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COMPOSITION AND DIVERSITY OF MACROINVERTEBRATES OF A TROPICAL RESERVOIR, SOUTHWESTERN NIGERIA

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Abstract
Benthic macroinvertebrates of Dandaru Reservoir were sampled at five stations monthly from April 2015 to March 2016 using van Veen grab (for open water biotope) and modified kick sampling method(for bankroot biotope). A total of 20 macroinvertebrates taxa comprising of 7,945 individuals were recorded in the reservoir during the period of study. Gastropods (76.4%) were the most abundant followed by insects (15.7%) and arachnids (0.89%) being the least. Melanoides tuberculata, Bellamya unicolor and Bulinus differed significantly ($p< 0.05$) between the sampling stations. More macroinvertebrates were encountered in the rainy season (51.6%). The Margalef's Index, Shannon diversity, Evenness index and Simpson dominance index were higher at station 1. The domination of the macroinvertebrate community structure of Dandaru Lake by pollution tolerant taxa and the low diversity index indicates the lake is under pollution stress.

Key Words: *Macrozoobenthos, Bankroot biotope, Diversity indices, Spatial variation*

Introduction

Dandaru Reservoir was created by weir construction on Ogunpa River, a third order stream draining the densely populated eastern part of Ibadan Nigeria. The Ogunpa River receives input from various anthropogenic activities (domestic activities, car wash, Mechanic workshop, cement/ block industry, Secretariat Office complexes) along its course and the reservoir also from the Agodi Zoological Garden and University College Hospital, Ibadan. Previous studies on the Dandaru Reservoir were on impact assessment of pollution from metal concentrations in water and fish (Abiona *et al*., 2012); microbial flora of the gastro-intestinal tract of *Clarias gariepinus* (Jimoh *et al*., 2014) and development of reproductive biomarkers for monitoring endocrine disruption in Tilapias (Ejirinde *et al*., 2014). There is paucity of information on the composition and diversity of macroinvertebrates of the lake. Macroinvertebrates as an integral part of the aquatic ecosystem are used as indicators of pollution, when testing for

the health status of a water body. This study was designed to investigate the macrobenthic fauna of the Dandaru Lake.

Materials and Methods

Study Area

Dandaru Reservoir located in Ibadan, Oyo State lies between latitudes 7°24 '16'N and 7°24' 27'N and longitudes 3°53' 32'E and 3°54' 30'E (Fig. 1). The total surface area of the lake is about 4 hectares. The reservoir has its source from Ogunpa River that flows through places including Ashi, Bodija, State Secretariat, before being dammed after the popular Agodi Zoological Gardens. It is stocked with different species of fish such as *Gymnarchus, Tilapia, Clarias and Heterotis* species and also supplies water to University College Hospital. The study area is located within the equatorial region which has a tropical wet and dry climate. Its wet season runs from March through October, while November to February forms the dry season. The mean total rainfall is 1420.06mm having a mean maximum temperature of 26.46°C, minimum 21.42 °C and the relative humidity is 74.55%. Macroinvertebrates were collected from the bankroot and open water biotopes of five stations.

Station 1: This station was located upstream of the reservoir (Latitude 07° 24.561'N; Longitude 003° 53.039'E). The substratum was composed of fine clear sand with small stones. It had an average depth of 38cm.

Station 2: This station was located close to the Agodi Zoological Garden (Latitude 07° 24.396'N; Longitude 003° 53.994'E). Effluents from University College hospital, Ibadan enter the reservoir here. Vegetation here includes *Ipomea aquatic* and *Panicum maximum.* The substratum was composed of mud, decaying organic matter, saw dust particles. It had an average depth of 37.6cm.

Station 3: This station was at the centre of the reservoir (Latitude 07° 24.371'N; Longitude 003° 53.884'E). Vegetation here includes plantain (*Musa paradisiacal*) and elephant grass. Fishing activities occurred here with average depth of 40.5cm. The substratum was composed of decaying plant particles and organic matters mixed with mud and clay.

Station 4: was 1.55 km downstream of station 3 (Latitude 07° 24.332'N; Longitude 003° 53.923'E). The substratum was composed of dark fine sand towards the bank of the river. The average depth of the reservoir was 33.2cm. Vegetation here includes *Ipomea aquatic* and *Panicum maximum.*

Station 5: This station was situated downstream of station 4. (Latitude 07° 24. 277'N; Longitude 003° 53.915'E). The substratum was dark fine sand with decaying leaves and fallen logs. The average depth was 48cm. Vegetation here includes *Ipomea aquatic* and *Panicum maximum.*

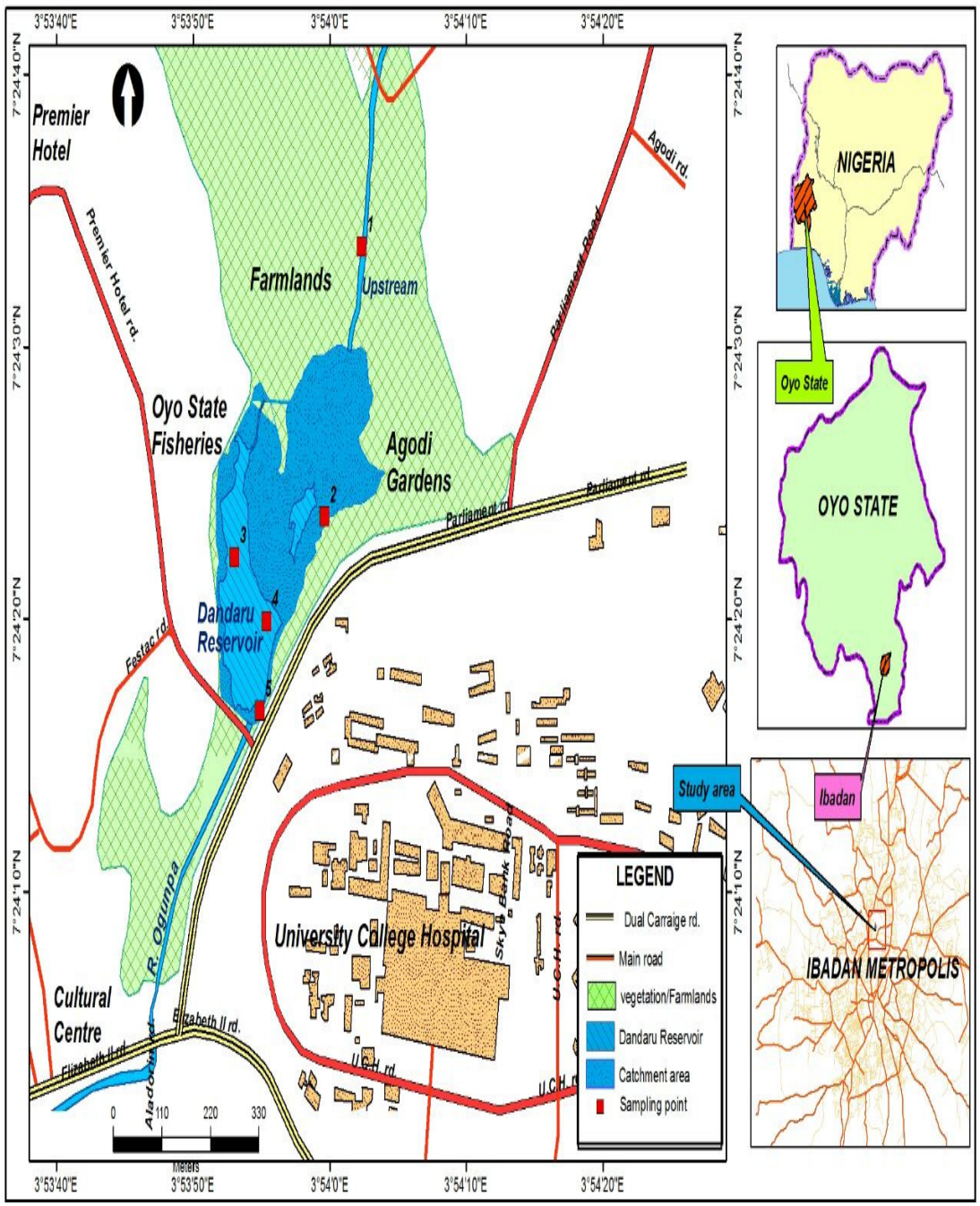

Fig.1: Map of Dandaru Reservoir

Macroinvertebrates Sampling

Macroinvertebrate samples were collected monthly from April 2015 to March, 2016 between 1000hr and 1500hr on each sampling day. The "kick sampling technique" by Lenat *et al*. (1981) and modified by Kellogg (1994) described by Ikomi *et al*. (2005) was used in collecting macroinvertebrates from the bankroot biotope of each station. The

substratum and emergent vegetation in each station were vigorously agitated by kicking for 3 minutes. The dislodged animals in the course of the agitation were collected with a cone shaped hand net with a detachable handle, by placing it opposite the flow direction of the reservoir. For the open water biotope, van Veen grab (36cm x 28cm) was used in collecting samples from each station due to the depth. All samples were fixed in the field with 10% formalin. In the laboratory, washing of the samples were done with 0.5mm mesh size sieve and invertebrates were picked from the substrate with aid of a hand lens. The macroinvertebrates were identified with aid of relevant keys and descriptions (Pennak, 1953; Ward and Whipple, 1959; Needham and Needham 1962; Victor and Ogbeibu, 1991). The identified macro-invertebrates were counted and preserved with 5% formalin.

Dominant and Subdominant Groups and Species

The dominant and subdominant taxa were determined using the system of Slack *et al*. (1979) which defines dominants as taxa constituting 15% or more of the total number of individuals and subdominants as those comprising at least 5% of the total number.

Data Analysis/Community Structure Indices

Significant differences in the fauna abundance between stations was determined by parametric analysis of variance (ANOVA) using the computer application SPSS 16.0 and Microsoft Excel for window. Margalef's Index (D), Shannon-Wiener diversity Index (H), Pielous Evenness (E) and Simpson Index of Diversity (SID) were used to analyze the community structure of the macroinvertebrates (Zar, 1984).

Result

Composition, Abundance and Distribution

A total of 20 macroinvertebrates taxa comprising of 7,945 individuals were recorded in the reservoir during the period of study. The highest number of taxa were Insecta (13, 65%), followed by Gastropoda (4, 20%), Arachnida (2, 10%) and Annelida (1, 5%) was the least. Table 1 lists the taxonomic categories of invertebrates, their distribution and abundance collected in the two biotopes of the five stations. The most abundant of the macroinvertebrates were gastropods (78.36%). They were followed by insects (15.7%), annelids (5.03%) and arachnids (0.89%) being the least (Fig. 2). *Melanoides tuberculata* (72.54%) and *Chironomus* sp. (5.49%) were the species with the highest abundance; *Lymnaea* sp. (0.03%) being the least.

Aquatic insects (only *Chironomus* sp. was encountered) and arachnids were not important components of macroinvertebrates in the openwater biotope. The gastropods and annelids encountered during the study were found in both biotopes.

Table 1: Abundance (%) and distribution of macroinvertebrates in the Dandaru Reservoir

	Station 1		Station 2		Station 3		Station 4		Station 5	
	Bank root	Open water	Bank root	Open water	Bank root	Open water	Bank root	Open water	Bank Root	Open water
ARTHROPODA										
INSECTA										
Coleoptera										
Hydrodamia sp	1(0.5)	-	2(0.9)	-	35(12.8)	-	37(15)	-	47(17.5)	-
Gyrinus sp	-	-	-	-	-	-	-	-	9(3.4)	-
Psephenus sp	6(2.9)	-	8(3.6)	-	-	-	4(1.6)	-	-	-
Hemiptera										
Gerris sp	14(6.7)	-	8(3.6)	-	6(2.2)	-	5(2)	-	16(6.0)	-
Hydrometra sp	11(5.3)	-	10(4.5)	-	11(4.0)	-	7(2.8)	-	8(3.0)	-
Belostoma sp	12(5.8)	-	16(7.2)	-	32(11.7)	-	10(4.1)	-	5(1.87)	-
Odonata										
**Aeschna* sp	29(13.9)	-	13(5.8)	-	35(12.8)	-	53(21.5)	-	60(22.4)	-
**Lestes* sp	30(1.4)	-	13(5.8)	-	12(4.4)	-	26(10.6)	-	32(11.9)	-
Ephemeroptera										
Cloeon sp	31(14.9)	-	30(13.5)	-	13(4.7)	-	6(2.4)	-	5(1.87)	-
Baetis sp	16(7.7)	-	15(6.7)	-	-	-	7(2.8)	-	-	-
Trichoptera										
Hydropsyche sp	14(6.7)	-	11(4.9)	-	-	-	8(3.3)	-	-	-
Diptera										
**Chironomus* sp	8(3.9)	11(30.6)	31(13.9)	140(35)	5(1.8)	55(1.2)	14(5.7)	30(6.8)	23(8.6)	119(8.7)
Hermetia sp	6(2.9)	-	6(2.7)	-	-	-	5(2)	-	15(5.6)	-
ARACHNIDA										
Hydracarina sp	7(3.4)	-	12/5.4	-	4(1.5)	-	15(6.1)	-	9(3.4)	-
Agyronecta sp	1(0.5)	-	4/1.8	-	5(1.8)	-	4(1.6)	-	10(3.7)	-
ANNELIDA										
**Tubifex tubifex*	9(4.3)	11(30.6)	19/8.5	94(23.5)	12(4.4)	102(2.3)	17(6.9)	33(7.5)	19(7.1)	85(6.3)
GASTROPODA										
**Melanoides tuberculata*	11(5.3)	11(30.6)	9/4.0	125(31.3)	93(33.9)	4050(90.3)	26(10.6)	368(83.1)	5(1.9)	1065(78.3)
Bellamya unicolor	1(0.5)	2(5.6)	1/0.4	18(4.5)	4(1.5)	276(6.2)	2(0.8)	11(2.5)	4(1.5)	84(6.2)
Bulinus sp	1(0.5)	1(2.8)	15/6.7	23(5.8)	7(2.6)	1(0.02)	-	1(0.23)	1(0.4)	8(0.6)
Lymnaea sp	-	-	-	-	-	2(0.04)	-	-	-	-
Total	**208**	**36**	**223**	**400**	**274**	**4486**	**246**	**443**	**268**	**1361**

(): % abundance; Italics indicate benthic macroinvertebrates that differed significantly between the stations

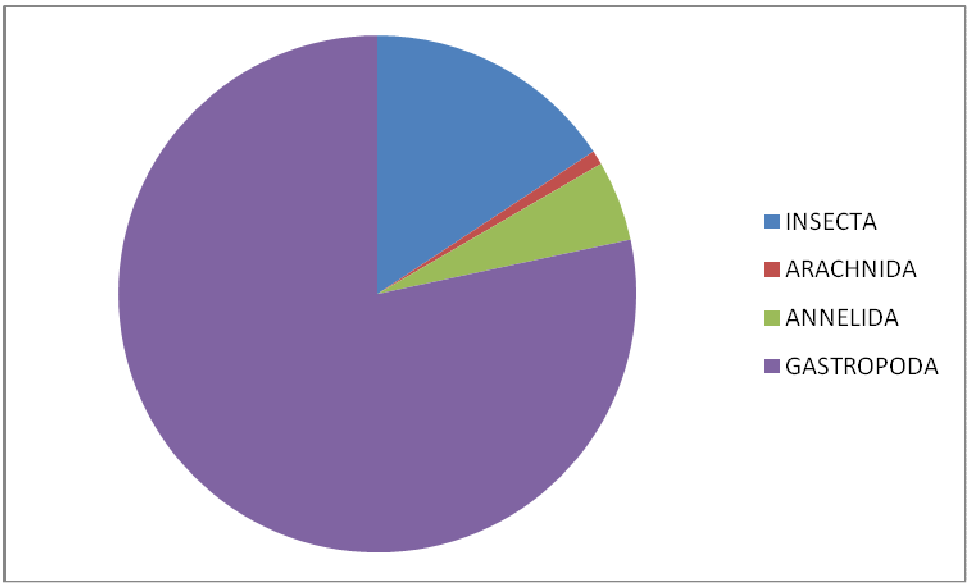

Figure 2: Percentage abundance of major taxonomic groups in Dandaru Reservoir

The highest number of macroinvertebrates (quantitatively) was recorded in sampling station 3 and accounted for 59.91%; followed by station 5(20.91 %), station 4(8.67 %), station2 (7.84 %) and station 1(3.07%). Qualitatively, the order of abundance was station 1 = station2> station 4> station 5> station 3. Most of the species encountered were common to all the stations except *Baetis* sp. (Ephemeroptera), *Hydropsyche* sp. (Trichoptera) and *Psephenus* sp. (Coleoptera) that were absent in stations 3 and 5 while *Gyrinus* sp. and *Lymnaea* sp. were found only in station 5 and 3, respectively. *Melanoides tuberculata*, *Bellamya unicolor* and *Bulinus* differed significantly between the sampling stations.

Seasonal Variation

All the macroinvertebrates taxa recorded in the dry season occurred in the rainy season except *Lymnaea* sp. Seasonal variation occurred in the abundance of all the macroinvertebrates taxa and Chironomous and Tubifex differed significantly. More macroinvertebrates were encountered in the rainy season (51.6%). Variations in the higher taxonomic categories with season are shown in Fig.3. Diptera and Annelida were more abundant in dry season, while the remaining taxa were more in the rainy season.

Diversity and Dominance

The summary of the diversity and dominance indices adopted in this study are given on Table 2. Adopting the Margalef's Index, the species richness across the stations was found to be minimal and maximal at stations 3 and 1. Station 2 is higher than station 4 and station 4 is more than station 5. The Shannon diversity (H), Eveness index (E) and Simpson dominance index followed the same trend.

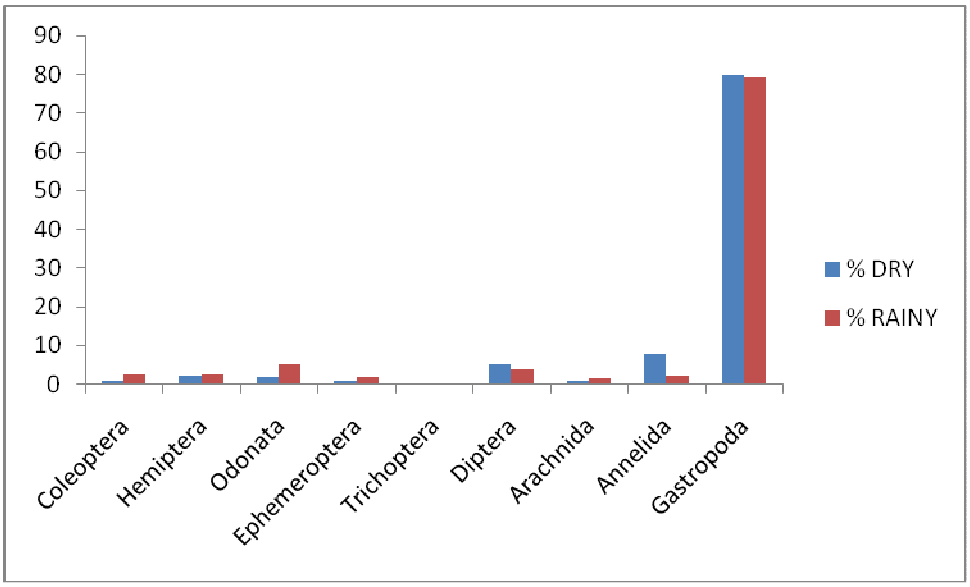

Figure 3: Seasonal variation in macroinvertebrates in Dandaru Reservoir

Table 3: Diversity of Macroinvertebrates in the Dandaru Reservoir

	Station 1		Station 2		Station 3		Station 4		Station 5		
	Bank root	Open water	Bank root	Open water	Bank root	Open water	Bank root	Open water	Bank root	Open water	Mean
No of individuals	208	36	223	400	274	4486	246	443	268	1361	
Margalef's taxa richness (D)	7.334	2.57	7.321	1.537	5.332	1.369	6.692	1.511	6.178	1.276	1.653
Shannon Weiner general diversity (H)	1.11	0.585	1.068	0.597	0.928	0.178	1.075	0.276	1.035	0.339	0.395
Pielous Evenness (E)	0.884	0.837	0.85	0.854	0.81	0.228	0.874	0.395	0.859	0.485	0.559
Simpson (SID)	0.912	0.737	0.916	0.721	0.832	0.181	0.894	0.299	0.883	0.373	0.462

Dominant and Subdominant Groups and Species

Coleopteran larvae were dominant in the bankroot biotopes of stations 4 and 5; hemipterans, dominant at stations 1, 2, and 3, subdominant at stations 4 and 5; odonatans, were dominant in all the stations except 2; ephemeropterans were only dominant in stations 1 and 2, subdominant in station 4; trichopterans, subdominant in station 1; arachnidans, were subdominant in stations 2, 4 and 5; dipterans' larvae were dominant in the open water biotopes of stations 1 and 2, subdominant in all the other stations except station 3; annelidans, dominant in open water biotopes of stations 1 and 2, subdominant in stations 4 and 5; gastropods were dominant in the open water biotopes of all the stations and subdominant in all the bankroot biotopes sampled except station 5.

The dominant species in the bankroot biotopes were *Hydrodamia* sp. (stations 4

and 5), *Aeschna* sp. (stations 4 and 5) and *Melanoides tuberculata* (station 3); in the open water biotope, dominant species were *Chironomus* sp. (stations 1 and 2), *Tubifex tubifex* (stations 1 and 2), *Melanoides tuberculata* (all the stations). The subdominant species in the bankroot biotope included *Belostoma* sp. (stations 1, 2 and 3), *Aeschna* sp. (stations 1 , 2 and 3), *Cloeon* sp. (stations 1 and 2), Baetis sp. (stations 1 and 2), *Chironomus* sp. (stations 2, 4 and 5), *Tubifex tubifex* (stations 2, 4 and 5), *Melanoides tuberculata* (stations 1 , 3 and 4).

Discussion

Twenty macroinvertebrate taxa recorded in this study is low compared with other inland water bodies for example in streams, 31 taxa was reported by Victor and Al-Mahrouqi, 1996; 46 taxa reported by Edema *et al*. (2002) ; 62 taxa reported by Egborge *et al*. (2003) and 59 taxa by Arimoro and Odhirin, (2005). The lower taxa number in this reservoir could be partly because diversity tends to be low in physically controlled systems (Odum, 1971). Higher macroinvertebrate taxa were also recorded in some lakes, 28 taxa recorded in Asejire Lake by Asibor (2015); 46 taxa were recorded in Obazuwa Lake by Olomukoro and Ovioije (2015); 66 taxa recorded in Nokoue Lake by Odountan and Adou (2016); however fewer taxa (9 each) was encountered by Adakole *et al*. (2008) in Kubanni Lake, Zaria and Tersoo *et al*. (2017) in some ponds in Makurdi. Length of sampling period in addition to various anthropogenic activities around the water bodies and collection method could be the cause of differences in taxa number. The low number of macroinvertebrates recorded here is due to deteriorating water quality caused by various urban and agricultural activities along the banks of the parent river and the reservoir. Most of the animals encountered in this study are also found in some other freshwater bodies Arimoro and Odhirin (2005), Asibor (2015), Olomukoro and Ovioije (2015).

The record of gastropods as the most abundant group with Melanoides tuberculata forming the largest percentage in this study is contrary to most other woks where insects were the most abundant as reported by Adeyemi *et al*., 2009; Tersoo *et al*., 2017 and Olomukoro *et al*., 2013. Molluscs are highly tolerant to many pollutants and exhibit high accumulations of them, particularly heavy metal (Gardenfors *et al*., 1998 and Lau *et al*., 1998)

The high taxa richness, general diversity, evenness and dominance index; also, more clean water species (Ephemeroptera and Trichoptera) recorded in station 1 which was upstream the Dandaru Reservoir is an indication that the environment is more favourable in terms of water quality, availability of food and substratum being more stable than the other stations. The low taxa richness, general diversity, evenness and dominance index recorded in station 3 (middle of the reservoir) suggests unstable substratum (due to dredging activities carried out in order to forestall flooding of adjacent surroundings during raining season) and unfavourable environmental parameters.

Aquatic insects (except *Chironomus*) and arachnids being encountered only in the bankroot biotope of the stations could be due to the shallower water, presence of aquatic macrophytes, substrate type and higher oxygen concentration.

Chironomus sp. occurrence in the openwater is due to Chironomidae being frequently associated with fine sediment, because they are able to burrow into the sediment (Waters, 1995). However, gastropods and annelids were dominant in the openwater biotopes of the stations.

The heterogenous distribution and abundance of taxa among the different biotopes at the same station is not unusual in temperate and tropical streams. This is a reflection of niche availability governed by physical, chemical and biological factors (Victor and Al-Mahrouqi, 1996).

The mean Margalef (1.653) and Shannon Weiner (0.395) water quality indices of Dandaru Lake suggest low to moderate pollution of the lake (Lenat *et al*., 1980; Plotka *et al*., 2009)

Conclusion

The domination of the macroinvertebrate community structure of Dandaru Lake by pollution tolerant taxa (*Melanoides tuberculata*, *Chironomus* sp. and *Bulinus* sp.) and the low diversity index indicates the lake is under pollution stress, thus steps should be taken to control the livelihood activities along the source river and the reservoir.

References

Abiona, O.O., Anifowose, A.J. Adedokun, M.A., Abdullah, J.O. and Bamigbelu, O.R. (2012). Impact Assessment of Pollution from Metal Concentrations in Water and Fish – A Case Study of Dandaru Reservoir in Ibadan, Nigeria. *Nature and Science*, 10(8):143-148.

Adakole, J.A., Abulode, D.S. and. Balarabe, M.L. (2008). Assessment of water quality of a man-made lake in Zaria Nigeria. In: Sengupta M. and Dalwani, R. (Editors). Proceedings of Taal2007: The 12th World Lake Conference 1373-1382.

Adeyemi, S.O., Adikwu, I.A., Akombu, P.M. and Iyela, J.T. (2009). Survey of Zooplanktons and Macroinvertebrates of Gbedikere Lake, Bassa, Kogi State, Nigeria. *International Journal of Lakes and Rivers*, 2(1): 37-44

Asibor, G.I. (2015). Seasonal Biodiversity Assessment of Benthic Macroinvertebrate of Asejire Reservoir, Southwest Nigeria. *Journal of Sustainable Development*, 8(2): 257 – 269.

Edema, C.U. Ayeni, J.O. and Aruoture, A. (2002). Some observations on the Zooplankton and Macrobenthos of the Okhuo River, Nigeria. *Journal of Aquatic Science*, 17(2): 145-149.

Egborge, A.B.M., Ezemonye, L.I. and Awoze, G.E. (2003). Macro invertebrate fauna of Udu-Ughievwen Wetlands, Sourthern, Nigeria. *Journal of Aquatic Science*, 18(1):1-8.

Ejirinde, S.A., Ibor, O.R., Azubuike, C., Fagbohun, O. and Adeogun, A. (2014). Development of reproductive biomarkers for monitoring Endocrine Disruption in Tilapias from Dandaru Lake, Ibadan, Nigeria. West African Society of Toxicology Conference 2014

Gardenfors, U., Westermark, T., Emanuelsson, U., Mutvei, H. and Walden, H. (1988). Use of land-

snail shells as environmental archives: preliminary results. *AMBIO*. 17(5): 347–349.

Ikomi, R.B., Arimoro, F.O. and Odihirin, O.K. (2005). Composition, distribution and abundance of macroinvertebrates of the upper reaches of river Ethiope, Delta State Nigeria. *Zoologist*, 3: 68-81.

Jimoh, M.O., Oladele-Bukola, M. D., Adebayo, A.A, Yusuff, F. A. and Salami, O.O. (2014). Microbial flora of the gastro-intestinal tract of Clarias gariepinus caught from river Dandaru Ibadan, Nigeria. *Sokoto Journal of Veterinary Sciences*, 12(2): 19 – 24

Kellogg, L.L. (1994). Save our streams. Monitor's guide to aquatic macroinvertebreates. Izaak Walton League of America, Gaithersburg, Maryland 60pp

Lau, S., Mohamed, M., Tan Chi Yen, A. and Su'Ut, S. (1998). Accumulation of heavy metals in freshwater molluscs. *Science of the Total Environment,* 214(1–3):113–121

Lenat, D.R., Penrose, D.L. and Eaglesor, K.W. (1981). Variable effects of Sediment addition on stream benthos. *Hydrobiologia*, 79:187-194.

Lenat, D.R., Smock, L.A. and Penrose, D.L. (1980). Use of benthic macroinvertebrates as bio-indicators of environmental quality. In: Biological Monitoring for Environmental Effects. Douglass, L.W. (Ed). Published by Lexington Books. Toronto. pp. 97-114.

Needham, J.G. and Needham, P.R (1962). A guide to the study of Freshwater Biology. Comstock, Ithaca, New York.

Odountan, H. and Abou, Y. (2016) Structure and Composition of Macroinvertebrates during Flood Period of the Nokoue Lake, Benin. *Open Journal of Ecology*, 6: 62-73

Odum, E.P. (1971). Fundamentals of Ecology. 3rd ed. London,W.B. Sanders. 546p.

Olomukoro, J.O., Osamuyiamen, I.M. and. Dirisu, A. (2013.). Ecological Survey of Macrobenthic Invertebrates of Selected Ponds in Agbede Flood Plain, Southern Nigeria. *Journal of Biology, Agriculture and Healthcare*. 3(10): 2224-3208.

Olomukoro, J.O. and Oviojie, E.O. (2015). Diversity and distribution of benthic macroinvertebrate fauna of Obazuwa Lake in Benin city, Nigeria. *Journal of Biology, Agriculture and Healthcare,* 5(1): 94 – 100.

Pennak, R.W. (1953). Freshwater Invertebrates of the United States. Ronald Press Company, New York. pp 1053.

Plotka, N., Ebrahmi, M., Hui, Z., Crisosto, T., Pajak. G. and Spychala, E. (2017). Ecological Status of the Lake Durowskie in Poznan Based on Benthic Macro invertebrates [Online]. [cited 2017 0ct. 4]; Available from: URL: http://www.restlake.amu.edu.pl/download/archive-2009/Report_Benthic_Macro-invertebrates.pdf

Slack, K.V., Nauman, J.W. and Tilley, L.J. (1979). Benthic invertebrates in a North-flowing stream and a South-flowing stream, Brook range, Alaska. *Water Resource bulletin*. 51(1): 108-135.

Tersoo, A.D., Terngu, I.S. and Akogwu, A.E. (2017). Survey and Identification of Macroinvertebrates Found in Some Ponds in Makurdi, Benue State Nigeria. *International Journal of Ecotoxicology and Ecobiology*. 2(1): 26-32.

Victor, R. and Ogbeibu, A.E. (1991). Macro invertebrate Communities in the erosional biotope of an urban stream in Nigeria. *Tropical Zoology*. 4:1-12.

Victor, R. and Al-Mahrouqi, A.I.S. (1996). Physical, chemical and fauna characteristics of a perennial stream in arid northern Oman. *Journal of Arid Environments*, 23: 465 – 476.

Ward, H.B. and Whipple, G.C. (1959). Freshwater Biology. 2nd edition. John Wiley and Sons Inc. USA. Pp. 1248.

Waters, T.F. (1995). Sediment in Streams Sources, Biological Effects and Control. Maryland, USA: American Fisheries Society.

Zar, J.H. (1984). Biostatistical Analysis Prentice-Hall, New Jersey.