Biological Monitoring of Pollution



Final Research Report – Section 5

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Executive Summary

A pilot scale study was carried out to develop a suitable tool for the community and local government to measure the water quality and biological condition of wetland areas, particularly those polluted industrial waste. Macroinvertebrate and water quality samples were collected on four occasions (February, May, October and December) from the Kaliakoir industrial area. The sites were distributed along Ratanpur *Khal* and Mokesh *Beel* from the main industrial area to the Turag River.

Results of study indicated that Ratanpur *Khal* is biologically dead at its upper reaches where it is fed almost entirely by factory effluent. In these areas very few macroinvertebrate families were found and water quality parameters showed high concentrations of almost all the pollutants analysed for. By contrast sites at the far end of the *beel* furthest from the factory outlets had much higher family diversity.

The correlation between low levels of pollutants and high species diversity suggests that macroinvertebrate sampling would be a suitable and simple option for monitoring the condition of water bodies that are polluted with industrial effluent. This tool could easily be adapted for use by both local government officials, the Department of Environment and local community members with very little educational background, technical knowledge and financial resources.

1 Introduction

Industry and associated industrial pollution has been gradually increasing in Bangladesh with the manufacturing sub-sector growing at a rate of five percent between 1972 and 1992 (Bhattacharya *et al.*, 1995) and a similar or higher growth rate still being experienced. The consequence is that water bodies are receiving large quantities of industrial effluent and becoming increasingly polluted. For example in Kaliakoir Thana, Gazipur District, to the north east of Dhaka, there are currently over 270 industries discharging effluent into Mokesh Beel and the Turag-Bangshi River. This has led in the past two years to fish kills in the river during the dry season (Daily Star, 6th April 2004).

Policing of industrial pollution in the country is complicated by the constant increase in the number of factories and the limited resources of the Department of Environment (DoE), which does not have the financial, human or technical capacity to monitor water pollution using traditional water quality analysis methods. To overcome these problems and to ensure that pollution levels can be satisfactorily monitored and evidence collected to prosecute industries that are not complying with the Government of Bangladesh Environmental Conservation Act (1995) and Rules (1997), a monitoring methodology and protocol is required that is less costly, less time consuming and does not need such technical equipment.

Those who suffer from the increased agricultural pollution are the people who traditionally depend on the water bodies for livelihoods activities, including bathing, fishing and irrigation. These people are aware of the pollution and its impacts but are rarely in a position to address the situation due to lack of information or empirical evidence (Zafar *et al.*, 2005). A methodology that enabled local communities to monitor their water bodies in a cost effective but scientific and systematic way would be extremely beneficial to them.

Biological monitoring using macroinvertebrates is one option that may be viable to meet the needs of both the DoE and local communities. It is generally considered that macroinvertebrate monitoring is of considerably lower cost than water quality analysis, needs much less sophisticated equipment and depending on the methodology employed, less technical expertise. It also has the advantage that it can provide an indication of longer-term, chronic pollution problems. This can not always be achieved by water sampling, which only indicates the level of pollution at the instant that the sample is taken and may miss pulses of pollution (Moss, 1998).

This methodology was therefore tested in Kaliakoir to determine whether it was possible to; collect samples of macroinvertebrates, identify them and adequately link them with pollution levels as measured by water quality parameters. Furthermore the work was used to determine whether or not it was a methodology that could be effectively used by community members or relatively unskilled staff members of DoE.

2 Macroinvertebrate Monitoring

Biological monitoring of water quality using macroinvertebrates has been used in Europe, the United States, Canada, Australia and South Africa. It has been developed primarily to monitor the quality of flowing water and thereby allows environment agencies to track pollution, especially where there is concern over illegal dumping of pollutants and to provide evidence of environmental improvement after the introduction of pollution reduction measures.

The principle of the approach is that the presence or absence of macroinvertebrate families and their relative proportions may be indicative of the "state" of the water body. The approach has been developed around how organic pollution affects the oxygen, nutrient and aquatic life of a flowing water body down stream of a pollution outfall (Figure 1). As the quality of water improves with distance from the outfall, it is expected that species that are less pollution tolerant will return to previously polluted sites and that species diversity will improve (Parivesh, 2002). Although the method was developed for organic pollution in flowing streams it is increasingly being used and adapted for static water bodies and for other forms of pollution.

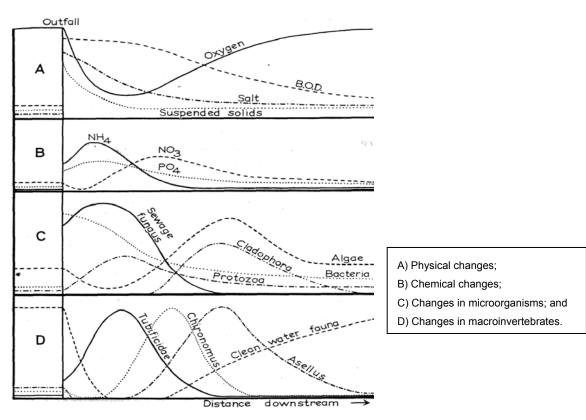


Figure 1: Representation of the changes in water quality and populations of organisms in a river below a discharge of organic effluent (Source: Hynes, 1960).

Macroinvertebrate monitoring is not however without its criticisms and much research has been undertaken to determine the effectiveness of the various biotic indices that have been developed. In days when chemical waste and sewage waste were not much, the Saprobic system was widely used for monitoring invertebrate population. The *system* classified pollution pattern into four types. These are Oligosaprobic, when water is clean, Betamesosaprobic when there is dominance of *Gammarus pulex*, Baetis rhodani and various Caddis flies, not *Chironomus* or *Asellus*., Alpha-meso-saprobic when *Chironomus* and *Tubifex*, *Asellus*, *Herpobdella* and other pollution tolerants were present.

After the Saprobic system, the Trent biotic index was used in a number of countries (Haslam, 1991). This Index classifies the biological samples into key indicator groups, diversity of fauna and total number of groups (for example each species of Platyhelminthes or family of Chironomidae).

Presently, two of the more prominent indices used in the United Kingdom are the Biological Monitoring Working Party (BMWP) Score System and the Average Score Per Taxon (ASPT) (Hallawell, 1978; Armitage *et al.*, 1983). The BMWP score system is based on families of benthic macroinvertebrates that have been given a score between 1 and 10 based on their pollution tolerance, with the most sensitive receiving the highest score. The scores for each family are added and an overall BMWP score is obtained, which gives an indication of the biological condition of the water body (Walley and Fontama, 1998). Advantage of BMWP score is that it gives a reasonable correspondence between chemical classification and biological scores and it can measure the effect of pollution over a period. In addition, BMWP score can detect short discharge of pollution, which is usually missed by chemical sampling (Moss,1998).

There are two main limitations of BMWP score system. The first is that it is sensitive not only to pollution but also other environmental factors such as the type of water body, as it is based on macroinvertebrate populations of streams, not static water bodies. Secondly the system was originally developed for European benthic macroinvertebrate families and can not therefore be immediately translated to Asian water bodies without knowledge of the sensitivity of the benthic macroinvertebrate families found in that region. There are however a few examples of the use of the BMWP score system being used in tropical and subtropical areas. One good example is in Thailand where application of the BMWP system was tested in two main rivers and canals. It was found some families that occur in Thailand are absent in the original BMWP system and some families that are present in the original BMWP system are The system was therefore modified and called the BMWP^{THAI} score. absent in Thailand. Similar type of study was also conducted in Indian rivers where the BMWP was also needed to be modified (Mustow, 2002). Does this reference relate to the India study or only the Thai Study? If only the Thai then put it at the end of the last sentence and put the India reference here.

Nishat, please put in a table of the families and their scores

The ASPT method in which the BMWP score is divided by the number of different families identified has similar limitations but is less sensitive to sampling effort and seasonal

variations. It can however yield a relatively high score from sites that would be considered polluted (some explanation of why this is) and it is therefore advisable to use the BMWP and ASPT together (Walley and Fontama, 1998).

In the United States, the U.S. Environmental Protection Agency (USEPA) has developed the Rapid Bioassessment Protocol and in In India the Biological Water Quality Criteria (BWQC) is used by the Indian Central Pollution Board (Diener *et al.*,unpublished).

3 Methodology

Samples have been collected from selected sites in Mokesh Beel and Ratanpur Khal, which runs through the *beel,* since 1993 (Figure 2 and Table 1).

Sample name	Sample location
Site 1	River Turag
Site 2	Khal below tributary
Site 3	Tributary khal
Site 4	Khal above tributary
Site 5	Khal at Matikata
Site 6	Railway bridge
Site 7	Khal at Shafipur
Site 8	Khal under factory outlet

Table 1: Sample labels and locations

In this component of the work water samples continued to be taken from those sites but in addition kick samples were taken to collect macroinvertebrates. Four sets of samples were taken at intervals though out the year to ensure that they were collected when there were various water levels in the *beel* as this would effect pollution loads and the locations where benthic macroinvertebrates could survive. It was also important as the life-cycles of macroinvertebrates in Bangladesh are not as well understood as those in the northern hemisphere and there was the possibility that this may influence the samples. The exception was "Above tributary site", which was a location on the tributary from Kalidaho *beel* flowing into Mokesh *beel*, from which biological samples were collected only three times.

In Bangladesh pre-monsoon occurs from March to May when rainfall is less than 20 percent of typical monsoon rainfalls. The monsoon occurs from May to October and the dry period occurs from November to April when rain fall is on average less than 3 percent. Based on this fact sampling was conducted in February, May, October and December. On the first occasion (February) water samples were not collected as on that occasion it was necessary to test out the methodology and to see whether the proposed methodology was feasible.

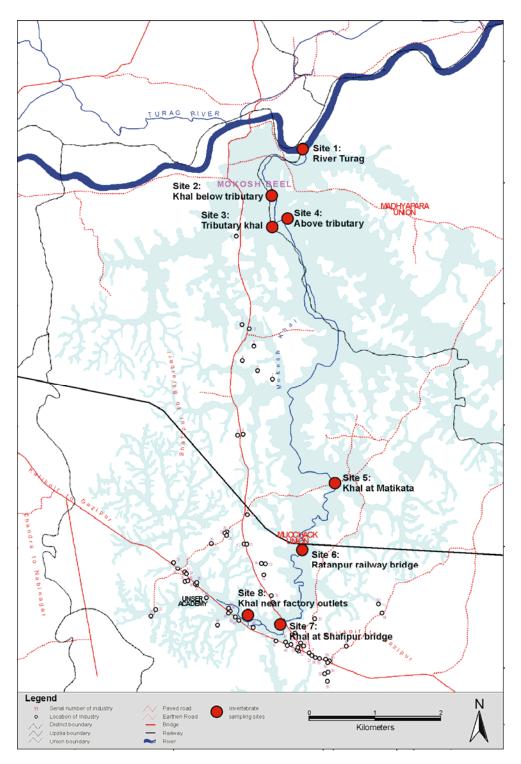


Figure 2: Sampling sites along Mokesh Khal and Mokesh Beel, Kaliakair, Bangladesh

Temperature and pH were measured on site using a simple "pen type" pH metre manufactured by HANNA. Total Dissolved Solid (TDS), Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD), sulphate an, sulphide were measured at the Bangladesh University of Engineering and Technology (BUET), Environmental laboratory, Dhaka. From December dissolved oxygen (DO) was also measured onsite using and in addition ammonia also measured at BUET for the December sample. These parameters were added because there were some major fish kills that were believed to be caused by

inadequate levels of DO. Ammonia was measured because it affects fish health in several different ways, acting as a strong irritant, especially to the gills. Prolonged exposure to sublethal levels of ammonia can cause skin and gill hyperplasia.

Macroinvertebrate samples were collected from the shallow sites using a kick sampling technique in which the substrate is disturbed by kicking and the benthic macroinvertebrates that are dislodged are caught in a net. This technique was used because it is the most common technique for benthic macroinvertebrate sampling in shallow waters and is extremely simple. During sampling standard kick sampling techniques was applied first. This was basically, holding the net for a period of time and shuffling through the substrate moving in the opposite direction to the flow of the water and collecting invertebrate samples. In site 1, the Turag River was very hard and compact, and it was immediately evident that standard kick sampling would be difficult. Therefore, a couple of options were tried. Finally, the sampling frame of the kick net was placed against a desired downstream edge location and sediment was scraped five times with the metal edge of the net before collecting a sample from the water. After collection of the sample, it was tipped into a large plastic box and the net was washed thoroughly with water to ensure nothing was left behind on the net (Figure 3). This method was repeated in all other sampling sites but was made difficult in site 3 during the summer where the water flow had been reduced to a trickle.



Figure 3: Macroinvertebrate sampling at site 4, Ratanpur Khal, Kaliakoir

During the October sampling even, a hand operated Ekman grab sampler was used as the water level was very high and it was impossible to do kick sampling. The grab was thrown out from the boat into the water and allowed to hit the substrate. After it hit the substrate, the

clamp on the bottom of the grab was closed. A sample of substrate held within the grab was then emptied into the large plastic box.

Samples were sorted at the project laboratory. The sorting involved a portion of the sample being filtered through a fine strainer into a white inspection tray. The sample was then sorted through using a hand lense, a dropping pipette and tweezers. Some samples contained a great deal of sediment and required rinsing thoroughly but all rinses were done over a tray to ensure no macroinvertebrates were lost. Many of the macroinvertebrates present in the sample were extremely small and were only visible when moving, though their fast movement made them difficult to catch. These were collected by isolating them by removing most of the water and then killing them with alcohol prior to collection.

All the macroinvertebrates collected were stored in alcohol in plastic containers labelled according to the sampling site. Identification of the preserved samples were carried out in the Department of Zoology, University of Dhaka.

4 Results

1.1 Macroinvertebrate Analysis

Sites 1, 2, 3 and 4, which are furthest from the factories, had the greatest variety of macroinvertebrate families ranging from one to 11 over the year but between five and eight families in February and eight to 11 families in December compared to none or one for the other four sites (Table 2 and Annex A). The count is slightly complicated by the fact that the biologists at Dhaka University were unable to identify many individuals and these have therefore not been included in the count of families (except in site 5 where only one unidentified individual was found in the whole sample and was therefore included). However this may not cause that much of a problem as in most cases the unidentified macroinvertebrates were found in sites 1-4 and not in sites with low family counts.

On all occasions no macroinvertebrates families were found in Site 6. It is likely that the reason for this is that samples were collected from the immediate vicinity of discharge point where there is high BOD, COD and temperature (Table 2).

Well known pollution indicator families Culicidae (mosquito larvae), Naidae (blood worms), Chironomidae (midge larvae) and Tubificidae (blood worms) families were found in the study area. These families are often considered to be pollution indicators because they can tolerate very high levels of BOD and low DO levels and are therefore the last to be removed from a highly polluted site and the first to return as pollution declines (Figure 1). Culicidae, or mosquito larvae, for example achieve their pollution tolerance by hanging from the surface of the water with a breathing tube protruding into the air, they can therefore survive in waters with zero dissolved oxygen.

Site 1 to Site 4 consistently had the highest number of pollution indicator families ranging from one to three but they also had the greatest species diversity, suggesting that these a the least polluted of the sites (Table 1). Tubificidae and Culicidae were found in sites close to factory outlet (Site 5, 7 and Site 8) and were often the only families found. This indicates that these sites were suffering from severe organic pollution (APHA, 1998, Anwara et al., 1986).

The seasonal macroinvertebrate family count of each site was tested using Kruskal-Wallis test. The results of the analysis did not show any seasonal variation in macroinvertebrate family counts (Chi-square = 3.046; df = 3; p > 0.01).

Month	Site ID	Family count	Indicator Family Count	Water Condition
February	Site 1	5	1	Moderate pollution
	Site 2	8	2	
	Site 3	8	2	
	Site 4	8	3	
	Site 5	1 ¹	1	High pollution
	Site 6	0	0	Severe pollution/dead
	Site 7	1	1	High pollution
	Site 8	0	0	Severe pollution
Мау	Site 1	2	2	Moderate pollution
	Site 2	3	2	
	Site 3	5	1	
	Site 5	3	1	
	Site 6	0	0	Severe pollution/dead
	Site 7	1	0	
	Site 8	1	1	High pollution
October	Site 1	1	2	It is difficult to interpret the level
	Site 2	3	?	of pollution as the samples were
	Site 3	1	?	collected in deep water
	Site 5	3	0	
	Site 6	0	0	
	Site 7	4	0	
	Site 8	0	0	
December	Site 1	11	1	Moderate pollution
	Site 2	8	2	
	Site 3	8	2	
	Site 4	9	2	
	Site 5	2	0	
	Site 6	0	0	Severe pollution
	Site 7	0	0	
	Site 8	0	0	

Table 2: Results Biological water quality of different sites

¹ "Unidentified" in site 5 is included in the family count as it was the only individual in the sample.

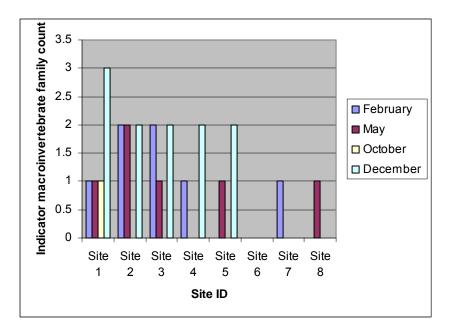


Figure 4: Indicator macroinvertebrate family count

Using this data a set of criteria were developed with which to categorize the condition of the sites (Table 2). This was based on the following:

- A site with no pollution indicator species and no family count indicates severe pollution.
- A site with pollution indicator families and < 2 family counts indicates high pollution.
- A site with or without pollution indicator species and ≥ 2 family counts indicates moderate pollution.

1.2 Water Quality Analysis

The results of the water quality analysis indicated levels of BOD significantly above the national standards in sites close to the factory outlets (Site 5 to Site 8). These were also the sites with the lowest family diversity. The sites located in or near the Turag river had low BOD levels on all occasions. The lowest concentration of BOD was observed in October suggesting that dilution may have occurred due to flood water. The highest concentration of BOD (185 mg/L) was observed in December at Stie 5, possibly due to the receding of water in the area or because factory effluent flow had increased (Table 3). COD was also consistently higher in Sites 4-8 than in Sites 1-4, where it never exceeded the national environmental quality standards, compared to a 50 percent compliance rate in Sites 4-8 (Table 3).

	Parameter	National standard	Unit	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8
February	Family count			5	8	8	8	Unknown family	0	1	0
2005	No chemical	parameters	tested	_							
May 2005	Family count			2	3	5	No sample	3	0	1	1
	pН	6.5-9		8.4	8.3	9.4		8.8	9	9.1	7.9
	TDS	2100	mg/L	1454	1482	1201		1659	1095	1697	768
	BOD5	50	mg/L	35	34	33		160	120	105	107
	COD	200	mg/L	69	68	66		322	226	168	172
	Sulfate	400*	mg/L	400	400	325		390	280	570	140
	Sulfide	1	mg/L	0.007	0.004	0.001		0.121	0.123	0.063	0.015
	Temp		°C								
October 2005	Family count			1	3	1	No sample	3	0	4	0
	pН	6.5-9	pН	7.8	9	9.3		8.9	9.7	9.2	9.8
	TDS	2100	mg/L	88	267	276		396	1514	1535	1609
	BOD5	50	mg/L	1.6	8	9		21	103	105	100
	COD	200	mg/L	3	20	22		44	167	169	153
	Sulfate	400	mg/L	8	37	39		74	475	325	875
	Sulfide	1	mg/L	0.007	0.003	0.001		0.005	0.038	0.045	0.029
	Temp		°C	31	31	32		35	35	35	35
December 2005	Family count			11	8	8	9	4	0	0	0
	pН	6.5-9		8.5	8.7	7.9	8.7	9.4	9.5	9.2	9.5
	TDS	2100	mg/L								
	BOD5	50	mg/L	50	45	25	50	185	130	110	140
	COD	200	mg/L	72	62	34	70	370	257	216	270
	Sulfate	400	mg/L	155	145	4	165	310	450	525	300
	Sulfide	1		0.02	0.015	0.01	0.02	0.125	0.15	0.105	0.155
	DO		mg/L	1.6	3.1	1.2	3.3	0.3	0.3	0.2	4.8
	NH3		mg/L	0.5	0.45	0.45	0.55	3.15	4.5	4.35	4.6
	Temp		°C	23	23.3	23.4	23.1	27.9	29.9	29.3	35.4

Table 3: Key chemical, physical and biological characteristics of the study sites²

Simple correlations in Microsoft Excel show a negative correlation between family count and almost all chemical water quality parameters in all sampling events (Table 4).

² In all site unidentified families were not treated as a family count

Chemical parameters	Мау	October	December
рН	0.304303	-0.2507	-0.80853
TDS	0.224088	-0.18625	
BOD5	-0.47647	-0.16294	-0.75122
COD	-0.36949	-0.13324	-0.77178
Sulfate	0.103481	-0.47078	-0.84112
Sulfide	-0.44129	-0.04174	-0.91864
Temp		-0.00747	-0.90289
DO			0.163159
NH3			-0.97405

Table 4: Correlation between family count and chemical parameters

5 Discussion

The purpose of this study was to determine whether or not biological monitoring of water bodies in Bangladesh using macroinvertebrates as indicators, is a viable option for determining the condition of the water bodies in terms of pollution levels, and whether the approach would be suitable for adoption by the DoE or community groups.

The results of the research indicated that Mokesh Khal is biologically dead at its upper reaches where it is fed almost entirely by factory effluent. This is likely to be due to the presence of a number of pollutants and absence of oxygen restricting life in the water body. The relatively diverse macroinvertebrate families found at sites 1, in the river (site 1), in the khal at the far end of the beel (sites 2 and 4) and in a "clean" tributary canal coming from Kalidaho Beel (Site 3), suggest that these sites are comparatively less polluted than the upper end of Mokesh Beel, which is closest to the industry outlets. High species diversity was observed in sites where water quality parameters indicated that the water was less polluted. The high correlation between biological diversity and lower pollution levests suggests that macroinvertebrate monitoring could be a good replacement for expensive and technical water quality analysis.

Well known pollution indicator families Culicidae, Naidae, Chironomidae and Tubificidae were found in the study area. These indicator species were easily detectable with the naked eye, though Naidae and Tubificidae cannot be distinguished and treating these two families collectively as Oligochaeta (blood worm) may be of greater use. These families are likely to be present in samples where no or few other families exist, when this is the case it is possible to say that the water is highly polluted.

The work clearly shows that a simplified methodology can easily be employed for monitoring the condition of water bodies using macroinvertebrates that can be adopted by people with relatively low education levels and with very basic equipment. All that is required is: a D-net of standard specification - the project had one make in Bangladesh from a wooden pole, a shaped metal bar and a fishing net of very fine mesh size; a white tray (to make it easier to see the invertebrates); and a handlense, although even this is not necessary. To undertake the analysis all that is required is that macroinvertebrates are separated into "things that look alike" (Table 1). The samples with the greatest number of these has the greatest species diversity and is therefore the least polluted.

Table 5: Example of simple family count

Description	Site 1	Site 2	Site 3	Site 4
Round hard body, six legs	Yes		yes	
Red, small worm	Yes	Yes	Yes	Yes
Round head, curled body, spike on tail	Yes	Yes	Yes	
Long thin body, six legs, antennae	Yes			
Pollution level	Low	Medium	Medium	High

Obviously this is highly simplistic and needs to be tested with the community and with further analysis of chemical properties of the water. However these initial results suggest that it will be viable.

For more experienced and better educated people, such as those employed by the DoE, it would be possible to further refine the method so that they identify families. Good keys exist to make this relatively simple.

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Annex A

Site 1: River Turag Site 2: Khal below tributary Site 3: Tributary khal Site 4: Khal above tributary Site 5: Khal at Matikata Site 6: Railway bridge Site 7: Khal at Shafipur

Site 8: Khal under factory outlet

Family	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8
Corbiculidae	√ (3)							
Viviparidae	√ (2)							
Thiaridae	√ (4)			√ (7)				
Bithynidae		√ (7)	√ (4)	√ (3)				
Chironomidae	√ (1)		√ (3)	√ (61)				
Palaemonidae	√ (2)							
Naididae		√ (1)	√ (1)					
Hydrophilidae		√ (20)	√ (1)					
Forcipomyidae		√ (5)	√ (3)	√ (4)				
Corynoneura		√ (29)						
Culicidae		√ (7)		√ (10)			√ (21)	
Corixidae		√ (12)						
Unionidae		√ (4)	√ (3)					
Thiaridae			√ (8)					
Viviparidae				√ (4)				
Tubificidae				√ (1)				
Pleidae			√ (1)	√ (5)				
Unknown	√ (7)	√ (40)	, , ,	√ (4)	√ (1)		√ (1)	
Sum of families	5	8	8	8	0	0	1	0

Table A-1 List of macroinvertebrate families in dry season (February, 05)

Family	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8
Bithynidae	√ (2)	√ (1)	√ (48)	No sample				
Chironomidae		√ (1)		collected				
Palaemonidae	√ (1)			CONECIEU				
Culicidae		√ (3)						
Unionidae			√ (4)					
Thiaridae			√ (6)					
Viviparidae					√ (2)			
Tubificidae			√ (2)		√ (2)			√ (1)
Lymnaeidae			√ (1)				√ (1)	
Tabanidae					√ (1)			
Unknown		√ (3)	√ (1)					
Sum of families	2	3	5		3	0	1	1

Family	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8
Viviparidae		√ (9)	√ (4)		√ (1)		√ (4)	
Thiaridae		√ (5)	(+)		(1)		√(1)	
Bithynidae					√ (1)			
Culicidae	√(1)							
Corixidae								
Lymnaeidae							√(1)	
Planorbis					√(1)		√(1)	
Unidentified		√(1)						
Sum of families				No				
	1	3	1	sample	3	0	4	(

Table A-3: List of macroinvertebrates families in late monsoon (October 2005)

Table A-4: List of macroinvertebrates families in dry season (November 05)

Family	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8
Corbiculidae	√(1)							
Viviparidae	√(1)	v(83)	(16)	√ (14)				
Thiaridae	√(71)	v(12)	√ (1)	√ (3)				
Unioinidae	√(11)	√ (6)	√ (2)	√ (1)				
Planorbidae	√(1)	√ (1)						
Pilidae	√ (2)		√ (19)	√ (44)				
Notonectidae	√ (19)		√ (9)	√ (187)				
Naidae		√ (171)	√ (6)	√ (4)				
Chironomidae		√ (9)	√ (3)	√ (4)				
Cobitidae	√ (2)	√ (2)						
Palaemonidae		√ (1)	√ (26)	v(1)				
Culicidae	√(1)							
Psephenidae					√ (5)			
Dytiscidae								
Pleidae	√(1)			√ (53)	√(1)			
Tendipedidae	√(1)							
unidentified		√ (1)		√ (4)	√ (2)			
Sum of familes	11	8	8	9	2			

(---) denotes invertebrate count

 $\sqrt{\mathrm{denotes}}$ presence of particular family

Annex B



















Family: Culicidae