ISSN : 0974 - 7532 Volume 10 Issue 1

Research & Reviews in

BioSciences

Regular Paper

RRBS, 10(1), 2015 [008-015]

Leachate simulated from municipal open dump induces biochemical changes in *Clarias gariepinus*

Olalekan Adeyemi

Department of Environmental Science, Federal University of Petroleum Resources Effurun. PMB 1221 Effurun, Delta State, (NIGERIA)

E-mail: adeyemi.olalekan@fupre.edu.ng

ABSTRACT

The effect of simulated-leachate on growth, haematological and serum biochemical properties of *Clarias gariepinus* over a period of 56 days was studied. Various dilutions (5, 10, 15 and 20% v/v) were made using dechlorinated tap water. Fish cultivated in the various simulated-leachate concentrations were designated groups A, B, C, D and E respectively. Serum aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), acid phosphatase (ACP), glucose, total cholesterol and triglycerides were the serum biochemical properties assayed. However, relative to the control, growth rate of catfish cultivated in water contaminated with simulated-leachate was found to be significantly lower ($p<0.05$). Haemoglobin concentration of fish in group E was about half that of group A. AST activity of serum of group E fish was about three folds that of group A while ALT and ALP activities of group E were about two folds those of group A. Conversely, total protein level of serum of fish in group E was 1/3 that of group A. Significant ($p<0.05$) increase in serum ALP activity and glucose level of fish exposed to simulated-leachate including 5% v/v dilution provided compelling evidence of tissue damage and increased energy demand as the main mechanism of stress induced by simulated-leachate.

KEYWORDS

Simulated-leachate;
Clarias gariepinus;
Open dump;
Biochemical;
Municipal.

INTRODUCTION

Runoffs and leachates from open dumps lead to pollution of aquatic environments including rivers, ponds and lakes. Pollution of water bodies does not only impact the water adversely but also the lives inside it[1]. Many lives have been driven to extinction while some have migrated to a less polluted stream or river. By survival of the fittest some species tend to remain in the polluted water thereby accumulating the pollutants in their tissues for onward transmission to their consumers[2].

There are also substantial risks from illegal sites and ad-hoc sites used by criminal gangs to dispose of waste materials. In Nigeria, in particular, waste leachates contaminate bodies of water and endanger aquatic lives and public health[3]. Leachate streams and runoffs are mixtures of various chemicals and contaminants, investigations had shown that leachate streams and runoff running directly into the

aquatic environment have both an acute and chronic impact on the environment which may be very severe and can severely diminish bio-diversity and greatly reduce populations of sensitive species[2,4]. Where toxic metals and organics are present this can lead to chronic toxin accumulation in both local and far distant populations. Rivers impacted by leachate and runoff are often yellow in appearance and often support severe overgrowths of sewage fungus.

Clarias species are air breathing fishes due to the presence of accessory assistant respiratory organs beside the gills enabling it to survive for long time outside the water, otherwise debilitating hypoxic environments. Catfish is freshwater, belonging to the genus *Clarias*. There are 32 species of catfish in Africa. *Clarias gariepinus* is the most popular members of the inland water fishes found in Nigeria. *Clarias gariepinus* are tolerant to a wide range of water and laboratory conditions and has detritivorous behavior. This means that the fish can be in contact with xenobiotics from different ways of interacting with algae from stone or sediment. These characteristics make this particular specie an interesting model for ecotoxicological and biochemical studies. Moreover, catfish are valuable bio-indicators of contamination because of their large distribution, being open swimmers, capacity to react against ecological pollution and food source for human.

The contamination of aquatic ecosystem by leachate streams and runoffs from open dumps has gained increasing attention in recent decades. The acute and chronic exposure and accumulation of these pollutants can result in tissue burdens that produce adverse effects not only in the exposed organisms, but also organisms including human beings; therefore, it seems essential to study detrimental effects of such hazardous pollutants so as to formulate the strategies for safe guarding aquatic organisms. In assessing the toxic effects of chemicals in aquatic organisms the use of growth, heamatological and biochemical parameters have become more useful in recent times, as a result of the intimate relationship between fish and its aqueous environment[5-6]. Sampath et al.[7] observed that haematological studies in fish, lies in the possibility that the blood will reveal anomaly within the body of the fish long before there is any outward manifestation of symptoms of disease or effects of unfavourable environmental factors.

The present study was designed to assess the effect of simulated-leachate on several growth, haematological and serum biochemical parameters of catfish (*Clarias gariepinus*) as a model for toxicological studies. The paucity of information on such studies coupled with the need to elucidate effects of mixtures of pollutants on aquatic organisms necessitated this study.

MATERIALS AND METHODS

Chemicals and solvents are of analytical grade and are products of Sigma-Aldrich Inc, St. Louis, USA.

Solid wastes were collected from the official dump site along Delta Steel Company (DSC) Expressway, Udu, Delta State, Nigeria. Leachate simulation was carried out following the ASTM method[3]. The physicochemical properties of simulated-leachate were carried out following standard method[8] and Atomic Absorption Spectrophotometer was used for the determination of heavy metals. Microbial analysis involving isolation and identification of bacteria in the simulated-leachate was done using the procedure described by Olutola et al[9].

One hundred and fifty species of *C. gariepinus* with the mean weight of 67.9±5.8 g and standard length mean length of 21.4±3.9cm were used for the experiment. They were purchased from a reputable fish farm in Delta State, Nigeria. The fish were kept in transparent plastic tanks filled with dechlorinated tap water and made to acclimatize in laboratory conditions for two weeks. The experimental fish were managed in accordance with the guidelines for handling experimental animals. They were fed (3% w/w) with commercial feeds. Water quality was measured according to the method of APHA/AWWA/WEF[8]. The temperature of the experimental water was 25.9±0.8°C, pH was 7.2±0.4 dissolved oxygen was 7.1±0.2 mg/L, free carbon dioxide was 5.7±0.3 mg/L and alkalinity was 106.4 mg/L. Water was changed every day.

Regular Paper

Five plastic aquaria (56 x 28 x 28cm) with 30 L of dechlorinated water were contaminated with varying concentration of simulated-leachate, and designated as follows:

A: dechlorinated tap water free of simulated-leachate
B: water contaminated with 5% v/v simulated-leachate
C: water contaminated with 10% v/v simulated-leachate
D: water contaminated with 15% v/v simulated-leachate
E: water contaminated with 20% v/v simulated-leachate

After the period of acclimation, the fish were randomly distributed into the five plastic aquaria (A – E) ten fish per aquarium. Each of these treatments had three replicates. The control group of fish were kept in aquarium A while aquaria B-E contained the test group of fish reared in water contaminated with varying volumes of simulated-leachate. The experiment lasted for fifty-six days.

Afterwards, the fish were removed from the water weighed, length measured and left for a while after which the blood was obtained by severing the caudal peduncle. A portion of the blood was collected in heparinised bottles and others in non-heparinised bottles. Blood samples in the non-heparinised bottle were thereafter centrifuged at 3,500 rpm for about 15 min using refrigerated centrifuge RC650s and the serum samples obtained were preserved at -8^{o}C until required for analyses.

Haemoglobin concentration of the blood of experimental fish was determined following the method described by Mitruka and Rawnsley[10]. The RBC and WBC was done by the method of manual counting, PCV by Microhaematocrit method, described by Mutheyya[11]. Other haematological parameters were determined as described by Tiez[12]. Serum concentrations of cholesterol and triglycerides of experimental fish were determined following the method described by National Cholesterol Education Programme (NCEP)[13]. Serum glucose concentrations were estimated based on glucose oxidase method described by Morgan and Iwana[14]. Activities of aspartate transaminase (AST) and alanine transaminase (ALT) were determined using the method described[15]. The method of Bessey et al.[16] as modified by Wright et al.[17] was employed in the determination of alkaline and acid phosphatases.

STATISTICAL ANALYSIS

The statistical analysis of data was done using SPSS11.5, Mean ± SEM. Student t was used to compare physicochemical propertied between tap water and simulated leachate while Post-hoc comparison using Duncan Multiple Range Test (DMRT) was employed for other data. The significance of the test result was observed at $P<0.05$ level.

RESULTS

Physicochemical properties of simulated-leachate and dechlorinated tap water is shown in TABLE 1. Chemical properties of simulated-leachate was found to be statistically higher than the dechlorinated tap water ($p<0.05$). Relative to the tap water, simulated-leachate was found to contain remarkably high concentrations of total hardness, total solids, total dissolved solids (TDS), chemical oxygen demand (COD), biochemical oxygen demand (BOD). Heavy metals including, Pb, Cr, Cd, Ni were detected in the simulated-leachate but not in the tap water.

Characteristics of bacteria detected in the simulated-leachate TABLE 2 were found to be those of *Streptococcus faecalis, E.coli, Pseudomonas, Shigella sp and Salmonella sp.* as shown in TABLE 3.

Fish in all experimental groups showed positive group response Figure 1. However, relative to the control, growth rate of catfish cultivated in water contaminated with simulated-leachate was found to be significantly lower ($p<0.05$). Interestingly, the growth rate of fish in groups B-E was found to be inversely related to the concentration of the simulated-leachate. Similarly, standard and total lengths Figures 2 and 3 respectively were of fish cultivated in water contaminated with simulated-leachate was significantly lower ($p<0.05$) compared with fish in group A. However, standard and total lengths of fish in groups B-C were not significantly different

Regular Paper

TABLE 1 : Physicochemical characteristics of simulated-leachate and dechlorinated water

Parameter (md/L)	Tapwater (dechlorinated)	Simulated-Leachate
Colour** (HU)	6.00±0.10	600.13± 12.71*
pH**	7.20±0.40	8.50±0.20*
BOD	-	5,890.20±13.44*
COD	-	4,770.21±17.12*
Total hardness	26.40±2.40	810.20±10.70*
Total solids	220.50±12.60	8,237.32±9.74*
Total dissolved solids	123.70±5.60	6,960.30±8.87*
Chlorides	nd	500.20±5.62*
Ammonium	0.01±0.00	830.15±7.82*
Nitrates	0.17±0.01	28.33±2.54*
Nitrites	nd	3.73±0.32*
Calcium	16.31±2.34	159.15±5.46*
Magnesium	9.76±1.96	254.40±7.41*
Sodium	12.39±3.77	156.30±4.38*
Potassium	6.96±1.12	358.50±6.37*
Iron	0.20±0.01	12.10±1.03*
Copper	nd	17.20±1.68*
Manganese	nd	0.12±0.02*
Zinc	nd	310.60±3.58*
Lead	nd	7.30±0.54*
Cadmium	nd	0.90±0.01*
Chromium	nd	8.90±0.75*
Nickel	nd	0.64±0.03*
Total bacteria	nd	512.30±6.33*

****Not in mg/L; nd: Not detected**

***Significantly different ($p<0.05$). Results are means of five dterminations±SEM.**

($p>0.05$) from one another.

TABLE 4 shows the changes in haematological properties of *Clarias gariepinus* cultivated in water contaminated with simulated-leachate. Excluding eosinophils and basophils, haematological properties of fish in group B is not significantly different ($p>0.05$) from group A. The MCH and monocytes of fish in all experimental groups are not significantly different ($p>0.05$) from one another. Other haematological parameters showed some level of simulated-leachate concentration-dependent significant different ($p<0.05$) relative to group A. Haemoglobin concentration of fish in group E, in particular, is about half that of group A. Conversely, WBC value of fish in group E is about twice that of group A.

Changes in serum biochemical parameters of *Clarias gariepinus* cultivated in water contaminated with simulated-leachate is presented in TABLE 5. Only ALP activity and glucose level of fish in group B were significantly different ($p<0.05$) from group A, other biochemical properties are not. However, all the biochemical parameter studied were found to be significantly higher ($p<0.05$) in fish in groups C-E relative to group A, except for total protein and ACP activity which are significantly lower ($p<0.05$). AST activity of serum of group E fish was about three folds that of group A while ALT and ALP activities of group E were about two folds those of group A. Conversely, total protein level of serum of fish in group E is 1/3 that of group A. levels of total cholesterol and triglycerides increased in fish as the concentration of simulated-leachate increased (A=B<C<D<E).

Regular Paper

TABLE 2 : Characteristics of bacteria isolated from simulated-leachate

Culture morphology	Gram stain	Catalase	Coagulase	Oxidase	Urease	Glucose	Lactose	Sucrose	Mobility	Indole	Probable identity
Flat, circular colonies	-ve, rod	nd	nd	-ve	+ve	+ve	+ve	+ve	-ve	-ve	*E. coli*
Smooth, circular colonies	+ve, cocci in chain	-ve	+ve	nd	nd	nd	nd	nd	nd	nd	*Streptococcus faecalis*
Circular colonies	-ve, rod	nd	nd	-ve	+ve	+ve	+ve	+ve	-ve	-ve	*E. coli*
Creamy, smooth, circular colonies	+ve cocci in chain	-ve	+ve	nd	nd	nd	nd	nd	nd	nd	*Streptococcus faecalis*
Metallic, sheen colonies	-ve, rod	nd	nd	-ve	+ve	+ve	+ve	+ve	-ve	-ve	*E. coli*
Whitish, pin point	-ve, rod	nd	nd	-ve	+ve	+ve	+ve	+ve	+ve	+ve	*Pseudomonas*
Convex, circular colonies	-ve, rod	+ve	nd	-ve	+ve	+ve	-ve	+ve	-ve	+ve	*Shigella sp.*
Flat, circular colonies	-ve, rod	+ve	nd	-ve	+ve	+ve	-ve	+ve	+ve	+ve	*Salmonella sp.*

nd: not detected; -ve: negative; +ve: positive

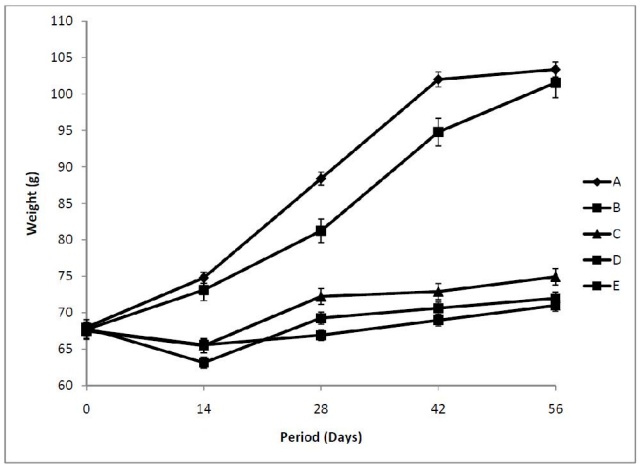

Figure 1 : Growth response of *Clarias gariepinus* cultivated in water contaminated with simulated-leachate

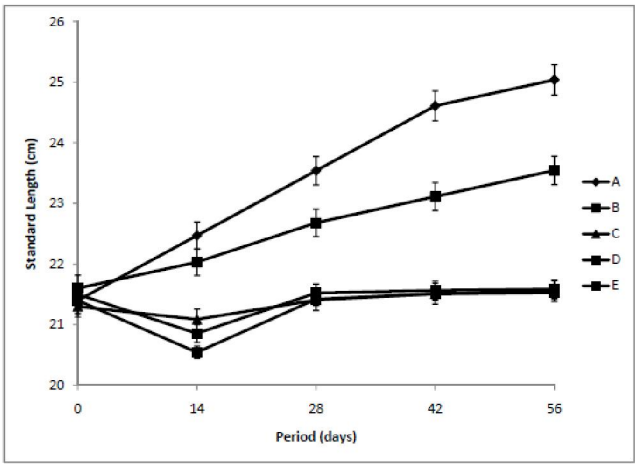

Figure 2 : Standard length of *Clarias gariepinus* cultivated in water contaminated with simulated-leachate

DISCUSSION

This study provides the first documented report on the effects of simulated-leachate on growth, haematological and serum biochemistry of *Clarias gariepinus*. The physicochemical properties of the simulated-leachate TABLE 1 revealed that it is contaminated[18]. The bacteria load in the simulated-leachate TABLES 2 and 3 agrees with previous studies[3,19]. *Clarias gariepinus* has a wide tolerance of relatively poor water quality conditions in which other freshwater fishes would find it difficult to survive. The ''hardiness'' of the fish makes it an ideal candidate for highly intensive culture, without prerequisite pond aeration or high water exchange rates[6].

Clarias gariepinus is able to withstand adverse environmental conditions; it is highly fecund and easily spawned under captive conditions. It is gen-

Regular Paper

TABLE 3: Bacteria isolated from simulated-leachate

Bacteria	Tapwater	Simulated-leahctae
Streptococcus faecalis	-	+
E. coli	-	+
Pseudomonas	-	+
Shigella sp.	-	+
Salmonella sp.	-	+

+: present; -: absent

TABLE 4 : Changes in haematological properties of *Clarias gariepinus* cultivated in water contaminated with simulated-leachate

Haematological properties	A	B	C	D	E
RBC ($x10^6/mm^3$)	5.89 ± 1.05^a	5.06 ± 0.87^a	3.23 ± 0.56^b	2.87 ± 0.52^b	2.65 ± 0.46^b
Hb (g/dL)	12.05 ± 1.15^a	11.89 ± 0.98^a	9.79 ± 0.55^b	7.34 ± 0.67^c	6.03 ± 0.54^d
MCV (μ^3)	96.12 ± 3.10^a	94.76 ± 2.75^a	93.39 ± 2.33^a	91.34 ± 2.17^{ab}	87.33 ± 2.15^b
MCH ($\mu\mu g$)	27.33 ± 2.00^a	27.01 ± 2.05^a	27.12 ± 1.99^a	27.10 ± 2.12^a	26.98 ± 2.10^a
MCHC (%)	25.16 ± 1.50^a	24.99 ± 1.20^a	24.87 ± 0.96^a	23.14 ± 0.88^{ab}	22.17 ± 0.87^b
PCV (%)	30.35 ± 2.30^a	27.19 ± 2.10^{ab}	25.73 ± 1.88^{bc}	23.71 ± 1.87^c	20.11 ± 1.50^c
WBC ($x10^3/mm^3$)	10.82 ± 1.60^a	12.34 ± 1.25^{ab}	13.27 ± 1.70^{ab}	15.89 ± 1.88^{bc}	19.45 ± 2.00^c
NEU (%)	13.43 ± 1.13^a	13.89 ± 1.34^a	14.83 ± 1.56^{ab}	16.19 ± 1.76^b	19.62 ± 1.88^c
EOS (%)	7.56 ± 0.44^a	5.88 ± 0.34^b	3.42 ± 0.28^c	2.18 ± 0.24^d	1.59 ± 0.22^e
BAS (%)	2.40 ± 0.06^a	2.00 ± 0.05^b	1.77 ± 0.03^c	1.46 ± 0.03^d	0.94 ± 0.02^e
LYM (%)	42.56 ± 2.23^a	40.13 ± 2.17^{ab}	39.35 ± 1.87^{ab}	38.78 ± 1.56^b	37.33 ± 1.43^b
MONO (%)	5.18 ± 0.54^a	5.12 ± 0.51^a	5.01 ± 0.44^a	4.88 ± 0.43^a	4.50 ± 0.42^a
ESR (mm/h)	2.03 ± 0.06^a	2.00 ± 0.10^a	2.78 ± 0.15^b	3.13 ± 0.17^c	3.89 ± 0.22^d

Tabulated results are means of ten determinations ± SEM. Values on the same row bearing different superscripts are significantly different ($p<0.05$).

erally recognized as an altricial species, yet it displays a remarkable degree of phenotypic plasticity. However, vendors of *C. gariepinus* attach importance to weight and length of the fish as these factors determine the selling price. By their standard, the heavier the fish the more expensive it is. The reduced weight and length observed in catfish cultivated in water contaminated with simulated-leachate Figures 1-3 was suggestive of adverse effect of simulated-leachate. It also portends that the fish in simulated-leachate utilized more energy for survival instead of growth because of their high tolerance to withstand adverse conditions.

The ability of *Clarias gariepinus* to withstand temperature ranges, low dissolved oxygen and high salinity might have accounted for insignificant ($p>0.05$) changes in the oxygen transport vehicles (Hb, PCV, RBC, MCV, MCH and MCHC) of catfish in groups A and B TABLE 4. This data agrees with earlier study by Ozmen et al[20]. However, the significant difference ($p<0.05$) in haematological properties observed among fish in groups C-E relative to A might be a protective response to stress induced

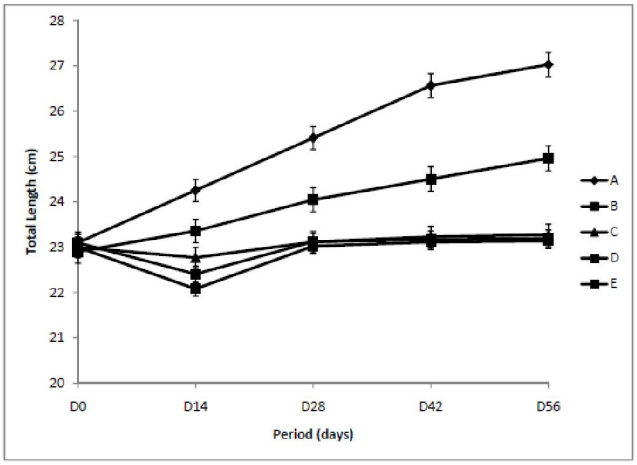

Figure 3 : Total length of *Clarias gariepinus* cultivated in water contaminated with simulated-leachate

Regular Paper

TABLE 5: Changes in serum biochemical parameters of *Clarias gariepinus* cultivated in water contaminated with simulated-leachate

Biochemical	A	B	C	D	E
AST (U/L)	18.89±2.12^{a}	22.34±2.11^{a}	33.76±3.21^{b}	42.18±3.76^{c}	55.24±3.98^{d}
ALT (U/L)	29.86±1.87^{a}	33.12±2.01^{a}	39.45±2.33^{b}	48.16±2.17^{c}	63.04±3.42^{d}
ALP	32.50±2.34^{a}	41.63±2.88^{b}	52.17±3.70^{c}	57.88±3.81^{c}	64.79±3.99^{d}
ACP	12.80±0.89^{a}	11.83±0.78^{a}	9.76±0.67^{b}	8.86±0.62b^{c}	7.77±0.54^{c}
Glucose (mg/dL)	134.36±7.29^{a}	154.12±6.87^{b}	166.0±7.88^{b}	187.5±7.32^{c}	193.66±7.44^{c}
Total protein (g/L)	32.09±4.50^{a}	26.33±3.17^{ab}	18.14±2.56^{bc}	13.27±2.43^{c}	9.78±1.88^{d}
Total triglycerides (mg/dL)	34.17±3.77^{a}	41.06±3.80^{a}	49.71±3.67^{b}	57.28±4.10^{c}	66.40±4.17^{d}
Total Cholesterol (mg/dL)	72.68±5.12^{a}	77.49±4.88^{ab}	85.18±5.70^{b}	100.11±6.37^{c}	112.56±6.84^{d}

Tabulated results are means of ten determinations ± SEM. Values on the same row bearing different superscripts are significantly different ($p<0.05$).

by the simulated-leachate as suggested by earlier studies[21-22] as well as a consequence of tissue damage. The presence of Pb, Cd, Cr and Ni in the simulated-leachate may likely cause physiological, biochemical and cellular alterations in the exposed fish. This underscores the likelihood of cellular damage[23].

Elevated serum activities of AST, ALT and ACP of the fish in groups C-E could have also resulted from cellular damage which might have arisen from the toxic pollution induced by the simulated-leachate TABLE 5. This is because serum enzymes are cytoplasmic in nature and are only released into the blood circulation after cellular damage[24]. It has also been reported that alterations in serum enzyme activities directly indicates major pathologic changes in cell membrane permeability or hepatic cell rupture a signal of underlying pathological process[25]. ACP hydrolyzes large variety of organic phosphate esters with formation of an alcohol and a phosphate ion. The decreased profile of this enzyme estimated in this study is attributed to adverse effect of simulated-leachate on cell and its organelles.

Glucose is one of the most sensitive indices of stress. The significant hyperglycaemic response under the influence of simulated-leachate indicates high energy demand and utilization of energy reserves. The elevated blood glucose level is attributed to glycogenolysis at tissue level especially liver and muscle. Under stress conditions, the chromaffin cells release catecholamines, adrenalin and noradrenalin towards blood circulation and such stress hormones together with cortisol mobilize and elevate glucose production in fish through gluneogenesis or glycogenolysis to cope with the energy demand[26].

The observed significant decrease ($p<0.05$) of total protein TABLE 5 appears to be a consequence of direct action of simulated-leachate on hepatocytes since serum proteins have hepatic origin. This may be associated with either partial inhibition of protein synthesis or breakdown of protein to free amino acids for utilization as supplementary energy source. In contrast, the simulated-leachate induced elevated cholesterol and triglycerides in this study could be attributed to leakage from liver or disruption in formation of lipoprotein or to decrease rate of steroid biosynthesis as a result of liver damage[6].

CONCLUSION

In accordance with the obtained physiological and biochemical results, it could be concluded that effect of simulated-leachate on growth, haematological and serum biochemical properties of *Clarias gariepinus* exposed to it is more pronounced at concentrations above 5% v/v dilution. However, significant ($p<0.05$) increase in serum ALP activity and glucose level of fish exposed to simulated-leachate including 5% v/v dilution provided compelling evidence of tissue damage and increased energy demand as the main mechanism of stress induced by simulated-leachate. Further investigations are underway to confirm or modify this submission.

REFERENCE

[1] Bagchi; Design of Landfills and Integrated Solid Waste Management: Landfill Design, 3rd Edition, Wiley and Sons, USA, **(2004)**.

[2] O. Adeyemi, J.O.Ajayi, A.M.Olajuyin, O.B.Oloyede, A.T.Oladiji, O.M.Oluba, O.Adeyemi, I.A.Ololade, E.A.Adebayo; Food Chem.Toxicol., **47**, 885 **(2009)**.

[3] O.Adeyemi, O.B.Oloyede, A.T.Oladiji; Asian J.Biochem., **2(5)**, 343 **(2007)**.

[4] A.A.Bakare, A.A.Mosuro, O.Osibanjo; J.Environ.Biol., **21(3)**, 263 **(1999)**.

[5] O.A.Akinrotimi, O.M.G.Abu, E.O.Agokei, B.Uedeme-Naa; Animl.Res.Intl., **7(2)**, 1199 **(2010)**.

[6] O.Adeyemi, O.Oginni, A.P.Igbakin, O.Adeyemi, C.C.Osubor; Exptal. and Toxicol.Pathol., **63**, 61 **(2011)**.

[7] K.Sampath, S.K.Velamnia, R.James; Acta Hydriobiology, **35**, 73 **(1993)**.

[8] APHA/AWWA/WEF, Standard methods for the examination of water and wastewater, American Public Health Association, 20th Editoin, New York, **(1998)**.

[9] P.O.Olutola, O.Famurewa, H.G.Sonntag; Measurement of microbial growth, in An introduction of general microbiology, A practical approach.Bolabay Publications, Lagos, Nigeria, 78-122 **(2000)**.

[10] B.M.Mitruka, M.Rawnsley; Materials and methods in hematology and clinical biochemistry, In clinical biochemical and hematological reference values in normal experimental animals, Masson Publishing Inc., USA, 41-58 **(1977)**.

[11] N.M.Muthayya; Blood.Human Physiology, 3rd Edition New Delhi, India: Jaypee Brothers Medical Publishers (P) Ltd., **(2002) (2002)**.

[12] N.W.Tiez; Clinical guide to laboratory tests, 2nd Edition.Philadelphia,: W.B.Saunders Company, USA, **(1990)**.

[13] National Cholesterol Education Programme (NCEP), Expert panel on detection, Evaluation, And treatment of high blood choletserol in adults (Adult Treatment Panel III), JAMA, **285**, 24866 **(2001)**.

[14] J.D.Morgan, G.K.Iwana; Measurement of stressed states in the field, In iwana GK, Pickering AD, Sumpter JP, Schreck CB, Editor, Fish stress and health in aquaculture, Society of Exploratory Biology Seminar Series, **62**, 247-268 **(1997)**.

[15] S.Reitman, S.Frankel; Am.J.Clin.Petol., **28**, 56 **(1957)**.

[16] D.A.Bessey, O.H.Lowry, M.D.Brock; J.Biol.Chem., **164**, 321 **(1946)**.

[17] P.J.Wright, A.A.Leatwood, D.T.Plummer; Enzymological, **42**, 317 **(1972)**.

[18] WHO, Facing American's Trash, What's next for municipal solid waste? OTA-O-424, Office of Technology Assessment, US Government printing office, Washington DC., **(1989)**.

[19] A.A.Bakare, A.A.Mosuro, O.Osibanjo; Landfill leachate-induced toxicity in mice.J.Envtal Biol, **24(4)**, 429 **(2003)**.

[20] M.Ozmen, A.Gunhordu, F.Z.Kucukbay, R.E Gular; Ecotoxicol., **26**, 191 **(2006)**.

[21] M.K.Das; Studies on the effects of some pesticides and commonly used chemicals on Indian major carp and their ecosystem., Ph.D. dissertation, University of Agriculture and Technology Bhubaneswas, India.

[22] S.Dhanekar, S.Srivastava, K.S.Rao, S.S.Pandya; Matsya, **11**, 75 **(1985)**.

[23] M.Atamanalp, E.M.Kocaman, A.Ucar, G.Alak; J.Anim.Vet.Adv., **9(16)**, 2167 **(2010)**.

[24] V.Palanivelu, K.Vijayavel, S.Ezhilarasilbalasubramanian, M.P.Balasubramanian; J.Environ.Biol., **26**, 191 **(2005)**.

[25] P.C.Hayes, K.J.Simpson, O.J.Garden; Liver and biliary tract disease, in Davidson's Principles and Practice of Medicine, 19th Edition, Churchil Living Stone., **2**, 865 **(2002)**.

[26] G.Choudhary, B.S.Jha; The Bioscan., **8(4)**, 1343 **(2013)**.