RBP II (Rapid Bioassessment Protocol II) as a tool to assess the sediment and water quality in a treated textile effluent receiving stream ecosystem.

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Research Article

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Abstract

Macrobenthos are important bioindicators of organic and nutrient pollution. The present study used the macrobenthos based rapid bioassessment protocol (RBP II) to study the effects of treated textile effluent input into a natural stream ecosystem. Three reference sites and three sites with point source inputs from a textile effluent treatment plant were selected. The physical and chemical parameters and the abundance and diversity of macrobenthos at each site were assessed during the rainy and dry seasons of 2020. Although the water quality parameters at the point source inputs did not exceed the standard textile effluent discharge limits, a significant difference in the microbenthic community composition was observed at the effluent discharge receiving sites. The dominant macrobenthos in the ecosystem (*Baetis sp., Leptophlebia sp* and *Tubifex* sp.) showed significant correlations with Pb, Cu, Cr and Cd concentrations of the water and sediments. The Shannon-Weiner diversity index, family biotic index, EPT Index and EPT/C ratio which are used in rapid bioassessment protocol indicated significantly strong correlations with the water and sediment quality parameters and reflected their suitability to be used as a tool of biological measurements in the textile effluent receiving aquatic ecosystems.

Introduction

Macrobenthos are invertebrates living within or on the substrate of the aquatic ecosystems. They are significant components in benthic food web and aids in nutrient cycling (Sarker Md, 2016). Their metabolic and behavioral activities help to improve the sediment quality. Further, macrobenthos are very sensitive to changes in sediment or water quality and their responses are reflected as changes in the abundance and community structure (Wijeyaratne and Kalaotuwawe, 2017).

Due to their high sensitivity to ecosystem changes, macrobenthos are widely used as biological indicators in monitoring aquatic ecosystem health (Koperski, 2011). Rapid bioassessment protocol II is a macrobenthos based biological assessment method of ecosystem health and this concept is introduced by the USEPA. In this protocol, multiple biological indices that reflects the abundance and distribution of macrobenthos are used to predict the sediment and water quality characteristics of stream ecosystems. The taxa richness, Ephemeroptera, Plecoptera, and Trichoptera (EPT) index, ratio of EPT and Chironomid abundance (EPT/C), modified Hilsenhoff family biotic index (FBI) and percent contribution of dominant taxa are the indices that are commonly used in the RBP II protocol. It is recommended to use at least four of these biotic indices in order to successfully interpret ecosystem health using RBP II (Singh et al., 2019; Wijeyaratne and Kalaotuwawe, 2017). Macrobenthos based RBP have been widely used in many parts of the world as they are cost effective screening tools that can be used to assess the cumulative effects and severity of impairment in the aquatic ecosystems (Bhadrecha et al., 2016; Copatti et al., 2013; Springe et al., 2006; Uyanik et al., 2005). However, in the Sri Lankan context the use of macrobenthos based RBPs in ecological studies are very rare. Several published researches have assessed the biodiversity and community structure of the benthic macroinvertebrates in coastal and freshwater ecosystems (Dahanayaka and Wijeyaratne, 2010; Madhushankha et al., 2015; Pathiratne and Weerasundara, 2004; Walpola et al., 2011). But these studies have not incorporated RBP II in the ecosystem health assessment studies. However, in a pilot study conducted by Wijeyaratne and Kalaotuwawe (2017), RBP II has been identified as a as a possible bioassessment protocol to predict water and sediment quality in stream ecosystems in Sri Lanka (Wijeyaratne and Kalaotuwawe, 2017).

The objective of the present study was to use the RBP II protocol and together with multivariate statistics to assess the biological health of water and sediments in a stream receiving treated textile waste water compared to that of the reference sites.

Methodology

Study area and Sampling sites

The study area is a stream located in the Western Province of Sri Lanka (7°7'29.28” N, 79°51'35.29" E) which receives point source inputs of treated textile waste water effluents. Six sampling sites were selected from the study area. The upstream sampling sites (Site A, Site B and Site C) were the reference sites as there were no point source discharges of textile effluent treatment plants. The downstream sites (Site C, Site D and Site E) were receiving point source inputs of textile waste water treatment plants.

Water and sediment quality parameters

Surface water and shallow sediment samples (0.5 m depth) were collected monthly during 2020. In each sampling event, time integrated composite surface water and shallow sediment samples were collected in ten replicates from each site to represent a particular subsample at 2 hour intervals during the sampling period from 09:00 h to 14:00 h.

Water pH, temperature, conductivity, total dissolved solids (TDS), dissolved oxygen concentration (DO), salinity, sediment pH and sediment conductivity were measured in situ using a calibrated digital multi parameter (YSI Environmental Model-556 MPS). In the laboratory, biochemical oxygen demand 5 days after incubation (BOD_5) and chemical oxygen demand (COD) were analyzed according to the standard methodologies described by the American Public Health Association (American Public Health Association (APHA), 1999). Sediment organic matter content was measured in the laboratory using the loss on ignition method and the percentages of sand, silt, and clay content of the sediments were measured using the sedimentation jar.

Water samples were acidified and were analyzed for Pb, Cr, Cd and Cu concentrations in the Atomic Absorption Spectrophotometer (Analytic jena (Model NovAA 400p)) on graphite furnace mode (American Public Health Association (APHA), 1999). and Freshwater BCR480 (Sigma Aldrich, USA) was used as the CRM for water analysis.

Concentration of Pb, Cr, Cd and Cu in the sediments were measured from the Atomic Absorption Spectrophotometer (Analytic jena (Model NovAA 400p)) on graphite furnace mode after acid digestion at 270°C for 3 hours in the Kjeldatherm digestion system using concentrated HNO_3 and $HClO_4$ in 1:1 (v/v) ratio. Certified reference materials (CRM) were included in the digestion and analysis process to verify the accuracy of the methods. Freshwater sediment CRM 016 (Sigma Aldrich, USA) was used as the CRM for sediment analysis. Minimum detection limits for the analyzed metals were: Pb - 0.2 mg/L; Cu - 0.7 mg/L; Cr - 1 mg/L and Cd - 0.7 µg/L. Continuing control verification was done after every 10 samples to check that variability was within 10%.

Sampling and identification of benthic macroinvertebrates

Shallow (0-4 m depth) benthic sediment samples (in ten replicates) were collected from the sampling locations using the Peterson grab sampler. The collected sediment samples were preserved immediately in 5% Rose Bengal and Formalin solution. The preserved samples were transported to laboratory. In the laboratory samples were subjected to wet sieving through 4 mm, 2 mm, 1 mm and 500 µm mesh sieves to sort macro benthic invertebrates. The organisms retained in each sieve were collected and preserved in 10% formalin and then transferred into ethyl alcohol to prevent dehydration. Collected macro benthic invertebrates were identified using to the Genus level using relevant keys (De Moor, 2003; Fernando, 1974; Merritt et al., 2019)and their abundance was recorded. Species richness, the dominant species of each site and the percentage contribution of each species, Shannon Weiner Diversity Index, Family Biotic Index (FBI), EPT (Ephemeroptera, Plecoptera, Tricoptera) index and ratio of EPT taxa to Chironomidae were calculated using the abundance data for each site during rainy and dry seasons.

Statistical analysis

After confirming for normality using Anderson Darling test, the water quality and sediment quality data were analyzed using one way ANOVA followed by Tukey’s pairwise comparison to determine the significance of spatial and temporal variation. The values of diversity indices used in RBP II protocol for each site, calculated for rainy and dry seasons were also confirmed for normality using Anderson Darling test and subjected to one way ANOVA followed by Tukey’s pairwise comparison. Pearson’s correlation analysis was performed among the abundance of dominant taxa and water and sediment quality parameters.

Principal Component Analysis (PCA) was applied to determine water and sediment quality parameters that describe the distribution of macrobenthos. The linear relationship of PC1 score with Shannon-Weiner diversity index, Family Biotic index, EPT Index and EPT/C ratio were determined using regression analysis. R statistical software package was used in the statistical analysis.

Results And Discussion

Spatial variation of the mean values of water quality parameters among the study sites during the rainy and dry seasons are presented in the Table 1. There were no significant temporal variations in the water quality parameters among study sites. The water quality parameters at the effluent discharge receival sites were within the textile effluent discharge limits stipulated by the Central Environmental Authority (CEA) of Sri Lanka. However, Significant spatial variations in some water quality parameters were observed during the study period. The sites receiving treated textile effluents (Sites D, E and F) recorded significantly high amounts of water conductivity, total dissolved solids, chemical oxygen demand and significantly low dissolved oxygen concentration compared to those in the reference sites in both rainy and dry seasons (Table 1, ANOVA, Tukey's pairwise comparison, $p < 0.05$). The water pH, temperature, nitrate concentration, phosphate concentration and BOD_5 were not significantly different among the study sites in both rainy and dry seasons (Table 1, ANOVA, $p > 0.05$). The Pb, Cd, Cr and Cu concentrations in the water samples collected from the treated textile effluent receiving study sites did not show significant variations among the study sites in both rainy and dry seasons. Further, the heavy metal concentrations in the water samples collected from the reference sites were not detectable within the instrument detection limits (Table 1).

Table 1
Spatial variation of shallow water quality parameters in each sampling site during the study period.

	site A	site B	site C	site D	site E	site F
Rainy Season						
Water pH	6.89 ± 0.1^{a}	6.42 ± 0.1^{a}	6.15 ± 0.1 ^{a}	6.17 ± 0.1 ^{a}	6.00 ± 0.1 ^{a}	5.94 ± 0.1 ^{a}
Water Conductivity (µS/cm)	45.2 ± 3.2^{a}	52.8 ± 5.2 ^{a}	49.8 ± 5.4 ^{a}	75.7 ± 1.2^{b}	68.9 ± 2.3^{b}	70.6 ± 1.9^{b}
Temperature (^{0}C)	31.0 ± 0.4^{a}	31.9 ± 0.2^{a}	32.05 ± 0.2^{a}	31.42 ± 0.3^{a}	29.84 ± 0.2 ^{a}	29.82 ± 0.1 ^{a}
Water TDS (mg/L)	32.3 ± 4.3^{a}	44.5 ± 4.4 ^{a}	44.58 ± 8.1 ^{a}	88.7 ± 5.9 ^{b}	84.8 ± 7.2 ^{b}	79.5 ± 7.9 ^{b}
Water DO (mg/L)	7.7 ± 1.7^{a}	6.4 ± 1.6^{a}	6.7 ± 1.3^{b}	4.3 ± 0.41 ^{b}	4.5 ± 0.13 ^{b}	4.2 ± 0.08^{b}
Water Nitrate (mg/L)	0.5 ± 0.09^{a}	0.4 ± 0.06^{a}	0.4 ± 0.06^{a}	0.4 ± 0.06^{a}	0.3 ± 0.07^{a}	0.4 ± 0.07^{a}
Water Phosphate (mg/L)	0.04 ± 0.006 ^{a}	0.09 ± 0.01^{a}	0.05 ± 0.007 ^{a}	0.05 ± 0.008 ^{a}	0.04 ± 0.004 ^{a}	0.04 ± 0.008 ^{a}
Water BOD_5 (mg/L)	1.44 ± 0.2^{a}	1.30 ± 0.19^{a}	1.18 ± 0.14^{a}	1.87 ± 0.26^{a}	1.77 ± 0.34^{a}	1.28 ± 0.19^{a}
Water COD (mg/L)	41.0 ± 5.8 ^{a}	52.0 ± 9.8 ^{a}	44.8 ± 7.4 ^{a}	269.1 ± 26.5^{b}	326.6 ± 21.8^{b}	322.1 ± 21.3^{b}
Water Salinity ($^{0}/_{00}$)	0.04 ± 0.001^{a}	0.05 ± 0.001 ^{a}	0.04 ± 0.001 ^{a}	0.04 ± 0.001 ^{a}	0.03 ± 0.001 ^{a}	0.03 ± 0.001 ^{a}
Pb (mg/L)	ND	ND	ND	12.2 ± 0.1	15.9 ± 0.1	14.3 ± 0.1
Cu (mg/L)	ND	ND	ND	15.2 ± 0.5	17.5 ± 0.1	17.5 ± 0.5
Cr (mg/L)	ND	ND	ND	8.3 ± 0.1	9.8 ± 0.3	8.2 ± 0.3
Cd (µg/L)	ND	ND	ND	6.1 ± 0.2	5.2 ± 0.1	6.3 ± 0.1
Dry Season						
Water pH	6.7 ± 0.1^{a}	6.3 ± 0.1 ^{a}	6.2 ± 0.1 ^{a}	6.2 ± 0.1 ^{a}	6.00 ± 0.1 ^{a}	6.1 ± 0.1 ^{a}

Data are presented as Mean ± Standard Deviation (SD). Results indicated by different superscript letters in each row at each season are significantly different from each other (n = 10, ANOVA, Tukey's test, $p < 0.05$). Sites A, B and C are reference sites and Sites D, E and F are treated textile effluent discharge receival sites.

	site A	site B	site C	site D	site E	site F
Water Conductivity (µS/cm)	45.7 ± 8.3^{a}	46.3 ± 3.1 ^{a}	49.60 ± 3.34 ^{a}	78.7 ± 6.5^{b}	69.9 ± 8.2^{b}	70.61 ± 5.2^{b}
Temperature (^{0}C)	31.07 ± 0.4^{a}	31.98 ± 0.2^{a}	32.06 ± 0.2^{a}	31.92 ± 0.3^{a}	29.44 ± 0.2^{b}	29.52 ± 0.1^{b}
Water TDS (mg/L)	23.8 ± 4.5^{a}	27.4 ± 4.4 ^{a}	24.58 ± 2.81 ^{a}	75.73 ± 1.5^{b}	72.01 ± 1.1^{b}	72.9 ± 0.9^{b}
Water DO (mg/L)	7.5 ± 0.7^{a}	6.8 ± 0.6^{a}	6.8 ± 0.3 ^{a}	4.6 ± 0.41 ^{b}	5.5 ± 0.13 ^{b}	5.6 ± 0.08 ^{b}
Water Nitrate (mg/L)	0.5 ± 0.09^{a}	0.6 ± 0.06^{a}	0.7 ± 0.06^{a}	0.4 ± 0.06^{a}	0.3 ± 0.07^{a}	0.4 ± 0.07^{a}
Water Phosphate (mg/L)	0.04 ± 0.006 ^{a}	0.09 ± 0.013^{a}	0.05 ± 0.007^{a}	0.05 ± 0.008^{a}	0.05 ± 0.004 ^{a}	0.05 ± 0.008 ^{a}
Water BOD_5 (mg/L)	1.54 ± 0.17^{a}	1.58 ± 0.19^{a}	1.68 ± 0.14^{a}	1.88 ± 0.2^{a}	1.85 ± 0.3^{a}	1.8 ± 0.2^{a}
Water COD (mg/L)	51.0 ± 12.8 ^{a}	48.0 ± 9.3 ^{a}	52.8 ± 8.4 ^{a}	267.1 ± 46.5^{b}	266.2 ± 31.8^{b}	262.0 ± 25.3^{b}
Water Salinity ($^{0}/_{00}$)	0.04 ± 0.004^{a}	0.04 ± 0.002 ^{a}	0.04 ± 0.001 ^{a}	0.04 ± 0.001 ^{a}	0.03 ± 0.001 ^{a}	0.03 ± 0.001 ^{a}
Pb (mg/L)	ND	ND	ND	15.2 ± 0.1	17.5 ± 0.1	15.9 ± 0.1
Cu (mg/L)	ND	ND	ND	18.6 ± 0.5	18.5 ± 0.1	16.5 ± 0.5
Cr (mg/L)	ND	ND	ND	9.3 ± 0.1	8.8 ± 0.3	9.6 ± 0.3
Cd (µg/L)	ND	ND	ND	8.1 ± 0.2	7.2 ± 0.1	7.3 ± 0.1

Data are presented as Mean ± Standard Deviation (SD). Results indicated by different superscript letters in each row at each season are significantly different from each other (n = 10, ANOVA, Tukey's test, $p < 0.05$). Sites A, B and C are reference sites and Sites D, E and F are treated textile effluent discharge receival sites.

Spatial variation of the mean values of sediment quality parameters among the study sites during the rainy and dry seasons are presented in the Table 2. There were no significant temporal variations in the sediment quality parameters among study sites. sites. The sites receiving treated textile effluents (Sites D, E and F) recorded significantly high sediment conductivity compared to the reference sites in both rainy and dry seasons (Table 2, ANOVA, Tukey's pairwise comparison, $p < 0.05$). Other sediment quality parameters did not show significant variations among study sites during both seasons. The Pb, Cd, Cr and Cu concentrations in the sediment samples collected from the reference sites were not detectable within the instrument detection limits (Table 2). The heavy metal concentration of the water of the effluent receiving sites varied as Cu > Pb > Cr > Cd where as in sediments it was Pb > Cu > Cr > Cd (Table 1,

Table 2). Sediments can function as both sources and sinks of heavy metals as they tend to adsorb and release heavy metals at different environmental conditions. Further, the heavy metals can be distributed to the non-polluted areas by sediment transport (Voica et al., 2017). The results of present study indicate significantly high sediment concentrations of Pb, Cd, Cr and Cu in the treated textile effluent receiving study sites compared to the respective water samples during both rainy and dry seasons (Table 1, Table 2). Therefore, it is possible that long term input of heavy metals to this region of the water body may have caused accumulation of heavy metals in the sediments overtime in significantly higher concentrations.

Table 2
Spatial variation of shallow sediment quality parameters in each sampling site during the study period.

	site A	site B	site C	site D	site E	site F
Rainy season						
Sediment sand %	50.7 ± 4.5^{a}	57.0 ± 1.3^{a}	50.9 ± 1.9^{a}	52.4 ± 2.5^{a}	51.1 ± 3.1^{a}	50.8 ± 3.1^{a}
Sediment silt %	28.6 ± 5.0^{a}	24.0 ± 4.0^{a}	26.4 ± 2.0^{a}	26.9 ± 3.9^{a}	24.0 ± 2.5^{a}	21.7 ± 3.0^{a}
Sediment clay %	20.7 ± 2.2^{a}	19.0 ± 2.4^{a}	22.7 ± 1.7^{a}	20.7 ± 4.9^{a}	24.9 ± 2.2^{a}	27.5 ± 1.8^{a}
Shallow sediment TOM%	2.32 ± 0.24^{a}	2.24 ± 0.11^{a}	2.61 ± 0.17^{a}	2.32 ± 0.12^{a}	2.48 ± 0.27^{a}	2.11 ± 0.18^{a}
Shallow sediment pH	5.56 ± 0.30^{a}	5.86 ± 0.22^{a}	5.85 ± 0.27^{a}	5.62 ± 0.27^{a}	5.11 ± 0.09^{a}	4.71 ± 0.07^{a}
Shallow sediment conductivity (µS/cm)	35.3 ± 28.1^{a}	36.36 ± 4.03^{a}	35.29 ± 2.12^{a}	557.0 ± 72.7^{b}	420.6 ± 64.1^{b}	535.7 ± 80.6^{b}
Pb (mg/L)	ND	ND	ND	41.5 ± 0.1	38.3 ± 0.2	39.3 ± 0.4
Cu (mg/L)	ND	ND	ND	28.4 ± 0.3	27.1 ± 0.1	22.4 ± 0.5
Cr (mg/L)	ND	ND	ND	16.8 ± 0.5	21.8 ± 0.3	18.3 ± 0.6
Cd (µg/L)	ND	ND	ND	16.2 ± 0.4	17.6 ± 0.3	17.3 ± 0.1
Dry season						
Sediment sand %	48.9 ± 1.5^{a}	56.2 ± 2.1^{a}	51.2 ± 2.1^{a}	50.2 ± 2.3^{a}	51.6 ± 2.9^{a}	51.6 ± 2.7^{a}
Sediment silt %	26.5 ± 4.2^{a}	24.7 ± 3.8^{a}	27.8 ± 3.5^{a}	28.4 ± 2.4^{a}	26.1 ± 3.7^{a}	27.5 ± 3.5^{a}
Sediment clay %	24.6 ± 1.2^{a}	19.1 ± 1.4^{a}	21.0 ± 1.8^{a}	21.4 ± 2.9^{a}	22.3 ± 3.2^{a}	20.9 ± 1.8^{a}
Shallow sediment TOM%	3.32 ± 0.24^{a}	3.21 ± 0.11^{a}	2.98 ± 0.17^{a}	3.28 ± 0.12^{a}	2.74 ± 0.27^{a}	2.68 ± 0.18^{a}

Data are presented as Mean ± Standard Deviation (SD). Results indicated by different superscript letters in each row at each season are significantly different from each other (n = 10, ANOVA, Tukey's test, $p < 0.05$). Sites A, B and C are reference sites and Sites D, E and F are treated textile effluent discharge receival sites.

	site A	site B	site C	site D	site E	site F
Shallow sediment pH	5.74 ± 0.25[a]	5.76 ± 0.26[a]	5.85 ± 0.47[a]	5.74 ± 0.14[a]	5.69 ± 0.19[a]	5.12 ± 0.25[a]
Shallow sediment conductivity (µS/cm)	39.2 ± 14.8[a]	38.54 ± 7.07[a]	36.9 ± 3.56[a]	625.0 ± 54.7[a]	625.6 ± 62.1[a]	578.2 ± 72.6[a]
Pb (mg/L)	ND	ND	ND	44.8 ± 0.9	47.5 ± 0.4	45.2 ± 0.4
Cu (mg/L)	ND	ND	ND	32.5 ± 0.1	30.5 ± 0.8	31.3 ± 0.4
Cr (mg/L)	ND	ND	ND	17.5 ± 0.8	21.3 ± 0.4	20.5 ± 0.5
Cd (µg/L)	ND	ND	ND	17.4 ± 0.8	17.1 ± 0.4	17.5 ± 0.1

Data are presented as Mean ± Standard Deviation (SD). Results indicated by different superscript letters in each row at each season are significantly different from each other (n = 10, ANOVA, Tukey's test, $p < 0.05$). Sites A, B and C are reference sites and Sites D, E and F are treated textile effluent discharge receival sites.

Abundance and diversity of benthic macroinvertebrates.

The mean of number of benthic macroinvertebrates recorded per m^2 from the study sites during rainy and dry seasons are given in Table 3. The mean values for Shannon-Weiner Diversity index (H'), Hilsenhoff Family Biotic index (FBI), Species richness, EPT index, EPT/C index, dominant species and its percentage contribution for each sampling site in rainy and dry seasons are given in Table 4. Total of 16 species belonging to three phyla were recorded during the study.

Table 3

Spatial variation of abundance of benthic macroinvertebrates (Number(x10) per m^2) in each sampling site during the study period .

	site A	site B	site C	site D	site E	site F
Rainy Season						
Phylum Arthropoda, Order Diptera						
Family Simuliidae Simulium jennings.	47 ± 5^a	48 ± 2^a	52 ± 5^a	68 ± 5^a	45 ± 2^a	50 ± 5^a
Family Tabanidae Tabanus sp.	17 ± 1^a	26 ± 2^a	32 ± 2^a	16 ± 1^a	28 ± 2^a	22 ± 3^a
Family Ceratopogonidae Palpomia sp.	28 ± 3^a	22 ± 2^a	21 ± 2^a	28 ± 5^a	24 ± 3^a	25 ± 3^a
Family Chironomidae Chironomus sp.	16 ± 2^a	21 ± 3^a	22 ± 2^a	87 ± 6^b	98 ± 5^b	120 ± 6^b
Family Culicidae Culex sp.	4 ± 0^a	8 ± 1^a	5 ± 0^a	2 ± 0^a	2 ± 0^a	8 ± 2^a
Family Blepharoceridae Blepharicera sp	3 ± 1^a	2 ± 0^a	2 ± 0^a	5 ± 0^a	1 ± 0^a	4 ± 0^a
Phylum Arthropoda, Order Tricoptera						
Family- Hydropsychidae Hydroptilla sp.	59 ± 3^a	65 ± 4^a	62 ± 5^a	5 ± 0^b	8 ± 0^b	4 ± 0^b
Family- Helicopsychidae Helicopsyche sp.	59 ± 2^a	62 ± 3^a	68 ± 5^a	12 ± 2^b	12 ± 2^b	14 ± 2^b
Phylum Arthropoda, Order Ephemeroptera						
Family- Baetidae Baetis sp.	88 ± 5^a	79 ± 6^a	78 ± 5^a	11 ± 1^b	5 ± 1^b	12 ± 2^b
Family- Leptophlebiidae Leptophlebia sp	74 ± 5^a	78 ± 5^a	89 ± 7^a	13 ± 1^b	12 ± 1^b	10 ± 1^b

Data are presented as Mean ± Standard Deviation (SD). Results indicated by different superscript letters in each row at each season are significantly different from each other (n = 10, ANOVA, Tukey’s test, $p < 0.05$). Sites A, B and C are reference sites and Sites D, E and F are treated textile effluent discharge receival sites.

	site A	site B	site C	site D	site E	site F
Phylum Arthropoda, Order Plecoptera						
Family- Perlidae Neoerla sp.	58 ± 5 [a]	54 ± 3 [a]	55 ± 5 [a]	2 ± 0 [b]	3 ± 0 [b]	5 ± 0 [b]
Phylum Annelida						
Family Tubificidae Tubifex sp.	21 ± 1 [a]	24 ± 2 [a]	23 ± 2 [a]	136 ± 8 [b]	125 ± 8 [b]	152 ± 8 [b]
Phylum Mollusca						
Family Thiaridae Melonoides tuberculate	1 ± 0 [a]	5 ± 0 [a]	6 ± 0 [a]	140 ± 12 [b]	126 ± 8 [b]	110 ± 8 [b]
Family Thiaridae Thiara scabra	27 ± 1 [a]	27 ± 2 [a]	28 ± 2 [a]	16 ± 1 [b]	14 ± 1 [b]	12 ± 1 [b]
Family Ampullariidae Pila globose	74 ± 5 [a]	75 ± 6 [a]	78 ± 5 [a]	25 ± 2 [b]	24 ± 4 [b]	25 ± 1 [b]
Family Bithnidae Bithynia tentaculata	32 ± 1 [a]	28 ± 2 [a]	27 ± 1 [a]	3 ± 0 [b]	5 ± 0 [b]	7 ± 0 [b]
Dry Season						
Phylum Arthropoda, Order Diptera						
Family Simuliidae Simulium jennings	26 ± 1 [a]	38 ± 5 [a]	24 ± 1 [a]	110 ± 10 [b]	121 ± 12 [b]	137 ± 12 [b]
Family Tabanidae: Tabanus sp.	10 ± 1 [a]	12 ± 2 [a]	10 ± 2 [a]	16 ± 2 [a]	8 ± 1 [a]	5 ± 1 [a]
Family Ceratopogonidae Palpomia sp.	18 ± 6 [a]	16 ± 4 [a]	21 ± 5 [a]	18 ± 4 [a]	41 ± 5 [a]	45 ± 5 [a]
Family Chironomidae Chironomus sp.	17 ± 1 [a]	19 ± 3 [a]	14 ± 3 [a]	92 ± 8 [b]	155 ± 37 [b]	94 ± 15 [b]

Data are presented as Mean ± Standard Deviation (SD). Results indicated by different superscript letters in each row at each season are significantly different from each other (n = 10, ANOVA, Tukey's test, $p < 0.05$). Sites A, B and C are reference sites and Sites D, E and F are treated textile effluent discharge receival sites.

	site A	site B	site C	site D	site E	site F
Family Culicidae Culex sp.	6 ± 0 [a]	7 ± 0 [a]	2 ± 0 [a]	3 ± 0 [a]	17 ± 2 [a]	6 ± 1 [a]
Family Blepharoceridae Blepharicera sp	2 ± 0 [a]	5 ± 0 [a]	4 ± 0 [a]	2 ± 0 [a]	4 ± 0 [a]	3 ± 0 [a]
Phylum Arthropoda, Order Tricoptera						
Family- Hydropsychidae Hydroptilla sp.	75 ± 12 [a]	68 ± 9 [a]	80 ± 12 [a]	3 ± 0 [b]	7 ± 0 [b]	6 ± 0 [b]
Family- Helicopsychidae Helicopsyche sp.	56 ± 8 [a]	48 ± 6 [a]	54 ± 6 [a]	8 ± 0 [b]	10 ± 1 [b]	11 ± 1 [b]
Phylum Arthropoda, Order Ephemeroptera						
Family- Baetidae Baetis sp.	82 ± 5 [a]	78 ± 6 [a]	77 ± 5 [a]	12 ± 2 [b]	8 ± 0 [b]	6 ± 0 [b]
Family- Leptophlebiidae Leptophlebia	88 ± 4 [a]	81 ± 5 [a]	88 ± 4 [a]	13 ± 1 [b]	10 ± 1 [b]	14 ± 3 [b]
Phylum Arthropoda, Order Plecoptera						
Family- Perlidae Neoerla sp.	59 ± 12 [a]	54 ± 8 [a]	62 ± 4 [a]	3 ± 0 [b]	6 ± 0 [b]	3 ± 0 [b]
Phylum Annelida						
Family Tubificidae Tubifex sp.	27 ± 2 [a]	36 ± 4 [a]	32 ± 4 [a]	170 ± 12 [b]	225 ± 14 [b]	212 ± 12 [b]
Phylum Mollusca						
Family Thiaridae Melonoides tuberculate	2 ± 0 [a]	8 ± 1 [a]	0 ± 0 [a]	134 ± 14 [b]	157 ± 15 [b]	114 ± 13 [b]
Family Thiaridae Thiara scabra	45 ± 9 [a]	37 ± 4 [a]	32 ± 4 [a]	8 ± 1 [b]	12 ± 3 [b]	14 ± 3 [b]

Data are presented as Mean ± Standard Deviation (SD). Results indicated by different superscript letters in each row at each season are significantly different from each other ($n = 10$, ANOVA, Tukey's test, $p < 0.05$). Sites A, B and C are reference sites and Sites D, E and F are treated textile effluent discharge receival sites.

	site A	site B	site C	site D	site E	site F
Family Ampullariidae Pila globosa	72 ± 6 [a]	59 ± 6 [a]	68 ± 7 [a]	24 ± 6 [b]	22 ± 4 [b]	20 ± 3 [b]
Family Bithnidae Bithynia tentaculata	28 ± 1 [a]	25 ± 2 [a]	35 ± 4 [a]	12 ± 1 [b]	10 ± 1 [b]	8 ± 1 [b]

Data are presented as Mean ± Standard Deviation (SD). Results indicated by different superscript letters in each row at each season are significantly different from each other ($n = 10$, ANOVA, Tukey's test, $p < 0.05$). Sites A, B and C are reference sites and Sites D, E and F are treated textile effluent discharge receival sites.

Table 4
The mean ± standard deviation values of Shannon-Weiner Diversity index (H`), Hilsenhoff Family Biotic index (FBI), Taxa richness (TR) and EPT index, EPT / C ratio, dominant species and percentage contribution of dominant species of benthic macroinvertebrates at each study site during the study period.

	site A	**site B**	**site C**	**site D**	**site E**	**site F**
Rainy Season						
H’	2.48 ± 0.8 [a]	2.53 ± 0.6 [a]	2.51 ± 0.7 [a]	2.11 ± 0.7 [a]	2.12 ± 0.7 [a]	2.14 ± 0.6 [a]
FBI	3.24 ± 0.3 [a]	3.44 ± 0.3 [a]	3.45 ± 0.3 [a]	3.76 ± 0.4 [a]	3.71 ± 0.3 [a]	3.78 ± 0.3 [a]
Species Richness	16 [a]	16 [a]	16 [a]	16 [a]	16 [a]	16 [a]
EPT index	57.1 ± 0.2 [a]	54.9 ± 0.3 [a]	55.70 ± 0.4 [a]	7.6 ± 0.4 [b]	7.5 ± 0.2 [b]	7.8 ± 0.2 [b]
EPT / C ratio	22.32 ± 0.5 [a]	16.57 ± 0.2 [b]	16.91 ± 0.5 [b]	49.42 ± 0.3 [c]	48.82 ± 0.3 [c]	47.53 ± 0.2 [c]
Dominant species	*Baetis sp.*	*Leptophlebia sp*	*Leptophlebia sp*	*Tubifex sp*	*Tubifex sp*	*Tubifex sp*
% contribution of dominant species	14.01 ± 0.5 [a]	21.26 ± 0.5 [b]	23.92 ± 0.3 [b]	24.91 ± 0.4 [b]	23.68 ± 0.2 [b]	26.21 ± 0.3 [b]
Dry Season						
H’	2.47 ± 0.2 [a]	2.55 ± 0.2 [a]	2.47 ± 0.3 [a]	2.03 ± 0.4 [a]	2.021 ± 0.3 [a]	2.00 ± 0.4 [a]
FBI	3.95 ± 0.2 [a]	3.94 ± 0.3 [a]	4.10 ± 0.2 [a]	5.48 ± 0.3 [a]	5.49 ± 0.3 [a]	5.37 ± 0.2 [a]
Species Richness	16 [a]	16 [a]	15 [a]	16 [a]	16 [a]	16 [a]
EPT index	57.1 ± 0.2 [a]	53.2 ± 0.2 [a]	55.3 ± 0.4 [a]	6.2 ± 0.4 [b]	5.7 ± 0.3 [b]	5.8 ± 0.3 [b]
EPT / C ratio	20.23 ± 0.2 [a]	15.74 ± 0.3 [a]	21.51 ± 0.2 [a]	48. 23 ± 0.3 [b]	46.32 ± 0.4 [b]	45.52 ± 0.2 [b]
Dominant taxon	*Neoerla* sp	*Neoerla* sp	*Hydroptilla* sp.	*Tubifex* sp.	*Tubifex* sp.	*Tubifex* sp.

Data are presented as Mean ± Standard Deviation (SD). Results indicated by different superscript letters in each row at each season are significantly different from each other (n = 10, ANOVA, Tukey’s test, $p < 0.05$). Sites A, B and C are reference sites and Sites D, E and F are treated textile effluent discharge receival sites.

	site A	site B	site C	site D	site E	site F
% contribution of dominant taxon	13.3 ± 0.6 ^{a}	24.7 ± 1.2 ^{b}	26.5 ± 1.8 ^{b}	27.3 ± 0.8 ^{b}	27.7 ± 1.1 ^{b}	30.4 ± 1.4 ^{b}

Data are presented as Mean ± Standard Deviation (SD). Results indicated by different superscript letters in each row at each season are significantly different from each other (n = 10, ANOVA, Tukey's test, $p < 0.05$). Sites A, B and C are reference sites and Sites D, E and F are treated textile effluent discharge receival sites.

Tubifex sp. was the dominant taxa in all the treated textile effluent receiving sites during both dry and rainy seasons. However, in the reference sites, *Baetis sp.* and *Leptophlebia sp* showed highest dominance during both seasons. The percentage contribution of *Tubifex* sp. was significantly lower in the reference sites compared to the effluent receival sites. Pollution by organic matter and heavy metals can influence the abundance of *Tubifex* sp. (Martins et al., 2008) Therefore, *Tubifex* sp. is an indicator of organic pollution in the aquatic ecosystems and some studies have shown that *Tubifex* sp. is less tolerant to heavy metal pollution (Kaonga et al., 2010). However, in the present study, *Tubifex* sp. showed the highest abundance in treated textile effluent receival sites, indicating their possibility to use as bio monitor of inorganic pollution as well.

Shannon Wiener diversity index and the Family Biotic Index did not show significant spatial variations among study sites in both seasons. The Shannon-Wiener Index and the Family biotic Index are among the most reliable indices in assessing stream water quality using benthic macroinvertebrates. (Ghosh and Kumar Biswas, 2015; Wijeyaratne and Kalaotuwawe, 2017). The criteria for water quality classification using FBI described by Hilsenhoff 1988 identifies 7 categories of water quality based on the level of organic pollution: $0 \leq FBI \leq 3.50$: Excellent; $3.51 \leq FBI \leq 4.50$: Very good; $4.51 \leq FBI \leq 5.50$: Good; $5.51 \leq FBI \leq 6.50$: Fair; $6.51 \leq FBI \leq 7.50$: Fairly poor; $7.51 \leq FBI \leq 8.50$: Poor; $8.51 \leq FBI \leq 10.50$: Very poor (Hilsenhoff, 1982; Wijeyaratne and Kalaotuwawe, 2017). According to the FBI based categorization, the water quality in the reference site is in the "excellent category" whereas the treated textile effluent receiving sites is in the "very good" category (Table 4). However, EPT index and EPT/C index in the reference sites were significantly high compared to those in the effluent receiving sites during both seasons (Table 4). The EPT index based water quality assessment identifies 5 categories of water quality (Couceiro et al., 2012; Dahanayaka and Wijeyaratne, 2010; Suhaila and Che Salmah, 2017; Wijeyaratne and Kalaotuwawe, 2017): $0 \leq EPT \leq 6$: poor; $6.1 \leq EPT \leq 13$: Fair; $13.1 \leq EPT \leq 20.0$: Good-Fair; $20.1 \leq EPT \leq 27.0$: Good; $EPT > 27$: Excellent. Therefore, according to EPT based classification the water quality in the effluent receival sites were categorized as "Fair" whereas the reference sites were categorized as "Excellent" (Table 4). The percentage of the representatives from family Chironomidae in a sample indicates whether the stream is oligotrophic or eutrophic. Percentage of representatives from Family Chironomidae will increase with decreasing water quality. A sample representing more than 50% of EPT/Chironomidae ratio suggests eutrophic conditions. Some species of Chironomidae are also tolerant to heavy metals as well. In the present study, the EPT/C ratio in the reference sites were ranged less than 25% (16.5–22.3). However, the EPT/C ratio in the effluent receiving sites were very close to 50% (47.5–49.4) indicating near eutrophic conditions (Table 4). The present study identifies EPT and EPT/C indices as very useful tools to directly assesses the cumulative effects on the watershed and to identify the composite impacts of potential nonpoint and point sources of pollution.

Two principal components displaying a cumulative variance of 86.6% were obtained by applying PCA on water and sediment quality parameters during rainy and dry seasons. Ordination of the study sites based on PC1 and PC2 scores of PCA of the water and sediment quality parameters is given in Fig. 1. The eigenvalues of the first two principal components, eigenvectors of the water and sediment quality variables and the principal component scores for the study sites are given in Table 5. According to the results of the PCA, the study sites were categorized in to 4 groups. The reference site A during both seasons and site C during rainy season were categorized together and was characterized by high dissolved oxygen concentration (Fig. 1, Table 5). The reference site B during both seasons and site C during dry season were categorized together and was characterized by high water conductivity, temperature and salinity (Fig. 1, Table 5). The effluent receiving sites E and F during the dry season and Site D during both seasons were categorized together and were characterized by high % sand, silt and clay contents, high sediment conductivity, high sediment pH, high sediment Pb, Cu and Cd contents, high TDS, phosphate, nitrate contents, high COD, BOD_5 and highwater Cu and Cd contents (Fig. 1, Table 5). Sites E and F during the rainy season were separated from other groups and they were characterized by high sediment Cr concentration, high water pH, high Pb and Cr concentration in water (Fig. 1, Table 5).

Table 5
Summary of the PCA of water and sediment quality parameters of the study sites.

	PC 1	PC 2
Eigenvalue	15.27	2.78
%Variance	71.2	15.4
Cum.%Variance	71.2	86.6
Eigen vectors		
Sediment sand %	-0.168	0.358
Sediment silt %	0.245	0.114
Sediment clay %	0.249	0.093
% TOM	0.246	0.134
Sediment pH	0.252	0.032
Sediment conductivity	0.252	0.052
Sediment Pb	0.008	0.198
Sediment Cu	0.239	0.141
Sediment Cr	0.244	-0.07
Sediment Cd	0.24	0.025
Water pH	0.227	-0.033
Water Conductivity	-0.177	0.084
Water temperature	-0.111	0.238
Water TDS	0.14	0.396
Water DO	-0.251	-0.018
Water Nitrate	0.191	0.209
Water Phosphate	0.25	0.076
Water BOD5	0.246	0.13
Water COD	0.249	0.016
Water Salinity	-0.244	0.117
Water Pb	0.077	-0.173
Water Cu	0.047	0.354

	PC 1	PC 2
Water Cr	0.112	-0.457
Water Cd	0.064	0.288

The results of the linear regression analysis between benthic macro invertebrate based Shannon Weiner Diversity index, Family Biotic Index, EPT, EPT/C and PC score1 of sediment and water quality characteristics are given in Fig. 2. Considering, that the coefficients of determination (R^2) being greater than 60% indicating a strong relationship between the variables, the results showed significantly strong correlations of PC score 1 with Shannon Weiner Diversity index, Family biotic index EPT index and EPT/C ratio at 95% level of significance ($p < 0.05$, Fig. 2).

The correlation between the dominant taxa and water and sediment quality parameters are given in Table 6. *Baetis* sp and *Leptophlebia* sp showed significant negative correlations with water and sediment Pb, Cr, Cu, Cd concentrations, sediment and water conductivity, total dissolved solids, and biochemical oxygen demand. These species showed significant positive correlation with the dissolved oxygen concentration and nitrate concentration of water. Further, *Tubifex* species showed significant positive correlations with water and sediment Pb, Cr, Cu, Cd concentrations, sediment and water conductivity, total dissolved solids, and biochemical oxygen demand and significant negative correlation with the dissolved oxygen concentration and nitrate concentration of water (Pearson's correlation, $P < 0.05$, Table 6).

Table 6

– Pearson's correlation coefficients (r) between the abundance of dominant species and water and sediment quality parameters of the study sites during the study period.

	***Baetis* sp.**	***Leptophlebia* sp.**	***Tubifex* sp.**
Sediment sand %	0.125	0.196	-0.231
Sediment silt %	0.237	0.171	-0.368
Sediment clay %	-0.314	-0.327	0.523
% TOM	0.346	0.406	-0.393
Sediment pH	0.595	0.582	-0.777*
Sediment conductivity	-0.95*	-0.911*	0.862*
SedimentPb	-0.962*	-0.926*	0.895*
SedimentCu	-0.95*	-0.921*	0.879*
SedimentCr	-0.963*	-0.926*	0.933*
Sediment Cd	-0.967*	-0.931*	0.942*
Water pH	0.748	0.602	-0.69
Water Conductivity	-0.953*	-0.916*	0.883*
Water temperature	0.624*	0.639*	-0.669*
Water TDS	-0.974*	-0.968*	0.943*
Water DO	0.921*	0.851*	-0.93*
Water Nitrate	0.757*	0.864*	-0.665*
Water Phosphate	0.271	0.342	-0.411
Water BOD5	-0.469	-0.395	0.414
Water COD	-0.959*	-0.914*	0.976*
Water Salinity	0.645	0.588	-0.702
Water Pb	-0.965*	-0.929*	0.926*
Water Cu	-0.954*	-0.918*	0.884*
Water Cr	-0.947*	-0.914*	0.927*
Water Cd	-0.947*	-0.911*	0.862*

* significant at 95% level of significance (Pearson's correlation test, $p < 0.05$).

The results of the present study agrees with Gower et al 1994 and Cadmus et al 2016, which identified Cu as a major stressor to the *Baetis* and *Leptophlebia* communities in the natural ecosystems (Cadmus et al., 2016; Gower et al., 1994). Moreover, some studies have also indicated that *Tubifex* sp can tolerate high concentrations cu contaminated effluent input in the natural ecosystems (Raj et al., 2005). Further, in addition to Cu, the results of the present study indicate that sediment and water Pb, Cr and Cd have an ecological significance in determining the community structure of the benthic macroinvertebrates.

Conclusion

The biotic indices used in RBP II have a strong relationship with water and sediment quality characteristics of the study sites ($R^2 > 60\%$) confirming that these biotic indices can be successfully used to monitor pollution associated changes in the textile effluent receiving water bodies. Further, the results of the present study confirms that the treated textile effluent receiving portion of the stream is dominated by the pollution tolerant taxa, whereas the reference areas are dominated by pollution sensitive taxa of benthic macroinvertebrates. In addition, correlation of the abundance of *Tubifex sp* recorded in the present study to the water and sediment heavy metal concentrations reflects the possibility of using *Tubifex* sp as an indicator of heavy metal contamination in the aquatic ecosystems.

Declarations

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Conflicts of interest/Competing interests: The authors declare that they have no conflicts of interest /competing interests (include appropriate disclosures)

Availability of data and material: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Code availability: Not applicable

Authors' contributions:

W.M. D.N. Wijeyaratne: Designed the experiment, analyzed the data and wrote the manuscript. U.P. Liyanage: Assisted in experimental design, collected and analyzed the samples and assisted in writing the manuscript.

The authors read and approved the final manuscript.

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Figures

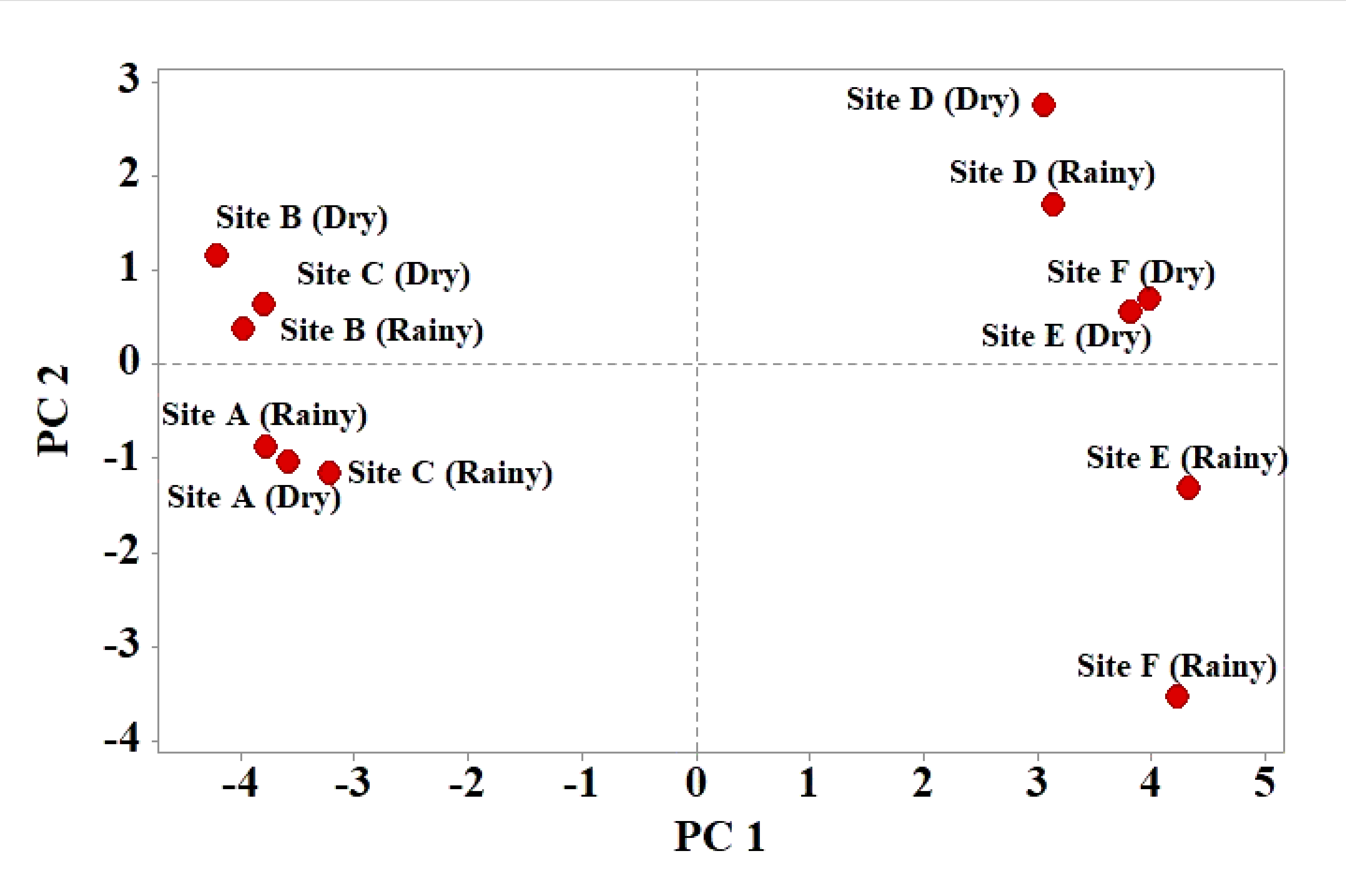

Figure 1

Ordination of the study sites based on PC1 and PC2 scores of PCA of the water and sediment quality parameters.

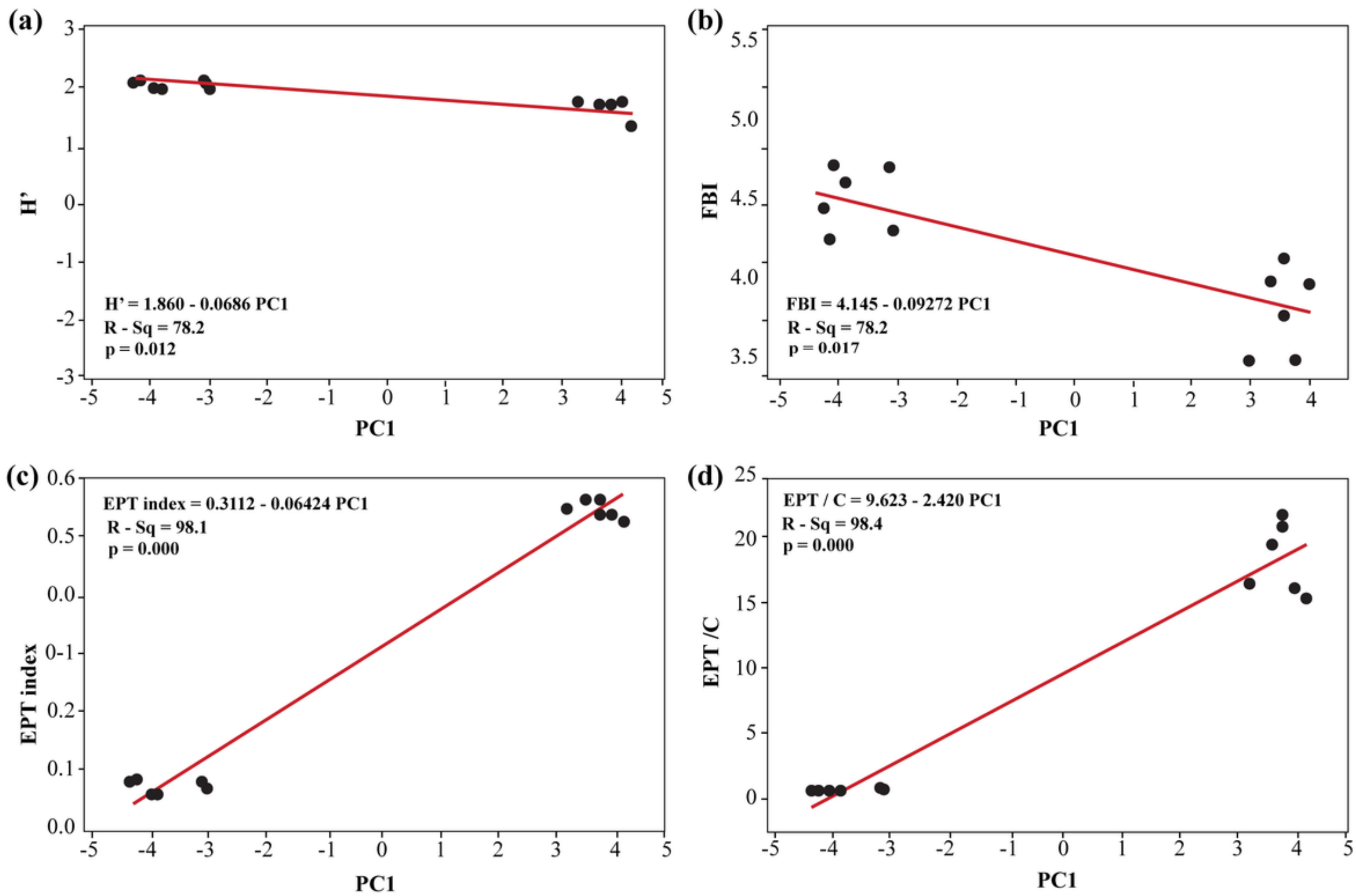

Figure 2

Linear regression of biotic indices used in Rapid Bioassessment Protocol II against the PC1 score for water and sediment quality parameters. (a) Shannon Wiener diversity index ; (b) Family Biotic Index; (c) EPT Index; (d) EPT/C ratio.