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Ecotoxicological effects of downstream wastewater samples on *Cloeon perkinsi* larvae, *Rana temporaria* tadpoles and *Clarias gariepinus* frys and fingerlings

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Abstract

Indiscriminate dumping of refuse and direct defaecation into surface water bodies alter physico-chemical qualities of the water and adversely affect the existence of certain fauna. Downstream water samples were collected from five streams (Irefin, Gege, Gbagi, Odinjo and Omi Adio) receiving domestic wastes in Ibadan and a control stream within the University of Ibadan, Oyo state, Nigeria. Physico-chemical qualities of the downstream water were analysed using standard methods. Acute toxicity of contaminated water was investigated on some fauna. The 48-hour-LC50 of stream samples was determined for Cloeon perkinsi (mayfly) larvae (CP); while 96-hour-LC50 for Rana temporaria tadpoles, Clarias gariepinus frys (CG1) and fingerlings (CG2) were determined using Probit method. Zero dissolved oxygen values were recorded for Irefin and Gege while 1.07 mg/L was recorded for Odinjo and these were lower than permissible limit for aquatic life. The values of biochemical oxygen demand (43.34 – 160.66 mg/L), chemical oxygen demand (136 – 487.10 mg/L) and phosphate (81.63 – 1877.60 mg/L) recorded for all the samples were significantly higher ($P<0.05$) than the control sample and were higher than limits set by National Environmental Standards and Regulatory Enforcement Agency (NESREA). The 48-hour-LC50 of contaminated stream water to CP (Irefin, 12.67%; Gege, 8.63%), and 96-hour-LC50 to CG1 (Gege, 0.79%; Gbagi, 2.75%) and CG2 (Gege, 3.33%; Irefin, 5.64%) indicated high toxicity of the sites. High toxicity of the study sites revealed that the streams were polluted in Ibadan. Therefore, there is need for adequate management and disposal of solid wastes to prevent further environmental contamination.

Keywords: Ibadan, Stream samples, Physico-chemical qualities, Acute toxicity.

1. Introduction

Population increase and urbanization in Nigeria have led to serious waste management problems. Indiscriminate dumping of refuse and direct defaecation are common practices amongst indigenous people of Ibadan living close to water bodies or streams. These practices affect the physico-chemical qualities of such receiving water bodies and adversely affect the existence of certain fauna inhabiting such areas. When there is large amount of organic matter

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and soil materials washed into water bodies, it raises the biochemical oxygen demand of the water and this can deplete the water of dissolved oxygen, therefore creating an anoxic condition (Adewoyin, 2016).

Mayflies are sensitive indicators of water qualities (Pontasch and Cairns, 1988; Short *et al.*, 1991; Williams and Williams, 1998), particularly to contaminants such as metals and ammonia (Peckarsky and Cook, 1981) and they have been regarded as the most sensitive order of aquatic invertebrates (Echols *et al.*, 2010). Their presence in an aquatic ecosystem is a strong indication that the water quality conditions are good and a healthy water body (Krieger, 1997; Voshell and Reese, 2002). They are specific in their choice of habitat requirements and tolerance of environmental conditions such as water temperature and chemistry (Krieger, 1997). They also play an important role in the food web because they acquire energy from decomposed plant material, move it to higher consumers like macroinvertebrates, fish, birds, and so on. More mayflies result in more fish (Krieger, 1997). Mayflies' nymphs are very sensitive to pollutants in the water and the sediments because of their constant contact with the sediments where the pollutants accumulate. If there is high concentration of pollutants, the mayflies could die or their development could be altered. If the sediments and the water qualities are good, they complete their life cycle. They are sensitive indicators of metal pollution (Simon, 2002).

Amphibians are bioindicators of environmental stress (Hall and Mulhern, 1984; Freda, 1991; Dunson *et al.*, 1992). They are very important components of different ecosystems worldwide. The deposition of their unshelled eggs in aquatic environment, development into a gill-respiring, swimming herbivorous/detritivorous larval stage and an adult stage which is semi-aquatic climbing/hopping insectivorous make them highly vulnerable to numerous stressors in both aquatic and terrestrial environment (Wassersaug, 1997). They are highly sensitive to many pollutants such as aluminium, cadmium, iron, lead and zinc and pesticides such as atrazine and DDT (Gupta, 2009). They can take up toxicants by both dietary ingestion and dermal absorption; this makes them to potentially accumulate significant body burdens (Gupta, 2009). Their highly permeable skin that is involved in dermal respiration also facilitates potential uptake of contaminants.

Anuran amphibians are susceptible to the uptake of heavy metals due to their highly permeable skin that allows the rapid absorption of metal ions (Ficken and Byrne, 2013). Tadpoles frequently ingest sediments that have accumulated heavy metals; this is due to the microphagous feeding habit of most species (Hopkins and Rowe, 2010). Ecological relevant concentrations of metals are lethal to amphibian embryos, larvae and adults (Linder and Grillitsch, 2000; Hopkins and Rowe, 2010). Sublethal concentrations can have harmful effects on tadpoles, which include reduced growth rates, delayed metamorphosis and impaired behavioural responses (Hopkins *et al.,* 2000; Hopkins and Rowe, 2010).

Fishes are a key unit in many natural aquatic food webs and they can also serve as environmental indicators of polluted water (APHA, AWWA, WEF, 1998). *Clarias gariepinus* is a major fish species for aquaculture in Africa (Sotolu, 2010). It has omnivorous feeding habit, high growth rate, and resistance to handling and stress (Fagbenro and Davies, 2001). Naturally, it is found in fresh water bodies such as streams, rivers, floodplains, swamps and lakes. It is of commercial importance in aquaculture due to its positive attributes like resistance to diseases, high fecundity and ease of larval production (Hogendoorn, 1980; Haylor, 1991; Kestemont *et al.*, 2007). However, in the larval stages of catfish, there is high mortality attributed to infectious diseases caused by parasites.

Due to the strong implications of waste dump into streams, this study was carried out to investigate some physico-chemical qualities of the water and assess the ecotoxicological effects of the wastes by using some animals as test models. This inferred the use of larvae of aquatic insects (*Cloeon perkinsi*), amphibian tadpoles (*Rana temporaria*) and frys and fingerlings of *Clarias gariepinus*.

2. Materials and Methods

Study Area

Ibadan city is the capital of Oyo state, Nigeria. It is located on longitude 3^{o}5'East of Greenwich Meridian and latitude 7^{o}23' North of the Equator. The city is made up of ten local government areas. For this study, downstream water samples from five streams receiving domestic wastes were collected in five local government areas. These were Irefin from Ibadan Northeast, Gege from Ibadan Southwest, Gbagi from Egbeda, Odinjo from Ibadan Southeast and Omi Adio from Ido local government areas. Water was also collected from a control stream (Awba stream) within the University of Ibadan.

The downstream water samples were tested for pH, dissolved oxygen (DO), biochemical oxygen demand (BOD), chemical oxygen demand (COD), total dissolved solids (TDS), electrical conductivity (EC), total alkalinity (T.Alk), anions (chloride, phosphate, nitrate, sulphate), cations (calcium and magnesium) and trace and heavy metals (Fe, Mn, Cu, Zn,Pb, Cd, Ni, and Cr). Physico-chemical qualities were carried out according to standard methods described by APHA (1998) and ANOVA was used to compare their means using SPSS version 17.

Cloeon perkinsi larvae were collected from the Opa dam of the Obafemi Awolowo University Ile-Ife. In the laboratory, 9th instar larvae were selected and acclimated to the laboratory conditions for twelve hours at a stocking density of 3 g/L at a temperature of 25^{0}C. Tadpoles were hatched in the laboratory while *Clarias gariepinus* frys and fingerlings were obtained from AgricMatta fish farm in Osogbo. All the test organisms were acclimated to laboratory conditions for seven days and were fed ad libitum.

The mayflies' toxicity assay was carried out according to the method described by Ogbogu (2003) while other assays were according to Obuotor and Onajobi (2000). After acclimization, 10 larvae of *Cloeon perkinsi* were assigned to test chambers in quadruplicates. Each test chamber contained 150 ml appropriately diluted test solutions (downstream water samples) to give final concentrations of 50 %, 25 %, 12.5 %, 6.25 %, 3.125 % and 1.56 %. The larvae were exposed for 48 hours while the mortality was monitored every 2 hours for the first 24 hours. The tadpoles and fishes toxicity assay involved the same method for mayflies except that 1.56 % concentration was not included in test solutions for tadpoles and fishes. The organisms were exposed in the test solutions for 96 hours.

Estimation of median lethal concentrations (LC_{50} values) and 95 % confidence interval were carried out, the mortality data were analysed using EPA Probit Analysis Program, version 1.5 (USEPA, 1997).

3. Results and Discussion

The result of the downstream waste water samples are presented in Table 1. The pH values were within the limit stipulated by NESREA (2011). Zero DO values were recorded in the downstream wastewater from Irefin and Gege while 1.07 mg/L was recorded from Odinjo which were far below the limit (4.0 mg/L) stipulated by NESREA (2011). NESREA does not have set limits for TDS, EC and T.Alk. Values of BOD, COD, chloride (except Omi Adio and Control), phosphates, sulphates (except Omi Adio and Control), iron (except Irefin, Gege and control), copper and lead (except Gege, and Omi Adio) were all higher than the NESREA standards. The physico-chemical values obtained from all the downstream wastewater samples were lower than the ones obtained from the control site (Awba stream).

Mortalities of all test organisms occurred in all test chambers except the control. The mortalities were concentration-dependent. As pollution intensity increases, in most cases, there is a decrease of all biological indices of environmental health (algae, invertebrates, fish) (Cuffney *et al.*, 2000; Hill *et al.,* 2000). In heavily contaminated soil and water, there is a decrease in the population, growth and function of biota present (Kaonga and Monjerezi, 2012). This could be inferred from the results except for the pollution tolerant species.

Mayflies' mortalities are represented in Figure 1, LC_{50} values and 95 % confidence intervals are in Table 2. The 48hour LC_{50} values of the stream-water samples obtained using Probit method were 12.67 %, 8.63 %, 72.13 %, 33.32 %, 49.55 % and 181.07 % for Irefin, Gege, Gbagi, Odinjo, Omi Adio and Awba streams respectively. The contaminated stream samples were highly toxic to *Cloeon perkinsi's* (mayflies') larvae. This is attributed to the fact that the presence of mayflies in water indicates clean water sources because they prefer cleaner waters (Science, Olympiad, 2013) and they are sensitive to chemical pollutants, decreased dissolved oxygen levels, and increases in suspended solids (erosion) (Sigurdson, 2010). They are sensitive to acidification, heavy metal contamination and low pH levels (Capinera, 2008). The reason for the high mortalities recorded during the test could probably be due to the fact that all the metals analysed did not meet up with the regulatory standards as stipulated by NESREA (2011).

Sublethal effects of pollution result in altered enzyme function, poor growth, behavioural change or lack of reproductive success (Capinera, 2008). Since they are important in the food chain, there is pollution effect on other organisms such as overgrowth of algae due to death of herbivorous nymphs, over-abundance of prey species due to scarcity of predacious nymphs (Capinera, 2008). However, mayflies help in removing pollutants from aqueous systems by processing a lot of great quantity of organic matter and transferring a lot of nitrates and phosphates to terrestrial environments when they emerge as adults from the water (Dominguez, 2006).

Death of tadpoles only occurred at 50 % and 100 % concentrations of Gege wastewater (Table 3). Death also occurred at 100 % concentration of Irefin wastewater (At 24 hours exposure, 10 mortalities occurred while 11 mortalities were maintained from 48 hours to 96 hours). Tadpoles growth and survival can be affected by alterations in environmental factors such as limitation of resources, predation, crowding and dessication of habitat (Shi, 2000). Exposure of tadpoles to contaminants can lead to frogs with many types of malformation. For example, frogs without eyes, with extra or missing legs, or deadly malformations (U. S. Fish and Wildlife Service, 2000). If the tadpoles exposed to stream test solution of Gbagi, Odinjo and Omi Adio were left for more than 96 hours and the growth to frogs observed, it is possible that some of these malformations would occur.

High electrical conductivity impacted the growth, development and survival of various anuran tadpoles of brown tree frog, *Litoria ewingii* exposed to high saline condition (Chinathamby *et al.*, 2006). Electrical conductivity above 3 000 µS/cm had negative impacts on anurans species presence (Smith *et al.*, 2007). However, the electrical conductivity values of the test solutions were not up to 3 000 µS/cm, death of the tadpoles observed might be due to the high values of electrical conductivity.

Chronic exposure of calcium phosphate significantly reduced the survival of *Litoria aurea* tadpoles of a fertilizer-applied agricultural land in southeastern Australia (Hamer *et al.*, 2004). The death of tadpoles exposed to the contaminated-stream water of Irefin and Gege might be due to the high phosphate contents of the samples.

Clarias gariepinus is very hardy, however, the frys and fingerlings cannot tolerate pollution stress since the organs are not well developed. This led to the mortalities of the frys and fingerlings observed because of the high contamination level of the streams. Mortalities of frys were observed in all test chambers (Figure 3; Table 4). However, deaths of fingerlings were only observed in test chambers containing Irefin and Gege downstream wastewater (Table 5).

According to NESREA (2011), the limit of oxygen for effluent discharged into a stream should not be less than 4 mg/L for organisms to be able to survive in it. Irefin and Gbagi test solutions had zero (0 mg/L) DO, Odinjo had 1.07 mg/L, this could be the cause of stress of the frys and fingerlings exposed to the test solution which eventually led to death of the frys.

Table 1: Physico-chemical qualities of downstream wastewater samples**.**

Parameters/Samples	**Irefin**	**Gege**	**Gbagi**	**Odinjo**	**Omi Adio**	**Control**	**NESREA Limit**
pH	6.41**a**	7.11**b**	7.25**c**	7.40**d**	6.79**e**	6.60**f**	6.5 - 8.5
DO (mg/L)	0**a**	0**a**	4.22**b**	1.07**c**	4.78**d**	5.18**e**	4
BOD (mg/L)	129.20**a**	160.66**b**	97.86**c**	97.06**d**	43.34**e**	10.87**f**	6
COD (mg/L)	396.60**a**	487.10**b**	299.60**c**	298.00**d**	136.00**e**	34.62**f**	30
TDS (mg/L)	600.00**a**	1430.00**b**	299.60**c**	1144.00**d**	270.00**e**	235.00**f**	NIL
EC (µS/cm)	1000.00**a**	2390.00**b**	499.33**c**	1907.00**d**	451.00**e**	392.00**f**	
T. Alk (mg/L)	601.80**a**	749.30**b**	295.00**c**	814.20**d**	300.80**e**	98.00**f**	
Chloride (mg/L)	107.80**a**	110.08**b**	43.58**c**	231.66**d**	36.60**e**	29.82**f**	350
Phosphate (mg/L)	1122.4	1877.6	383.67	359.18	81.63	ND	3.5
Nitrate (mg/L)	6.94**a**	12.05**a**	6.03**a**	38.99**a**	5.55**a**	5.42**a**	40
Sulphate (mg/L)	2900.00**a**	14069.00**b**	1931.30**c**	1500.00**d**	308.33**e**	133.33**f**	500
Calcium (mg/L)	82.909	62.29	ND	15.746	2.18	ND	180
Magnesium (mg/L)	36.038**a**	36.4232**b**	34.637**c**	36.756**d**	36.089**e**	9.04**f**	40
Iron (mg/L)	0.3106**a**	0.3244**b**	1.7687**c**	1.8557**d**	8.2208**e**	0.02**f**	0.5
Copper (mg/L)	0.1125	0.2269	0.1129	0.5339	0.2843	ND	0.01
Zinc (mg/L)	0.0249	0.1418	0.0172	0.0055	0.023	ND	0.2
Lead (mg/L)	0.126	0.0672	0.5432	0.6483	0.0053	ND	0.1
Nickel (mg/L)	0.0619	0.0355	ND	0.0449	0.0581	ND	0.1

NOTE: Post hoc tests are not performed for phosphate, copper, zinc, lead and nickel because at least one group has fewer than two cases.
Means with similar letters are not significantly different at $P<0.05$.

Table 2 : Acute toxicity of contaminated downstream water samples on 9th instar larvae of *Cloeon perkinsi*.

Duration	**Parameters**	**Samples**					
		Irefin	**Gege**	**Gbagi**	**Odinjo**	**Omi Adio**	**Control**
24 hrs	LC_{50}	2.75	15.8	125.44	82.94	143.76	402.27
	95 % confidence interval	18.50 - 51.33	11.98 - 21.48	53.89 - 2086.81	35.02 - 1313.71	>0.1 - 32.59	>0.1 - 86.52
48 hrs	LC_{50}	12.67	8.63	72.13	33.32	49.55	181.07
	95 % confidence interval	9.17 - 18.10	6.42 - 115.94	32.55 - 748.76	18.72 - 110.27	18.75 - 5927.56	>0.1 - 38.58

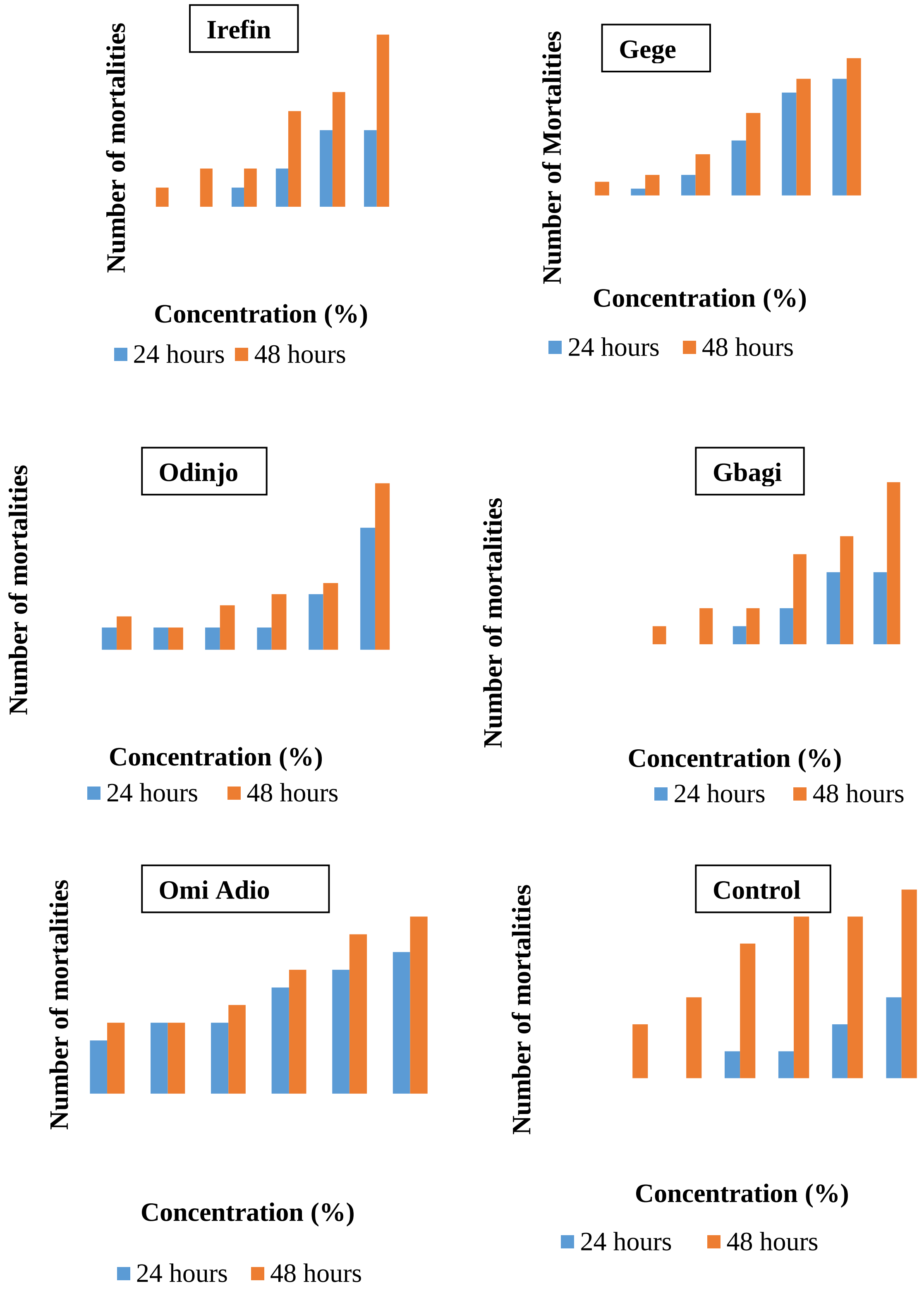

Figure 1: Mortalities of *Cloeon perkinsi'*slarvae exposed to different concentrations of Irefin ,Gege, Gbagi, Odinjo, Omi Adio and Contol stream water.

Table 3: Acute toxicity of contaminated downstream Gege sample on tadpoles of *Rana temporaria*.

Concn	Exposure Duration	No of Mortalities	Concn	Exposure Duration	No of Mortalities	LC_{50}	95 % Confidence Interval
50%	24 hours	11	100%	24 hours	40	58.78	52.55 – 65.74
	48 hours	11		48 hours	40	58.78	52.55 – 65.74
	72 hours	13		72 hours	40	56.12	49.81 – 63.24
	96 hours	18		96 hours	40	53.59	47.34 – 60.66

Table 4: Acute toxicity of contaminated downstream water sampleson frys of *Clarias gariepinus*.

Duration	Parameters	Samples					
		Irefin	Gege	Gbagi	Odinjo	Omi Adio	Control
24 hrs	LC_{50}	0.30	9.91	11.73	1.35	1.49	10.23
	95 % confidence interval	0 –2.45	7.80–12.05	0.76 – 24.59	0 – 3.78	0–4.47	2.75–17.51
48 hrs	LC_{50}	0.30	7.09	4.65	0.86	1.33	7.45
	95 % confidence interval	0 –2.45	0.51 – 8.75	0.46 – 9.56	0 - 3.26	0–4.28	3.65 – 105.06
72hrs	LC_{50}	0.30	4.76	4.00	0.86	2.63	6.70
	95 % confidence interval	0 –2.45	2.03 – 6.51	7.41 – 7.45	0 - 3.26	0 – 4.602	27.45 – 9.89
96hrs	LC_{50}	0.79	2.75	3.00	0.79	-	4.27
	95 % confidence interval	0 – 1.32	0.04 – 4.92	4.37 – 5.74	0 – 3.20	-	0.54 – 7.68

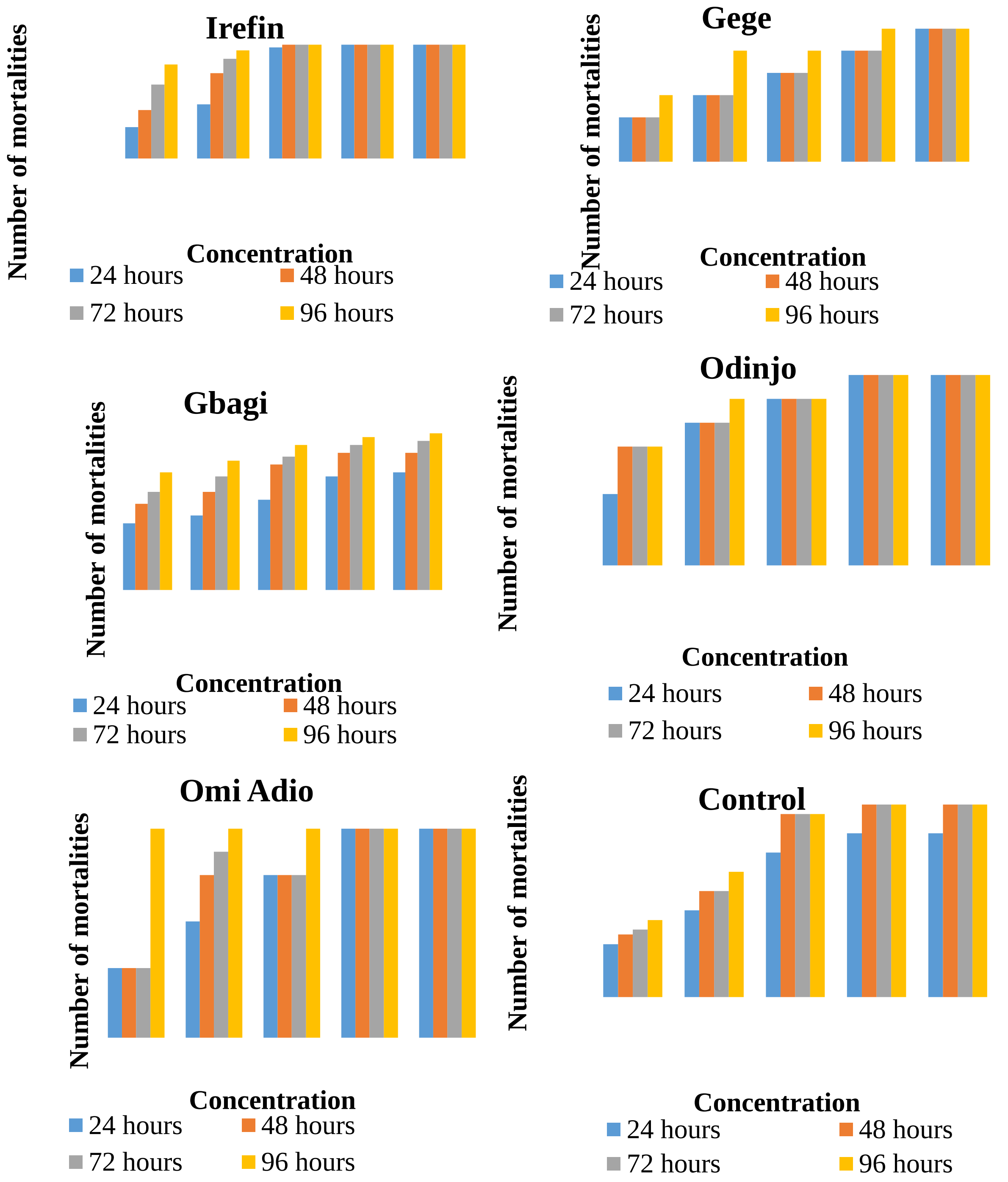

Figure 2: Mortalities of *Clariasgariepinus*frysexposed to different concentrations of downstream water (Irefin, Gege, Gbagi, Odinjo, Omi Adio and Control).

Table 5: Acute toxicity of contaminated Irefin and Gege downstream samples on fingerlings of *Clarias gariepinus.*

Sample	Exposure Duration	LC_{50}	95 % Confidence Interval	Sample	Exposure Duration	LC_{50}	95 % Confidence Interval
Irefin	24 hours	1089.22	216.59 – 1181094.60	Gege	24 hours	42.20	33.85 – 54.31
	48 hours	338.80	112.38 – 15753.70		48 hours	18.22	14.00 – 23.84
	72 hours	24.10	16.40 – 37.65		72 hours	9.48	5.98 –13.57
	96 hours	5.64	3.05 – 8.37		96 hours	3.33	1.14 – 5.78

4. Conclusion

The results indicated that the streams in Ibadan were polluted. These adversely affected the existence and distribution of certain fauna. Therefore, there should be adequate waste management and disposal of solid wastes to prevent further environmental contamination and preserve the biota inhabiting the streams. People living in the neighborhood should be enlightened on the disposal of wastes and should be encouraged to provide toilet facilities so that faeces will not be dumped into the water bodies.

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