Toxicity Assessment of Bindare Stream Sediment on Chironomid Nutrient Composition and Growth Using Sediment Contact Bioassay: Zaria, Nigeria

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Abstract-**Toxicity of four polluted sites and a reference site in the Bindare stream basin were investigated by a 10-day sediment contact bioassay. The test organism, the fourth instar of Chironomus spp (midge larvae) weighed 60.00 ± 13.00µg. Chironomid growth, crude proteins, and carbohydrate content were determined by conventional methods, as well as sediment metals and water physiochemical parameters. The stream water physicochemical parameters varied with the exception of dissolved oxygen concentration at sites 3 (1.25 ± 2.10mg/L) and 4 (3.20 ± 2.30mg/L), which were within the range suitable for survival of aquatic life. Site 4 sediment demonstrated the highest concentration levels of Zn (4.47 ± 0.11mg/kg), Cu (5.34 ± 0.61mg/kg) and Pb (4.32 ± 0.26mg/kg) while the highest levels of Cd (0.04 ± 0.01 mg/kg) and Fe (26.41 ± 0.81 mg/kg) were obtained at sites 3 and 1, respectively. Chironomid least mean weight gain (12.40 ± 5.46µg) was obtained at site 4, while the highest (22.00 ± 4.47µg) was obtained at site 5. Specific growth rate among the sites were as follows: site 5 > site 2 > site 3 > site 1 > site 4. Chironomids exposed to site 2 sediment had the least carbohydrate content (7.50 ± 1.20 %). Those exposed to site 1 were the most proteinous (19.60 ± 1.10%) while those at site 4 were the least (14.18 ± 1.25%). The measured end points/effects were compared to sediment contaminations and discussed. The results demonstrate differences in sensitivity of Chironomus species and the need for a sediment contact bioassay, when estimating effects of aquatic sediment pollution on benthic communities.**

Keywords- Contaminated Sediment; Bioassay; Chironomid Sp; Toxicity; Nutrient Composition; Growth

I. INTRODUCTION

Water basin sediments are formed as a result of the sedimentation of allochthonous and autochthonous substances [1]. Bottom sediments constitute a habitat for vegetable and animal life because they are rich in nutritional compounds. Therefore, they constitute an important component in the circulation of matter and energy in water basins [2]. They are composed of mineral and organic particles, with water contained between those particles. The development of ecotoxicological studies coincided with an increasing awareness that sediments act as a major pollutant reservoir and that there are a number of sites worldwide in which contaminated sediments represent a significant problem [3]. Toxicity testing has proved extremely useful for environmental and chemical hazard assessments because it can be done relatively quickly and inexpensively compared to chemical analysis [4].

Early toxicity testing of contaminated sediments focused primarily on acute toxicity, but short-term exposure, which measures effects on survival, can generally identify only high levels of contaminated sediments ([5, 6]). Recent studies have shown that selected sensitive sublethal endpoints (e.g., growth, reproduction, etc.) may provide better estimates of the responses of benthic organisms to contaminants under laboratory ([2, 7]) and field [8] conditions. Suitable test species for sediment toxicity tests are the oligochaete, *Tubifex tubifex* [9] and the larvae of the dipteran *Chironomus riparius* ([2, 4]); thus, these are commonly used to evaluate the toxicity of freshwater sediments in Africa, Europe and in the United States, because they have a wide sensitivity to contaminants ([7, 10]). Due to differences in their ecology (e.g., feeding behavior) and sensitivity, these species are used in bioassays of varying durations. In tests with these two organisms, the toxicity of sediments is assessed by measuring the growth of surviving larval insects in a sub-chronic test, and reproductive endpoints of tubificid worms are used to assess long-term contamination.

The nutritional value of farm-raised fish depends on the chemical composition of the fish diet. Industrial fish food tends to mimic natural food, containing approximately 50% protein (including all of the essential amino acids), 10-15% carbohydrates (without significant fiber content) and 12-15% lipids (including the necessary essential fatty acids). Yet, some authors [11] prefer and recommend natural food for fish diet, especially in the nutrition of younger fish. The outlined advantages of the natural food compared to the industrial ones include: a high digestibility (particularly of proteins), high water content (85-95%), a soft and elastic food structure which allows its deformation more quickly after ingestion, and the food moveability, allowing fish to react to the "food" motions. Additionally, unconsumed industrial food, which contains a high dry matter content, contaminates water in manifold ways compared to natural food.

Among the possible sources of natural food in Nigeria are the *Chironomus* species (Diptera: Chironomidae). Chironomids are a common group of aquatic insects living in running and still waters and represented by a large number of individuals. The duration of the larval phase lasts from several weeks up to one year, depending on water temperature [12]. At the pupae stage and as an adult fly, they live for only a few days. Chironomid larvae have elongated cylindrical bodies varying in size between

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1 and 25 mm. They move in a characteristic twitch, resembling the letter "S". Their feeding on green algae or detritus is species diversified [13].

The principal objective of this study was to assess the toxicity and characterize four polluted sediments and a single reference sediment in the Bindare stream basin in Zaria, Nigeria, through a 10-day sediment contact bioassay. In addition, our research evaluated the growth and nutritional quality/suitability of *Chironomus* species for fish diet, by determination of its basic chemical composition.

II. MATERIALS AND METHODS

A. Study Area

The Bindare stream (Fig. 1) flows in a west to east direction along a gully situated to east of Sabon Gari and the Chikaji Industrial Area, Zaria. Most of the gully contains water throughout the year, likely because it cuts through a perched water table. Additionally, the gully receives various urban effluents [14]. The source of the Bindare stream, which is approximately 6.0 km long, is from the Kwangila hills and it empties into River Galma. Large quantities of refuse, human feces and cattle dung were found on the slopes of the valley. Municipal and industrial effluents are frequently channelled into the same drains and subsequently into the stream, irrespective of their quality.

Five sampling stations were chosen for the current study. Station 1 was located upstream, before the Chikaji Industrial Estate. Station 2 was approximately 20m after the Bindare stream receives all the effluents from the Chikaji Industrial Estate, approximately 700m downstream from station 1). Station 3 (approximately 900m downstream of station 2) was located just after the stream begins to receive domestic effluents. Station 4 (approximately 900m from station 3) was located after the stream had received some of the municipal effluents. Station 5 (approximately 700m from station 4), was located just before the stream joins the River Galma.

B. Sample Collection and Analysis

Water and sediment samples were collected in triplicate from a relatively unpolluted station (station 1) which served as a reference station, and from four pollution impacted stations (stations 2-5) on the Bindare stream in July, 2013. Bottom sediments were collected with a steel scoop from the top 5-7-cm layer of sediment in the stream basin. Most of the stream's sediment was muddy.

The water temperature (ºC, 1m below water surface) was determined in the field using a mercury thermometer. Electrical conductivity and hydrogen ion concentration (pH) were determined using a Jenway 4010 conductivity meter and a Kent Eil 7055 pH meter, respectively. Total hardness and dissolved oxygen content were determined by burette titration, while sediment metals were determined using a Unicam 919 atomic absorption spectrophotometer after drying and digestion [15]. The metals analysed were Zinc (Zn), Copper (Cu), Lead (Pb), Cadmium (Cd) and Iron (Fe).

C. 10-Day Chironomus Species Bioassay

The tests were conducted according to OECD guideline 233 [16] with minor modifications. All the Chironomis riparius used in this investigation was collected from the sediment along shallow pools of a slow moving (0.01m/sec) stream in Zaria, by sieving sediment through a 250 μm mesh-size net. The animals were acclimatized for 12 hours at room temperature by keeping them in dechlorinated tap water in a tank, which was continually aerated. Mean body length and head capsule width of the larval instars of Chironomus spp were 7.5 ± 1.2 mm and 410.00 ± 60.00 μm, respectively. The mean chironomid wet weight was 3.70 ± 0.65 mg. The color of the animals was red.

The sediment from each sampling station was sieved twice through a 250 μm mesh-size sieve into a tank in order to remove any macro-fauna and larger sediment particles and ensure a standard particle size for the sediment in all the experiments. 20.00g of the sieved sediments were placed in transparent plastic-glass bioassay containers measuring 10.00cm x 5.00cm x 5.00cm. Stream water was added to the containers up to 1.00cm from the top to allow the sediment and water to equilibrate to test conditions and allow suspended sediment to settle before the addition of the test animals. Each set-up consisted of three replicate containers for each of the five sampling stations.

At the beginning of the test, 100 second instar larvae were chosen at random and transferred to each bioassay tank. The larval instars were separated from the stock tank on the basis of body length and head capsule width, measured by light microscope. Tests were performed under a 16:8 hour light: dark photoperiod for 10 days, with constant aeration. The containers were examined daily; dead larval instars were removed and not replaced. During the period of the bioassays, the number of Chironomus that had avoided the sediment, either floating on the water surface or lying on top of the sediment, was also recorded daily. Every other day, larvae were fed with prepared finely ground fish food with 1.0mg × larvae–1 × day–1., and water lost to evaporation was added. The pH and dissolved oxygen content were measured in all the beakers before and at the conclusion of each test. At the end of the test, the number of surviving chironomids and their wet weights were determined.

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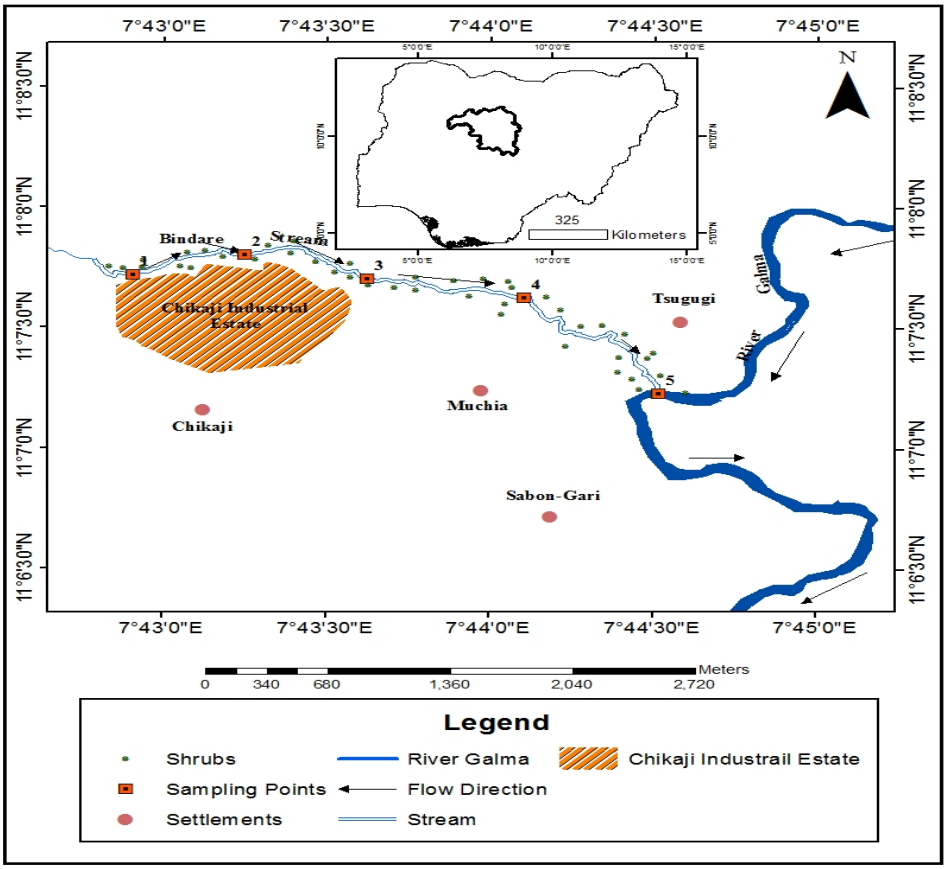

Fig. 1 Sabon Gari, Zaria, Nigeria, showing study area and locations of sampling stations along Bindare stream

Weight gained was calculated from the differences between initial and final weights, and growth during the bioassay period is expressed as:

$$SGR = \frac{LogW_t - LogW_0}{t - t_0}$$

where Log = logarithm, W_o = initial weight, W_t = weight at time t, and t = time at start of test.

Crude protein content was determined by the Kjeldahl method using the Kjel-Foss 16200 nitrogen analyzer. A Chironomid dry sample of approximately 100 mg was oxidized by concentrated sulphuric acid in the presence of a digestion catalyst. The ammonia collected was titrated directly with 0.02N HCL using a mixed indicator containing 6 ml methyl red; 12 ml bromo-cresol green and 6 ml 95% alcohol to a pink colour [17].

$$\%\ protein = \frac{Titre\ value \times 0.02 \times 0.014 \times 6.25}{wt.of\ sample\ in\ g} \times 100.$$

For crude carbohydrate, 0.4g of dry sample, digested in a 250 ml reagent bottle using 13 ml perchloric acid was filtered with a vacuum pump, the resulting solution adding up to 250 ml. Absorbance at 620 nm of 1 ml resultant solution in test tube was read after treatment with freshly prepared anthrone using a model 260 Colorimeter. After determination of glucose concentration from the calibration curve [17], carbohydrate content was calculated as % glucose according to the following equation:

$\%\ carbonhydrate = \frac{2.5 \times G}{W} 100$. *Where G = glucose concentration in mg/l and W = wt of chironomids taken for analysis.*

Each sample was analysed in triplicate.

III. RESULTS AND DISCUSSION

Variation in the physiochemical characteristics of surface sediment samples collected from five collection stations from the Bindare stream is presented in Table 1. The data indicates that anthropogenic activities, such as urbanization, industrial, and agricultural practices have affected the quality of the Bindare stream ecosystem. The temperature varied from 23.70 ± 3.11°C at station 1 to a peak 26.40 ± 0.9°C at station 4, and then dropped slightly at station 5 (25.80 ± 1.09°C). However, analysis of

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variance (ANOVA) revealed no significant differences ($p < 0.05$) in water temperature across the various sites. Temperature is a factor of great importance for aquatic ecosystem, as it affects the organisms, as well as the physical and chemical characteristics of water [18]. The lowest concentration of DO was recorded at station 3 (1.25 ± 2.10 mg/L) while station 5 demonstrated the highest (7.20 ± 0.88 mg/L). When DO is below 2.0 mg/L, many aquatic organisms perish. Low DO levels results from biological respiration and decomposition processes which reduce the concentration of DO in water bodies [19]. Alkalinity increased progressively from station 1 (16.00 ± 3.00 mg/L.$CaCO_3$) to a peak at station 5 (39.00 ± 3.00 mg/L.$CaCO_3$). The levels of total alkalinity and total hardness for good fish/aquatic organism cultures should fall within 20 to 300 mg/L. $CaCO_3$ [19]. The results of both total alkalinity (with the exception of station 1) and total hardness in the present study fell within this range. Sampling stations 3, 4 and 5 were acidic, with station 3 being the most acidic (pH 4.46) while station 2 was alkaline (pH 8.03).

TABLE 1 STATISTICAL SUMMARY OF SOME PHYSICOCHEMICAL PARAMETERS OF BINDARE STREAM AND NUTRIENT COMPOSITION OF CHIRONOMID LARVAE

Sample	Parameters	Stations				
		1	2	3	4	5
Water	Temperature(°C)	23.70 ± 3.11	24.00 ± 2.10	25.01 ± 3.30	26.40 ± 0.92	25.80 ± 1.09
	D. O (mg/L)	6.85 ± 0.90	7.2 ± 0.80	1.25 ± 2.10	3.20 ± 2.30	7.20 ± 0.88
	Alkalinity(mg/L-$CaCO_3$)	16.00 ± 3.00	25.00 ± 3.40	32.00 ± 1.80	38.00 ± 2.00	39.00 ± 3.00
	Hardness (mg/L-$CaCO_3$)	84.00 ± 6.20	80.00 ± 5.20	100.00 ± 3.22	168.00 ± 5.33	175.00 ± 1.16
	Conductivity (µS/cm)	276.00 ± 8.00	255 .00 ± 9.50	552.00 ± 11.00	428.00 ± 12.60	475.00 ± 5.38
	pH	7.99 ± 0.30	8.03 ± 0.81	4.46 ± 0.77	6.20 ± 0.25	6.90 ± 0.68
Sediment	Zinc (mg/kg dry wt.)	1.939 ± 0.20	0.9228 ± 0.01	4.4729 ± 0.11	4.8361 ± 0.31	3.3753 ± 0.22
	Copper (mg/kg dry wt.)	0.1417 ± 1.10	0.1033 ± 0.80	0.1379 ± 0.81	5.3458 ± 0.61	0.1161 ± 0.32
	Lead (mg/kg dry wt.)	1.9278 ± 0.20	0.7118 ± 0.22	0.9789 ± 0.24	4.3154 ± 0.26	0.9491 ± 0.11
	Cadmium (mg/kg dry wt)	0.0227 ± 0.01	0.0450 ± 0.02	0.0454 ± 0.01	0.0413 ± 0.01	0.0358 ± 0.01
	Iron (mg/kg dry wt.)	26.4116± 0.81	24.6575 ±0.91	22.1914 ± 1.30	23.7378 ± 0.13	21.0862 ±0.22
Chironomid larvae	Crude Protein (%)	19.60 ± 1.10	16.98 ± 0.90	16.45 ± 0.51	14.18 ± 1.25	18.55 ± 1.83
	Carbohydrate (%)	8.75 ± 0.51	7.50 ± 1.20	8.125 ± 0.82	8.60 ± 0.66	10.35 ± 0.73

Station 2 had the lowest concentrations of Zn (0.9228 ± 0.01mg/kg), Cu (0.1033 ± 0.80 mg/kg) and Pb (0.7118 ± 0.22 mg/kg). The trend of these metals was such that an initial concentration decreased at station 2, rose gradually to peak at station 4 and decreased at station 5, suggesting that the most severe pollution occurred at the point where urban wastewater is discharged. Cu concentrations varied from 0.1033 ± 0.80 to 5.3458 ± 0.61 mg/kg. The highest Cu content was detected in the downstream station 4, which is close to the main wastewater discharge point, suggesting an anthropogenic contribution to total Cu concentrations in Bindare stream sediment. The ranges of all other stations recorded were comparable to those reported for local and regional streams and rivers ([18, 19]). Fe exhibited the highest concentrations among the five metals in all the sampling stations, varying between 21.0862 ± 0.22 mg/kg (station 5) and 26.4116 ± 0.81 mg/kg (station 1). The concentration of Cd varied between 0.0227 ± 0.01to 0.0454 ± 0.02 mg/kg. The level of Cd obtained in the sediment samples were within South African Target Water Quality Range (TWQR) for irrigation purposes [20]. Cd is an important factor in aquatic monitoring studies, because it has been found to be toxic to fish and other aquatic organisms [21]. Also, Cd has been implicated in endocrine disrupting activities which could pose serious health problems [14, 22]. Apart from natural sources such as runoff from agricultural fields where phosphate fertilizer might be in use, other sources may include leaching from Ni-Cd based batteries [14]. The abundance of measured heavy metals decreases as follows: Fe>Zn>Pb>Cu>Cd. Most of the elements show a similar distribution pattern, thus suggesting a common source and similar enrichment mechanisms of the stream. Many studies have reported detrimental effects of metals to benthic communities [19, 23, 24, 25]. The degree of metal toxicity to aquatic organisms is determined by metal speciation, pH, hardness, uptake site, previous exposure, and differences among species in regulation [26].

The nutritional status of an aquatic organism, both prior to and during testing, can significantly modify the apparent toxicity of a chemical [7, 14]. The nutritional status of an organism is related to the quantity and quality of the organism's diet, as well as the level of contaminants present in the diet [27]. The obtained crude protein range (14.18 ± 1.25 to 19.60 ± 1.10 %) and carbohydrate content range (7.50 ± 1.20 to 10.35 ± 0.73 %) in fresh larvae are in accordance with the values reported in other chironomid species [12, 28]. Tests with *C. riparius* demonstrated that nearly the entier length of the Bindare stream sediments sampled caused toxic stress, affecting both chironomid growth and weight gain (Fig. 2).

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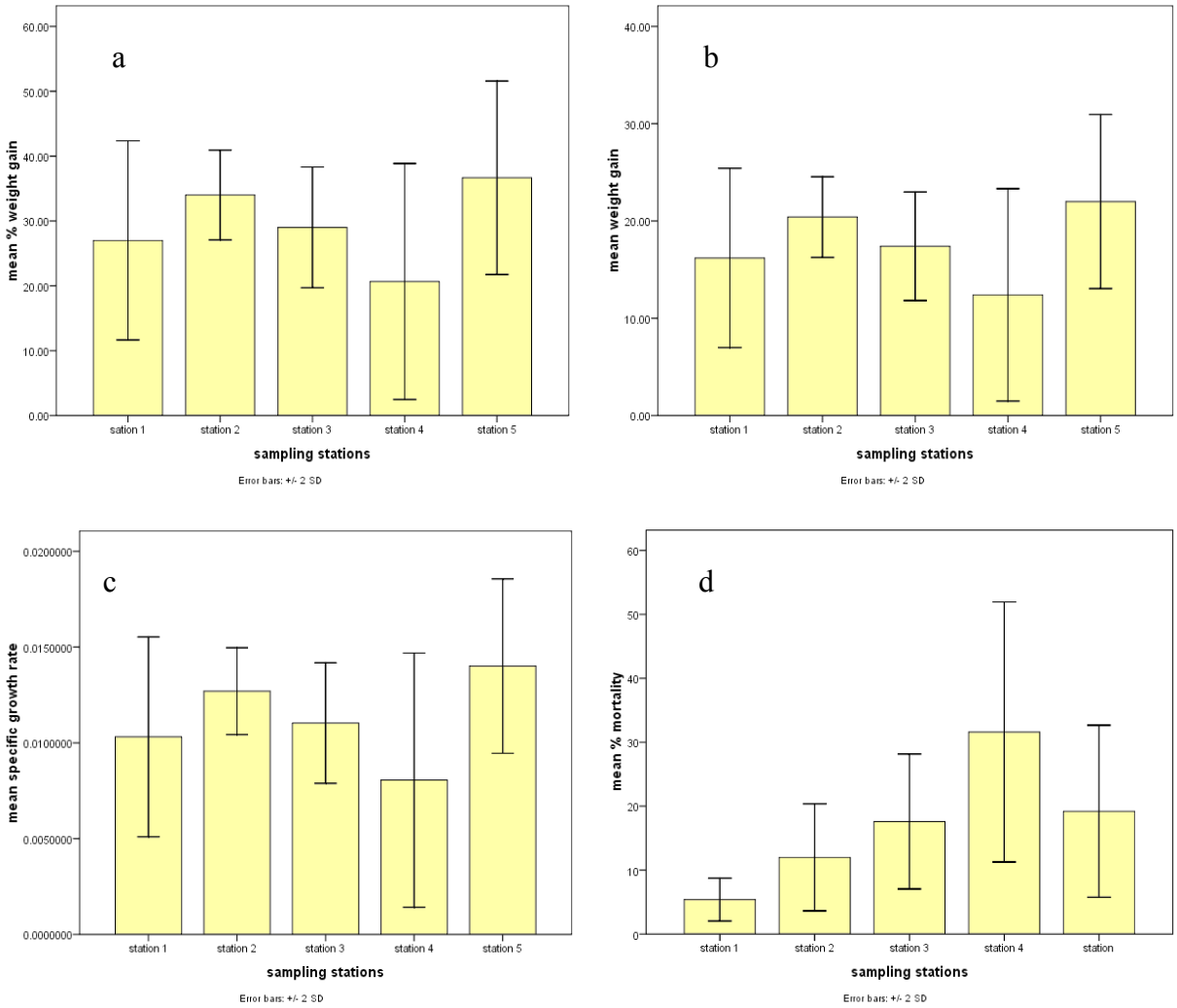

Fig. 2 Effects of sediment toxicity of Bindare stream on: (a) mean weight gain (µg); (b) mean % weight gain; (c) mean specific growth rate; and (d) mean % mortality of Chironomus species

Mean weight gain (20.4 ± 2.07 µg) at station 5 decreased progressively to the lowest level (12.40 ± 5.45 µg) at station 4 before leaping drastically to peak at station 5 (22.00 ± 4.47 µg). Percentage weight gain and specific growth rate closely follows a similar trend. The least mean % mortality (5.60 ± 2.07 µg) occurred at station 1 (reference station), rose to a peak (34.80 ± 14.04 µg) at station 4 and then demonstrated a slight decline at station 5 (19.20 ± 6.72 µg). These toxic effects that emerged from the 10-day survival test with *C. riparius* point toward the potential toxicity of Bindare stream sediment.

The trend of the sediment toxicity was in agreement with that of the concentration pH and comparatively higher heavy metal load, which caused more stress to chironomid mean weight gain, specific growth rate and % mortality rate. Exposure of chironomid larvae to sediments from the Bindare midstream also caused a decrease of carbonhydrate content and crude protein percentage. Long-term toxicity tests have proven to be particularly useful in characterizing the potential toxicity of sediment, providing a more precise indication of the trend of contamination in rivers [3]. Several authors recommend the *Chironomus* spp 10-day growth test as a useful bioassay for evaluation of sediment toxicity [4, 29, 30, 31, 32], under standard protocols ([10, 16]). Growth reduction in C. *riparius* larvae has been considered a sensitive response criterion [30].

IV. CONCLUSIONS

Toxicity assessments of aquatic bottom sediments have been successfully realized since the 1970's. Nigeria is clearly lagging in this area, both compared to other developing countries and world trends in ecotoxicology. From the biological responses that were monitored, growth was most susceptible to sewage/metal contamination, proving to be the most suitable endpoint to detect and assess the impact of this type of contamination on rivers. We hope that the results of the present study will contribute to an understanding of the sources and effects of pollutants along the course of the stream and provide the first data to lead to its restoration.

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