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Identifying Pollution Classes and Macroinvertebrate Sensitivities in an Afrotropical Riverine System: A Multivariate Approach

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ABSTRACT
Measuring the level of degradation in riverine systems is paramount to assessing their current health status. In this study, we identified pollution classes and the sensitivities of macroinvertebrates in River Ringim, northwestern Nigeria. We marked four sites in the study area based on their level of human influence and accessibility. Physicochemical variables and macroinvertebrates were sampled monthly for a period of five months between November 2018 and March 2019. Physicochemical variables were used to categorize the four sites into pollution categories, while the 12 taxa of macroinvertebrates recorded were also classified into biological categories. The constructed principal component analysis (PCA) showed that physicochemical variables, such as total dissolved solids, conductivity, and pH, were positively associated with Site 2, whereas Sites 3 and 4 were negatively associated with water temperature, air temperature, and flow velocity. Further analysis based on the extracted coordinate scores from the PCA showed that Sites 2–4 were heavily polluted, whereas Site 1 was slightly polluted. The result of the canonical correspondence analysis (CCA) revealed that macroinvertebrate taxa, such as Leuctridae and Hydrometridae, were positively associated with conductivity, flow velocity, water depth, water temperature, air temperature, dissolved oxygen, and pH. Of the 12 taxa we categorized, nine were highly vulnerable based on our further analysis using the extracted coordinates from the CCA. The classified sites and macroinvertebrate taxa can be used for biomonitoring the ecological health of River Ringim and other river systems subjected to similar stressors within the northwestern region of Nigeria by river managers. Our study provides baseline information on the current state of River Ringim, and we recommend sampling of more rivers and sites within the northwestern region of Nigeria to confirm our present results.

KEYWORDS: Macroinvertebrates, Degradation, Anthropogenic stressors, Biomonitoring, Nigeria, Afrotropic region.

1. INTRODUCTION
Urban development, agricultural activities, and industrial undertakings are the major stressors increasing the pollution levels in riverine systems [1-3]. Degraded riverine systems suffer from dissolved oxygen depletion, increased nutrient concentrations, and accumulation of dissolved solids occasioned by agricultural activities, urban and industrial

developments [4-5]. The debilitating effects of increasing pollution in riverine systems include compromised water quality, loss of biodiversity, and reduced ecosystem service provision [6-8].

In the northwestern region of Nigeria, most catchments of riverine systems are used for farming (subsistence and nomadic) and developing residential settlements [9]. Previous studies have reported a considerable increase in the portion of river catchments used as informal settlements, urban development, and agricultural undertakings within the Hadejia-Nguru axis, where the current study area, River Ringim, is located [8, 11]. These have heightened the level of degradation occurring in riverine systems within the Hadejia-Nguru axis of the northwestern region of Nigeria [8, 10-11]. River Ringim catchments are rapidly urbanizing, with most of the river's reaches occupied by residential buildings and other anthropogenic activities. Based on the large-scale stressors impacting rivers in the northwestern region of Nigeria, the present study focuses on the level of degradation in selected sites in River Ringim and the consequent effects on the ecological conditions of the sites and their inhabitant biota. To achieve this, we classified sites into pollution categories using physicochemical variables and assessed the level of sensitivities of macroinvertebrates taxa in a bid to classify them into biological categories. The classified sites and taxa can be used for biomonitoring the ecological health conditions of river systems subjected to human disturbances. Moreover, river managers and other appropriate water resource regulators can use them to advise properly on possible management and sustainability measures.

Physicochemical variables have been used in different regions to assess the ecological health of riverine systems [12-14]. For instance, a proportionately increased level of biochemical oxygen demand and nutrients portrays poor water quality, whereas an increased dissolved oxygen concentration indicates that a water system is unimpaired [15-16]. Nonetheless, categorizing sites into a gradient of pollution using physicochemical variables alone cannot provide an all-embracing picture of the level of degradation in riverine systems. Hence, complementary identification and classification of taxa into biological categories could be a useful approach for properly profiling the level of pollution in rivers.

Several aquatic organism groups have been used to develop biological monitoring approaches (e.g., macrophytes, diatoms, planktons, macroinvertebrates, and fishes; [7, 9, 17, 18-20]). Among these organisms, macroinvertebrates are one of the most explored based on the following reasons: (i) they are easily sampled, (ii) they are ubiquitous, and (iii) they respond differentially to gradients of pollution [21-24]. The vulnerability and resilience to pollution levels in macroinvertebrates have been reported to vary [11, 25-28]. Different researchers have identified and classified macroinvertebrates taxa according to their level of vulnerability and resilience [16, 28, 29]. For instance, Osimen et al. [29] used a multivariate approach to identify and categorize macroinvertebrates taxa into biological classes in a reservoir in the Niger Delta region of Nigeria. They concluded that the categorization of macroinvertebrates taxa was helpful in monitoring and managing freshwater systems, especially in sub-Saharan Africa, where such a study is still at the infant stage. The present study adds to the growing literature on developing such tools for Nigeria and the northwestern region of Nigeria, where very few studies involving the development of monitoring approaches using the combination of physicochemical variables and macroinvertebrate taxa exist. Therefore, the present study aims to explore the level of degradation of River Ringim, northwestern Nigeria. To the best of our knowledge, this may be the first attempt in developing such approaches in the region.

2. Materials and Methods

Study Area

River Ringim is located in the northwestern region of Nigeria. The river is situated along the interception of latitudes $12^{o}17'$N- $9^{o}28'$E and longitudes $12^{o}283'$N- $9^{o}467'$E of the equator (Figure 1). The climate of the study area is tropical, with two seasons (dry and wet). The dry season is from October to May, and the wet season is from June to September. The dry season is characterized by an extremely cold weather condition called harmattan, and it usually occurs between November and February [15]. The vegetation of the study area is dominated by shrubs and sparsely distributed trees; the vegetation is of the Sudan savannah.

Study Area Sites

In this current study, four sampling sites were selected based on the level of human influences within the site's reaches. **Site 1** is in Yakasawa community (Plate 1). The site biotopes are mainly loam and clay, and typha grass dominates the bank of this site. Activities within the station include subsistence farming and fishing.

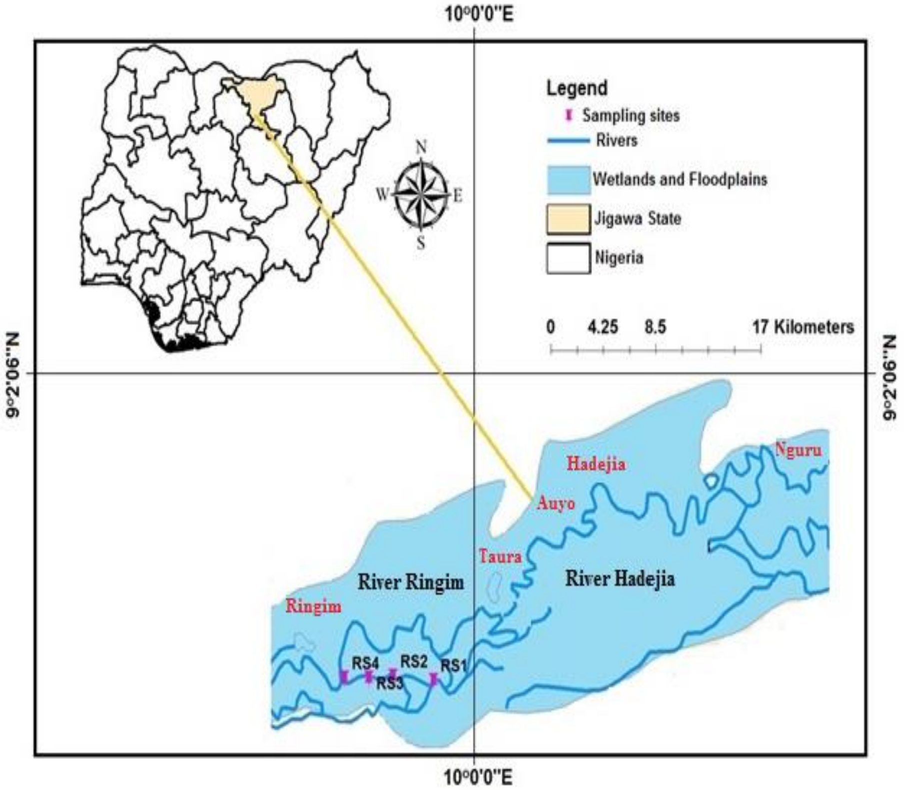

Figure 1: Study area map showing the four sampling stations (map of Nigeria and Jigawa State insert).

Plate 1: Site 1 (Yakasawa community).

Site 2 is about 2 km from Site 1 and is located in Ringim community (Plate 2). The biotopes here are mainly loam and silt, while mango and guava trees and few shrubs dominate the riparian zone. The human influences include farming, household washing, sand dredging, and bathing.

Plate 2: Site 2 (Ringim community)

Site 3 is located in Zangon Kanya village, and it is about 1 km away from Site 2 (Plate 3). The biotopes are mainly sandy and loamy, and the vegetation is characterized by shrubs and typha grasses. Human activities here include sand dredging, open defecation, farming, and bathing.

Plate 3: Site 3 (Zangon Kanya village).

Site 4 is located within the Yandutse community (Plate 4), and it is about 1.5 km away from Site 3. The biotopes are mainly loam and mud. Neem trees (*Azadirachta indica*) dominate this site with sparse distribution of shrubs. Open defecation, farming, and fishing are common human activities occurring within this site.

Plate 4: Site 4 (Yandutse community).

Sampling Expedition: Physicochemical Variables and Macroinvertebrates

Physicochemical Variables

The sampling expedition was carried out during the dry season for a period of five months (November 2018 to March 2019). Sampling was done once a month. Air and water temperatures (ºC) were determined using mercury in a glass thermometer, and flow velocity (m/s) was measured by floating a weighted cork along a given distance [30]. Secchi disc was used to determine transparency (m), while pH, conductivity (µS/cm), and total dissolved solids (TDS; mg/l) were measured using a portable conductivity meter (HANNA HI 9913001/1 instrument). A turbidimeter (Confab model 850) was used to measure turbidity (NTU) at each site. Dissolved oxygen (DO; mg/l) was determined using a DO meter (YSI 55 dissolved meter). Biochemical chemical oxygen demand (BOD; mg/l) was determined using the APHA method [31].

Macroinvertebrates

Macroinvertebrates were collected alongside physicochemical variables on each sampling expedition. A modified kick net of 500 µm mesh size was used in collecting macroinvertebrates along a wadeable distance of 40 meters, covering all available biotopes per site. All collections made per site were pooled together as one composite sample. At each site, preliminary sorting was done to reduce sorting properly in the laboratory. Sorting was done with forceps, a white enamel tray, and a hand lens on the field. Sorted samples were preserved in 10% formalin in a separate bottle, while the yet-to-be-sorted samples were preserved in 70% alcohol in a container for further sorting at the laboratory. Macroinvertebrates were identified to the family level following available identification keys and guides [32-34].

Data Analysis

Principal component analysis (PCA) was conducted to summarize physicochemical variables at sampling sites. Sites were categorized into pollution categories using the sampling sites' position along the first principal component (PC). We used the first PC, because it explained the highest variance (53%) in the data.

The proper pollution categorization was undertaken following the method of Edegbene [16], who used the first axis of PCA to delineate sites into a pollution gradient. First, the coordinate scores of the four sites were extracted. Second, the extracted scores were used to calculate the intersite distance of each site by subtracting the lowest scoring site from the remaining sites one after the other. Third, the intersite distance value was converted to percentages. Fourth, three-percentile distribution ranges were adopted in categorizing the sites based on the percentage values of sites. The percentile distributions were as follows: 90%–100% (slightly polluted, SP), 70%–<90% (moderately polluted, MP), and 0–<70% (heavily polluted, HP). The PCA was constructed using paleontological statistical package (PAST; Hammer et al. [35]).

The relationships between physicochemical variables and macroinvertebrate taxa among the four sites were visualized using the canonical correspondence analysis (CCA). Before concluding on the type of multivariate analysis to be used (e.g., CCA or redundancy analysis), we conducted a test for linearity and unimodality [36]. In doing this, we performed detrended correspondence analysis and the gradient length was >3. Hence, the macroinvertebrate abundance datasets were unimodal; thus, CCA was determined to be appropriate. A Monte-Carlo permutation test at 999 permutations was computed to show the significance level of both the physicochemical variables and macroinvertebrate taxa on the entire CCA axes. The CCA triplot and Monte-Carlo permutation test were computed on PAST [35].

Classifying Macroinvertebrate Taxa Based on the Vulnerability and Resistance Levels in the Study Area

The 12 macroinvertebrate taxa collected in the current study area were classified into four biological categories: V = vulnerable; HV = highly vulnerable; R= resistant; HR = highly resistant. The vulnerability and resistance of macroinvertebrate taxa were determined by correlating physicochemical variables with macroinvertebrate taxa along the four sampling sites using CCA. Macroinvertebrate taxa coordinate scores were extracted from the first axis of the CCA because it accounted for 62.39% of the axes of the CCA that were visualized. Biological categorization was computed following the thinking of Osimen et al. [29]. First, we subtracted the coordinate score of the lowest scoring taxa from the other taxa to give us the intertaxa distances. Second, the calculated intertaxa distances were converted into a percentage. Third, we adopted a percentile distribution of 100%, 70–<100%, 69.99–<70%, and 0–<69.99% for HR, R, V, and HV, respectively.

3. RESULTS

Categorization of Sites into Pollution Category

The results of the constructed PCA revealed that the first and second axes of the principal components explained 78.84% of the variance. The first component explained 53.8% with an eigenvalue of 5.92, and the second component explained 25.04% with an eigenvalue of 2.75.

Site 2 was positively associated with conductivity, DO, TDS, pH, transparency, and water depth (Figure 2) on component 1 of the PCA. On the other hand, Sites 3 and 4 were negatively associated with water temperature, air temperature, and flow velocity (Figure 2). Site 1 was positively associated with BOD and negatively associated with turbidity on the second component of the PCA (Figure 2).

The result of the intersite distance based on percentile distribution showed that Site 1, with a percentile of 100%, was slightly polluted (SP) and Site 3, with 37.57%, was heavily polluted (HP) (Table 1). Sites 2 and 4, with 16.99% and 0.00%, respectively, were moderately polluted (MP) (Table 1).

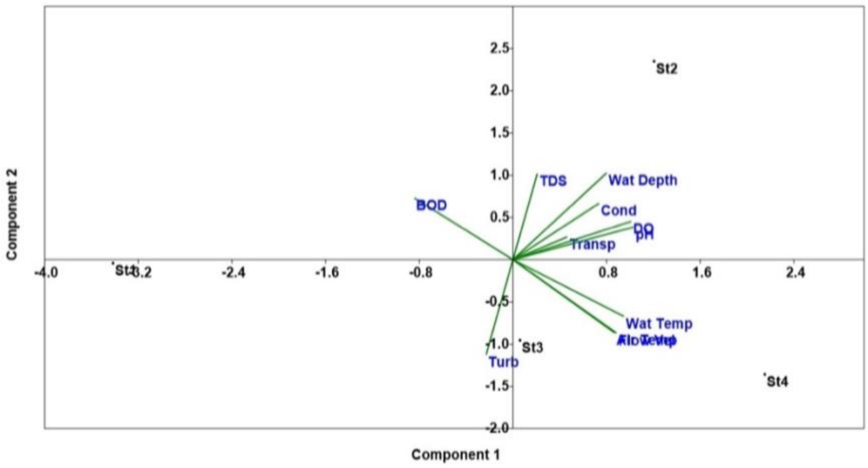

Figure 2: Principal component analysis (PCA) based on correlation matrix showing the relationship between physicochemical variables and sampling sites in River Ringim.

Physicochemical variables abbreviations: Air Temp = air temperature; Wat Temp = water temperature; Wat Dep = water depth; Flow Vel = flow velocity; Transp = Transparency; Cond = conductivity; Turb = turbidity; TDS = total dissolved solids; DO = dissolved oxygen; BOD = biochemical oxygen demand.

Table 1: River Ringim sites categorization into pollution category.

Sites	Extracted coordinates from PCA component 1	Intersite distance	Intersite distance (%)	Pollution category
St1	-3.4161	5.5671	100	Slightly polluted (SP)
St2	1.2054	0.9456	16.9855	Heavily polluted (HP)
St3	0.059679	2.091321	37.56572	Heavily polluted (HP)
St4	2.151	0	0	Heavily polluted (HP)

Identifying and Classifying the Sensitivities of Macroinvertebrate Taxa

The result of the first two axes of the CCA accounted for 86.09% of the inertia. Axis 1, with an inertia of 62.39% and an eigenvalue of 0.19, was the highest and was thus used to determine the level of vulnerability and resistance of macroinvertebrate taxa by extracting their coordinates from the axis for further calculation and categorization (Figure 3, Table 2). Axis 2 of the CCA had an inertia of 23.7% and an eigenvalue of 0.073. A Monte-Carlo permutation test with 999 permutations revealed that the first axis of the CCA had a P-value of 0.26 and the second axis P-value was 0.431, and this showed that the first two axes of the CCA were not statistically significant ($P > 0.05$) in correlations between macroinvertebrates taxa and physicochemical variables. In Site 4, Leuctridae and Hydrometridae were positively associated with conductivity, flow velocity, water depth, water temperature, air temperature, DO, and pH (Figure 3). Dytiscidae, Gerridae, Notonectidae, and Bithynidae were negatively associated with turbidity, TDS, and transparency in Sites 2 and 3 (Figure 3). Unionidae, Hydraenidae, and Atyidae were negatively associated with BOD in Site 1, and Chironomidae were positioned at the center of the CCA triplot and was not associated with any variable (Figure 3).

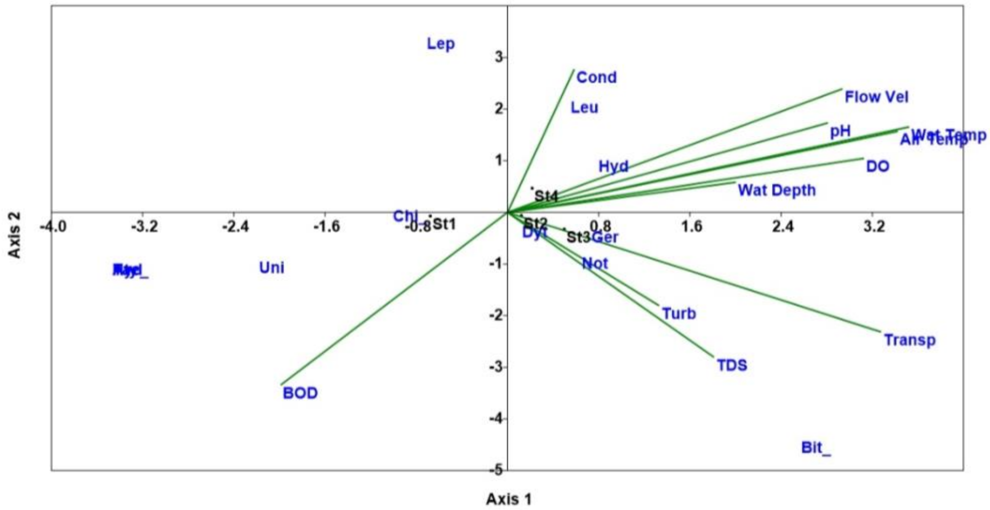

Figure 3: Canonical correspondence analysis showing the first two axes of macroinvertebrate taxa, physicochemical variables, and the sampling sites of River Ringim.

Physicochemical variables abbreviations: Air Temp = air temperature; Wat Temp = water temperature; Wat Dep = water depth; Flow Vel = flow velocity; Transp = Transparency; Cond = conductivity; Turb = turbidity; TDS = total dissolved solids; DO = dissolved oxygen; BOD = biochemical oxygen demand.
Macroinvertebrate codes: Ger = Gerridae; Hyd = Hydromitridae; Not = Notonectidae; Leu = Leutridae; Tae = Taeniopterygidae; Uni = Unionidae; Lep = Leptophlebiidae; Chi = Chironomidae; Dyt = Dytiscidae; Hyr = Hydraenidae; Aty = Atyidae; Bit = Bithynidae.

In categorizing the sensitivities of macroinvertebrate taxa, we extracted the coordinates of each taxon from the first axis of the CCA, being that it accounted for the highest CCA variance (62.39%) compared to the remaining axes. Nine of the 12 macroinvertebrates recorded were highly vulnerable (HV), including Notonectidae, Leuctridae, Taeniopterygidae, Unionidae, Lepthophlebiidae, Chironomidae, Dytiscidae, Hydraenidae, and Atyidae (Table 2). The remaining three taxa, Hydrometridae, Bithynidae, and Gerridae, were resistant, highly resistant, and vulnerable (V), respectively (Table 2).

Table 2: Biological category of macroinvertebrate taxa in River Ringim. Taxa biological categories were derived from the extracted coordinates on the first axis of the CCA constructed (see Figure 3). Note: V = vulnerable, HV = highly vulnerable, R= resistant, HR = highly resistant

Macroinvertebrate taxa	Macroinvertebrate taxa codes	Extracted taxa coordinates from CCA Axis 1	Intertaxa distance	% intertaxa distance	Taxa biological category
Gerridae	Ger	0.718655	4.20202	69.5624139	V
Hydrometridae	Hyd	0.781152	4.26451	70.5970228	R
Notonectidae	Not	0.632483	4.11584	68.1358763	HV
Leuctridae	Leu	0.535199	4.01856	66.5253847	HV
Taenioptergygidae	Tae	-3.48336	0	0	HV
Unionidae	Uni	-2.19716	1.2862	21.2924458	HV
Leptophlebiidae	Lep	-0.726096	2.75726	45.6452296	HV
Chironomidae	Chi	-1.02223	2.46113	40.7428683	HV
Dytiscidae	Dyt	0.110113	3.59347	59.4882827	HV
Hydraenidae	Hyd	-3.48336	0	0	HV
Atyidae	Aty	-3.48336	0	0	HV
Bithynidae	Bit	2.55728	6.04064	100	HR

4. DISCUSSION

The level of degradation of River Ringim, northwestern Nigeria, was explored by categorizing sites and taxa using physicochemical variables and macroinvertebrates abundance data. PCA and CCA were employed for the sites and taxa categorization, respectively. The result of the PCA revealed that the first two axes of the PCA explained a total variance of 78.84%, making it a good ordination [37-38]. A PCA variance of between 70% and 90% has been considered good enough to decide the reliability of environmental variables used in constructing ordination plots [27]. Keke et al. [38] reported 70% ordination variance for the first two axes of the PCA they constructed for a river subjected to industrial and urban activities in the southern part of Nigeria, which corroborates with the result of the current study.

On the PCA biplot, most physicochemical variables indicating pollution (e.g., TDS, conductivity, and pH) were positively associated with Site 2, portraying the level of degradation the site is subjected to. Similarly, relationships between environmental variables and sites have been reported by other authors [39-40].

Based on the result of the sites' categorization, three sites (Sites 2, 3, and 4) were heavily polluted. The deteriorated state of most of these sites in the current study portends their pollution state, which also confirms the structural assemblage of macroinvertebrates in the river system.

Our CCA triplot showed that macroinvertebrate taxa such as Leuctridae and Hydrometridae, were positively associated with conductivity, water and air temperatures, and flow velocity. On the other hand, taxa, such as Dytiscidae, Bithynidae, and Unionidae, were negatively associated with TDS and turbidity on the CCA triplot. Similar studies have reported variation in the association of organisms with environmental variables due to the level of sensitivities of the organisms and their ability to adapt to changing environmental factors [3, 26, 38, 41, 42]. For instance, in this study, taxa such as Leuctridae and Hydrometridae can be considered tolerant of environmental degradation, whereas Dytiscidae, Bithynidae, and Unionidae are vulnerable to environmental degradation. Generally, Leuctridae (Trichoptera) and Hydrometridae (Hemiptera) are widely known pollution-sensitive taxa [16, 29]. Their classification as tolerant taxa may be due to the very few taxa of Leuctridae and Hydrometridae recorded in the present study. Furthermore, the constraint of identifying to the family level may be the reason for the tolerance of the taxa, as genus or species levels identification is more appropriate to decide the sensitivities of macroinvertebrate taxa. Also, the number of sampled sites was relatively small; thus, a more robust sampling exercise is required to confirm the current categorization in this study. It has been reported that pollution-sensitive macroinvertebrate taxa inhabit less impaired or unimpaired sites, whereas the pollution-tolerant taxa inhabit impaired sites [25, 42]. Fu et al. [26] have reported that sediment accumulation and heavy metals explained the distribution patterns of macroinvertebrates in a river system in China, as pollution-sensitive taxa were significantly affected by the increasing level of pollution in the river system.

From the taxa categorization and classification result, Gerridae was resistant and Hydrometridae was highly resistant, while the remaining macroinvertebrate taxa were highly vulnerable, except for Gerridae that was vulnerable. Other authors in Nigeria have reported a similar level of sensitivities of freshwater macroinvertebrates [38, 43]. Furthermore, the biomonitoring significance of aquatic insects, such as Ephemeroptera, Plecoptera, Trichoptera, and Odonata, have been widely reported by several aquatic scientists globally [26, 28, 41, 44]. Our present study portrays the importance of identifying and categorizing river sites and macroinvertebrate taxa into pollution categories. To the best of our knowledge, it is the first attempt to develop such approaches in northwestern Nigeria. Overall, the methods we used to categorize and classify sites and taxa are a first step toward developing more robust tools for biomonitoring similar Nigerian rivers.

5. CONCLUSIONS

We explored the ecological health of River Ringim in northwestern Nigeria. Physicochemical variables were used to delineate sites into pollution categories based on PCA ordination. Variables such as conductivity and TDS were positively associated with Site 2, portraying the deteriorated state of the site. Further analysis of the categorization procedures showed that Sites 2, 3, and 4 were heavily polluted. Of the 12 taxa categorized and classified, nine were highly vulnerable to pollution: Notonectidae, Leuctridae, Taeniopterygidae, Unionidae, Leptophlebiidae, Chironomidae, Dytiscidae, Hydraenidae, and Atyidae. This study has some limitations: the sampling was carried out for five months and seasonal fluctuation in physicochemical variables and macroinvertebrate community structure were not considered, which may have limited the robustness of our findings. Hence, we recommend that future studies should consider spatiotemporal variations in physicochemical variables and macroinvertebrate assemblage when developing monitoring tools for riverine systems. More rivers and sites should also be explored to confirm our present findings.

Overall, the study was a preliminary step in identifying and classifying potential biological indicators for effective monitoring and management of freshwater ecosystems, most especially in sub-Saharan Africa, where such research is still scarce.

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