml showing severe necrosis (destruction) of both the primary and secondary lamellae X400mg

On the hand, no observation was recorded for various volumes of the leachates in other organs examined (Kidney-Plate 2a; Liver-2b and Muscle-Plates 2c) from control to 500ml of leachates.

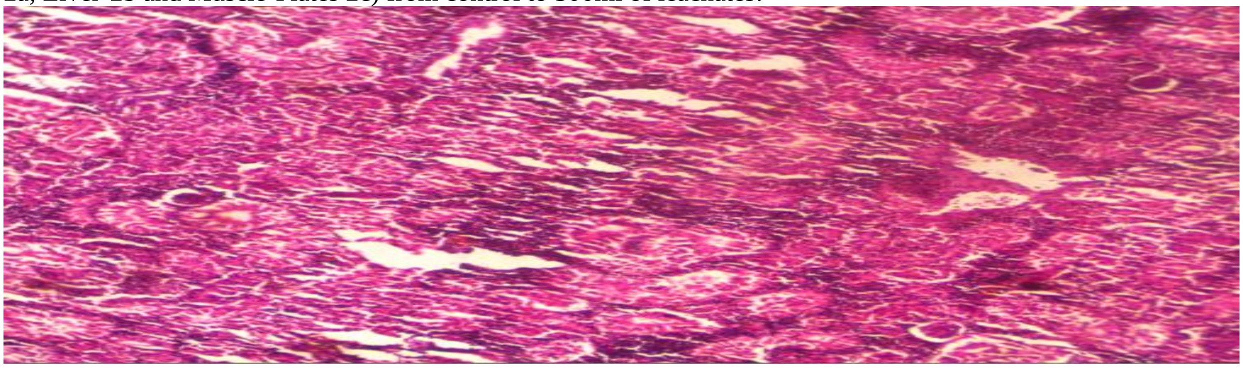

Plate 2a: A histological profile sections of the kidney at control to 500ml from the exposed fish with no recorded observation at the selected volume of exposure X400mg

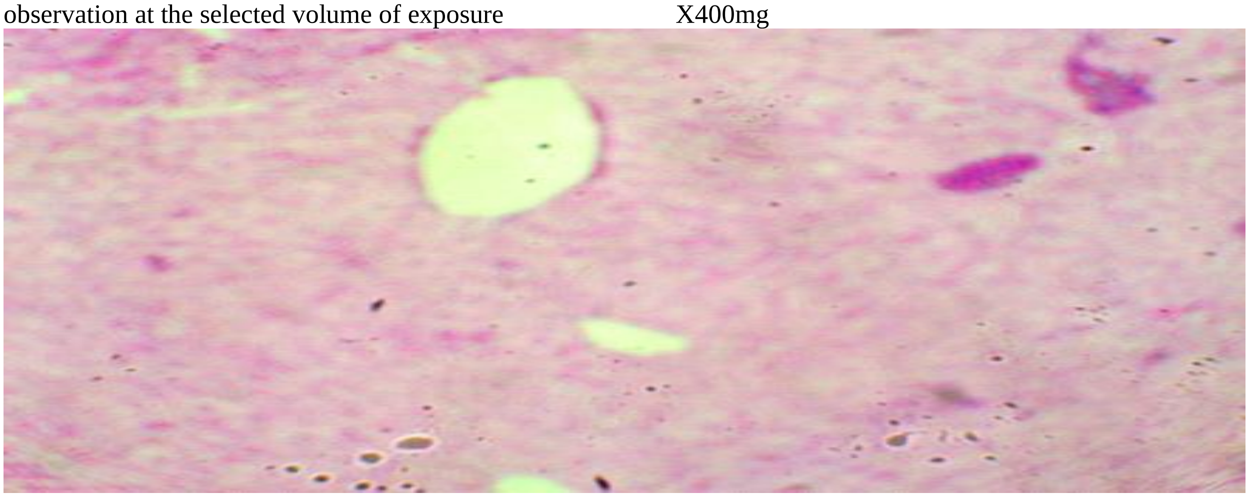

Plate 2b: A histological profile of the liver at control to 500ml of the leachates showing radially arranged plates of hepatocytes X400mg

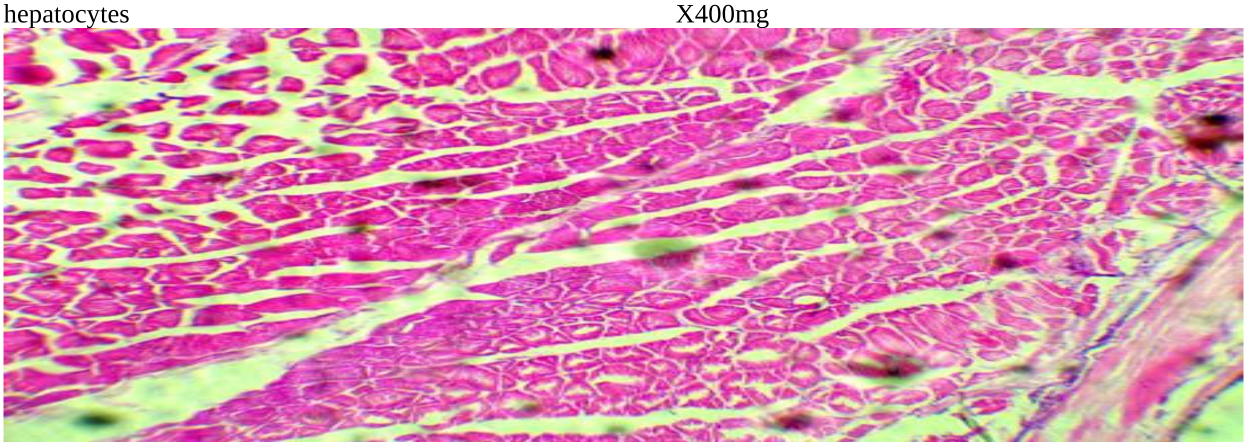

Plate 2c: A histological profile of the muscle at control to 500ml showing interlacing fascicles of striated, unbranched and the smooth un-striated, musculature. X400mg

While the liver at 550ml inclusion (Plate 3) showed a sinusoidal congestion

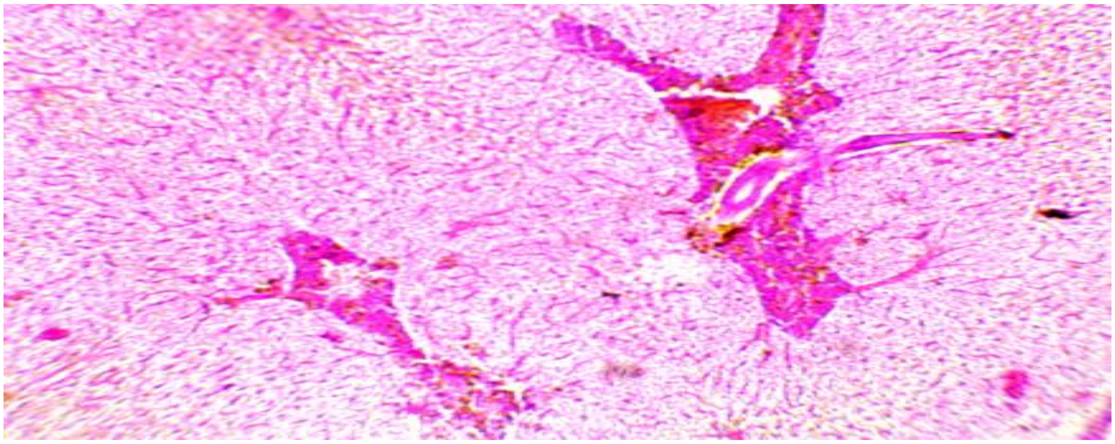

Plate 3: A hhistological profile of liver excised from the fish in the 550ml tank showing radial plates of hepatocytes. X400mg

Discussion

The log concentration and probit values observed in the definitive test at 96 hours was used to generate the lethal concentration at 96hours LC_{50}. For this study, the 96hr LC_{50} is on the Log 2.785 at 500 ml. The exposed fish samples displayed various behaviors and became inactive at higher doses, alongside prolonged exposure to the leachates. At LC_{50} (2.785), this value is higher than what were obtained by Saravanan *et al.*, (2011) and Akinjogunla *et al.*, (2022) who recorded LC_{50} (1.035 g/L) and LC_{50} (1.511g/L) on the toxicity level of *Azadirachta indica A. Juss* on Indian Major Carp, *Cirrhinusmrigala* spp. and Nile Tilapia, *Oreochromis niloticus* respectively but lower than LC_{50} (4.80g/l) documented by Tiwary and Pandey, (2010). However, differences in lethal toxicity values recorded by various researchers could be as a result of variation in age, size, sex, type of fish, quality of water and / or methodology. The lethal toxicity values of the leachates documented in this research indicates its potent toxicity on *Oreochromis niloticus* with the observed fish behaviour and appearance at the introduction of contaminants into the environment, such as sudden movement, grasping for air, change in fish colour, fish drowning, and so on. These behavioural changes observed in the exposed fish samples agrees with documented results from Ajani and Ayoola (2010) who worked on '*Sarotherodon gallilaeus* using *Adenia cissampeloides* extract'; Ayoola *et al.*, (2011), who studied the effect of aqueous and ethanolic extracts of *Ipomoea* aquatic leaf on *Oreochromis niloticus*; Umi *et al.*, (2014) who worked on Landfill Leachate toxicity analysis with *Oreochromis mossambicus* and Akinjogunla *et al*., (2022) who worked on ;Cytogenotoxicity of Aqueous *Azadirachta indica A. Juss* Extracts on O*reochromis niloticus*'.

The presence of pollutants in aquatic environment exerts its effect at cellular or molecular level which result in significant changes in biochemical responses. For monitoring of aquatic environment, analysis using biochemical methods serves as important biomarkers (Authman *et al.*, 2013). Biodegradable pollutants are some of the most-active polluting substances that causes serious impairment to life processes like circulatory, metabolic, physiological, and even structural systems when high concentrations are present in aquatic ecosystems (Yang and Chen, 2003).

Generally, the biochemical parameters analysed, showed no specific trend in the blood of each of the exposed fish tested, while urea level showed an increased in the blood as the concentrations increased. Due to the ammonium constituent of urea, this research agrees with Umi *et al.*, (2014) who reported that Ammonia is one of the inorganic compounds contained in leachate, alongside other organic compounds.

The gills, kidneys, livers and muscles were the organs collected for the histopathological examination of the Nile Tilapia – *Oreochromis niloticus* for this study. No histopathological changes were observed in the examined organs at 300ml to 450ml of leachates exposure. However, histopathological changes started manifesting at 500ml and 550ml leachates exposures to only the gills and livers. The histopathological analysis of the gills in this study which showed mild and severe necrosis at 500ml and 550ml leachates exposure partially agrees with the report of Ayoola (2008), who documented that the exposure of fish to toxicants with decreasing dissolved oxygen level of water may have negative respiratory impact on the gills of the exposed fish.

For the liver histopathological examination, it revealed sinusoidal congestion in the liver of the fish exposed to the highest concentration (550ml) during the sub-lethal assay. However, at the very same concentration i.e. 550ml, the kidney appeared quite normal under the photomicrograph, this differs from that of Ayoola (2008) which showed signs of necrosis, congestion and even haemorrhage when exposed to sub-lethal concentration of glyphosate. This indicates difference in the constituent compounds of glyphosate herbicide and leachate, with the former having compounds inimical to the kidney even at sub-lethal concentrations. Though it should be known that leachate is a combination of several pollutants which may include pesticides, such as glyphosate, it is different due to the presence of other contaminates or wastes present, which may either have synergistic or antagonistic properties. Although at 450ml of leachate volume, the liver, kidney and muscles appeared normal, while the gills showed mild lamellar necrosis, it should be known that below this particular concentration, all the tested organs appeared normal and unaffected under the photomicrograph. It is also necessary to state that the result of each parameter tested in the biochemical analysis does not follow a pattern of correlation, except in the urea and calcium levels.

The livers and kidneys have been proposed as one of the major target organs for environmental contaminants even as the kidney serves as an important organ for metabolic waste excretion and heavy metal elimination in fish (Hadi *et al.* 2009)). Increasing values found in the Kidney serums (AST, Urea, Creatinine, ALB, TP and ALP) indicates the presence of disturbances or toxicants as their values can be used as a rough index of the glomerular filtration rate (Elghobashy *et al.*, 2001). These results are in agreement with that of several authors (Elghobashy *et al.* (2001); Yang and Chen (2003), Zaki *et al.* (2009), Sayed *et al.*, 2011; Midhat *et al* 2013) who observed increase in kidney functions in different fish samples exposed to various pollutants.

Histopathological changes recorded in examined kidney of *O. niloticus* when exposed to 550ml of leachates infusion may be due to the presence of microorganisms, trace or heavy metals and /or pathogens in large quantities (Palavi and Srivastava (2006); Abdel-Tawwab *et al.* (2007); Tayel *et al.*, 2007; Tavel *et al.*, 2008; Oshode *et al.*, 2008; Zaki *et al.* (2009) and Authman *et al.* (2012).

Conclusion

This study demonstrated that high concentrations and prolonged exposure to leachates have clastogenic effects on the gills and kidneys of *Oreochromis niloticus* (*O. niloticus*), highlighting their toxic impact on aquatic life. The findings reinforce the significant environmental risks associated with leachate contamination, emphasizing the need for strict waste management practices. The indiscriminate discharge of effluents and solid waste into aquatic ecosystems should be strictly regulated and prevented, as these environments often act as sinks for various pollutants. Due to the chemical and biological toxicity of landfill leachates, their uncontrolled release can severely affect aquatic organisms, disrupting ecological balance and threatening biodiversity. Existing research has consistently demonstrated the detrimental effects of improper landfill management on water quality and aquatic species, necessitating continued studies on mitigating these environmental hazards. To minimize the adverse impacts of leachate pollution, sustainable waste disposal practices should be implemented, including controlled dumping, sanitary landfills with daily cover, leachate circulation, and advanced leachate treatment systems. These measures will help reduce contamination risks, ensuring the protection of aquatic ecosystems and the sustainability of fisheries resources.

Recommendation

The dumping of municipal solid wastes on indiscriminately especially close to residential area and water bodies. should be discouraged to ensure environmental sustainability. There is need for government, policy makers and appropriate stakeholders to make laws or should develop strategies that will ensure that the dumping sites are restored to their pristine states in order to have a pollution-free environment. Also, landfills should be sited far away from water bodies, so even if leachate permeates through the soil, the toxicity will be reduced when it enters an underlying water body. There is urgent need to improve waste management practices to prevent soil and ground water contamination. Finally, further works are suggested to be carried out in order to provide information for its after-effects on humans via bioaccumulation through consumption of such exposed fish species.

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