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RESEARCH PAPER

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A New Model for Organic Contamination Assessments Using Benthic Macroinvertebrates as Biological Indicators

Nadeesha Dilani Hettige[1,3], Rohasliney Hashim[1,*], Ahmad Abas Kutty[2], Zulfa Hanan Ashaari[1]

[1]Universiti Putra Malaysia, Faculty of Forestry and Environment, Department of Environment, 43400 UPM, Serdang, Selangor, Malaysia.
[2]Universiti Kebangsaan Malaysia, Department of Earth Science and Environment, Faculty of Science and Technology, 43600 UKM, Bangi Selangor, Malaysia.
[3]National Aquatic Resource Research and Development Agency (NARA), Environmental Studies Division, Crow Island, Colombo 15, Sri Lanka.

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Corresponding Author
Tel.: +60179777055
E-mail: rohasliney@upm.edu.my

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Abstract

The main goal of this study was to develop a model for organic pollution assessment. Seven sampling sites in six rivers in the Rawang sub-basin, Selangor River, Malaysia, were selected with one reference site. The sampling sites near the fish farm were used to develop the model. SR2 was used for the validation of the developed model. Benthic macroinvertebrates and water sampling were conducted from April 2019 to March 2020. The Principal Components Analysis (PCA) and regression were conducted to select the most representing benthic macroinvertebrates family. Based on the score value (variance coefficient) of each benthic macroinvertebrates family, the cumulative score value of each sampling site was calculated (i.e., 18=6 sampling sites x 3 replicates). The nine benthic macroinvertebrate families (Baetidae, Libellulidae, Protoneuridae Chironomidae, Curbicullidae Hydropchysidae, Tubificidae, Lumbriculiade, and Naididae) were identified using PCA and regression. The cluster analysis and mean confidence intervals were used to classify water quality classes precisely. Finally, three different value scales were produced to represent the level of contamination (i.e., <0.69 as organically polluted, 0.69-0.87 as slightly organic polluted, and >0.87 as clean status). The newly developed model was validated. The results produced after validation were better than the water quality status from other studies based on the BMWP/BMWP^{Thai} score. This study concludes that the developed model can evaluate river organic contamination successfully. model can evaluate river organic contamination successfully.

Introduction

Among the water pollution types, organic contamination of waterways by wastewater released from anthropogenic activities affects humans and ecosystems globally via the global sanitation crisis (Wen et al., 2017). Fish farming is one of the main activities contributing to organic pollution. Most fish farms in Malaysia use river water as their primary water source. The Selangor River is one example. The environmental impacts of fish farming arise due to the release of excess nutrients and antibiotics to the surrounding environment and the introduction of invading species (Kawasaki et al., 2016). Among the several anthropogenic activities, fish farming facilitates organic pollution. Organic pollution can be determined by assessing and integrating water quality parameters and benthic macroinvertebrates.

The most biotic water index model was developed in 1980 based on a scoring system (Armitage et al.,

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1983), and the first multimetric index was developed by Karr in 1981 using fish. After that, various types of the multimetric index were developed using other aquatic organisms such as plankton and macrobenthos (Herman and Nejadhashemi, 2015). Likewise, several researchers have developed water quality standards using macrobenthos. First, it was initiated in European member state countries (Musonge et al., 2020), and then, some tropical countries attempted to develop a WQI considering their geographical conditions (Lakew and Moog., 2015).

Consequently, it was moved to the tropical rivers. Using several multivariate statistical approaches, Lakew and Moog (2015) developed water quality standards for Ethiopian highland rivers and obtained five water quality classes: high, good, moderate, poor, and bad. Similarly, Blakely et al. (2014) established macrobenthos-based water quality standards for the Singapore streams and canals. They disclosed four water quality criteria, i.e., poor, intermediate, good, and very good water for biomonitoring. They concluded that this criterion applies to other Southeast Asian rivers with similar taxa and land use characteristics. Likewise, Sirisinthuwanich et al. (2016) developed a multimetric index using macrobenthos and physiochemical parameters of river water in the large rivers (Phong and Cheon rivers) in Thailand. Moreover, Tumusiime et al., (2019) have found the suitability of the Tanzania River scoring system macrobenthos index for the Mapanga River basin, Uganda. Furthermore, Musonge et al. (2020) developed macrobenthos-based water quality standards using tolerance score-based techniques and multivariate statistical tools.

Many models have been developed in Malaysia to assess river water quality using physicochemical parameters (Fulazzaky et al., 2010; Ahmed, 2014; Chowdhury et al., 2018). Arman et al. (2019) developed a multimetric index based on macrobenthos using four different catchments in Malaysia. However, a model must be developed to assess organic pollution using benthic macroinvertebrates as bioindicators in Malaysian rivers. Hence, proposing a new model indicating organic contamination is significant in determining river organic pollution.

Hence, this study's main objective was to develop a new water quality model to evaluate organic pollution. Developing water quality standards using local macroinvertebrates is vital due to the fluctuation of benthic macroinvertebrates in different geographical regions. Therefore, such results are essential for the effective management and restoration of river ecosystems, especially for Malaysian rivers in the future.

Methodology

Study Site and Sampling Design

Seven sampling sites were selected along the rivers in the Rawang sub-basin, Selangor River, namely Guntong River (SR1) and its tributary (SR2), Kuang River (SR3 and SR7), Gong River (SR4), Buaya River (SR5), and Serendah River (SR6) (Figure 1). The Guntong River's tributary (SR2) was chosen as a reference site for this study because of its least disturbance in its surrounding area and the absence of upstream fish farms.

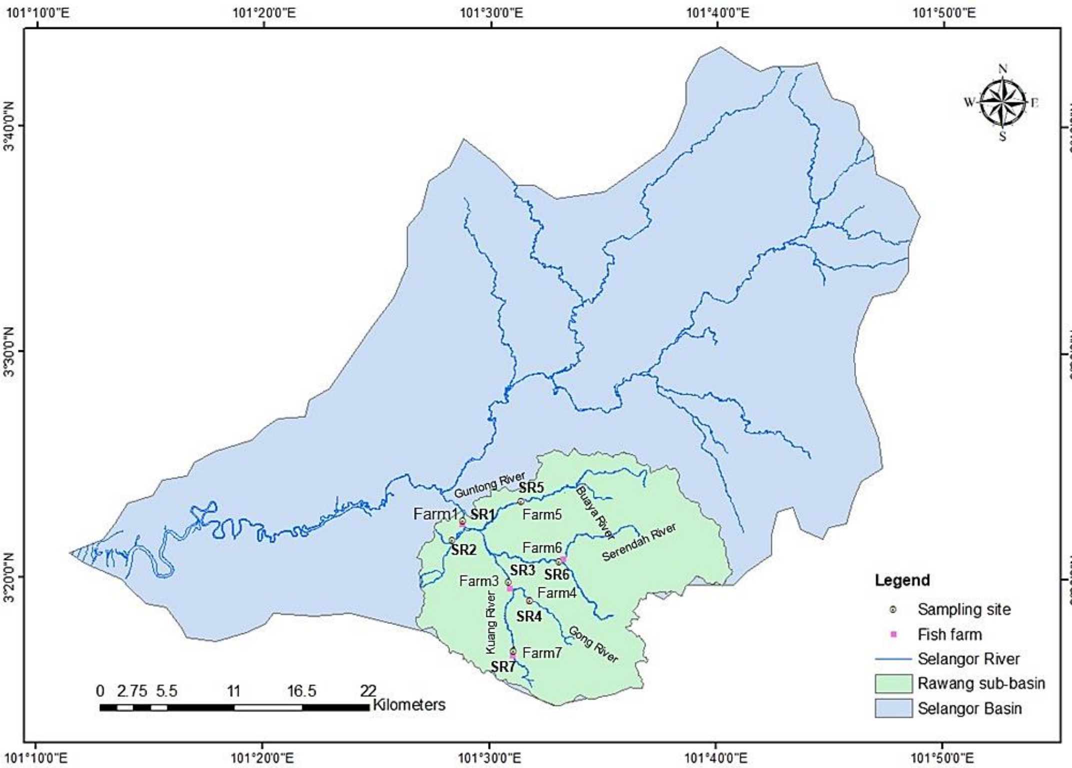

Figure 1. Map of the Selangor River and the study area's selected sampling sites

Moreover, all the sampling sites were chosen based on the random sampling method close to the riverbank. Sampling sites SR1 and SR3, SR4 and SR5, SR6 and SR7 are approximately 200 m, 20 m, and 400 m downstream from the effluent discharge points of the freshwater fish farm. Therefore, there were no directly discharged point sources of pollution into the river between the fish farm wastewater outlets and the sampling sites. Since all fish farms operated as land-based farms, fish farming in the Rawang sub-basin was managed for aquaculture (SR1, SR3, and SR6) and sportfishing activities (SR4, SR5, and SR7).

Water Quality Analysis

Before the macroinvertebrates sampling, water sampling was conducted once in two months, from April 2019 until February 2020. Water samples were also collected concurrently during the sampling of macroinvertebrates in March 2020 (N=7 sampling trips x 7 sampling sites x 3 replicates=147 samples/sub-basin). The dissolved oxygen (DO) and pH of the water samples were measured in-situ using YSI 52 (USA) and Thermo Scientific Orion 3-Star (Indonesia) portable meters, respectively. A temperature of 4°C was maintained during the transport of water samples from the sampling site to the laboratory for analysis. The standard method was utilized for the measurement of Total Suspended Solids (TSS) at the laboratory (American Public Health Association [APHA] 2012). In addition, a UV spectrophotometer (DR 2800, HACH, Germany) was utilized to measure ammoniacal-nitrogen and Chemical Oxygen Demand (COD), while a Biochemical Oxygen Demand (BOD) probe was measured using a BOD probe meter (YSI 5905, USA). The initial BOD values were recorded when the samples were collected. Then, the samples were incubated under 20°C for five days, after which the BOD values of the samples were measured again. The difference in the BOD values of each sample was calculated as the concentration of BOD_5.

Benthic Macroinvertebrates Analysis

Benthic macroinvertebrates were sampled every alternate month from April 2019 to February 2020. To obtain a proper rarefaction curve, additional sampling visits were undertaken in March 2020 (N=7 sampling trips x 6 sampling sites x 3 gears x 5 replicates=630 samples/sub-basin). The sampling gears used to obtain the said samples were the D-frame dip net, aquatic kick net, and hand spade. The five replicate samples were composited in the laboratory and then considered as one sample. A sieve with a 0.5 mm fine mesh size (APHA 2012) was used to wet sieve the samples, after which they were separated according to particle size. After separation and before further analyses were conducted, benthic macroinvertebrates were sorted and subsequently stored and preserved in a solution comprising 70% ethanol. After mounting a temporary prepared slide to a compound microscope, Chironomids larvae and oligochaete worms were observed. Other macroinvertebrates were also observed using a dissecting microscope. Next, via the utilization of standard identification keys provided by various sources in literature, all of the taxa present were identified and categorized to family level (Brinkhurst, 1971; Brinkhurst and Jamieson, 1971; Hong, 1994; Xiufu, 1994; Merritt and Cummins, 1996; Yong and Yule, 2004; Sangpradub and Boonsoong, 2006; Thorp and Lovell, 2014).

Development of a Water Quality Index Model

A few families were retained for further analysis after trimming rare taxa with less than 5% of the total benthic macroinvertebrate population (Clarke, 1993; Kim et al., 2018). This was attributed to the small sample size of some of the families. Hence, benthic macroinvertebrates with less than ten individuals were considered rare and excluded from modeling. If rare taxa are present in a particular site, it hinders the selection of good biological indicators for water pollution. Next, the reference site (SR2) was excluded from statistical tests because it showed a significant difference in the total number of benthic macroinvertebrates and water quality parameters compared to the other sites.

The PCA was then performed to select the most representing benthic macroinvertebrates families (good bioindicators) for the model development. PCA can assist in producing a good pattern in analyzed data. Hence, suitable benthic macroinvertebrates can be grouped with similar characteristics in the same group and significantly different macroinvertebrates in a different group. All benthic macroinvertebrates families were selected within the seven principal components by considering the component loading value of more than 0.6 (Tashtoush, 2015).

Also, the family Chironomidae and Tubificidae were manually added to the benthic macroinvertebrates list. These two families were consistently recorded in high abundance (total number of individuals) in every sampling month during the study period (Hettige et al., 2020). Thus, it was difficult for the PCA to produce high component loading values for these two families. Notwithstanding, several researchers have proven that the family Chironomidae and Tubificidae are good indicators of organic pollution (Azrina et al., 2006; Jenderedjian and Hakobyan, 2007; Martins et al., 2008). Therefore, based on the nearest characteristics of these two families, they were manually included within the same group of the family Naididae (under Component 5).

The general characteristics of each component were listed and described, such as habitat, pollution tolerance, and DO level for each selected benthic macroinvertebrate family. Then based on previous literature, families with similar characteristics were grouped while considering the different components leading to the selection of four groups of benthic macroinvertebrates.

Next, a backward multiple linear regression (MLR) was performed simultaneously for each family with all water quality parameters (pH, DO, BOD, COD, TSS, and ammoniacal-nitrogen) included in WQI, Malaysia. This procedure was carried out because these water quality parameters have been established as indicators of organic pollution.

The score value (variance coefficient) was computed only for benthic macroinvertebrates families that were statistically significant for water quality parameters using the original PCA formula shown below:

$$PC_m = a_{m1}X_1 + a_{m2}X_2 + \ldots + a_{mn}X_n$$

PC=Principal component

a_{mn}=component of weighted value m^{th} for variables n^{th}

X=variable

m=component

The score value was calculated following the PCA formula as shown below:

$a_{mn}X_n$ =variance coefficient x frequency of occurrence

$$a_{mn}=\text{variance coefficient (score value)}=\frac{\text{\% of variance}}{\text{Cumulative \% of the variance}}$$

Based on each benthic macroinvertebrate family's score value, each sampling site's cumulative score value was calculated by considering each replicate as a sampling site to increase the number of samples (i.e., 6 sampling sites x 3 replicates=18). Following the conversion of the original sampling sites to the replicates sampling sites, the statistical results were better, as reflected in a higher number of samples. Then, the cumulative score values of sampling sites were analyzed using hierarchical agglomerative clustering analysis (i.e., Ward's method with Euclidean distance as a measure of similarity) in IBM SPSS statistical software 25.0 software. The dendrogram is essential to cluster similar sets of data.

Then, the mean confidence intervals for each cluster range value were calculated using the following equation to obtain a good range of values for the water quality standards.

$$MCI= \overline{X} \pm t_{df,\alpha/2}\left(\frac{S}{\sqrt{n}}\right)$$

Where,

MCI: Mean confidence interval

$\overline{X}$: Mean

t: t value for the degree of freedom (df), number of samples (n)

S: Standard deviation

n: Number of samples

α: 0.05

The water quality standards were developed for three categories (clean, slightly organic polluted, and organically polluted). In addition, the established water quality standards were internally and externally validated. For internal validation, reference site and impaired site data in the current study (primary data) were used.

Furthermore, a systematic review was conducted to determine the relevant research works in Malaysian rivers for external validation using resources from journals from 2012 to 2020 (eight years) in primary scientific databases: Scopus, Science Direct, Springer, Wiley, and Google scholar. For the external validation, proposed water quality standards were compared with the water quality status of other studies that used the data for biotic indices formation, such as the biological monitoring working party (BMWP)/ biological monitoring working party in Thailand ($BMWP^{Thai}$) score to determine their applicability. The keywords used for the systematic review were biomonitoring, bio-indicators, bio-indices, benthic macroinvertebrates, aquatic insects, and Ephemeroptera, Trichoptera, Plecoptera (EPT) in Malaysia. For example, one previous publication selected from this systematic review was used for external validation (Ghani et al., 2018).

Results

Identification of Benthic Macroinvertebrates

A total of 7,677 individual macroinvertebrates belonging to 27 families were recorded from the Rawang sub-basin. These families are Aeolosomatidae, Naididae, Haplotaxidae, Tubificidae, Lumbriculidae, Unidentified Oligochaeta, Erpobdellidae, Chironomidae, Ephydridae, Aytidae, Gomphidae, Libellulidae, Corduliidae, Protoneuridae, Coenagrionidae, Caenidae, Baetidae, Leptophlebiidae, Dytiscidae, Hydropsychidae, Viviparidae, Lymnaeidae, Thiaridae, Planorbidae, and Corbiculidae. These results have been published comprehensively by Hettige et al. (2020). Some rare taxa recorded in this study are Cladocera, Coenagrionidae, Corduliidae, Dytiscidae, Ephydridae, Leptophlebiidae, Gomphidae, Lymnaeidae, and Planorbidae.

Benthic Macroinvertebrates Families Selection

The correlation matrix revealed coefficient values of 0.3 and above based on PCA's benthic macroinvertebrates composition outcomes. In the present study, the KMO and the χ2 of Bartlett's test values were 0.488 and 437.771, respectively. The χ2 of Bartlett's test was statistically significant (P<0.05), thus, confirming the suitability of the dataset for PCA (Bartlett, 1954; Tabachnick and Fidell, 2007). The principal components with a corresponding eigenvalue

≥1 were retained based on the KMO's criterion (Kaiser, 1958) (Table 1). As a result, the eigenvalues of the first, second, third, fourth, fifth, sixth, and seven components were higher than one, accounting for 12.235%, 11.265%, 9.910%, 8.760%, 7.849%, 7.098%, and 5.900% (correspondingly 63.017%) of the total variance, thus classifying the data into seven components (Table 1). Also, the scree plot shows a non-pronounced slope variation after the seven eigenvalues.

The component values of benthic macroinvertebrate families higher than 0.6 (Tubificidae and Chironomidae) were manually added (Table 1). Based on the previous literature, each family's general characteristics were compared (Table 2). Due to some components were not loaded with similar benthic macroinvertebrate characteristics, their general characteristics were observed through different components (Table 3).

Families Glossiphoniidae and Aeolosomatidae were excluded as they did not fit into any family groups based on their general characteristics. Finally, in contrast to the seven components initially extracted by the PCA, four groups of benthic macroinvertebrates with similar characteristics were obtained (Table 3).

Based on general characteristics, Haplotaxidae Lumbriculidae, Naididae Chironomidae Tubificidae, and Unidentified Oligochaeta were initially grouped in Group 1. However, four families (Naididae, Lumbriculidae, Chironomidae, and Tubificidae) were significantly affected by water quality parameters following the MLR analysis. Based on the regression outputs, only ammoniacal-nitrogen, DO, and COD values were favorable to the family Lumbriculidae. The determination of the family Naididae has been reported to be markedly influenced by ammoniacal-nitrogen. However, all the families in Group 2, comprising Libellulidae and Protoneuridae, were significantly affected by TSS. From Group 3, only the family Corbiculidae was significantly affected by ammoniacal-nitrogen. Two families (i.e., Hydropchysidae and Baetidae) were significantly affected by water quality in Group 4. In addition, an increase in DO resulted a proportional increase of the family Hydropchysidae and vice versa. Furthermore, the family Chironomidae was favorably influenced by BOD, COD, and TSS, whereas the family Tubificidae was influenced by water quality parameters, BOD and COD.

Proposed Model for Water Quality Classification

The calculated score values for each benthic macroinvertebrate family are presented in Table 4, and they were included based on their ecological role. The calculated cumulative score value (variance coefficient) for every sampling site was included in Table 5. The cumulative score values of the sampling sites were grouped into three clusters (Figure 2). Therefore, current study results showed highly correlated sampling sites which were clustered together based on their cumulative score values.

Cluster 1 was presented by sampling sites 15 and 12, with cumulative score values ranging from 0.981 to 1.007. These two sampling sites represented at least by one "pollution sensitive" benthic macroinvertebrate

Table 1. Principal components (PC) and Varimax rotated component matrix of benthic macroinvertebrates in PCA

	Eigenvalue explained by PCs						
	2.202	2.028	1.784	1.577	1.413	1.278	1.062
	Percentage of total variance explained						
	12.235	11.265	9.910	8.760	7.849	7.098	5.900
	Component matrix						
Benthic macroinvertebrates families/Variables	PC1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 7
Thiaridae	0.928	-0.028	-0.030	0.084	-0.037	-0.015	0.010
Viviparidae	0.864	0.048	0.005	-0.035	-0.007	0.043	-0.214
Corbiculidae	0.637	-0.042	-0.031	-0.087	-0.030	-0.111	-0.409
Haplotaxidae	-0.017	0.828	-0.081	-0.038	0.092	0.031	-0.011
Lumbriculidae	-0.089	0.801	0.041	0.117	0.037	-0.075	-0.084
Glossiphoniidae	0.082	0.642	0.040	-0.098	-0.035	0.139	-0.034
Libellulidae	-0.005	-0.025	0.901	-0.047	-0.009	0.032	0.035
Protoneuridae	-0.023	0.022	0.900	0.077	-0.067	-0.049	-0.019
Hydropsychidae	0.016	0.004	0.041	0.848	-0.090	-0.092	0.008
Caenidae	0.048	-0.070	-0.126	0.727	-0.095	0.185	0.081
Chironomidae	-0.151	0.057	0.333	0.566	0.277	-0.205	0.023
Naididae	-0.061	0.061	0.019	0.026	0.861	-0.058	-0.005
Aeolosomatidae	0.011	0.011	-0.069	-0.105	0.718	0.113	-0.047
Uindentified Oligochaeta	0.063	0.068	0.008	-0.030	0.004	0.670	-0.120
Atyidae	-0.006	0.062	-0.068	0.144	0.336	0.663	0.063
Erpobdellidae	-0.093	-0.011	0.004	-0.064	-0.100	0.597	0.018
Baetidae	0.017	0.049	-0.061	0.027	-0.096	-0.014	0.739
Tubificidae	0.038	0.142	-0.076	-0.061	-0.051	0.022	-0.594

Table 2. The general characteristics of selected families based on the previous literature and their principal component numbers

Component Number	Selected families	Type of group	General characteristics	References
Component 1	Viviparidae, Thiaridae, and Corbiculidae	Aquatic clams/bivalves	High tolerance against extremes of physico-chemical components of water, present in rivers that are substrate with rocks, submerged wood, sometimes in large aggregations, and under loose bark, or in sand or mud, they are known to feed above the suspended particles, has the capability of invading habitats.	(Sangpradub and Boonsoong, 2006; Weerakoon et al., 2021)
Components 2	Haplotaxidae, Lumbriculidae, Glossiphoniidae	Aquatic Oligochaeta Hirudinea	Pollution tolerant, prefer to low oxygen level. They can be found in the running and standing water in muddy and sandy conditions. They occupy depositional habitats of most aquatic organisms, functioning as decomposers of decaying organic matter and mixing and aerating the benthic substrates through burrowing. Free-living or parasitic annelids, and some are medicinally important. Not meeting the criteria as indicator due to least composition, no clear response to pollution, difficulty to sample and culture.	(Brinkhurst and Jamieson, 1971; Vivien et al., 2015)
Components 3	Libellulidae, and Protoneuridae	Aquatic odonatans	Moderately pollution tolerant taxa, are found in running and standing freshwater habitats, some species are found in streams clinging to rocks and vegetation.	(Merritt and Cummins, 1996; Bassa and Jimma, 2016)
Components 4	Hydropsychidae, Caenidae and Chironomidae	Aquatic insects	Present in rivers with rocks, sands, and buds, substrate and has high habitat variance, and they are mainly diversified in unpolluted running water. Can tolerate extremely low oxygen concentration and survive in different environmental gradients.	(Merritt and Cummins, 1996; Bassa and Jimma, 2016)
Component 5	Naididae Chironomidae Tubificidae Aeolosomatidae	Aquatic Oligochaeta	Pollution tolerant, prefer to low oxygen level. They can be found in the running and standing water in muddy and sandy conditions. They occupy depositional habitats of most aquatic organisms, functioning as decomposers of decaying organic matter and mixing and aerating the benthic substrates through burrowing. Not meeting the criteria as indicator due to least composition, no clear response to pollution, difficulty to sample and culture.	(Brinkhurst and Jamieson, 1971; Al-Abbad, 2012; Zhou et al., 2021)
Component 6	Atyidae Uindentified Oligocheata	Family of shrimp Aquatic Oligochaeta	Present in rivers with rocks, sands, and buds substrate, has high habitat variance, and they are mainly diversified in unpolluted running water. Detritus feeders and well-segmented worms. Pollution tolerant, prefer to low oxygen level. Pollution tolerant, prefer to low oxygen level. They can be found in the running and standing water in muddy and sandy conditions. They occupy depositional habitats of most aquatic organisms, functioning as decomposers of decaying organic matter and mixing and aerating the benthic substrates through burrowing.	(Merritt and Cummins, 1996; Bassa and Jimma, 2016) (Brinkhurst and Jamieson, 1971)
Component 7	Baetidae	Aquatic insects	Present in rivers with rocks, sands, and buds substrate, has high habitat variance, and they are mainly diversified in unpolluted running water.	(Merritt and Cummins, 1996; Bassa and Jimma, 2016)

family. Therefore, clean water quality was represented by cumulative score values ranging from 0.981 to 1.007. Cluster 2 accommodated the large groups of sampling sites, namely sites 17, 18, 16, 1, 7, 10, 6, 14, and 13. The "moderately pollution-tolerant" benthic macroinvertebrates were observed in these sampling sites. However, sampling sites 13 and 14 contain pollution tolerant, moderate pollution, and one sensitive taxon. Cluster 3 was represented by sampling sites 9, 11, 5, 2, 3, 4, and 8, with cumulative score values ranging from 0.544 to 0.711. The more "pollution-tolerant" benthic macroinvertebrates, namely Oligochaeta (Naididae, Lumbriculidae, and Tubificidae) and Chironomidae were observed in these sampling sites. Also, sampling sites 3 and 4 have one pollution tolerant taxa, while sampling site 8 has one moderate pollution taxa.

The calculated mean confidence interval values were arranged for each cluster range, followed by standards for determining different water quality statuses (Table 6). It was sufficient to split the responses of cumulative score values of the sampling sites into three groups, which were favorable for classifying water quality standards.

In Cluster 3, a value less than the upper limit of the mean confidence interval was chosen as the benchmark value of standards for polluted (<0.69) (Table 6). Therefore, water quality standards of less than 0.69 were categorized as "organically polluted." Based on Cluster 2, the confidence intervals varied from 0.830 to 0.87, indicating that the 0.87 value was the upper limit. Therefore, the water quality standards ranging from 0.69 to 0.87 were categorized as "slightly organic polluted" (Table 6). Furthermore, the lower limit of confidence interval (0.83) of cluster 1 and cluster 2 was the same. Therefore, this overlapping value (0.83) could not be considered as the benchmark for "clean" water quality since it lies within the "slightly organic polluted" status of water quality. The upper limit of the confidence interval for "slightly organic polluted" is 0.87, and any value above this estimate was considered "clean" water quality status (Table 6). Overall, Cluster 1 included two

Table 3. Selected groups from the eight components in the PCA based on similar characteristics

Group name	Family Name	Selected component	References
Group 1	Haplotaxidae Lumbriculidae, Naididae Chironomidae Tubificidae and Unidentified Oligochaeta	Component 2, Component 5 and Component 6	(Brinkhurst and Jamieson, 1971; Vivien et al., 2015; Zhou et al., 2021)
Group 2	Libellulidae, Protoneuridae	Component 3	(Merritt and Cummins, 1996; Martín and Maynou, 2016; Abdul et al., 2017)
Group 3	Viviparidae, Thiaridae and Corbiculidae	Components 1	(Sangpradub and Boonsoong, 2006; Tinoco-Pérez et al., 2019; Parra et al., 2021)
Group 4	Baetidae, Caenidae, Hydropsychidae, and Atyidae	Component 4, Component 6, and Component 7	(Merritt and Cummins, 1996; Bassa and Jimma, 2016)

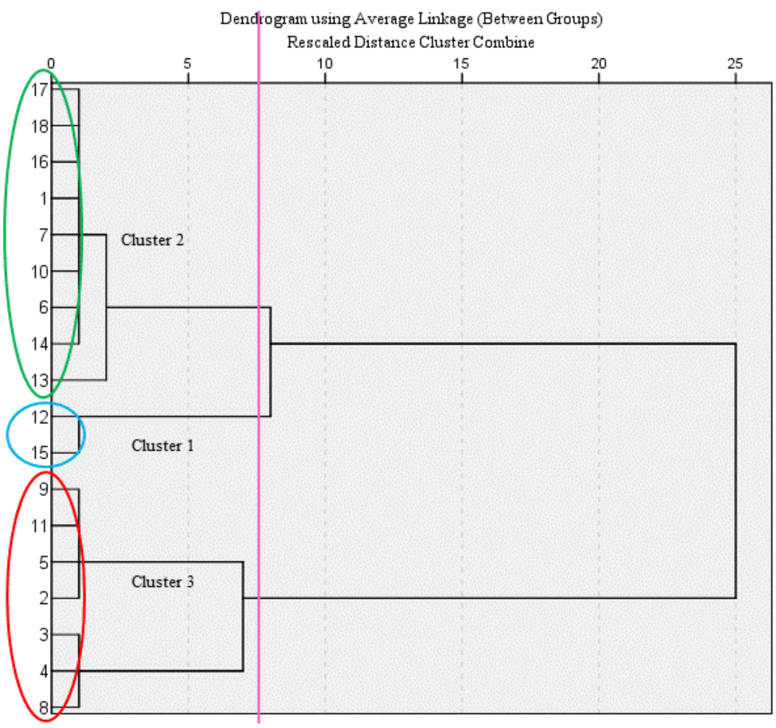

Figure 2. Dendrogram showing the cluster analysis of sampling sites based on cumulative score value

Table 4. PCA score values are based on the benthic macroinvertebrates families and their ecological role in the aquatic ecosystem

Group	Family	Ecological role	References	% of the variance for the component	Total percentage of variance	Coefficient of variation (score value)	Frequency of appearance	Coefficient of variation * Frequency of app	Family component based on PCA analysis
1	Naididae	Polluted water	(Chapman et al., 1981; Arimoro et al., 2007)	7.849	63.017	0.125	73	9.09	Comp 5
	Chironomidae		(Azrina et al., 2006; Jenderedjian and Hakobyan, 2007)	7.849	63.017	0.125	98	12.21	Comp 5
	Tubificidae		(Martins et al., 2008; Burnhill, 2006)	7.849	63.017	0.125	100	12.46	Comp 5
	Lumbriculiade		(Chapman et al., 1981; Arimoro et al., 2007)	11.265	63.017	0.179	53	9.47	Comp 2
2	Libellulidae	Moderate polluted water	(Martín and Maynou, 2016; Abdul et al., 2017)	9.91	63.017	0.157	5	0.79	Comp 3
	Protoneuridae			9.91	63.017	0.157	12	1.89	Comp 3
	Curbicullidae		(Tinoco-Pérez et al., 2019; Parra et al., 2021)						
				12.235	63.017	0.194	6	1.16	Comp 1
3	Baetidae	Clean water	(Hamid et al., 2011; Salmiati et al., 2017)	5.9	63.017	0.094	4	0.37	Comp 7
	Hydropchysidae			8.76	63.017	0.139	19	2.64	Comp 4

Note: Comp = Component

Table 5. The cumulative score values of each sampling sites

Family	Ecological role	Score value	SR1			SR3			SR4			SR5			SR6			SR7		
			Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Site 10	Site 11	Site 12	Site 13	Site 14	Site 15	Site 16	Site 17	Site 18
Naididae	Polluted water	0.125	17	38	24	122	83	120	64	126	235	48	87	35	5	7	8	21	134	49
Lumbriculidae		0.125	14	57	71	5	85	18	3	57	11	13	126	71	0	4	2	1	12	4
Tubificidae		0.125	118	293	187	96	308	330	24	876	56	42	179	130	6	6	9	10	56	82
Chironomidae		0.125	54	60	66	75	47	80	91	17	179	274	211	325	58	154	202	73	70	170
Hydropsychidae	Clean	0.139	0	0	3	4	0	4	2	0	0	45	0	31	18	0	47	0	0	0
Baetidae		0.094	0	0	0	0	0	0	0	0	0	0	0	0	2	40	16	0	0	0
Corbiculidae	Moderately Polluted	0.194	0	0	0	0	0	0	0	0	0	0	0	0	0	7	4	0	0	0
Libellulidae		0.157	1	0	0	0	0	0	0	0	0	0	0	10	0	0	0	1	1	3
Protoneuridae		0.157	1	0	0	0	0	1	1	1	0	2	0	9	0	0	0	1	1	2
Cumulative score value for each site			0.868	0.554	0.693	0.693	0.554	0.850	0.850	0.711	0.544	0.850	0.554	1.007	0.787	0.841	0.981	0.868	0.868	0.868

sampling sites (15 and 12), and these sites contained at least one "clean water" taxa. Nine sampling sites were present in Cluster 2, and eight contained at least one "moderately polluted water" taxa (Figure 2). Therefore, taxa in these two clusters support considering values greater than 0.87 as a benchmark for "Clean" water quality status.

Validation of the Proposed Water Quality Standards

Internal Validation

The validation process helps predict actual data by inputting values into the selected standards. The family Baetidae, Libellulidae, Chironomidae, Tubificidae, and Lumbriculiade were recorded at the reference site (SR2) in the present study, and the cumulative score value of the sampling site was 0.679 (0.7) (Table 7). Based on the proposed water quality standards, the reference site of this study was classified as "moderate polluted" (>0.69). The BMWP^{Thai} value, the reference site was classified as a "moderately polluted" condition.

Table 8 shows the internal validation results of the proposed water quality standards for 18 tested sampling sites. The water quality status of four sampling sites (2, 5, 9, and 11) was recorded as "organically polluted", 12 sampling sites were classified as "slightly organically polluted", whereas two sampling sites (12 and 15) showed "clean" water quality status.

For instance, the cumulative score value of sampling site 1 was 0.868. This result indicated that the site was "slightly organic polluted" based on the new water quality standards, which is equivalent to WQI water quality status (moderate condition) (Hettige et al., 2021). Based on the BMWP^{Thai} score, thirteen tested sampling sites (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 13, and 14) was recorded as "very poor", whereas five sampling sites (12, 15, 16, 17, and 18) showed "Moderate poor" water quality status.

When considering the original sampling sites, the calculated cumulative values for SR1, SR3, SR4, SR5, SR6, and SR7 were 1.007, 0.85, 0.85, 1.007, 0.981, and 0.868, respectively. Based on new water quality standards, SR1, SR5, and SR6 showed "Clean" water quality status,

Table 6. The final proposed water quality standards in this study

Cluster No	Range	Mean confidence intervals	Standards	Water quality status
Cluster 1	0.981-1.007	0.830-1.159	>0.87	Clean
Cluster 2	0.787- 0.868	0.830-0.870	0.69-0.87	Slightly organic polluted
Cluster 3	0.554 - 0.711	0.533-0.687	<0.69	Organically polluted

Table 7. Internal validation result of the reference sampling site (SR2) in the present study

Benthic macroinvertebrates families recorded	Score value
Baetidae	0.094
Libellulidae	0.157
Chironomidae	0.125
Tubificidae	0.125
Lumbriculiade	0.179
Total score	0.679 ≃ 0.7
Ecological status based on a new standard	Moderate polluted
BMWP^{Thai} score in the present study	70 (Moderate)

Table 8. Internal validation results of the tested sampling sites

Sampling site	Replicate sampling site	Cumulative score value	Ecological status based on new standards	Water quality status based on their BMWP score
SR1	Sampling site 1	0.868	Slightly organic polluted	14 (Very Poor)
	Sampling site 2	0.554	Organically polluted	5 (Very Poor)
	Sampling site 3	0.693	Slightly organic polluted	11 (Very Poor)
SR3	Sampling site 4	0.693	Slightly organic polluted	10 (Very Poor)
	Sampling site 5	0.554	Organically polluted	6 (Very Poor)
	Sampling site 6	0.85	Slightly organic polluted	14 (Very Poor)
SR4	Sampling site 7	0.711	Slightly organic polluted	16 (Very Poor)
	Sampling site 8	0.711	Slightly organic polluted	11 (Very Poor)
	Sampling site 9	0.554	Organically polluted	5 (Very Poor)
SR5	Sampling site 10	0.85	Slightly organic polluted	16 (Very Poor)
	Sampling site 11	0.554	Organically polluted	5 (Very Poor)
	Sampling site 12	1.007	Clean	22 (Moderate poor)
SR6	Sampling site 13	0.787	Slightly organic polluted	14 (Very Poor)
	Sampling site 14	0.841	Slightly organic polluted	12 (Very Poor)
	Sampling site 15	0.981	Clean	17 (Moderate poor)
SR7	Sampling site 16	0.868	Slightly organic polluted	17 (Moderate poor)
	Sampling site 17	0.868	Slightly organic polluted	17 (Moderate poor)
	Sampling site 18	0.868	Slightly organic polluted	17 (Moderate poor)

whereas SR3, SR4, and SR7 showed "Slightly organic polluted" (Table 9). This is justifiable according to the WQI index. Except for SR4, other sampling sites showed "Slightly polluted" water quality status based on the WQI index. Therefore, the present study findings was concurred with the data published by Hettige et al. (2021). Based on the BMWP^{Thai} score, these original sampling sites, SR1, SR3, and SR7, were categorized as "moderate" and other sampling sites (SR4, SR5, and SR7) indicated "Moderately poor" water quality status (Table 9).

External Validation

A selected previous study for external validation using systematic review is included in Table 10. Among the nine benthic macroinvertebrates families presented in Table 4, six of them (Baetidae, Hydropchysidae, Libellulidae, Chironomidae, Tubificidae, and Naididae) were reported by Ghani et al. (2018) following the study conducted in an urban river, Penchala River in Selangor State, Malaysia. Thus, the cumulative score values for the four selected sampling sites were 0.515, 0.375, 0.407, and 0.375, respectively (Table 10). Hence, the water quality status of all the sampling sites was classified as "organically polluted". However, in comparison to the BMWP, sampling site 1 showed "good" water quality, whereas other sampling sites indicated "very good" water quality (Ghani et al., 2018). Therefore, the new water quality standards were not equivalent to the BMWP water quality criteria for sampling site 1. However, the water quality status of other sampling sites was equivalent to the BMWP water quality status.

Discussion

In this study, the general characteristics of benthic macroinvertebrates assisted in categorizing similar groups, and MLR analysis determine whether they are statistically significant with the water quality parameters. Similar to the current study, a study conducted in Odra River, Poland, recorded a significantly high ($P<0.05$) ammoniacal-nitrogen concentrations with a residence of pollution-tolerant benthic macroinvertebrates taxa such as Oligochaeta (Krepski et al., 2014). Also, Odonata larvae (Libellulidae and Protoneuridae) have a relatively long history of being used as a bioindicator for river health assessment in Malaysia (Al-Shami et al., 2014).

Based on the study conducted in various streams, Gunung Tebu Forest Reserve, Terengganu, Malaysia, Md Rawi et al. (2014) found that species in the family Hydropsychidae were highly dependent on DO. Also, Shafie et al. (2017) reported a similar finding in the Liwagu River, Sabah, Malaysia. Overall, organic pollutants are known to reduce the oxygen concentration in water bodies. This event negatively impacts clean water taxa because they mainly depend on external gills for respiration, and their populations are reduced following depleted oxygen concentrations (Edegbene et al., 2019).

The proposed water quality standards failed to yield a wide range (i.e., slightly organic polluted: 0.69-0.870) for water quality standards like the BMWP (i.e., moderate: 41-70). This was due to the uncertainty of the differences between the clear and polluted conditions during sampling periods. Hettige et al. (2021) published these amalgamated data. Moreover, there was only a slight change in the water quality status during each sampling month of this study.

Based on the study conducted in the Teesta River Basin, India, Bhatt and Pandit (2010) found a narrow range of water quality (0 to 1), which was used to determine the level of organic pollution. However, a sensitive score criterion of the FBI was considered to obtain the biotic index, and the authors recommended the criteria for universal application. Therefore, the findings may be due to the high sensitivity of the data in their study. Furthermore, several other developed

Table 9. Internal validation results of the original sampling sites

Families	SR1	SR3	SR4	SR5	SR6	SR7
	Score values based on recorded families					
Naididae	0.125	0.125	0.125	0.125	0.125	0.125
Lumbriculidae	0.179	0.179	0.179	0.179	0.179	0.179
Tubificidae	0.125	0.125	0.125	0.125	0.125	0.125
Chironomidae	0.125	0.125	0.125	0.125	0.125	0.125
Hydropsychidae	0.139	0.139	0.139	0.139	0.139	-
Baetidae	-	-	-	-	0.094	-
Corbiculidae	-	-	-	-	0.194	-
Libellulidae	0.157	-	-	0.157	-	0.157
Protoneuridae	0.157	0.157	0.157	0.157	-	0.157
Water quality status based on their BMWP score	57 Moderate	70 Moderate	50 Moderate	41 Moderately poor	41 Moderately poor	53 Moderate
Cumulative score value	1.007	0.85	0.85	1.007	0.981	0.868
Water quality status based on a new standard	Clean	Slightly organic polluted	Slightly organic polluted	Clean	Clean	Slightly organic polluted

water quality standards are characterized by a broader range of water quality classifications, such as Sigscore and BMWP^{Thai}.

A combination of multivariate statistical analysis and cumulative score values of each sampling site helped establish the present study's water quality index model. Achieng' et al. (2017) and Milner and Oswood (2000) mentioned that multivariate techniques give more precise and accurate for biomonitoring assessment. However, several studies have applied different methods, such as multivariate and multimetric techniques, to build such models. For instance, previous studies used different macrobenthos metrics to develop water quality standards (Arman et al., 2019; Sirisinthuwanich et al., 2016; Lakew and Moog., 2015). In addition, some studies employed an average weighted score method to develop biotic indices and assess water quality status (Musonge et al., 2020; Blakely et al., 2014).

This study used the clustering method to develop water quality standards into three categories (clean, slightly organic polluted, and organic polluted). Similarly, Banda and Kumarasamy (2020) used the clustering method to develop WQI for South African watersheds. The same technique was used in Malaysia to classify score values into five water quality classes for creating the Malaysian FBI (Ghani, 2016). Some researchers have employed different statistical approaches to classify water quality classes. The box-and-whisker plots with various metric scores were commonly used to classify water quality class boundaries (Arman et al., 2019). Irrespective of the statistical technique used to define the class boundaries, a minimum of three distinguished classes are best for good water quality classification. The significant differences in water quality during the study period were responsible for this situation.

The family level identification was considered in this study to obtain the organic pollution determination model. As a result, family-level taxonomic identification is more reliable than genus and species level as there is an incomplete taxonomic identification guide in Southeast Asia. Similarly, Boonsoong et al. (2009) stated that family-level taxonomic resolution is essential to the benthic macroinvertebrates-based indices model because it is easy to use and less expensive. Therefore, several researchers have identified macrobenthos up to the family level to develop a multimetric index (Arman et al., 2019; Sirisinthuwanich et al., 2016; Lakew and Moog., 2015).

The present study excluded the reference sampling site due to the significant difference in macrobenthos composition and water quality parameters among the sampling sites. However, the reference sampling site's condition is recommended to be maintained and remain unchanged for better outcomes. Furthermore, in contrast to previous literature, various reference sites were considered for the macrobenthos-based model indices (Arman et al., 2019; Sirisinthuwanich et al., 2016; Lakew and Moog., 2015). This is because establishing stress gradient into reference and impaired site is one of the requirements of developing a multimetric index (Barbour et al., 1999).

According to available literature, this study is the first attempt to improvise a model for Malaysian rivers to determine organic pollution using local macrobenthos. However, Jumaat and Hamid (2020) also reported that the Malaysian FBI developed using local macrobenthos (Ghani, 2016) is applicable for assessing recreational rivers. Developing water quality standards using local macrobenthos is vital due to macrobenthos fluctuation in different geographical regions. The macrobenthos-based water quality standards model was widely developed to evaluate river health (Musonge et al., 2020; Hilsenhoff, 1988; Armitage et al., 1983). Hence, a few studies were conducted in Southeast Asia to achieve a similar task (Arman et al., 2019; Blakely et al., 2014; Mustow, 2002).

The proposed new water quality standards and water quality criteria based on the BMWPThai value showed equivalent results. Hence, the internal validation for the reference site was comparatively successful. Table 9 shows water quality status and condition category classification at the original sampling sites based on their corresponding indices. In the present study, the water quality status of new standards at many sampling sites was equivalent to the water quality status of BMWP^{Thai}. However, compared with

Table 10. External validation results for the study conducted in Penchala River, Selangor, Malaysia

	Sampling site 1	Sampling site 2	Sampling site 3	Sampling site 4
Families	Score values based on recorded families			
Baetidae	0.094	-	-	-
Hydropchysidae	0.139	-	-	-
Libellulidae	0.157	-	0.157	-
Chironomidae	0.125	0.125	0.125	0.125
Tubificidae	-	0.125	0.125	0.125
Naididae	-	0.125	-	0.125
Water quality status based on their BMWP average score	86 Good	7 Very poor	6 Very poor	6 Very poor
Water quality status based on new standard	0.515	0.375	0.407	0.375
Based on the new index	Organically Polluted	Organically Polluted	Organically Polluted	Organically Polluted

external validation, there are significant gaps in water quality status between new water quality standards and the the BMWP score in the first sampling site (Table 10). This is one of the standard practices when doing modeling. However, there are many variations in sample collection, sampling region, and methodology used for analysis in the present study. Also, there is a wide biological variation of benthic macroinvertebrates when considering their life cycle.

Similarly, Jumaat and Hamid (2020) reported inconsistent water quality status in selected rivers in Perak, Malaysia, using different water quality indices, namely BMWP and Malaysian FBI. The authors attributed the outcomes to the presence and high abundance of intolerant taxa such as Baetidae. It is important to note that the newly established water quality standards are relevant to and appropriate in some studies in Malaysia. Nevertheless, it may not apply to others, probably due to the good influence on water quality and organic contamination. Several studies conducted in recreational and upstream areas in Malaysia identified Pollution-sensitive macrobenthos. For instance, Jumaat and Hamid (2020) identified two pollution-sensitive taxa (Baetidae and Hydropsychidae), one moderate pollution-tolerant taxa (Libellulidae), and one pollution-tolerant taxon (Chironomidae), with a calculated cumulative score value less than 0.69. The water quality status of their study area was established as organically polluted. The macrobenthos in this study was sampled in the middle rivers in contrast to the upper stream sampling by Jumaat and Hamid (2020). According to the River Continuum Concept, the macrobenthos composition and structure are diverse, and this study did not capture all. Therefore, the water quality status of previous studies differed when using the proposed water quality standards.

Like worldwide reorganized biotic indices, the proposed water quality standards have advantages and disadvantages. The advantages of the newly estimated standards are that they are easy to use, classify the main characteristics of polluted waters, it does not require a rigorous sampling technique. It does not require a rigorous sampling technique, is easily understood by non-biologists, is sufficient for identification up to the family level, and is suitable for determining organic pollution. The disadvantages are that they are insensitive to moderate changes in water quality, only seven families were considered for the development of a standard due to statistical significance, provides only a narrow range of values due to the small sample size, and some species and genera of the same family group often exhibit different tolerance levels and ecological traits.

The limitations of this study are well-acknowledged because only one sub-basin in the river basin was sampled in contrast to previous studies that considered larger sampling areas (Sirisinthuwanich et al., 2017). Boonsoong et al. (2009) used a small sample size. However, their results produced an acceptable and more comprehensive range of standards. The current proposed standards are mainly based on the presence and absence of benthic macroinvertebrate taxa. Thus, the abundance of the taxa widely used as benthic macroinvertebrates-based water quality standards were not considered. Nevertheless, the currently proposed water quality standards are advantageous and applicable in determining organic pollution in Malaysian rivers.

Conclusion

In conclusion, the developed new water quality index model using benthic macroinvertebrates can be used to evaluate organic pollution in the future.

Ethical Statement

Not applicable

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Author Contribution

NDH performed the literature search, data analysis and reported findings. RH supervised the research, and critically revised the work. AAK had the idea for the article, critically reviewed the article and verified the reference sources. ZHA also critically revised the work. All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest regarding the publication of this article.

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