Identifying and categorizing potential indicator macroinvertebrate taxa in a southern Nigerian reservoir using a multivariate approach

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ABSTRACT

Anthropogenic activities within and around freshwater ecosystems are serious threats to the structural and functional assemblages of aquatic biodiversity. In the present study, an identification and categorization of the potential sensitive and tolerant macroinvertebrate taxa in a southern Nigerian reservoir were assorted, using multivariate approach. Macroinvertebrates and selected physico-chemical variables were sampled monthly in four sites for a period of two years (January 2009 to December 2010). PCA performed classified Site 1 as less impacted, and Site 4 as heavily impacted. Elevated mean values of pollution indicating physico-chemical variables such as BOD_5, phosphate and TSS were recorded in Site 4, and an increased concentration of DO was recorded in Site 1. Diptera dominated the 28 taxa of macroinvertebrate that were recorded in the entire study period, noting that the absolute abundance of Diptera taxa was higher at Site 4. NMDS revealed Sites 1 and 2 to form cluster B portraying similarity in the assemblage structure of taxa recorded in both sites, and cluster A formed a mixture of macroinvertebrate taxa assemblages from all the sampled sites. CCA and further categorization analyses performed identified and classified *Centroptilum* spp. to be very sensitive to pollution, while *Mytilus dubia* was the only taxon that was identified and classified as very tolerant of pollution. Overall the study pointed out the usefulness of using multivariate approach in identifying and classifying potential indicator taxa for effective monitoring and management of freshwater ecosystem most especially in Sub- Saharan Africa where studies of this kind are still scanty.

INTRODUCTION

Anthropogenic activities within and around freshwater ecosystems e.g. rivers, streams, lakes, and reservoirs are seriously affecting the structural and functional assemblages of aquatic biodiversity (Parienté, 2017; Edegbene *et al.,* 2020). Traditionally, the effects of anthropogenic activities resulting in pollution in freshwater ecosystems are assessed by measuring and analyzing values of physico-chemical variables (**Adakole & Anunne, 2003; Palmer *et al.,* 2004**). The relationship of physico-

chemical variables and aquatic biota have also been estimated for decades to evaluate the impacts of anthropogenic activities in aquatic ecosystems (**Palmer** *et al.,* **2004; Arimoro** *et al.***, 2015; Edegbene** *et al.,* **2015**). In recent times, biological monitoring tools have replaced phisico-chemistry, taxa abundance, and diversity to   assess and monitor freshwater ecosystems in developed countries; e.g. biological monitoring working party(BMWP) (**Walley & Hawkes, 1996**), south Africa scoring system(SASS) in sub-Sahara Africa (**Dickens & Graham, 2002),** and Tanzania scoring system(TARIS) (**Kaaya** *et al.,* **2015**). In Nigeria, such biological monitoring tools have not been developed for evaluating the negative impacts of anthropogenic activities on aquatic ecosystems except the recent macroinvertebrate-based multimetric indexes particularly developed for urban rivers in the Niger Delta region of Nigeria (**Edegbene** *et al.,* **2019a**) and River Chanchaga in North Central region of Nigeria (**Edegbene** *et al.,* **2019b**). The above mentioned biomonitoring tools are important in terms of predicting gradients of pollution in freshwater ecosystems (**Odume** *et al.,* **2016**).

Despite the fact that physico-chemical variables are significant to assess the health of aquatic ecosystems, high financial implication and requisite expertise are needed coupled with the rigorous analytic procedures and chemicals required in analyzing physico-chemical variables. Therefore, aquatic health assessment method of using physico-chemically-based approach is not economical and in most times very difficult to analyze. Furthermore, the effects of anthropogenic activities on freshwater ecosystems cannot be assessed entirely depending on physico-chemically-based approach due to the fact that physico-chemically-based approach is sticktly contributed to the time and spot in which the water sample was collected for the analysis (**Palmer** *et al.,* **2004; Odume** *et al.,* **2016; Edegbene** *et al.,* **2019a**). Hence, the need to identify and classify biological parameters for rapid and easy biomonitoring of aquatic health is deeply required. Biomonitoring takes into account a holistic measure of multiple parameters, e.g. physical, chemical and biological stressors, and their effects on the structural and functional assemblage of freshwater ecosystems and their inherent biota (**Bonada** *et al.,* **2006**).

Several aquatic biota; e.g. fish, macroinvertebrate, plankton (**Ogidiaka** *et al.,* **2013; Katsiapi** *et al.,* **2016; Gieswein** *et al.,* **2019**) have been examined in assessing the effect of anthropogenic activities on freshwater ecosystem. Among the biota used, macroinvertebrates are the most explored due to their key position in aquatic food chain and food web (**Odume** *et al.,* **2016; Edegbene** *et al.,* **2019b**). Macroinvertebrate-based biological monitoring is done by compiling the structural and functional assemblage of several groups of the macroinvertebrates (**Lenat & Resh, 2001; Adakole & Anunne, 2003; Edegbene** *et al.,* **2020b**). Furthermore, due to the fact that taxa of macroinvertebrates respond differently to anthropogenic influences, a more sophisticated approach is required to determine their level of sensitivity and tolerance to pollution in freshwater ecosystems, including rivers, streams, lakes and reservoirs (**Lenat & Resh, 2001; Edegbene & Arimoro, 2012**). For instance, rivers, streams, lakes and reservoirs sites with high abundance of Diptera and Oligochaeta are mainly interpreted as being polluted as Diptera and Oligochaeta are tolerant to pollution (**Adeogun & Fafioye 2011; Arimoro** *et al.,* **2015**). However, recent studies have shown some species in the Diptera families of Simuliidae and Chironomidae to be sensitive to pollution as their absence were noticed in gold mining and agriculturally impacted sites (**Edegbene** *et al.,* **2015;**

Shimba & Jonah, 2016). Based on variation recorded in the sensitivity and tolerance of some group of macroinvertebrates, identifying and classifying macroinvertebrate taxa as potential biological indicators is of paramount importance at this stage most especially for reservoirs in Sub-Saharan Africa, Nigeria, inclusively, where there is paucity of information regarding the sensitivity and tolerance of macroinvertebrate taxa (**Odume, 2016; Edegbene, 2020**). Therefore, this study was carried out in Ojirami reservoir to address the following specific objectives: (i) to assess the level of impairment to which each sampling site has been subjected; (ii) to visualize the structural assemblage pattern of identified macroinvertebrate taxa in relation to sampling sites and months; and (iii) to identify and classify macroinvertebrate taxa into biological classes for biological health assessments purposes of aquatic ecosystems.

MATERIALS AND METHODS

Description of study area and sites

Ojirami Reservoir in Ojirami Dam is located in Akoko-Edo, Edo State, south-south Nigeria. The reservoir is the oldest man-made lake in the State, constructed between 1971 and 1974, to supply water to the communities of Akoko Edo, Owan and Etsako Local Government Areas at an output capacity of 245m^3/hour. The reservoir lies between latitude $7^o18''$ to $7^o20''$ North and longitude $6^o\ 09''$ to $6^o\ 11$ East of the Equator. It is fed by River Onyami and River Ese which flow from Kogi State down south, in addition to four tributary streams; Somorika, Ebukumu, Ekenireme and Ikekpa (Figure 1). The climatic condition of Ojirami Reservoir falls within the warm-humid tropical climate with two seasons: wet season (April - October) and dry season (November - March). Mean air temperature during the study was 30.5^oC, and total annual rainfall was 1,250.22 mm in 2009 and 1,439.47 mm in 2010 (**Osimen *et al.,* 2017**). The reservoir stores water for Ojirami Dam water treatment plant under the management of the Edo State Urban Water Board.

Human activities within and around the reservoir include swimming, bathing, washing of clothes and utensils. The reservoir is a center of attraction for eco-tourism, and a site for students' excursion and research. Fishing and crop farming activities are the main agricultural activities within the reservoir catchment. According to fishers, fish catches have reduced drastically due to siltation and constant release of water from the reservoir (a management strategy to prevent dam collapse). Other sources of anthropogenic influence around the reservoir catchment include run-off from settlements that are informal, unauthorized domestic use of water in the reservoir, and few industrial sites (upstream) for exploitation of metallic minerals. Pollution from these anthropogenic activities is threatening the structural composition and diversity of aquatic biota and the general health of the Ojirami Reservoir. Vegetation around the reservoir comprises of grasses and woody trees. Dominant macrophytes species in the reservoir include *Leersia hexandra, Polygomum salicifolium and Rhyncospora* species (**Adesina *et al.,* 2010**). The pelagic zone of the reservoir is free of aquatic macrophytes except for few *Nymphae lotus* in the creek. Different shrubs and woody species such as *Danielia oliveri, Albiza zygia* and *Isoberlina doka*, and few oil palm trees are found on the hilly side. Cashew and oil palm (*Elaeis guineensis*) plants are some of the riparian vegetation around the reservoir catchment.
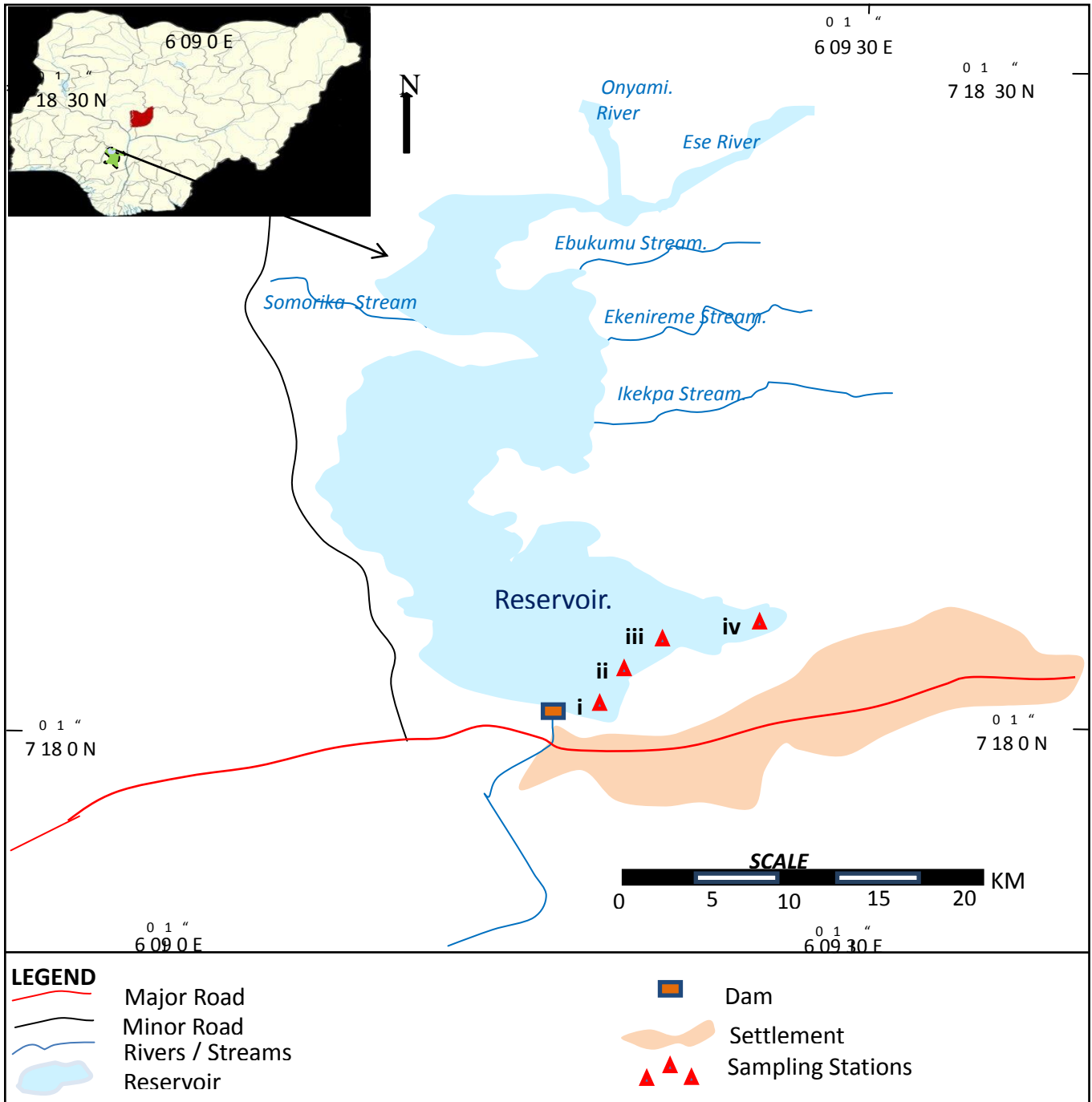
Figure 1: Ojirami Reservoir showing sampling sites (map of Nigeria insert)

Four sites were selected for this study including Sites 1 – 4 along a one kilometer stretch of the eastern part of Ojirami reservoir bankroot biotope. Site 1 was located about 300 m from the Ojirami Dam embankment. The site was located in a section of the reservoir with intense anthropogenic impact in which fetching of water for domestic use, washing of clothes, bathing and swimming, and fishing/fish landing are the major human activities noticed there. There is a foot path from the village which serves as access route by the fish mongers to patronize the fishermen and the villagers for other activities. Site 2 was

located about 200 m after Site 1 with evidence of mild anthropogenic influences such as fishing, bathing, swimming, and washing by inhabitants within the reservoir catchment. Site 3 was located at the middle reach of the reservoir with intense fishing and other agricultural cropping activities. Also pronounced in Site 3 are activities such as bathing, swimming, washing and open defecation. Site 4 was situated at the downstream of the reservoir which is very close to farmlands, and intense fishing activities. Site 4 is also close to industries that engage in metallic mineral exploitation. Additionally, anthropogenic activities evident in Site 3 were all present in Site 4 at an elevated rate.

*Analysis of physico-chemical variables and sampling of macroinvertebrate*s

Physico-chemical variables and macroinvertebrates data were collected once per month for a period of two years (January 2009 – December 2010). Physico-chemical variables analyzed for the study include water temperature, depth, electrical conductivity (EC), turbidity, pH, dissolved oxygen (DO), five day biochemical oxygen demand (BOD_5), total dissolved solids (TDS), total suspended solids (TSS) and phosphate. Water temperature and depth were measured using mercury-in-glass-thermometer (measured in ^oC) and a calibrated stick (measured in metre), respectively. Electrical conductivity and pH were measured with a portable hand held HANNA HI 9913001/1 instrument. Dissolved oxygen was measured at each sampling site per sampling expedition using DO metre (YSI 55), while turbidity was measured using a WGZ-B model turbidity metre. Water samples were collected in a sterile 50ml sample bottles on each sampling occasion for the determination of phosphate, TDS, TSS and BOD_5 in the laboratory following **APHA (1998)** methods.

Simultaneously, with physico-chemical variables sampling, macroinvertebrates were sampled monthly by using a D-frame kick net of 500 μm mesh size as modified in the study of **Lazorchak *et al.* (1998)**. Macroinvertebrate samples were collected in various biotopes which include bank roots, silt, sand and stones. Three different samples of macroinvertebrate were collected for a period of five minutes at each biotope, thus a total of 12 samples (three samples X four biotopes) were collected from each site. On each sampling occasion all samples collected from different biotopes were pooled together as one composite sample, and thereafter preserved in 10% formalin in transparent well labeled containers before transferring to the laboratory for sorting, identification and counting. Identification of macroinvertebrates were estimated to lowest possible taxa using a binocular microscope under a magnification of X10 using available macroinvertebrate pictorial guides of **Merritt and Cummins (1996); Day *et al.* (2003)** and **de Moor *et al.* (2003)**, and consultation of expert macroinvertebrate taxonomist. Voucher samples of macroinvertebrates were deposited at the Laboratory of Zoology Department of Ambrose Alli University, Ekpoma, Nigeria for future reference.

Descriptive statistic (mean $\pm$ standard deviation, and range) of physico-chemical variables were computed using Palaentological statistical package (PAST) (**Hammer *et al.,* 2001**). Global significant differences of each physico-chemical variable between sampled sites were tested using one-way permutational multivariate analysis of variance (PERMANOVA). Confirmation of significant differences between physico-chemical variables and sampled sites was recorded using one way analysis of variance (ANOVA) based on the calculated mean values of physico-chemical variables. Following the results

of ANOVA, a post hoc test, using Turkey Honestly Significant difference (HSD), was computed to confirm sites that differed, based on means of the physico-chemical variables. Assumption and homogeneity of variance of the physico-chemical variables across the four sites were checked using Shapiro-Wilk's and Levene's tests (**Derrick** *et al.,* **2018**). PERMANOVA was computed using Primer software package version 6, and ANOVA, HSD and test for homogeneity (Shapiro-Wilk's and Levene's tests) were computed using PAST statistical package (**Hammer** *et al.,* **2001**). The correlation between the sampled sites and physico-chemical variables were visualized with Principal Component Analysis (PCA) using correlation matrix function. PCA was computed using vegan package within the R-programming environment (**R Development Core Team, 2019**).

Structural assemblage of macroinvertebrates in relation to sampling sites and months

The structural assemblage patterns of macroinvertebrate taxa throughout the study period in the four sampling sites were visualized using non-metric multidimensional scaling (NMDS) based on Bray-Curtis similarity. Macroinvertebrate taxa data were logarithmically transformed (log x +1) to avoid skewness of highly abundant taxa with regards to sampling sites. The NMDS were computed using PAST statistical package (**Hammer** *et al.,* **2001**).

Relating taxa of macroinvertebrate and physico-chemical variables

Canonical correspondence analysis (CCA) was used to correlate macroinvertebrate taxa and physico-chemical variables in a bid to visualize their pattern of distribution along the four sampling sites. Macroinvertebrate datasets were log (x+1) transformed and subjected to a test of unimodality using detrended correspondence analysis (DCA), and the DCA confirmed the dataset to be unimodal by returning a gradient length of >3.0 (**ter Braak, 1995**), hence CCA was employed for computing the ordination. A Monte Carlo permutation test at 999 permutation arguments was further computed to reveal the significant differences among the taxa, physico-chemical variables and sites on the CCA axes (**Legendre & Legendre, 2012**). The CCA ordination and Monte Carlo permutation test were conducted on the vegan package using R-statistics (**Oksanen** *et al.,* **2019; R Development Core Team, 2019**).

Identifying and categorizing potential sensitive and tolerant macroinvertebrate taxa

The 28 macroinvertebrate taxa identified in the studied reservoir were classified into eight biological classes which include very tolerant (VT), tolerant (T), moderately tolerant (MT), slightly tolerant (ST), slightly sensitive (SS), moderately sensitive (MS), sensitive (S) and very sensitive (VS). Taxa sensitivity and tolerance were derived by correlating physico-chemical variables with macroinvertebrate taxa along the four sampling sites using CCA. Macroinvertebrate taxa scores were extracted from the CCA first axis owing to the fact that the CCA first axis explained over 99% variance of the total CCA variance. The exact biological classification was performed by calculating the inter-taxa distance of each macroinvertebrate taxa following a three-step approach. Firstly, by subtracting the coordinate scores of the lowest scoring taxa from the highest scoring taxa among the 28 identified macroinvertebrate taxa, and subsequently coordinate

scores of the remaining taxa that were subtracted from the highest scoring taxa. Secondly, the calculated inter-taxa distances were further converted to percentage distances, then thirdly, a percentile distribution was used to classify macroinvertebrate taxa into one of the eight biological classes; namely, VT, T, MT, ST, SS, MS, S and VS with corresponding percentile distribution of 100th, $<100 - 90^{th}$, $<90 - 70^{th}$, $<70 - 50^{th}$, $<50 - 40^{th}$, $<40 - 20^{th}$, $<20 - 10^{th}$ and $<10 - 0^{th}$, respectively. The approach employed in this study in classifying macroinvertebrate taxa into biological classes has earlier been used by **Murphy et al. (2013)** and **Odume et al. (2016)** who calculated distances of species along Axis 1 of CCA ordination. Similarly, **Edegbene *et al.* (2019a and 2020b)** recently applied this approach in categorizing river sites into pollution impact categories by extracting the coordinate scores of river sites on the first axis of PCA ordination.

RESULTS

### *Descriptive statistical significance and correlation of physico-chemical variables with sampled sites*

The mean values of physico-chemical variables were not significantly different ($P > 0.05$) in all the sites sampled, except for DO and phosphate (Table 1).The decrease in value of DO in Site 4 (3.5 ± 1.23 mgl^{-1}) corresponds to an exceptionally increased value of nutrient (phosphate) in Site 4 (17.38 ± 5.93 mgl^{-1}). Marked increase in the mean values of TSS and BOD$_5$ was observed in Site 4 (Table 1) compared to the remaining three sites. Mean values of water temperature and pH were relatively similar in all the four sampled sites. Turbidity was lower in Site 3 (9.25 ± 10.45 NTU) compared to Sites 1, 2 and 4. Generally, physico-chemical, variables analysed in the present study, showed a fast deteriorating water quality in Site 4 compared to the other three sampled sites. Site 1 showed a relatively less impacted water quality condition among the four sites sampled.

Axis 1 of the PCA ordination explained a total variance of 99.84%, with an Eigen value of 3.99. Site 1 was strongly positively correlated with depth, DO and water temperature on Axis 1, although the influence of depth and DO in structuring Site 1 was relatively higher than water temperature (Figure 2). Further on Axis 1, Sites 3 and 4 were strongly correlated with increasing concentration of BOD$_5$, phosphate, turbidity, EC and TDS (Figure 2). Specifically, the structuring of Sites 3 and 4 level of impact were mainly influenced by increasing concentration of BOD$_5$, phosphate and turbidity. Axis 2 of the PCA ordination explained 0.082% of the total PCA variance. Site 2 was strongly positively correlated with TSS and pH on Axis 2 (Figure 2). Based on the result revealed by the descriptive statistic and PCA ordination computed, Site 1 indicates a relatively less impacted site, and Site 2 was moderately impacted and Sites 3 and 4 were heavily impacted.

Table 1: Mean± Standard Deviation (excluding pH) of physico-chemical variables sampled in Ojirami Reservoir between January 2009 and December 2010. P-value and F-value as revealed by one-way analysis of variance (ANOVA). Physico-chemical variables per site with the same letters indicate no significant differences (P>0.05) as revealed by post hoc test (Turkey honestly significant difference, HSD).

| Physico-chemical variables	Site 1	Site 2	Site 3	Site 4	P-value	F-value
Water temperature (^{o}C)	28.92±1.62 (26 – 31)[a]	28.92±1.56 (27 – 31) [a]	29.42±2.94 (24 – 34)[a]	29.25±2.42 (25 – 34)[a]	0.9269	0.1535
Depth (m)	2.60±0.91 (0.65 – 3.5)[a]	2.14±0.72 (0.62 – 3.0)[a]	1.96±0.57 (0.73 – 2.8)[a]	2.22±0.81 (0.62 – 3.5)[a]	0.2198	1.531
pH	(5.2 – 8.2)	(5.7 – 8.0)	(6.9 – 8.5)	(6.0 – 8.5)	0.2403	1.453
Electrical Conductivity µScm^{-1}	110±32.47 (50 – 150)[a]	110.83±31.75 (50 – 140)[a]	108.33±31.57 (50 – 150)[a]	106.67±36.01 (50 – 150)[a]	0.9901	0.03762
Turbidity (NTU)	16.58±21.99 (0 – 76)[a]	17±22.25 (1 – 66)[a]	9.25±10.45 (0 – 29)[a]	15.08±21.35 (1 – 65)[a]	0.7544	0.399
TDS (mgl^{-1})	54.08±15.92 (25 – 70)[a]	55.83±16.73 (25 – 77)[a]	54.67±16.37 25 – 80)[a]	52.58±19.08 (25 – 79)[a]	0.973	0.07522
TSS (mgl^{-1})	8.58±16.54 (0 – 52)[a]	9.25±16.09 (0 – 46)[a]	8.92±13.59 (0 – 48)[a]	15.42±31.31 (0 – 105)[a]	0.8247	0.3007
DO (mgl^{-1})	6.2±1.15 (4.3 – 6.7)[a]	4.43±0.83 (3.4 – 5.8)[b]	4.23±0.73 (3.1 – 5.4)[b]	3.5±1.23 (2.9 – 5.7)[c]	0.746	0.4108
BOD$_5$ (mgl^{-1})	1.51±0.9 (0.1 – 2.9)[a]	1.52±0.98 (0.4 – 3.5)[a]	1.39±0.78 (0.4 – 2.8)[a]	1.60±1.34 (0.5 – 5.2)[a]	0.63	0.5819
Phosphate (mgl^{-1})	7.57±6.14 (1 – 20.1)[a]	7.55±5.48 (0.5 – 14.2)[a]	7.41±5.37 (1.28 – 14.8)[a]	17.38±5.93 (15.9 – 17.9)[b]	0.9997	0.003448
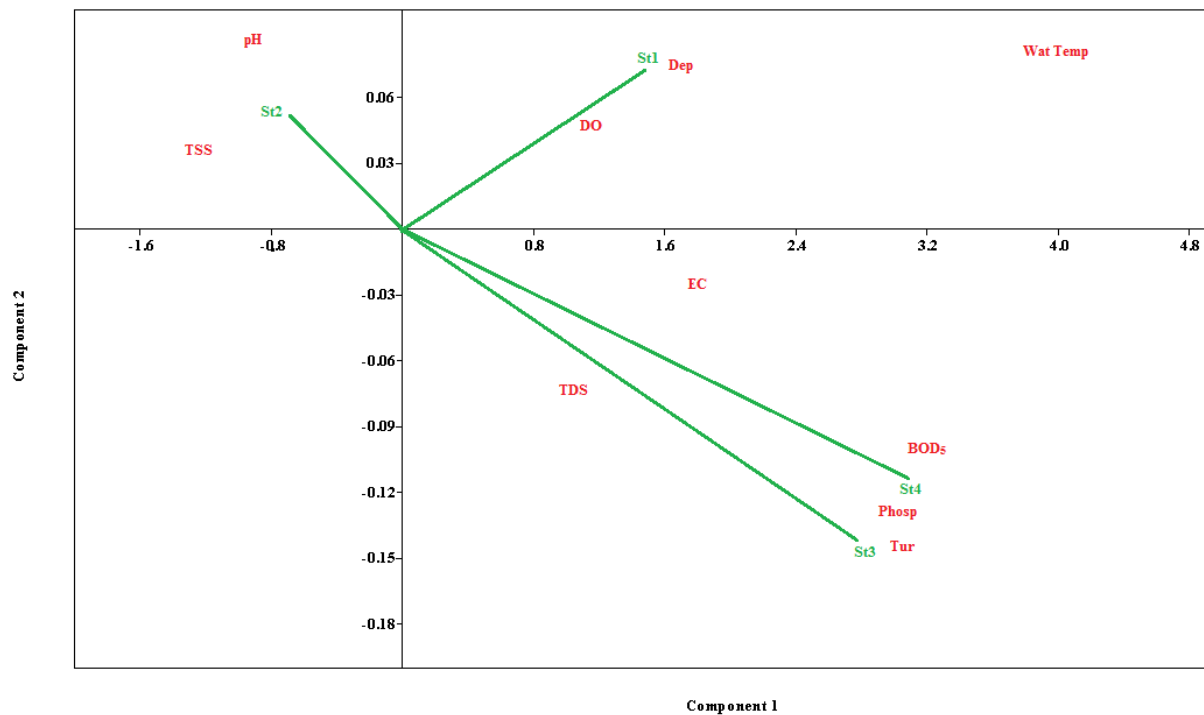
Figure 2: PCA showing correlation matrix between sampled sites and physico-chemical variables in Ojirami Reservoir during the study period (January 2009 – December 2010).

Physico-chemical variables abbreviation: Wat Temp = water temperature, Dep = depth, Tur = turbidity, EC = electrical conductivity,   DO = dissolved oxygen, BOD_5 = five day biochemical oxygen demand, TDS = total dissolved solids, TSS = total suspended solids, Phosp = phosphate.

Structural assemblage of macroinvertebrates

Twenty-eight (28) taxa of macroinvertebrate in seven phyla/orders were recorded during the entire study period (Appendix). Order Diptera dominated the macroinvertebrate assemblage represented by nine taxa, followed by Order Ephemeroptera represented by seven taxa. The absolute abundance of Diptera taxa was generally higher at Site 4. *Chironomus* spp. and *Cricotopus* spp. were the most preponderant taxa in Site 4 among the recorded Diptera taxa (Appendix). Mollusca taxa absolute abundance were exceptionally higher at Site 4 with *Mytilus* spp. , having 416 individuals which represent 47.4% of the total number (878) of macroinvertebrate taxa individuals recorded in Site 4. Sites 1, 2 and 3 had 370, 326 and 408 individual taxa, respectively (Appendix).

On the NMDS computed, macroinvertebrate taxa assemblage in Sites 1 and 2 formed cluster B portraying similarity in the assemblage structure of taxa recorded in Sites 1 and 2 (Figure 3). Cluster C was formed by taxa assemblages in Sites 3 and 4, and Cluster A formed a mixture of macroinvertebrate taxa assemblages from all the sites sampled with Sites 3 and 4 distinctly forming most of the Cluster A assemblage (Figure 3).
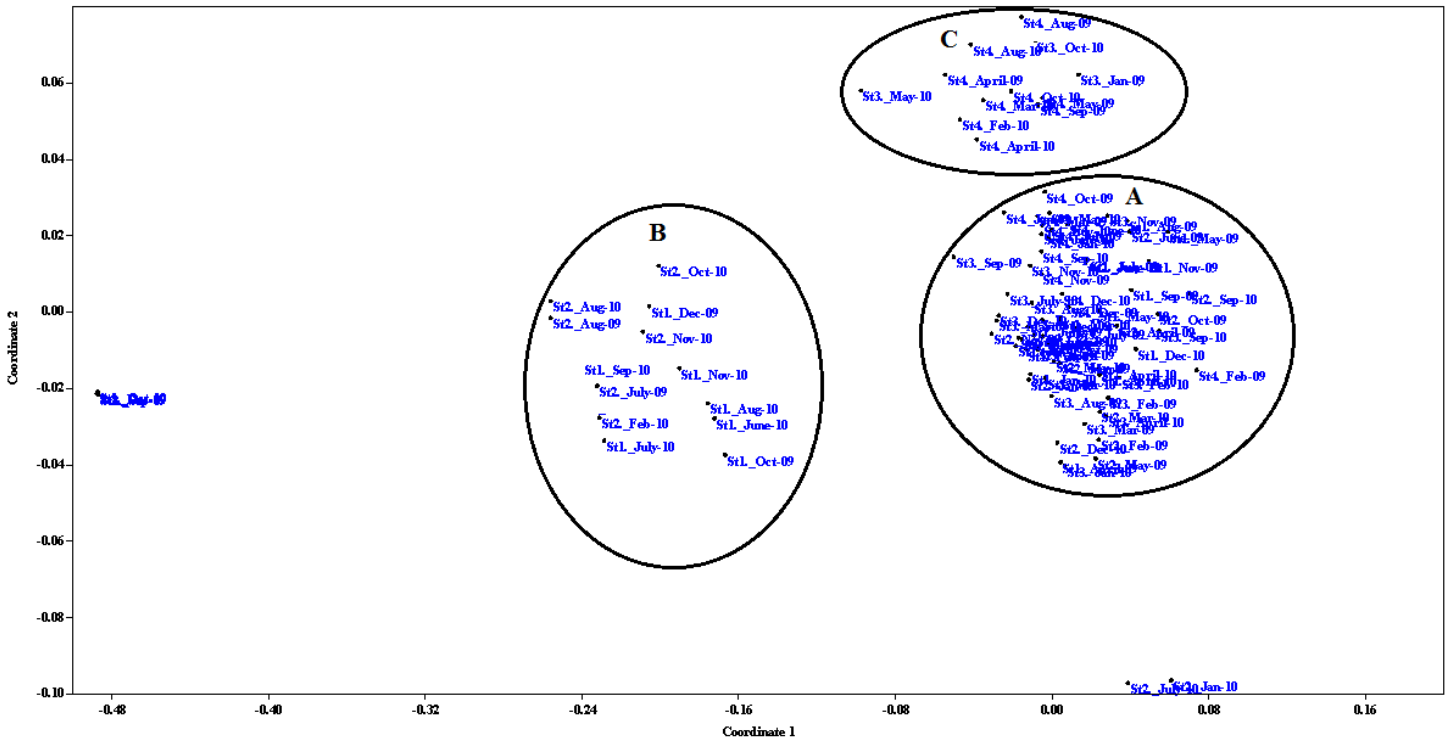
Figure 3: Non-metric multidimensional scaling based on Bray-Curtis similarity index showing clusters of sites based on assemblage of macroinvertebrate taxa in Ojirami Reservoir between January 2009 and December 2010. Cluster A formed mainly samples collected at Sites 1 and 2; Cluster B formed samples collected majorly from Sites 3 and 4 and Cluster C consist mainly of samples collected at Site 4**.**

Relationship between macroinvertebrate and physico-chemical variables

The Eigen values of Axes 1 and 2 of the CCA triplot was 0.030 and 0.0013, respectively (Figure 4). Axis 1 explained 99.56% of total variance and Axis 2 explained 0.4413% variance of the CCA biplot. Monte Carlo test at 999 permutation argument revealed a P-value of 0.54 and 0.173 for Axes 1 and 2 respectively which shows that no significant difference existed (P>0.05) between the first two axes of the CCA biplot, with regards to correlation of macroinvertebrate taxa and physico-chemical variables analysed during the study period. Depth and DO were strongly positively correlated with *Caenis* spp., *Hydrophlebia* spp., *Strictochironomus* spp., *Siphlonurus* spp., *Centroptilum* spp. on Axis 1 and *Cricotopus* spp., *Chironomus* spp., *Corynoneura* spp. and *Ephemerella* spp. were slightly positively correlated with depth and DO in Site 1 on the first axis of the CCA biplot (Figure 4). These taxa were negatively correlated with increasing concentration of turbidity and TSS (Figure 4). *Mytilus dubia* was strongly positively correlated with turbidity in Site 2 (Figure 4). Increasing concentration of BOD_5, EC and phosphate were strongly positively correlated with *Pseudagrion* spp.,

Caridinagabonensis, *Corixa* spp., *Culexpipiensfatigan*, *Notonecta* spp., *Ischura* spp. and *Chaoborus* spp. in Sites 3 and 4 on the first CCA axis (Figure 4).
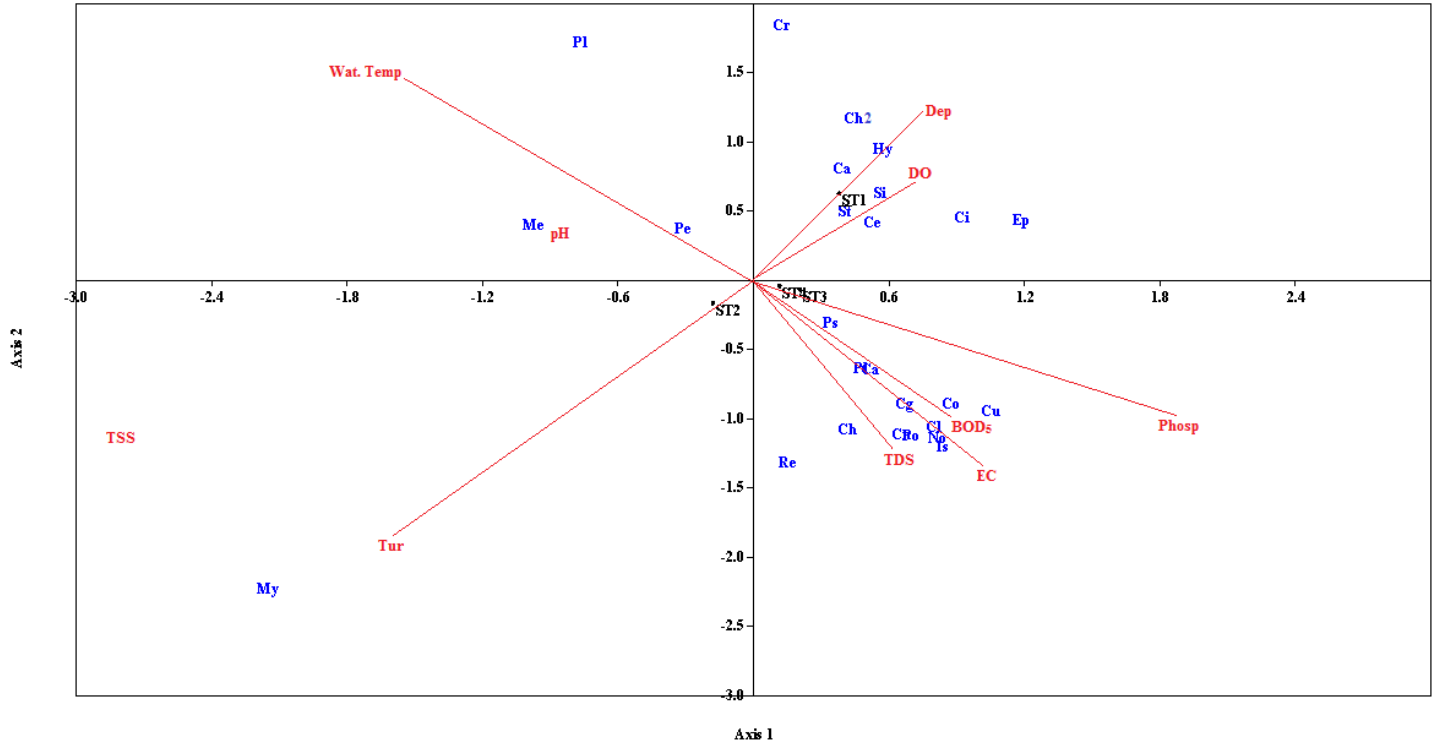
Figure 4: CCA ordination plot showing the first two axes of the correlation between analyzed physico-chemical variables and macroinvertebrate taxa in Ojirami Reservoir during the study period (January 2009 – December 2010). **See Table 2 for macroinvertebrate taxa code. Physico-chemical variables abbreviation:** Wat Temp = water temperature, Dep = depth, Tur = turbidity, EC = electrical conductivity, DO = dissolved oxygen, BOD_5 = five day biochemical oxygen demand, TDS = total dissolved solids, TSS = total suspended solids, Phosp = phosphate.

Identifying and classifying potential sensitive and tolerant macroinvertebrate taxa

Potential sensitive and tolerant macroinvertebrate taxa were determined using the taxa coordinate positions along the first axis of the CCA because the first axis of the CCA explained over 99% of the total variance explained by the CCA first two axes which indicates a good ordination. Taxa were classified from very tolerant (VT) to very sensitive (VS) using a percentile distribution range of 100th and $<10-0^{th}$,respectively. *Mytilus dubia* was the only taxon that was classified as very tolerant because it had a % inter-taxa distance of 100 corresponding to 100th percentile (Table 2). Two taxa; each was classified as tolerant (*Melanoides* spp. and *Corynoneura* spp.) and moderately tolerant (*Nais* spp. and *Pentaneura* spp. - Table 2).

For the sensitive taxa, only *Centroptilum* spp. was classified as very sensitive taxon, owing to the fact that it had a % inter-taxa distance of 0% corresponding to the percentile distribution range of $<10-0^{th}$ (Table 2). *Strictochironomus* spp. and *Siphlonurus* spp. were both classified as sensitive taxa having % inter-taxa distance of 13.14376 % and 18.9614 %, respectively, falling into the percentile distribution of $<20-10^{th}$ (Table 2). Overall, more of the recorded macroinvertebrate taxa were classified as moderately sensitive

(Table 2). Of the 11 taxa classified as moderately sensitive, four were in the Order Diptera which include *Protoplasa* spp., *Cricotopus* spp., *Chaoburus* spp., *Culexpipiens fatigan* (Table 2). Two taxa, each of which was moderately sensitive in the Orders Odonata, Hemiptera and Ephemeroptera. They include *Coenagrion* spp. and *Ischura* spp. for Odonata; *Notonecta* spp. and *Corixa* spp. for Hemiptera and *Ephemerella* spp. and *Cleon* spp. for Ephemeroptera (Table 2). *Caridina gabonensis* was the only Crustacea taxon that was moderately sensitive (Table 2).

Table 2: Identified and classified macroinvertebrate taxa in Ojirami Reservoir. Taxa biological classes were derived from the coordinate positions of taxa along CCA first axis in Figure 4.

Macroinvertebrate taxa	Taxa code	Coordinates of taxa on CCA Axis 1	Inter-taxa distance	% inter-sites distance	Taxa biological class
Mytilus dubia	My	-1.5837	3.3988	100	VT
Melanoides spp.	Me	-1.26505	3.08015	90.62463	T
Corynoneura spp.	Cr	-1.2518	3.0669	90.23479	T
Polypedilum spp.	Po	-1.16338	2.97848	87.63328	MT
Nais spp.	Na	-0.63047	2.445567	71.95384	MT
Pentaneura spp.	Pe	-0.55232	2.367417	69.6545	ST
Hydrophlebia spp.	Hy	-0.42072	2.23582	65.78263	ST
Chironomus spp.	Ch2	-0.07733	1.892431	55.67938	ST
Caenis spp.	Ca	0.092198	1.722902	50.69148	ST
Pseudagrion spp.	Ps	0.170669	1.644431	48.38269	SS
Renatra spp.	Re	0.270536	1.544564	45.44439	SS
Baetis spp.	Be	0.328868	1.486232	43.72814	SS
Plathemis spp.	Pl	0.41074	1.40436	41.31929	SS
Caridina africanus	Cf	0.436192	1.378908	40.57044	SS
Chaoborus spp.	Ch	0.496981	1.318119	38.78189	MS
Cricotopus spp.	Ci	0.534401	1.280699	37.68092	MS
Caridina gabonensis	Cg	0.613889	1.201211	35.34221	MS
Coenagrion spp.	Cn	0.672056	1.143044	33.63081	MS
Protoplasa spp.	Po	0.703866	1.111234	32.69489	MS
Notonecta spp.	No	0.789071	1.026029	30.18798	MS
Cleon spp.	Cl	0.793947	1.021153	30.04452	MS
Ischura spp.	Is	0.808573	1.006527	29.61419	MS
Ephemerella spp.	Ep	0.848827	0.966273	28.42983	MS
Corixa spp.	Co	0.869248	0.945852	27.829	MS
Culexpipiensfatigan	Cu	0.992233	0.822867	24.21052	MS
Siphlonurus spp.	Si	1.17064	0.64446	18.9614	S
Strictochironomus spp.	St	1.36837	0.44673	13.14376	S
Centroptilum spp.	Ce	1.8151	0	0	VS

Biological classes: VT = very tolerant, T =tolerant, MT = moderately tolerant, ST = slightly tolerant, SS = slightly sensitive, MS = moderately sensitive, S = sensitive, VS = very sensitive.

DISCUSSION

Physico-chemical variables

Potential bioindicator macroinvertebrate taxa were identified and classified in a reservoir in southern Nigeria. Twenty-eight (28) macroinvertebrate taxa were recorded and related to 10 physico-chemical variables using multivariate approach. Elevated pollution indicating physico-chemical variables such as BOD_5, nutrient (phosphate) and TSS were recorded in Site 4, which indicate that Site 4 is seriously deteriorating. Elevated values of pollution indicating physico-chemical variables can result in water quality deterioration in freshwater ecosystems by posing potential stress to the structural assemblages of aquatic organisms. Similar studies have stressed on the negative effects of elevated values of pollution indicating physico-chemical variables in freshwater ecosystems located in highly industrialized and urbanized catchment (**Odume *et al.,* 2016; Edegbene et al., 2019a**). Increasing concentration of nutrienst in freshwater ecosystems can result to eutrophication occasioned by algal bloom and its consequent alteration of the structural assemblage of aquatic organisms (**Marchand *et al.,* 2012**). The depletion of dissolved oxygen concentration in Site 4 may be explained by increasing concentration of nutrient and other pollution indicating physico-chemical variables in Site 4. Further buttressing the reason for DO concentration depletion in Site 4 in the present study can be explained by the negative correlation of DO with phosphate, BOD_5, turbidity, EC and TDS on the PCA ordination. The elevated human activities in the catchment of Site 4 could be a major contributor to the decreased DO concentration and increased concentration of nutrient and other pollution indicating physico-chemical variables. Similar study has reported elevated values of nutrients, EC, BOD_5 and heavy metals in an organic effluent discharge site of a river located in a highly industrialized area (**Odume *et al.,* 2016**). The strong positive correlation of increasing DO concentration with Site 1 on the PCA is a pointer to the fact that Site 1 is relatively less impacted compared to Sites 4, 3 and 2.

Macroinvertebrate structural assemblage and selection of potential indicator taxa

Assessing the ecological health of freshwater ecosystems is of paramount importance as this would aid in biomonitoring, managing and conservation of aquatic biodiversity within freshwater ecosystems. Therefore, a study of this kind which identifies and classifies simple and cost effective potential indicator macroinvertebrate taxa would help in reducing the cost of monitoring freshwater ecosystems, most especially in Sub Sahara Africa that is still behind in the science of developing biomonitoring tools for ecological assessment of freshwater ecosystems. Most developed countries have developed both regional and national biological tools for assessing freshwater ecosystems (**Gieswein *et al.,* 2019; Shull *et al.,* 2019**). Reversely is the case for countries in Sub Sahara Africa including Nigeria, as they still engage in the use of aquatic macroinvertebrate taxa abundance and diversity in judging the level of deterioration that freshwater ecosystems are undergoing (**Adakole & Anunne, 2003; Arimoro *et al.,* 2015**). In this study, a more sophisticated approach was explored in awarding the level of sensitivity and tolerance of macroinvertebrate taxa to pollution effects. The approach employed in this study has been successfully used to award

tolerance scores to macroinvertebrate taxa in Europe (**Murphy *et al.,* 2013**) and South Africa (**Odume *et al.,* 2016**). Further, **Edegbene et al. (2019a; 2020)** have also used similar approach in categorizing river sites into pollution impact categories in the Niger Delta area of Nigeria. The multivariate approach employed in the present study will add to and solidify on the existing potential macroinvertebrate taxa that have been suggested to be either sensitive to or tolerant of pollution gradient (**Edegbene *et al.,* 2015; Shimba & Jonah, 2016; Edegbene, 2020**).

Earlier studies had reported macroinvertebrate taxonomic metric, percent Ephemeroptera+Plecoptera+Trichoptera (%EPT) and percent Diptera+Oligochaeta (%DO) , sensitive to and tolerant of increasing pollution gradient respectively (**Andem *et al.,* 2014; Edegbene *et al.,* 2015**). However, the multivariate approach employed in the present study indicated four out of the 11 Diptera taxa recorded to be moderately sensitive, while the taxa include *Protoplasa* spp., *Cricotopus* spp., *Chaoborus* spp. and *Culex papiens fatigan*. This corroborates with **Shimba and Jonah (2016)** findings in an agriculturally disturbed river in Tanzania were some taxa in the Order Diptera were reported to be dominant in relatively undisturbed site.

Mytilus dubia (Mollusca) was classified as a very tolerant taxon, while *Nais* spp. (Annelida) and *Melanoides* spp. (Mollusca) as tolerant taxa. This is expected as several studies have reported most taxa in the Phyla Mollusca and Annelida to be tolerant of pollution (**Andem *et al.,* 2014**). The three Hemiptera taxa recorded were either slightly sensitive or moderately sensitive. Hemipterans are mainly predators making them specialist feeders, and their numbers are expected to decrease with increasing concentration of pollution indicating environmental variables (**Doledec & Statzner, 2008**). *Centroptilum* spp. in the Order Ephemeroptera was the only very sensitive taxon among the macroinvertebrate taxa recorded in Ojirami reservoir, and the remaining taxa of the Order Ephemeroptera were slightly sensitive, moderately sensitive and sensitive except for *Hydrophlebia* spp. and *Caenis* spp. that were slightly tolerant. Some families of the Order Ephemeroptera; e.g. Baetidae have been reported to be capable of becoming resilience in the face of organic pollution (**Merrit *et al.,* 1996**). The ability of Baetidae to feed by collecting-gathering and scrapping of exposed surfaces have been reported to make them confer resilience in polluted freshwater ecosystems (**Edegbene *et al.,* 2019b**). Therefore, the classification of *Caenis* spp. ,as slightly tolerant of pollution, can be explained by its multiple feeding habits just like the baetids.

Overall, the CCA model, showing the relationship between macroinvertebrate taxa and physico-chemical variables along the four sampling sites, further supports the categorization of potential indicator macroinvertebate taxa identified in this study. For example, on the CCA model increasing DO concentration was strongly positively correlated with *Centropitilum* spp. (very sensitive taxon), *Siphlonurus* spp. and *Strictochironomus* spp. (sensitive taxa). On the other hand, *Polypedilum* spp. (moderately tolerant),*Chironomus* spp. (slightly tolerant) were positively strongly correlated with increasing pollution indicating physico-chemical variables.

CONCLUSION

Potential sensitive and tolerant macroinvertebrate taxa were identified and classified in a reservoir in southern Nigeria. The multivariate approach employed in this study proved effective as the PCA categorized the four sampling sites of the reservoir along a pollution gradient. Site 1 was the less impacted site and Site 4 was the most impacted site. Elevated mean values of nutrient (phosphate), BOD_5, turbidity and TDS were noticed in Site 4, further buttressing the level of pollution Site 4 of the reservoir is undergoing. The CCA model computed showed that most of the taxa were deemed moderately sensitive to pollution. Diptera taxa such as *Chaoborus* spp., *Strictochironomus* spp., *Culex pipiens fatigan* and *Protoplasa* spp. were all moderately sensitive to pollution. *Centroptilum* spp. (Ephemeroptera) was the only very sensitive taxon, and *Mytilus dubia* (Mollusca) was the only very tolerant taxon. Overall, the study was able to elucidate the usefulness of identifying and classifying potential biological indicator for effective monitoring and management of freshwater ecosystem, most especially, in Sub Sahara Africa where studies of this kind are still scanty.

DECLARATION OF INTEREST

There is no conflicting interest to declare in this work.

AUTHORS' CONTRIBUTION STATEMENT

Dr. Osimen and Prof. Elakhame conceptualizes the work. Dr. Edegbene design the structure of the manuscript. Dr. Osimen did the macroinvertebrates and physico-chemical variables sampling and analyses. Dr. Edegbene performed the statistical/data analyses. The first draft of the manuscript was written by Dr. Osimen and Dr. Edegbene. Dr. Osimen, Dr. Edegbene and Dr. Izegaegbe reviewed and edited the manuscript and the manuscript was finalized by Dr. Edegbene. Prof. Elakhame supervised the entire work.

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APPENDIX

Appendix: Structural assemblage of macroinvertebrates in Ojirami Reservoir, Edo State, Nigeria during the study period (January 2009 – December 2010)

Phylum/Order	Taxa	Site 1	Site 2	Site 3	Site 4
Annelida	Nais spp.	0	8	18	38
Ephemeroptera	*Beatis* spp.	24	14	19	12
	Ephemerella spp.	7	7	17	13
	Siphlonurus spp.	0	2	2	9
	Caenis spp.	45	31	25	29
	Centroptilum spp.	13	7	0	0
	Cleon spp.	5	4	5	0
	Hydrophlebia spp.	8	0	0	0
Hemiptera	*Renatra* spp.	0	3	0	0
	Corixa spp.	4	0	3	0
	Notonecta spp.	0	0	0	2
Odonata	*Plathemis* spp.	3	0	0	0
	Pseudagrion spp.	7	2	0	4
	Coenagrion spp.	0	5	5	5
	Ischura spp.	0	3	3	9
Diptera	*Chironomus* spp.	48	21	30	87
	Polypedilum spp.	45	18	16	3
	Strictochironomus spp.	8	4	30	26
	Protoplasa spp.	0	0	0	1
	Corynoneura spp.	27	22	0	2
	Cricotopus spp.	23	8	22	27
	Pentaneura spp.	26	15	5	10
	Chaoborus spp.	0	7	7	0
	Culex pipiens fatigan	0	0	7	6
Crustacea	*Caridina africanus*	6	0	0	1
	Caridina gabonensis	3	0	0	3
Mollusca	*Melanoides*	68	145	194	175
	Mytilus dubia	0	0	0	416
Total no. of taxa		**370**	**326**	**408**	**878**