

MACROINVERTEBRATE FAUNA OF A NIGERIAN FRESHWATER ECOSYSTEM

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ABSTRACT

A survey of macro invertebrate fauna of Anambra River was carried out for 22 months at Otuocha, Ogurugu and Nsugbe. The macro invertebrates were sampled using kick sampling techniques and scoop nets. Sampled specimens were identified to generic level. During the study a total of 21 genera of macroinvertebrates belonging to 13 families were identified. The fauna was composed of Gyrius sp. (29.2%), Macrobranchium sp. (19.6%), Ranatra sp. (13.2%) and Agabus sp. (3.5%). The margalef's index of fauna richness showed that Otuocha station had the highest species richness (12.70), followed by Nsugbe (7.01), and Ogurugu (6.80) stations. The least fauna diversity of 0.21 was registered at Nsugbe as against 3.15 at Otuocha and 0.86 at Ogurugu. The Mc Naughton community dominance index was more pronounced at Nsugbe (53.1) than at Otuocha (49.69) and Ogurugu (47.04). Jackson's fauna similarity index showed that the fauna at Otuocha and Ogurugu were more closely related (0.64) than the fauna at Nsugbe.

Keywords: Macroinvertebrates, Anambra River, Nigeria

INTRODUCTION

The lotic and lentic inland water, as well as brackish and marine waters in the tropics are habitats for a variety of macroinvertebrate fauna. Work on macroinvertebrate fauna in the tropics has shown that the quantitative collection of key species from natural aquatic habitats or that modified by man can provide a means of estimating various ecological parameters, such as richness or evenness in diversity (Holloway and Barlow, 1983).

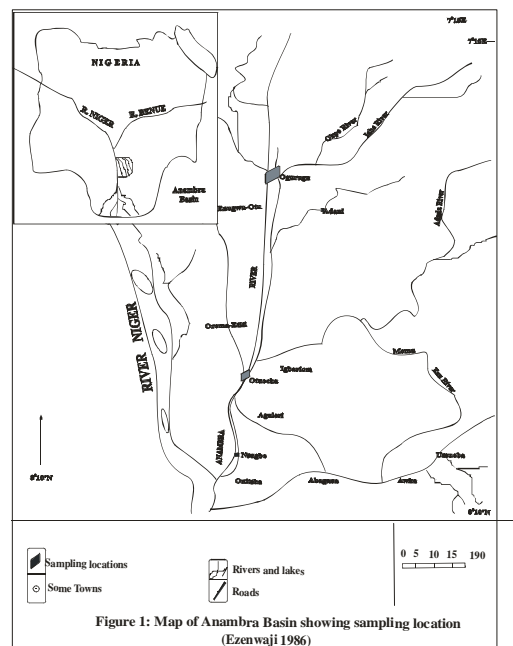
Pickavance (1991) reported that mayflies (Ephemeroptera) and Gyrius (Coleoptera) are predominant members of the fauna of polluted stream. The presence of large detrital material has profound effect on the macroinvertebrate community, especially in those deeper parts of the lake where the homogeneous sediments support only a limited diversity of species. In such cases animals which are normally only found in the littoral may extend into the benthic zone (Me Lachlan, 1974). This may be due either to the availability of food or the provision of a suitable substratum or a combination of these factors.

Recent faunistic, quantitative works include those leading to the provision of keys for the identification of tropical fresh water fauna (Miles and Graham, 1970), checklist of macroinvertebrates of Ikpoba rivers, (Ogbeibu and Oribhabor, 2001), and an x-ray of macroinvertebrate fauna of flood plain (fadama) of the Anambra River (Eyo and Ekwonye, 1995).

The primary aim of this paper is to provide quantitative information on aquatic macroinvertebrate fauna in the Anambra river to supplement the only existing information (Eyo and Ekwonye, 1995).

MATERIALS AND METHODS

Study Area: The study area was the Anambra River (Figure 1) covering about 14014 km² (Awachie, 1976). The Anambra river is about 207.4 km length, it rises from the Ankpa hills (ca. 305 - 610 m above sea level), flows in southerly direction through a narrow trough that gradually broaden as it courses down. It crosses the Kogi/Anambra State boundary a bit north of Ogurugu, then meanders through Ogurugu to Otuocha and Nsugbe. From there it flows down to its confluence with the Niger at Onitsha. The basin lies between latitude 6°10' and 7°20', longitude 6°35' and 7°40', east of the River Niger into which the Anambra river empties. There are two



main seasons the dry season October – March and the rainy season (April – September) approximately corresponding to the dry and flood phase, respectively of the hydrological regime. The vegetation is derived guinea savannah. The riparian vegetation, ecology and productivity of the river basin have been extensively studied (Awachie and Ezenwaji, 1981). The area it drains is one of the agriculturally rich area of this country. The macroinvertebrates were sampled from Anambra river at Ogurugu, Otuocha and Nsugbe. Samples were collected using scoop net and by “kick sampling techniques” (Ogbeibu and Oribhabor, 2001).

Macroinvertebrate Sampling: The kick sampling technique was used in collecting macroinvertebrates from the bank root biotope of each station. A hand scoop net (154 µm mesh size) was used in sampling 0.3m² of the substratum at four different points to form one composite sample per station.

The sampling period was from January 1998 – October 1999. Collected samples were preserved in formalin. Sampled specimens were identified to the generic level. Faunal abundance and biomass were computed thus; numbers per 0.3m² x total weight (mg) (Ogbeibu and Oribhabor, 2001).

Identification of the macroinvertebrate fauna to generic level was done by using the keys of Miles (1970), Needham and Needham (1962) and Mellamby (1963).

Margalef's index of taxa richness was used in computing taxa richness (Margalef, 1974) while the Shannon-Wiener (H) and Evenness (E) indices (Shannon and Wiener, 1963) were used to evaluate species diversity.

RESULTS

Composition, abundance and biomass of macroinvertebrate in Anambra river: A total of 21 genera belonging to 13 families of macroinvertebrates were identified (Table 1). The fauna was composed of *Hydrophilus sp.*, *Agabus sp.*, *Gyrinus sp.*, *Macrobranchium sp.*, *Nepa sp.*, *Hirudo sp.* and *Velia sp.* all of which constituted 87.02% abundance and 88.19% biomass. *Gyrinus sp.* were the most abundant group, accounting for 29.77% abundance and 6.35% biomass. *Caridina sp.* had 13.93% abundance and 17.79% biomass.

Agabus sp. 8.47% abundance and 3.67% biomass, occupied an intermediate rank. *Hydrophilus sp.*, 7.05% abundance and of 3.50% dominated the *Hydrophilidae*, *Ranatra sp.*, 13.93% abundance and 18.2% biomass, *Velia sp.*, 5.19% abundance and 14.75% biomass dominated the *nepidae* and *velidae* respectively.

Mesovelgia sp., 0.16% abundance and 0.34% biomass dominated the *Mesovelidae sp.*, *Rhabdolaimus sp.*, 0.60% abundance and 2.13% biomass dominated the *nematodes* and was among the least abundant group in the river system.

Taxa richness and diversity indices of macroinvertebrate in the Anambra river: The macro invertebrate richness as computed by Margalef's index showed that *Hydrophilus sp.*, had 31.9% taxa richness at Otuocha and ranked highest followed by 3.3% in Ogurugu and 0.4% in Nsugbe (Table 2). *Gyrinus sp.*, accounted for 14.8% taxa richness at Otuocha, 10.1% at Ogurugu and 5.0% at Nsugbe.

The *Bulinus sp.*, *Libyodrilus sp.*, and *Rhabdolaimus sp.*, were least available in the three sampled stations. *Hydrophilus sp.*, accounted for 35.6% taxa richness followed by *Gyrinus sp.* 29.8% taxa richness and *Caridina* 13.9% taxa richness. *Rhabdolaim sp.* had 0.1% taxa richness, *Bulinus sp.* 0.1% taxa richness and *Baetis sp.* 0.2% were among the least occurring macroinvertebrates.

The macroinvertebrate diversity deduced from Shannon-Weaver's diversity index was highest at Otuocha (9.93), closely followed by Nsugbe (8.01) and lowest in Ogurugu (6.29). Diversity Equitability (Evenness) Index (E) of 0.32 was recorded at Otuocha, 0.03 at Nsugbe and 0.01 at Ogurugu. The value for Otuocha was significantly different from Nsugbe and Ogurugu ($P < 0.05$).

The macroinvertebrate community similarity index indicated a pronounced variation in the similarity index among macroinvertebrates of Ogurugu, Otuocha and Nsugbe stations (Table 2). The Mc Naughton community dominance index was more pronounced at Nsugbe than at Otuocha and Ogurugu respectively. Seasonal variations in the relative abundance of the 21 genera of macroinvertebrates are shown in Table 3. *Gyrinus sp.*, *Hydrophilus sp.*, *Agabus sp.* and *Ranatra sp.* were more abundant in the dry season months of December and January than in the months of wet season (Table 3). *Rhabdolaimus sp.*, *Donacia sp.* and *Libyodrilus sp.*, showed a temporal trend that was the reverse of that *Gyrinus sp.* and *Agabus sp.* They were generally more prominent in the rainy season than in the dry seasons. The mean abundance and biomass of major macro invertebrate among the three stations of the river system are shown in Table 4. The *Gyrinus sp.* ranked highest in Otuocha and was closely followed by *Macrobranchium sp.* and *Ranatra sp.* In Nsugbe *Gyrinus sp.* was most abundant and was followed by *Ranatra* and *Macrobranchium sp.* respectively. In Ogurugu the least abundant species were *Velia* and *Nepa species*.

In all, *Gyrinus sp.* ranked highest followed by *Macrobranchium sp.* and *Ranatra sp.* The *Nepa sp.* and *Velia sp.* were the least occurring of all the major macro invertebrates collected during the study (Table 4).

DISCUSSION

Water quality and food availability are important factors governing abundance and distribution of macro invertebrate fauna in aquatic environment (Bishop, 1993, Dance and Hynes, 1970).

Table 1: The composition, percentage number, Abundance and Biomass of total macroinvertebrate fauna of Anambra river system

Family	Genera	Number (%)	Density (%)	Biomass (%)
Hydrophilidae	<i>Hydrophilus</i>	129(6.94)	430.0(7.05)	30.10(3.50)
Dytiscidae	<i>Hydrobius</i>	27(1.45)	90.0(1.47)	5.40(0.63)
	<i>Agabus</i>	155(8.34)	516.69(8.47)	31.53(3.67)
	<i>Hybius</i>	19(1.02)	63.33(1.04)	3.99(0.46)
	<i>Dytiscus</i>	30(1.61)	100.00(1.64)	16.00(1.86)
Gynnidae	<i>Gyrinus</i>	545(29.32)	1816.67(29.77)	54.50(6.35)
Chrysomelidae	<i>Donacia</i>	29(1.56)	69.67(1.58)	26.10(3.04)
Nepidae	<i>Ranatra</i>	247(13.29)	823.33(13.49)	156.43(18.21)
	<i>Nepa</i>	72(3.88)	240.0(3.93)	48.0(5.59)
Velidae	<i>Velia</i>	95(5.11)	316.67(5.19)	126.67(14.75)
	<i>Mesovelia</i>	31(1.67)	10.0(0.16)	2.9(0.34)
	<i>Lymphula</i>	11(0.59)	36.67(0.60)	5.87(0.68)
Chronomidae	<i>Chronomus</i>	3(0.16)	10.0(0.16)	0.3(0.03)
Baetidae	<i>Baetis</i>	5(0.27)	16.67(0.27)	6.50(0.76)
Palaemonidea	<i>Macrobranchium</i>	110(5.92)	366.67(6.01)	8.07(0.94)
	<i>Caridina</i>	255(13.72)	850.00(13.93)	170.0(19.79)
Lymnaeidae	<i>Lymnaea</i>	8(0.43)	26.67(0.44)	8.00(0.93)
	<i>Bulinus</i>	3(0.16)	10.0(0.16)	17.0(0.20)
	<i>Libyodrilus</i>	9(0.48)	30.0(0.49)	6.3(0.73)
Libyodridae	<i>Libyodrilus</i>	9(0.48)	30.0(0.49)	6.3(0.73)
Hirudidae	<i>Hirudo</i>	65(3.5)	216.67(3.55)	132.17(15.39)
Rhabdolaimidae	<i>Rhabdolaimus</i>	11(0.59)	36.69(0.60)	18.33(2.13)
Total		1859(100.00)	6103.36(100)	858.86(100.00)

Table 2: The percentage number, Taxa richness, diversity indices and faunal similarities of total macro invertebrates at Ogurugu, Otuocha and Nsugbe stations of Anambra river system January 1995 – October 1999

Family	Taxa	Ogurugu		Otuocha		Nsugbe		Total		
		N (%)	Taxa richness (%)	N (%)	Taxa richness (%)	N (%)	Taxa richness (%)	N	%	%TR
Hydrophilidae	<i>Hydrophilus</i>	61(3.28)	18.4(3.3)	59(3.17)	17.7(31.9)	9(0.48)	2.4(0.4)	129	6.94	35.6
	<i>Hydrobius</i>	6(0.32)	1.5(0.3)	21(1.13)	6.1(1.1)	0	0	27	1.45	1.4
Dytiscidae	<i>Agabus</i>	63(3.39)	19.0(3.4)	81(4.36)	24.5(4.4)	11(0.59)	3.1(0.6)	155	8.34	8.5
	<i>Hybius</i>	10(0.54)	2.8(0.5)	9(0.48)	2.4(0.4)	0	0	19	1.02	0.9
	<i>Dytiscus</i>	16(0.86)	4.6(0.8)	11(0.59)	3.1(0.6)	3(0.16)	0.6(0.1)	30	1.61	1.3
Gynnidae	<i>Gyrinus</i>	184(9.90)	56.0(10.1)	270(14.52)	82.3(14.8)	91(4.90)	27.5(5.0)	545	29.32	29.9
Chrysomelidae	<i>Donacia</i>	19(1.02)	5.5(1.0)	10(0.54)	2.8(0.5)	0	0	29	1.56	1.5
Nepidae	<i>Ranatra</i>	60(3.23)	18.0(3.2)	141(7.58)	42.8(7.7)	46(2.47)	13.8(2.5)	247	13.29	13.4
	<i>Nepa</i>	30(1.61)	8.9(1.6)	30(1.61)	8.9(1.6)	12(0.65)	3.4(0.6)	72	3.87	3.8
Velidae	<i>Velia</i>	5(0.27)	1.2(0.2)	56(3.01)	16.8(3.0)	34(1.83)	10.1(1.8)	95	5.11	6.4
Mesovelidae	<i>Mesovelia</i>	0	0	31(1.67)	9.2(1.7)	0	0	31	1.67	1.7
	<i>Lymphula</i>	5(0.27)	1.2(0.2)	6(0.32)	1.5(0.3)	0	0	11	0.59	0.5
Chronomidae	<i>Chronomus</i>	4(0.22)	0.9(0.2)	7(0.38)	1.8(0.3)	0	0	11	0.59	0.5
Baetidae	<i>Baetis</i>	0	0	5(0.27)	1.2(0.2)	0	0	5	0.29	0.2
Palaemonidae	<i>Macrobranchium</i>	110(5.92)	33.3(6.0)	0	0	0	0	110	5.92	6

	<i>Caridina</i>	0	0	215(11.57)	65.5(11.8)	40(2.15)	11.9(2.1)	255	13.72	13.9
Lymnaeidae	<i>Lymnaea</i>	0	0	5(0.27)	1.2(0.2)	3(0.16)	0.6(0.1)	8	0.43	0.3
	<i>Bulinus</i>	0	0	3(0.16)	0.6(0.1)	0	0	3	0.16	0.1
Lumbriculidae	<i>Lumbriculus</i>	0	0	3(0.16)	0.6(0.1)	6(0.32)	1.5(0.3)	9	0.48	0.4
Hirudidae	<i>Hirudo</i>	52(2.80)	15.6(2.8)	11(0.59)	3.1(0.6)	2(0.11)	0.3(0.1)	65	3.5	3.5
Rhabdolaimidae	<i>Rhabdolaimus</i>	0	0	2(0.11)	0.3(0.1)	1(0.05)	0	3	0.16	0.1

Table 3: Seasonal mean abundance and biomass of the major macroinvertebrates of Anambra river system

Taxonomic group	Dry season		Wet season	
	Abundance (Nm ⁻²)	Biomass (Mgm ⁻²)	Abundance (Nm ⁻²)	Biomass (Mgm ⁻²)
<i>Hydrophilus sp</i>	214	15	65	4.7
<i>Agabus sp</i>	250	4.21	63	1.68
<i>Gyrinus sp</i>	891	25.31	184	4.8
<i>Macrobranchium sp</i>	180	41.2	60	15.3
<i>Ranatra sp</i>	477	90.6	75	13.1
<i>Caridina sp</i>	403	88.8	76	16.06
<i>Nepa</i>	140	28.2	41	8.4

Table 4: Mean taxonomic abundance and biomass of main macro invertebrates per station, January 1998 - October, 1999 of Anambra river system

Taxonomic Group	Ogurugu		Otuocha		Nsugbe		Mean Value	
	Abundance (Nm ⁻²)	Biomass (Mgm ⁻²)	Abundance (Nm ⁻²)	Biomass (Mgm ⁻²)	Abundance (Nm ⁻²)	Biomass (Mgm ⁻²)	Abundance (Nm ⁻²)	Biomass (Mgm ⁻²)
<i>Hydrophilus sp</i>	203.33	14.23	196.67	13.77	30	2.1	143.33	10.03
<i>Agabus sp</i>	196.67	13.77	270	16.47	36.67	2.24	167.78	10.83
<i>Gyrinus sp</i>	613.33	18.4	900	27	303.33	9.1	605.33	18.17
<i>Ranatra sp</i>	200	38	470	42.3	153.33	29.13	274.44	22.38
<i>Macrobranchium sp</i>	366.67	154	716.67	301	133.33	56	405.56	170.33
<i>Velia sp</i>	16.67	5.57	186.67	74.67	40	8	81.11	29.78
<i>Hirudo sp</i>	173.33	105.73	36.67	2.01	113.33	45.33	107.78	65.11
<i>Nepa sp</i>	100	20	100	20	6.67	4.07	68.89	14.69

In Anambra river system there was pronounced diversity of species among the macroinvertebrates sampled.

Coleopteran, for instance, exhibited a reasonable diversity of aquatic fauna. They were found in all the bank root biotopes where collections were made. Majority of the Coleopteran were collected during the dry season period (October – April) when the river level was drastically reduced and the insects concentrated in small ponds that were formed along the river course during residing flood. The high concentration accounted for more specimens caught, per unit effort during the sample periods.

The large number of aquatic beetles (Coleopteran) sampled, especially from Otuocha 461 (24.79%) and Ogurugu 359 (19.11%) (Table 2) stations of the river may be attributed to their adaptation to the environment, availability of food, reproduction and time of collection (Mbah and Vajime, 1989).

Belostomid water-bugs (Hemiptera) found in Anambra river were of four types. The *Velia sp.* 95(5.11%) were common and more frequent than the *Mesovelia sp* 31(1.6%). The *Velia sp* were morphologically adapted to the aquatic environment by the possession of a short, retractile respiratory siphon. The female glues her eggs to the back of the male, which carries it until they hatch thus ensuring high percentage of hatchability and subsequent survival.

Specimens of water sticks insect, *Ranatra sp.* 247(13.29%) and *Nepa sp.* 72(3.87%) were found below the water surface clinging to aquatic vegetation with long respiratory siphon thrust upward to obtain oxygen at the water-air interface. This morphological adaptation contributed to their survival in the habit (Umeham, 1989).

The sampled aquatic fauna from the River basin suggest coleopteran and hemiptera dominance.

Baetis (Ephemeroptera) usually form a major part of the fauna of normal streams (Mba and Vajime, 1989). They were only collected in Otuocha station, the (Baetis) absence in Ogurugu and Nsugbe could be ascribed to the fact that the majority of the tree shrubs which provided ideal habitat for these fauna have decayed; thus eliminating the natural habitat.

Generally, the chironomids were periphytic. The absence of chironomid in Nsugbe station and 4(0.22%) at Ogurugu may be due to the ability of this fauna to colonize all kinds of aquatic environment especially those which are badly polluted (Burton, 1987). This is possible because the chironomid species possess haemoglobin used to extract oxygen from water in areas where the concentrations of oxygen is very low. *Hirudo* respond to organic enrichment macrophytes by increase in abundance. This clearly manifested in Ogurugu station that registered 52(8.3%) of *Hirudo*.

Their significantly higher density in Ogurugu than in Otuocha and Nsugbe stations could be

attributed probably to organic enrichment leading to growth of macrophytes.

Macrobranchium is a common taxon in the Anambra river. These macro invertebrates were available in Ogurugu, Otuocha and Nsugbe stations especially during the dry season, October – April. The abundance of this fauna could be attributed to favourable organic enrichment of the river.

Fewer numbers of macro invertebrates were collected from Ogurugu and Nsugbe stations than Otuocha. This could be attributed to the lotic and relatively lack of macrophytes in Ogurugu and Nsugbe stations.

The significant interaction ($P < 0.05$) between *Hydrophilus sp.*, *Agabus sp.*, *Ranatra sp* and *Gyrinus sp.* of the river and dissolved salts, oxygen concentration, water current, temperature indicated the importance of these parameters in the River system for growth and survival of the aforementioned fauna. The significant relationship signified that the variables concerned did not favour abundance and distribution of the fauna. The picture that emerges when species richness is compared in descending order is depicted below; Coleoptera, Hemiptera, Decapoda, Oligochaeta, Diptera and Ephemeroptera.

Hynes (1970) and Macan (1974), reported that presence or absence of aquatic fauna is associated with other factors such as predators, behaviour, food, concentration of dissolved salts, hydrogen ion concentrations, oxygen concentration, water current, water level and water temperature.

REFERENCES

- AWACHIE, J. B. E. (1976). Fish culture possibilities on the flood plain of the Niger Benue drainage system. *CIFA Technical Paper, 4*: 251 – 258.
- AWACHIE, J. B. E. and EZENWAJI, H. M. G. (1981). The fisheries development of the Anambra River Basin, Nigeria. *CIFA Technical Paper, 8*: 212 – 224.
- BISHOP, J. F. (1993). Limnology of a small Malayan river Surgai Gombak. Dr. W. JUNK, The Hague, 220 pp.
- BURTON, M. (1987). *Encyclopedia of Insect and Arachnid*. Evan Brothers Nigeria Ltd Ibadan, Nigeria 136 pp.
- DANCE, K. W. and HYNES, H. B. N. (1970). Some effects of agricultural land use of stream insect communities. *Environmental Population, 22*: 19 – 28.
- HOLLOWAY, Y. and BARLOW, H. (1983). The role of taxonomy, reference works and insect collection in tropical ecology. *Antenna, 7*: 50 – 53.
- EYO, J. E. and EKWONYE, U. C. (1995). The macroinvertebrate fauna of pool in the floodplain (fadama) of the Anambra River, Nigeria. *Freshwater Forum, 5(23)*: 160 – 162.
- EZENWAJI, H. M. G. (1986). The problems of the taxonomy of *Clarias* species (Pisces:

- Clariidae) in Africa and suggestions for the field worker. *Journal of Science Education*, 2: 22 – 34.
- HYNES, H. B. N. (1972). *The ecology of running waters*. University of Toronto Press, Toronto 555 pp.
- MACAN, T. T. (1974). *Freshwater Ecology*. 2nd edition Longman, London. 343 pp.
- MARGALEF, R. (1974). Estimating quantity and quality of biomass 2: *In: Vallen Welder, R. A manual for environment*. IBP handbook No 2: 225 pp.
- MBAH, C. E. and VAJIME, C. G. (1989). Preliminary Taxonomy Survey of fresh water insects from Northern Nigeria. *Journal of Aquatic Sciences*, 4: 27 – 39.
- MCLACHLAN, A. J. (1974). Development of some like ecosystems in tropical Africa with special reference to the invertebrates. *Biological Review*, 49: 365 – 397.
- MELLANBY, H. (1963). *Animal life in freshwater: A guide to freshwater invertebrate*. Methuen and Company Limited, 11 New Fetter Lane, ECA, London. 301 pp.
- MILES, M. P. and GRAHAM, V. E. (1970). *Tropical freshwater ecology*. Hutton Educational Publishers Limited, London. 136 pp.
- NEEDHAM, J. G. and NEEDHAM, P. R. (1962). A guide to the study of freshwater biology. Holder-Day Inc. San Francisco 180 pp.
- OGBEIBU, A. E. and EGBORGE, A. B. M. (1995). Hydrobiology studies of water bodies in the Okonu Forest Reserve (Sanctuary) S. Nigeria. *Tropical Freshwater Biology*, 4: 1 - 27.
- OGBEIBU, A. E. and ORIBHABOR, B. J. (2001). The Ecological impact of stream regulation using benthic macroinvertebrates as indices. *Journal Aquatic Sciences*, 16(2): 139 – 143.
- PICKAVANCE, J. K. (1991). Pollution of a stream in new Canada; Effect on invertebrate fauna. *Biological Conservations*, 34: 264 – 268.
- SHANNON, C. E. and WIENER, W. (1963). *The mathematical theory of communication*. University of Illinois Press, Urban. 117 pp.
- UMEHAM, S. N. (1989). Some aspects of the physiochemical limnology of Lake Chad. *Journal Aquatic Sciences*, 4: 21 – 26.