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Riverine biota as indicators of water quality in tropical Cagayan de Oro River, Philippines

Astrid L. Sinco, Judy P. Sendaydiego, Leolinda L. Saab, Geraldine R. Mojica, Giovanni G. Tampus, Adora S. Rondez

Biology Department, Xavier University-Ateneo de Cagayan, Corrales Avenue, Cagayan de Oro City, Misamis Oriental, Philippines. Corresponding author: A. L. Sinco, asinco@xu.edu.ph

Abstract. The quality of an aquatic habitat is an important factor that influences the biological assemblage of any river. Consequently, the characteristics of the biological assemblage determine the overall health of the aquatic system. Thus, characterization of the habitat and biological community are important in assessing and monitoring riverine systems. This study is an inventory of organisms that are considered as bioindicators of the water quality of river systems. These include the macroinvertebrates, diatoms and coliform. These organisms are sensitive to changes in physical and chemical conditions of river systems. The study area, Cagayan de Oro River is the biggest river within Cagayan de Oro City situated in the Southern part of the Philippines. The river basin has a vast economic and environmental significance and potentials as it is a source of water for domestic, industrial, agricultural, and even for hydroelectric power generation. There is no comprehensive baseline data of the physical, chemical and biological integrity of the river or an attempt to monitor these parameters. Thus, collection and analysis of biological assemblage from different sampling sites of Cagayan de Oro River provided holistic evaluation of its current condition. Among the macroinvertebrates, 9.93% are sensitive to poor stream conditions, 61.06% are moderately tolerant to degraded habitat and water quality, and 29.01% are most tolerant to degraded habitat and water quality. In terms of diatoms, the overall biological integrity of Cagayan de Oro River is fair which indicates reasonable degree of siltation and stress to diatom assemblage. The coliform load of the river falls within the standard range for natural bodies of water. Among the measured physico-chemical parameters, only total suspended solids and phosphates are beyond the acceptable standard values.

Key Words: bioindicator, coliform, diatoms, macroinvertebrates.

Introduction. A significant effort all over the world were done to assess water quality focusing not just to chemical parameters but also to biological indicators (Martin & Fernandez 2012; Resh et al 1995). Currently, most of the criteria for national standards of water quality assessment are based on chemical integrity which does not reflect the response to multiple stressors to aquatic resources (Uttaruk et al 2011). Biomonitoring is a precise and accurate method in assessing several kinds of water pollution because it provides complete spectrum of information for proper water management (Li et al 2010). In this study, biological assemblages of macroinvertebrates, diatoms and coliform were used to determine the water quality of Cagayan de Oro River.

Macroinvertebrates are ubiquitous in streams and rivers, and play a crucial role in the aquatic ecosystem. They help in cycling of nutrients, and provide the link in the energy flow between primary producers and other consumers. Macroinvertebrates are also widely used as indicators of water quality, especially pollution of rivers and streams (Goodnight 1973; Chessman & McEvoy 1998; Kalyoncu & Zebek 2011). Many species of different taxa such as mollusks, rotifers, worms, and insects among others have been found particularly useful as indicators of pollution because they show wide ranges of tolerance in their reactions to various degrees of pollution. Some are mobile enough to rapidly leave a polluted area (Goodnight 1973), while others are sedentary in nature which ensures exposure to pollutants and environmental stressors (Chessman et al

1998). Macroinvertebrates are used to assess and monitor water quality in the tropics and worldwide.

The taxonomic composition of diatom communities has been widely used for monitoring water quality. Changes in their abundance and diversity reflect water quality (Stevenson 1984). Assessment of human impact on aquatic biota included the listing of micro-algal species that are indicators of the saprobic level to assess the level of organic matter (Passy 2007). Monitoring water quality using diatoms has the following advantages: (1) the ease of permanent storage of specimen; (2) a relatively straight forward sampling which can be readily adapted to most geographical regions and circumstances; (3) their taxonomic diversity mirrors the extent of pollution and (4) basis of several developed indices of water quality (Kelly & Whitton 1998; Stevenson 1984).

Fecal coliform is a group of bacteria that are also commonly used as indicators of water quality in rivers and streams. These bacteria are found in the gut of mammals. Isolation and enumeration of this group of bacteria (Howell et al 1995; Thelin & Gifford 1983) may determine the extent by which natural waters have been impacted by fecal matter. As indicator organisms, the presence or absence of coliform bacteria assesses the extent of contamination and degree of pollution in the river. Monitoring coliform load helps direct pollution management and control.

Cagayan de Oro River is a major river system in Cagayan de Oro City situated in the Southern part of the Philippines. The river has its headwaters in the Kalatungan Mountain Range found in the central part of the province of Bukidnon. It flows northward towards Cagayan de Oro City traversing the municipalities of Talakag, Baungon, and Libona, picking up tributaries along the way and empties into Macajalar Bay stretching about 90 kilometers. The catchment area is approximately 152,000 hectares, eighty percent of which is located in the province of Bukidnon, and the rest is in Lanao del Norte and Cagayan de Oro City. The river basin has great social, physical, economic, and environmental significance and potentials as it is a source of water for domestic, industrial, agricultural, and even for hydroelectric power generation. The river has also been a popular site for white water rafting and kayaking in the Philippines. The river's full potential as a recreation area may be realized if water quality along the river stretch is maintained clean and safe.

This research is a baseline inventory of macroinvertebrates, diatoms and coliform which are bioindicators of the status of water quality in river systems. Specifically, the study aimed to determine the current status of the river based on the Family Biotic Index (FBI) for macroinvertebrates, overall biological integrity for diatoms and Most Probable Number (MPN) index for coliform. Physical and chemical conditions that existed at the time of field sampling were measured and compared to acceptable levels using Philippine and US-EPA (1994) standard values.

Material and Method. The research was conducted in 2010 to 2011 and comprised of field and laboratory components. Sixteen sampling sites were established along Cagayan de Oro River which includes sites in the main channel and tributaries. Sampling was done twice. Figure 1 shows the map of the sampling sites in Cagayan de Oro River.

The physical profile of each sampling station was measured including channel width, depth at the left, right and middle of the river channel; flow velocity at the left, right and middle of the channel; and elevation as well as the latitudinal and longitudinal location were determined using Magellan global positioning system (GPS). Samples for the analyses of nutrients such as phosphate, nitrate, nitrite and silicate were collected using acid washed bottles. Concentration of nutrients was analyzed using spectrophotometry. The total dissolved solids (TDS) and the hydrogen potential (pH) was measured *in situ* using Sartorius pH-TDS meter. Temperature, conductivity and dissolved oxygen (DO) was measured *in situ* using Jenway DO-Temperature-Conductivity meter. Biological oxygen demand (BOD) used dark bottles during sampling. In the laboratory, water samples were incubated at 20^{o}C for five days prior to determination of dissolved oxygen concentration. Total suspended solids (TSS) determination used 500-mL sampling bottles. Samples were filtered in a pre-weighed Whattman standard filter paper, GF/C with a pore size of 0.45 μm (Cat No. 1822047). TSS concentration is equal to the weight of the residue in the filter paper after drying at 103-105^oC.

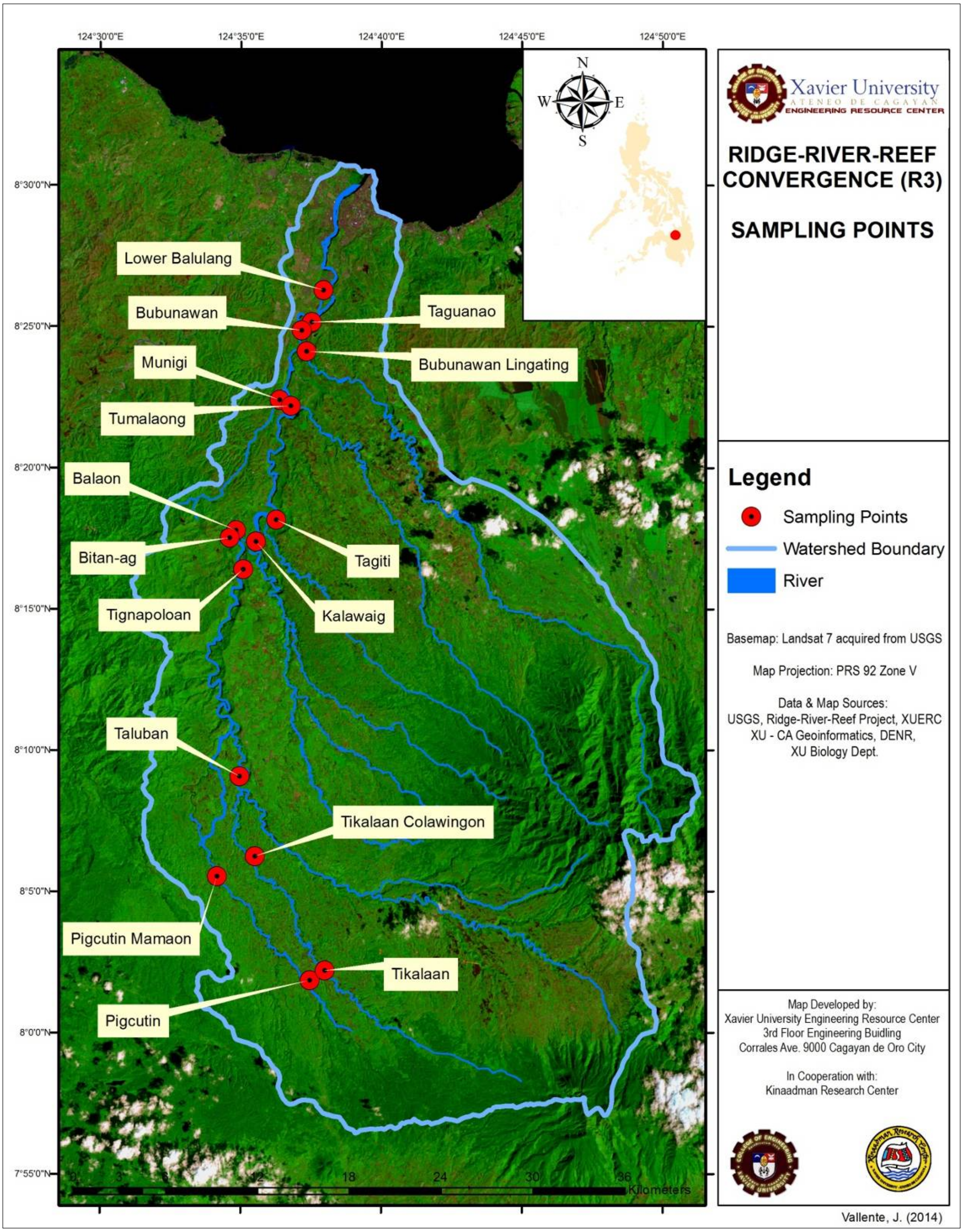

Figure 1. Map of sampling sites in Cagayan de Oro River.

Field sampling of macroinvertebrates employed the “kick” net method through a 1 m^2 kick net with mesh size of 0.3 mm. The method involved a 3-minute kicking of the stream substrate and other habitats to dislodged the organisms, only to be caught by the net placed 1 meter downstream. The process was repeated at every 50-meter interval of the 150-meter sampling site to obtain a pooled field sample of organisms and debris from each site. Each field sample was added with equal amount of 95% ethyl alcohol for preservation, and few drops of rose Bengal for staining. The macroinvertebrates from each field sample were picked and collected in the laboratory using medicine dropper or forceps with the aid of a stereo-binocular dissecting microscope. The collected organisms were brought to a volume of 100 mL by adding 95% ethyl alcohol, and obtained a sub-sample of 10 mL in 3 replicates. Organisms in each replicate sample were rehydrated, counted, and identified at Family level taxon using the taxonomic keys of Sangpradub & Bonsoong (2006). Biodiversity of macroinvertebrates was measured through relative abundance, taxa richness, and Simpson's index of diversity. Similarity of macroinvertebrate assemblage among sites was determined using the Bray-Curtis similarity index. Determination of organic pollution in the river using the macroinvertebrates as indicators employed the Modified Family Biotic Index (Hilsenhoff 1988; Barbour et al 2000).

Epilithic algal assemblages were collected in each transect by picking ten stones (20 cm or greater) from the stream bottom. A 1.5 inch delimiter was placed on the upper surface of each stone to define a 12 cm^2 area then a firm-bristle toothbrush was used to dislodge algae from the stone surface within the delimiter and fixed with 10% formalin. Each sampling bottle contained a composite of epilithon samples from the stones collected in each transect per sample site. Counting and identification of epilithon was done by a volume of 0.101 mL of the sample into a nanoplankton counting chamber. Samples were viewed and counted at 400x using Leica light microscope. Epilithon were identified down to genus level. Community indices such as species richness (S), Shannon's diversity (H'), and Pielou's Evenness index (J') were determined and computed.

Collection of water samples for coliform analysis was done by lowering sterile sampling bottles into the bottom of the water body at a depth of about 30 cm, and allowed to over flow before withdrawing. Bottles were stored in ice coolers while in transit to the laboratory for analysis. The water samples were immediately inoculated in lactose broth to determine the number of coliform bacteria. A three-tube MPN (Most Probable Number) method was used for this purpose. The number of positive reactions at each dilution was used to determine the MPN of fecal coliforms from a standard MPN table.

Results and Discussion. The macroinvertebrate assemblages comprised a total of 63 families as shown in Table 1, 36 of which are indicators of water quality and habitat degradation. Many of these families belong to the orders Ephemeroptera (13 families), Trichoptera (11 families), Coleoptera (8 families) and Diptera (7 families). Among the macroinvertebrates, 9.93% are classified as sensitive to poor stream conditions, 61.06% are classified as moderately tolerant to degraded habitat and water quality, and 29.01% are classified as most tolerant to degraded habitat and water quality.

The abundance of macroinvertebrate assemblage differs in relation to stream size and tributary position (Heino et al 2005). In many streams and rivers, the highest taxa richness is commonly observed among insect groups like the ephemeropterans (Rada & Puljas 2010; Tampus et al 2012) especially during summer when the ephemeropteran Family Baetidae and Family Baeticidae adult emerge. These taxa are moderate to highly intolerant of water pollution, and require swift flowing water to receive adequate oxygen (Barbour et al 1999). Although the *Baetis* group are common to several sites it had notable morphological differences among individuals collected in different areas (Buffagni et al 2001). The Modified Family Biotic Index (FBI) is a measure of the water quality using the tolerance value of the comprising macroinvertebrate family. The mean FBI of the sampling sites and the rating of the water quality are shown in Table 2. The FBI index was lowest at Tagiti with 3.67 and highest at Lower Balulang with 4.89.

Samples taken from Cagayan de Oro River (Figure 2) consisted of nineteen (19) genera belonging to Bacillariophyceae. Diatoms are organisms that stabilize substrata and serve as habitat for many other organisms (Hill et al 2000). Since diatoms are attached to the substrate, they are most likely affected by physical, chemical and biological disturbances that occur in the stream reach (Hobbs et al 2010). Diatoms are a large and diverse group of single-celled algae distributed throughout the world in nearly all types of aquatic systems, and are one of the most important food resources in freshwater ecosystems (Potapova & Charles 2002).

Table 1
Abundance of intolerant, intermediately tolerant and tolerant macroinvertebrates (Plafkin et al (1989) in Mandaville (2002))

Family of macroinvertebrates in Cagayan de Oro River	*Classification*	*Abundance (%)*
Leuctridae, Calamoceratidae, Glossomatidae, Perlidae, Philopotamidae, Psychomyiidae, Rhyacophilidae, Baeticidae, Ephemerellidae, Leptophlebiidae, Oligoneuridae, Polymitarcyidae, Potamanthidae,Tipulidae	Intolerant (tolerance value=0-3; sensitive to poor stream conditions)	9.93
Lymnaedae, Hydroptilidae, Hydropsychidae, Limnephilidae, Phryganeidae, Baetidae, Caenidae, Heptageniidae, Trichorythidae, Sialidae, Elmidae, Psephenidae, Corduliidae, Calopterygidae, Empididae, Certatopogonidae, Simuliidae, Pyralidae,	Intermediately tolerant (tolerance value=4-7; moderately tolerant to degraded habitat and water quality)	61.06
Physidae, Sphaeriidae, Libellulidae, Chironomidae	Tolerant (tolerance value= 8-10; most tolerant to degraded habitat and water quality)	29.01

Table 2
Family Biotic Index of macroinvertebrates as indicators of water quality in the sampling sites where the scores are based on Hilsenhoff (1998) (values ranged from 0.00-3.75 excellent with organic pollution unlikely; 3.76-4.25 very good with possible slight organic pollution; 4.26-5.00 good with some organic pollution probable; 5.01-5.75 fair with fairly substantial pollution likely; 5.76-6.50 fair poor with substantial pollution likely; 6.51-7.25 poor with very substantial pollution likely; 7.26-10.00 very poor with severe organic pollution likely)

Sampling sites	*Family Biotic Index (FBI)*
Taluban	4.61
Bitan-ag	4.32
Balaon	4.12
Kalawaig	4.00
Tagiti	3.67
Taguanao	4.76
Lower Balulang	4.89
Tikalaan	4.31
Pigcutin	4.17
Pigcutin at Mamaon	4.46
Tikalaan at Kolawingon	4.84
Tignapoloan	4.08
Tumalaong	4.53
Munigi	4.49
Bubunawan	4.40
Bubunawan at Lingating	4.58
Overall Family Biotic Index: 4.39	

In this study, 19 diatom genera were identified. The five most abundant genera are *Navicula, Fragillaria, Gomphonema, Cymbella* and *Cocconeis. Navicula* and *Surirella* are known to crawl towards the upper surface if they are covered by silt (Barbour et al 1999). *Cocconeis* density increases in response to general impairment in streams and rivers as well as to sediment, nutrient and metals impairment while *Surirella* increase in response to sediment impairment (Teply & Bahls 2006). Figure 2 suggests that Cagayan de Oro River being dominated with *Navicula* was tainted with siltation.

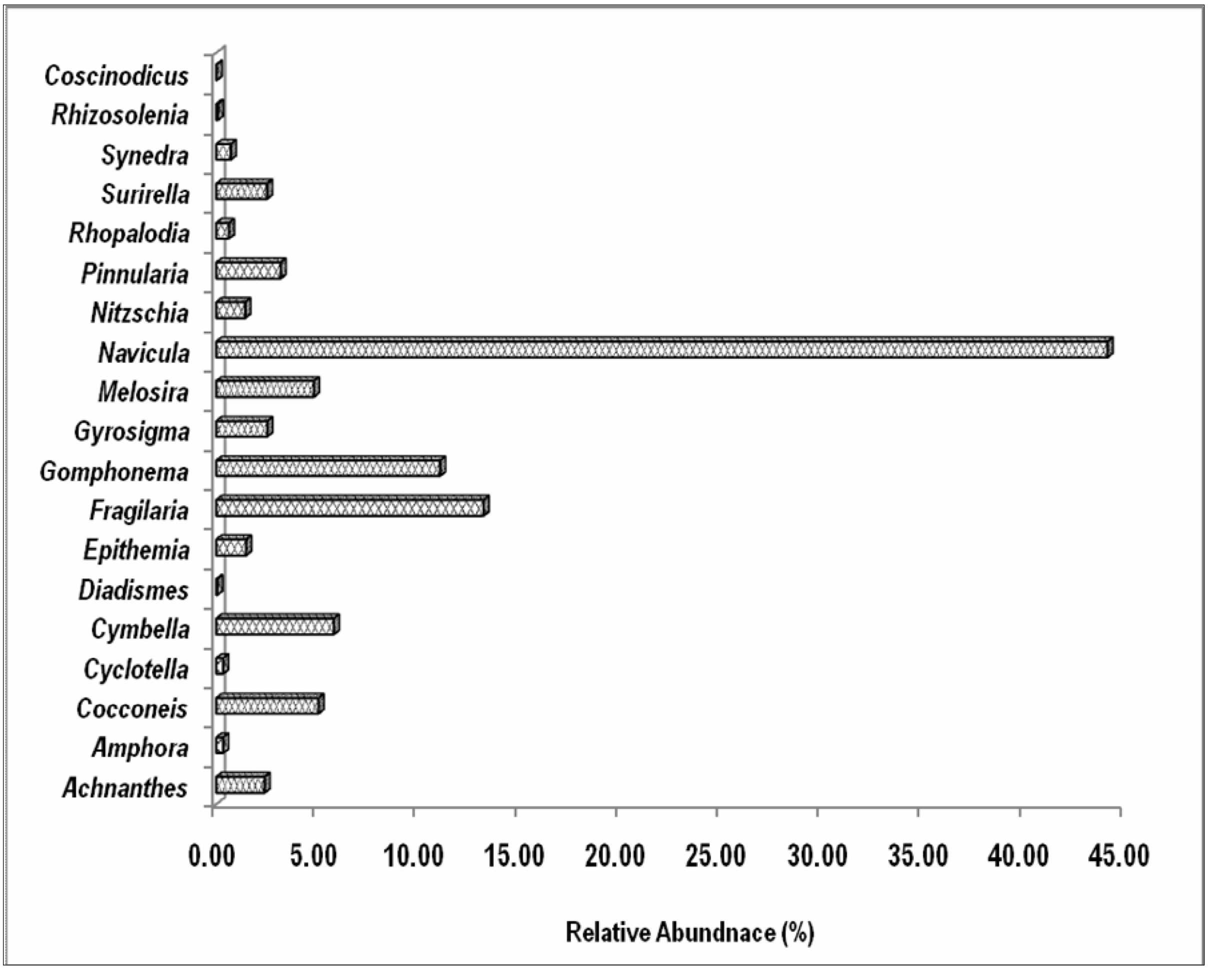

Figure 2. Relative abundance of diatoms in Cagayan de Oro river.

Diagnostic diatom metrics have been developed and tested in several countries of Europe and the United States (Descy 1979; Bahls 1993; Kelly & Whitton 1995) which are also the basis of the studies on diatom metrics in the tropics. These are used to diagnose environmental stressors affecting ecological health and to assess biotic integrity (Stevenson & Pan 1999; Teply & Bahls 2006). In this study three metrics was used to infer ecological conditions and biotic integrity namely diversity index, pollution tolerance index and siltation index. Results indicate that the diversity indices ranged from moderate to minor stress. It shows that sample sites consist of environmental variables that are potential stressors to the diatoms inhabiting Cagayan de Oro River.

The siltation index showed that most of the sampled sites had moderate to heavy siltation. Nearly all of the diatoms that live on sediments are biraphidean and highly motile (Bahls 1993). The total abundance of diatoms such as *Navicula, Nitzschia, Surirella* and *Gyrosigma* are high in most of the sampling sites. The high abundance of these diatoms reflects the amount and frequency of siltation in Cagayan de Oro River. These genera usually have high abundance in natural or human-inflicted siltation because they can just creep to the surface if covered by sediments or silt which characterize most of the sampling sites in Cagayan de Oro River.

The overall biological integrity rating considering pollution index, Shannon index and siltation index revealed that the rating is affected by the moderate to heavy siltation of the river is shown in Table 3. The siltation of Cagayan de Oro River may be caused by the (1) run-offs from agricultural lands surrounding the watershed, (2) degraded riparian vegetation, (3) periods of high rainfall in overland, (4) presence of several unpaved roads which are prone to erosional agents, and (5) instream disturbances one of which is quarrying which was mostly observed in the sampling sites.

Table 3

Diagnostic diatom metrics that infer ecological conditions

Sampling sites	*Diversity index (Shannon)*[1]	*Pollution index*[2]	*Siltation index*[3]	*Overall biological integrity*[4]
Taluban	1.79	2.46	50.49	Fair
Bitan-ag	1.71	2.53	43.66	Fair
Balaon	2.31	2.49	49.81	Fair
Kalawaig	2.09	2.49	47.73	Fair
Tagiti	2.27	2.61	36.01	Good
Taguanao	1.88	2.39	60.40	Poor
Lower Balulang	1.79	2.38	60.84	Poor
Tikalaan	2.11	2.56	41.71	Fair
Pigcutin	1.77	2.55	43.14	Fair
Pigcutin at Mamaon	1.90	2.55	44.43	Fair
Tikalaan at Kolawingon	1.99	2.51	44.78	Fair
Tignapoloan	2.17	2.46	52.13	Fair
Tumalaong	1.78	2.36	60.93	Poor
Munigi	1.37	2.21	79.43	Poor
Bubunawan	2.03	2.51	41.51	Fair
Bubunawan at Lingating	1.98	2.41	53.83	Fair
Overall	1.93	2.47	50.68	Fair

[1]>2.99 = no stress, 2.00-2.99 = minor stress, 1.00-1.99 = moderate stress, < 1.00 = high stress (Bahls 1993, 1993); [2]>2.50 = no pollution, 2.01-2.50 = minor pollution, 1.50-2.00 = moderate pollution, <1.50 = severe pollution (Bahls 1993); [3]<20.0 = no siltation, 20.0-39.9 = minor siltation, 40.0-59.9 = moderate siltation, >59.9 = heavy siltation (Bahls 1993); [4] The lowest rating for any one metric (diversity, pollution or siltation index) is the rating for that site: 4 = excellent, 3 = good, 2 = fair, 1 = poor (Bahls 1993).

The Department of Environment and Natural Resources, Philippine standards, classified Cagayan de Oro River as Class A waters (DENR 1990). The acceptable criteria values for coliform in class A waters should not exceed 1000 MPN/100 mL. In this study, results showed that coliform load in some sites of the river exceeded the acceptable standard for class A waters (Table 4). The mean MPN value of 646.28 however, falls within the acceptable standard. Class A waters, also known as Public Water Supply Class II is described as sources of water supply that will require complete treatment (coagulation, sedimentation, filtration and disinfection).

Table 4

Coliform load in Cagayan de Oro River based on the Most Probable Number (MPN) index

Sampling sites	*MPN index per 100 mL*
Taluban	1100
Bitan-ag	670
Balaon	1100
Kalawaig	670
Tagiti	670
Taguanao	655
Lower Balulang	670
Tikalaan	571.50
Pigcutin	350
Pigcutin at Mamaon	116.50
Tikalaan at Kolawingon	68
Tignapoloan	126.50
Tumalaong	596.50
Munigi	655
Bubunawan	1750
Bubunawan at Lingating	571.50
Overall mean MPN index of Cagayan de Oro River: 646.28	

Population of bacteria is flushed downstream by rapid water flow. Water transport from the upstream washes organic materials including coliform from livestock and human waste. It is likely that non-point sources are the most significant contributors of total coliform loadings in the river. In the Philippine waters, rapid inventory of pollution sources by the DENR Environmental management bureau revealed that domestic wastes are the major source of pollution, followed by livestock and industrial sources (DENR 1990). Non-point sources of pollution account for 11% of the organic load contribution to water bodies. Nutrient loading is a prerequisite to an increase in coliform density.

Previous studies have noted that changes in coliform concentration in streams and rivers is dependent upon differences in temperature, flow regime, amount of access by warm-blooded animals, and overall water quality (Goldreich et al 1955; Bukantis 1995). The MPN value indicates that the river carries a substantial load of coliforms from domestic, agricultural wastes, livestock and human wastes. Obstructions of water flow due to human activities especially quarrying and small-scale mining have possibly contributed to the sustained higher coliform count in the main channel. Interference of rainfall and increase in flow rate disturbed natural bacterial populations and distribute them in the downstream sites.

The physical and chemical characteristics namely: TSS, TDS, conductivity, pH, temperature, nitrate, nitrite, and silicate were within the standard values (Table 5).

Table 5

Physical and chemical conditions in Cagayan de Oro River

Indicators of River's physical and chemical conditions	*Acceptable level*	*Reference*	*Mean*	*Minimum & maximum values at the time of sampling*
Total Suspended Solids (TSS)	50 mg L^{-1}	DENR (1990) for Class A freshwaters	50.15	1.33- 637.00
Total Dissolved Solids (TDS)	1000 mg L^{-1}	DENR (1990) for Class A freshwaters	153.71	28.33- 894.67
Dissolved Oxygen (DO)	5 mg L^{-1}	DENR (1990) for Class A & B freshwaters	6.50	4.88- 9.22
Conductivity	600 μs cm^{-1}	Chapman (1996)	297.81	47.17- 961.00
Phosphate (PO_4)	<0.01 mg L^{-1}	Class A freshwaters DENR (1990)	0.13	0.01-1.18
Nitrate (NO_3)	rarely exceeds 10 mg L^{-1}	Class A freshwaters DENR (1990)	0.29	0.01-1.90
Nitrite (NO_2)	Typically present in extremely low concentration	US-EPA (1994)	0.02	0.01-0.04
Silicate (SiO_4)	1-100 mg L^{-1}	-	92.72	6.00- 263.33
pH	6.5-8.5	DENR (1990)	8.16	6.34- 9.80
Temperature	26-30^oC	DENR (1990)	26.43	20.47-30.22

Total suspended solids (TSS) refer to a mix of silt and organic waste particles, tinier than grains of sand. Since inorganic suspended solids can attenuate light through scattering, its high concentration can degrade optical water quality by reducing water clarity and decreasing light needed for photosynthesis. In most freshwater system the suspended solids originate from watershed sources, pollutant point sources, and sediment resuspension. The high river total suspended solids can impact water quality and deposition in downstream water bodies and reservoirs. The high overall TSS concentration was due to the accumulation of sediments from eroded tributaries.

Total dissolved solids (TDS) is the amount of soluble salts that yield ions such as sodium (Na^+), calcium (2^+), magnesium (Mg^{2+}), bicarbonate (HCO_3^-), sulfate (SO_4^{2-}), or chloride (Cl^-). The TDS concentration in the main channel and tributaries may be caused by many different factors. A high concentration of dissolved ions in freshwater bodies by itself is not an indication that it is polluted or unhealthy.

Silicon is a limiting factor for the growth of diatoms and other silicon-requiring algae. Due to diatom growth in natural waters, the silicate concentrations may be reduced seasonally. The sources of silicate in surface waters are the watershed discharges and the regeneration from bottom sediments.

Conclusions. The macroinvertebrate assemblages comprised a total of 63 families, 36 of which are indicators of water quality and habitat degradation. Many of these families belong to the orders Ephemeroptera (13 families), Trichoptera (11 families), Coleoptera (8 families) and Diptera (7 families). Among the macroinvertebrates, 9.93% are classified as sensitive to poor stream conditions, 61.06% are moderately tolerant to degraded habitat and water quality, and 29.01% are classified as most tolerant to degraded habitat and water quality. The Modified Family Biotic Index (FBI), a measure of the water quality using the tolerance value of the comprising macroinvertebrate family, was lowest at Tagiti with a value of 3.67 and highest at Lower Balulang with 4.89. This indicates that water quality is good with some probable organic pollution.

A total of 19 diatom genera were identified. The five most abundant genera are *Navicula, Fragillaria, Gomphonema, Cymbella* and *Cocconeis.* In terms of diatoms, the overall biological integrity rating considering pollution index, Shannon index and siltation index is fair which indicates reasonable degree of siltation and stress to diatom assemblage.

The Department of Environment and Natural Resources, Philippine standards, classified Cagayan de Oro River as Class A waters. Results of this study showed that coliform load in some sites of the river exceeded the acceptable standard for class A waters which is 1000MPN/100 mL. The mean MPN value however falls within the acceptable standard. This indicates that coliform load varies in different sites. The river may have carried a substantial load of coliforms from domestic, agricultural wastes, livestock and human wastes.

All measured physico-chemical parameters falls within the acceptable standards except for total suspended solids and phosphates.

This study proved that indicator organisms such as macroinvertebrates, diatoms and coliform can respond to water quality conditions of Cagayan de Oro River. In the Philippines monitoring of rivers is mostly based on physico-chemical methods. It should be noted that biological monitoring is more reliable because indicator organisms inhabit the area for a longer period of time and their presence or absence in the area may describe the water quality and general condition of the river. Monitoring based on physico-chemical parameters alone is fluctuating and may just give a snapshot picture of the river at the time of sampling.

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Authors:
Astrid L. Sinco, Biology Department, Xavier University, Corrales Avenue, Cagayan de Oro City 9000, Philippines, e-mail: asinco@xu.edu.ph
Judy P. Sendaydiego, Biology Department, Xavier University, Corrales Avenue, Cagayan de Oro City 9000, Philippines, e-mail: jsendaydiego@xu.edu.ph
Leolinda L. Saab, Biology Department, Xavier University, Corrales Avenue, Cagayan de Oro City 9000, Philippines, e-mail: lsaab@xu.edu.ph
Geraldine R. Mojica, Biology Department, Xavier University, Corrales Avenue, Cagayan de Oro City 9000, Philippines, e-mail: gmojica@xu.edu.ph
Giovanni G. Tampus, Biology Department, Xavier University, Corrales Avenue, Cagayan de Oro City 9000, Philippines, e-mail: gtampus@xu.edu.ph
Adora S. Rondez, Biology Department, Xavier University, Corrales Avenue, Cagayan de Oro City 9000, Philippines, e-mail: arondez@xu.edu.ph

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