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# Calibrating and Validating the Biomonitoring Working Party (BMWP) Index for the Bioassessment of Water Quality in Neotropical Streams

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Additional information is available at the end of the chapter

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## Abstract

The Biological Monitoring Working Party (BMWP) is among the most used bioassessment indices for aquatic ecosystems quality assessment, which assigns scores to each macroinvertebrate taxa according to their sensitivity to organic pollution. However, BMWP scores must be calibrated to each geographical and ecological conditions. In this study, we obtain statistically derived scores of sensitivity for macroinvertebrates taxa from Neotropical Mexican rivers, Apatlaco and Chalma-Tembembe rivers, Balsas Basin. We obtained water samples and aquatic macroinvertebrates in four sampling campaigns (dry and rainy seasons). Physicochemical parameters and the abundances of the aquatic macroinvertebrates were used for the BMWP index calibration, which was performed in steps obtaining: the physicochemical quality index (*Pcq*), incorporation of abundances classes of macroinvertebrates taxa in the corresponding *Pcq* interval and the determination of bioindication values for each macroinvertebrate family. The BMWP calibrated index was validated and tested for the geographical range extension. The BMWP scores for Chalma-Tembembe River (located in agricultural areas) showed bad polluted to regular and moderated polluted categories. The urban river zone of Apatlaco River showed: bad, very polluted to very bad categories. The BMWP calibrated is a suitable biomonitoring tool, allowing the detection of those zones that needs urgently a management and recovery plan.

**Keywords:** water quality assessment, biomonitoring tool, macroinvertebrate sensitivity, land use, bioindication values, water quality categories

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## 1. Introduction

In the framework of the World Economic Forum, the “Water Crisis” is positioned as the highest concern global risk for the next 10 years [1]. In this sense, water quality and management of freshwater ecosystems are one of the main challenges worldwide [2]. However, these ecosystems face impacts and degradation that are result of human population increase and agricultural and industrial development [3]. Consequently, freshwater ecosystems and their biota are considered as the most endangered and threatened worldwide [4]. In developing countries, there is an extremely high population growth, increasing industrialization and urbanization processes, with severe and constant changes in land use, whereby the freshwater ecosystems are highly impaired [5]. Rivers crossing different land uses (urban, industrial and agricultural) are the most threatened by anthropogenic activities [5]. The threat to freshwater systems, in particular in developing countries, make evident an urgent need for developing tools for the assessment and classification of aquatic conditions in order to manage water resources and bring them a sustainable management.

Biomonitoring is considered as the most appropriate method for environmental studies and for the control of water quality, due to that living organisms are excellent biosensors of the physicochemical and biological characteristics of water [6]. The aquatic macroinvertebrates have been used as bioindicators because they have a wide range of habitats and sensitivity to environmental pollution and other types of stressors, including sediment [7]. Thus, the macroinvertebrate assemblages change in response to environmental disturbances in predictable ways including a strong reduction in species and abundance in impacted areas and more tolerant species predominate; whereas, sensitive species are only present in environments with the least impact or un-impaired conditions. Moreover, biomonitoring integrate information over longer periods of time and better represent the responses of aquatic habitats providing information concerning the present state and the past trends in environmental conditions [8].

The Biological Monitoring Working Party (BMWP) is among the most used bioassessment index in Europe, which was originally developed in the UK in 1976 [9] and it has been used by the regulatory authorities in the UK as the basis of their river invertebrate status classification system since 1980. This index assigns scores to each macroinvertebrate taxon according to their responses to oxygen deficits caused by organic pollution. The analysis of these pollution-induced responses allows the calculation of sensitivity values by the different groups of organisms. Because of its ease of use and low cost, the BMWP index has been used in many other countries in Africa, Asia, Oceania and Latin America [10]. Nevertheless, the BMWP scores for each taxon must be calibrated to each ecological region since the taxonomic composition, ecological, zoogeographic and anthropogenic conditions promote important geographical differences.

Additionally, the scale to ranking water conditions must be adapted for each particular condition. In Latin America, attempts have been made to develop regional indices [5]. In México, the water quality indicators used by the National Commission of Water are fecal coliforms, biochemical oxygen demand, chemical oxygen demand and total dissolved solids [11]; unfortunately, biomonitoring is not included in the current legislation, while information

on bioindication is scarce [12]. However, main urban zones of Mexico exert a high rate of changes in land use and deforestation for agricultural, industrialization and urban expansion provoking serious damages in water bodies [13]. Consequently, there is a need for a tool that considers both, biotic and abiotic variables and their relationships to assess the river water quality.

The aim of this study is to obtain the statistically derived scores of sensitivity for aquatic macroinvertebrates taxon for Neotropical Mexican rivers (Apatlaco and Chalma-Tembembe rivers, Balsas Basin). This chapter presents the calibration of the BMWP index based on Riss et al. [14] with some modifications, using physical, chemical and biological data from Apatlaco and Chalma-Tembembe rivers. This index, besides being an easy-to-use tool, allows for the implementation of a permanent biomonitoring network.

## 2. Materials and methods

### 2.1. Study area

Apatlaco and Chalma-Tembembe rivers are located in the Balsas Basin (**Figure 1a**), one of the largest catchment areas in Mexico (area of 117,405 km<sup>2</sup>) [15]. Both rivers are in the same zoogeographic region (Neotropical), which belongs to the Ecoregion Balsas Complex and belongs to the Biogeographic Province "Depresión del Balsas," particularly to the "Alto Balsas" [15]. The Chalma-Tembembe River is formed by the Chalma and Tembembe rivers; the first has a length of 70 km and the second of 50.72 km. The Chalma River joins the Tembembe River at its lower reaches. The Chalma-Tembembe subbasin has a mean annual rainfall around 600 mm. The area consists of tropical deciduous forest ( $\approx 47\%$  of landcover), with some areas of rain-dependent and irrigated agricultural use. The strong pressure from agricultural activities has favored land use changes and the loss of the original vegetation [15]. Six study sites were selected in Chalma-Tembembe: El Arco (I), La Loma (II), El Platanar (III), Casa de la Escuela (IV), Coatlán (V) and Hacienda de Cuautlita (VI) (**Figure 1a**). The Apatlaco River has a length of 63 km, with annual rainfall from 850 to 1500 mm. The natural vegetation has been highly fragmented and transformed, with only 27% of the original area of tropical deciduous, coniferous and oak forest remaining. Moreover, an important urban-industrial corridor (Cuernavaca corridor), runs alongside the river. The activities in the vicinity of the river include agriculture, lumber forestry, hunting and fishing [15]. Nine study sites along the main river channel (Apatlaco River) were selected: Las Truchas (VII), El Pollo (VIII), El Rayo (IX), El Encanto (X), Salida Panochera (XI), Xochitepec (XII), Alpuyeca (XIII), Xoxocotla (XIV) and Zacatepec (XV) and two more along the westerly tributary: Buenavista 1 (XVI) and Buenavista 2 (XVII), study sites located before and after the effluent of a wastewater treatment plant and three more along the easterly tributaries: El Texcal (XVIII), La Gachupina (XIX) and Las Juntas (XX), resulting in a total of 14 sampling sites (**Figure 1a**). In both rivers, four sampling campaigns were undertaken (the dry season in December 2012 and February-March 2013 and the rainy season in August-September 2012 and June 2013).

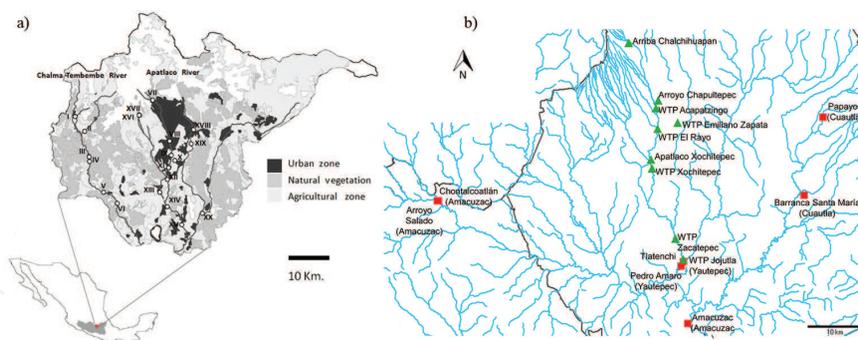


Figure 1. Study area location. (a) Study sites. (b) Validation and range extension study sites.

## 2.2. Water quality measurements and water quality index

For each site and sampling period, some variables were recorded: altitude (masl), water temperature ( $^{\circ}\text{C}$ ), conductivity ( $\mu\text{S}/\text{cm}$ ), pH, salinity (PSU), dissolved oxygen ( $\text{DO mg}/\text{L O}_2$ ) and turbidity (NTU), using a Quanta<sup>®</sup> multiparameter probe. Air temperature was recorded with a 45118 EXTECH anemometer. Water samples were transported to the laboratory in refrigerated and dark conditions. Biochemical oxygen demand ( $\text{BOD}_5 \text{ mg}/\text{L O}_2$ ), chlorides ( $\text{mg}/\text{L Cl}$ ), alkalinity ( $\text{mg}/\text{L CaCO}_3$ ) and total and fecal coliforms (MPN/100 mL) were determined according to [16]. Nitrite ( $\text{mg}/\text{L NO}_2$ ), nitrate ( $\text{mg}/\text{L NO}_3$ ), ammonia ( $\text{mg}/\text{L NH}_3$ ), total nitrogen ( $\text{mg}/\text{L TN}$ ), orthophosphate ( $\text{mg}/\text{L PO}_4$ ), total phosphorus ( $\text{mg}/\text{L TP}$ ), hardness ( $\text{mg}/\text{L CaCO}_3$ ), sulfates ( $\text{mg}/\text{L SO}_4^{2-}$ ) and color (U Pt-Co) were analyzed using a Hach DR 2500 spectrophotometer. Additionally, the DO saturation (%) was computed. The water quality index (WQI) proposed by Dinius [17] was calculated, which range from 0 to 100; 100 is excellent and 0 is strongly polluted.

## 2.3. Macroinvertebrate sampling

Aquatic macroinvertebrates were sampled at each sampling site and season with a multi-habitat monitoring system [18], along a section of 100 m length to incorporate local habitat variation (fast flowing riffles, pools, submerged vegetation and riparian vegetation). An area of  $1 \text{ m}^2$  was sampled for each type of habitat. Triplicate samples for each type of habitat, with a 10-min collecting effort, were collected and pooled for analysis. The samples were taken using a kick net for fast-flowing riffles and pools, while type-D nets for submerged and riverine vegetation, all nets with a mesh size of  $500 \mu\text{m}$ . Organisms collected were preserved with 70% alcohol. Taxonomic identification at the family level was conducted using stereomicroscopes (Nikon C-Leds) and with the use of keys [19, 20].

## 2.4. BMWP index calibration

### 2.4.1. Data processing

The BMWP index is calculated by adding up the individual tolerance scores of aquatic macroinvertebrates at family taxonomic level present at a sample site. We calibrate the

tolerance scores of the aquatic macroinvertebrates in several steps [14]: (1) obtaining a physicochemical quality index (*Pcq*) for all the study sites; (2) assessing the bioindication values to each macroinvertebrate family according to the *Pcq* and their abundance class; and (3) with the scores calibrated for each family of macroinvertebrate from Apatlaco and Chalma Tembembe rivers, we assessed the BMWP. Additionally, we define the water quality categories following the procedure of Ref. [21].

#### 2.4.2. Mathematical formulation

The physicochemical quality index (*Pcq*) is a value that describes water quality at each sample site on a scale from 0 to 10; 0 corresponds to a highly impacted site and 10 to a site with excellent water quality. Its calculation utilizes a data matrix with the mean values of each physicochemical parameters obtained from the four study periods for each sample site. Parameters recorded in situ, as well as quantified in the laboratory, were included. Values for each parameter were normalized:  $C_i = \ln(i + 1)$  and for percentages  $C_i = (2/\pi) * \arcsin \sqrt{i}$ . Where  $C_i$  is a physicochemical variable and  $i$  is the mean value of a physicochemical variable. The data matrix was subjected to a factor analysis with the software XLstat version 2013. Parameters that showed a significant correlation ( $p < 0.05$ ), either positive or negative, in the first two factors were considered as qualifying variables or  $C_{i2}$ ; these variables were arranged in a new data matrix of sampling sites *vs* the  $C_{i2}$ . The maximum ( $C_{i2max}$ ) and minimum ( $C_{i2min}$ ) values for each qualifying variable were determined and assigned taking into account the environmental legislation for the water management in México (Mexican Official Standards: [22, 23]), the USA [24], Canada [25], Central and South American countries [26, 27] and a worldwide level [28]. With  $C_{i2max}$  and  $C_{i2min}$  values for each qualifying variable the  $C_{i2}$  were standardized:

$$C_{i3} = \frac{C_{i2} - C_{i2min}}{C_{i2max} - C_{i2min}} \quad (1)$$

Each  $C_{i3}$  value was adjusted to fit the BMWP scale that goes from 0 to 10, using the following formula:  $C_{i4} = (1 - C_{i3}) * 10$ . In the case of the qualifying variables associated with good water quality (DO and DO% saturation), the inverse procedure was followed by applying the formula:  $C_{i4} = C_{i3} * 10$ . For each study site, an average was assessed with the  $C_{i4}$  values for the selected variables to determine the physicochemical quality:

$$P_{cq} = \frac{\sum_{i=1}^n C_{i4}}{ni} \quad (2)$$

The *Pcq* values fluctuate from 0 to 10, for all 20 sampling sites and were incorporated into 10 categories of quality or *Pcq* intervals.

### 2.4.3. BMWP scores for macroinvertebrates families

To assign bioindication values to the different macroinvertebrates families, a data matrix of sampling sites *vs* abundance was constructed using the mean abundance values for each aquatic macroinvertebrate family from all sampling and study sites. The mean abundances were standardized to six abundance classes: class 0 (0 organisms), class 1 (1–3 organisms), class 2 (4–10 organisms), class 3 (11–33 organisms), class 4 (34–100 organisms) and class 5 (>100 organisms). This standardization was carried on following [14] to reduce the possible effect of the overvaluation by the local dominance of some groups due to the nonhomogeneous natural distribution of the macroinvertebrates.

For each family of macroinvertebrates, the abundance class data were pooled within each *Pcq* interval. In cases where a family appeared in more than one study site within the same *Pcq* interval, the abundance classes of such sites were averaged in order to obtain a single abundance value per family for each *Pcq* interval. The value of class abundance obtained by a *Pcq* interval indicates the number of times you have to replicate the value of the superior limit corresponding to the *Pcq* interval; for example, if a family obtained a value of 1 for its abundance class within the 0–1 *Pcq* interval, a 3 for the 3–4 *Pcq* interval, a 4 for the 5–6 *Pcq* interval and a 2 for the 6–7 *Pcq* interval, the data for calculating the fifth percentile would be 1, 4, 4, 4, 6, 6, 6, 6, 7 and 7. The bioindication values for each aquatic macroinvertebrate family were calculated by obtaining the fifth percentile of the abundance class distributions along the *Pcq* intervals where that family was present. This value represents the minimum tolerance value of this family in relation to organic pollution; in the case of this example, the fifth percentile would be 2, which is the family BMWP bioindication value.

## 2.5. Definition of BMWP water quality categories

The water quality category ranges for the BMWP values were assigned following [21]. The median value of the data set of the reference sites was calculated. Scores above this median value will correspond to the “Excellent” quality category, while values that fall between the median and the tenth percentile of that distribution are considered to be in the “Good, not sensible affected” quality category. Values below the tenth percentile were subdivided into four equal parts, which correspond to the categories “Regular,” “Bad, polluted,” “Bad, very polluted,” and “Bad, extremely polluted.” The names assigned to each of the water quality categories with some modifications were those proposed by Alba-Tercedor [29]. The selection of reference conditions included physical, chemical and biological criteria (WQI, *Pcq*, land use and macroinvertebrate community) following [30].

## 2.6. BMWP statistical validation

The validation process was performed using three approaches. First, a score prediction test, proposed by Armitage et al. [31], with the average per study site of  $BMWP_{\text{observed}}$  *vs*  $BMWP_{\text{expected}}$  values. The expected values were calculated with a multiple linear regression (best model procedure) with the qualifying variables for each study site. The observed and expected values were plotted and confidence intervals ( $\alpha = 0.05$ ) were calculated using the XLSTAT software

2013. The goodness of fit of the model was evaluated using the coefficient of determination ( $R^2$ ) and  $p$  values.

For the second validation approach, the degree of fit of the model for the  $BMWP_{observed}$  vs  $BMWP_{expected}$  was tested using two independent methods: the [32] efficiency model (NSE) (range  $-\infty$  to 1.0), a widely used statistic for hydrological models

A third approach for the index validation and for the assessment of the geographical extension of the BMWP calibrated was performed with additional information that was obtained from the National Agency for Water in México (CONAGUA), data included aquatic macroinvertebrates collected in the county of Morelos. For the index validation, nine sites within the Apatlaco subbasin were considered: Arriba Chalchihuapan, Arroyo Chapultepec, water treatment plant (WTP) Acapatzingo, WTP Emiliano Zapata, WTP El Rayo, Apatlaco-Xochitepec, WTP Xochitepec, WTP Zacatepec and Tlaltenchi (**Figure 1b**). For the geographical extension, data from CONAGUA of seven sites in three subbasins were considered: Amacuzac subbasin, sites Chontalcoatlán, Amacuzac and Arroyo Salado; Cuautla subbasin, sites Barranca Santa María and Papayos; and Yautepec subbasin, sites Pedro Amaro and WTP Jojutla. These sites belong to the Balsas Basin and the last three subbasins are adjacent to the two rivers monitored in this study (**Figure 1b**). The monitoring team of CONAGUA used D-nets (mesh size of 500  $\mu\text{m}$ ), a multihabitat sampling and each habitat was sampled in 1  $\text{m}^2$ , with three replicates. Based on the calibrated scores for aquatic macroinvertebrates, the BMWP scores were calculated for each site of the data set from CONAGUA and these scores were included in the previous model generated with *observed vs expected* data for the BMWP with confidence limits ( $\alpha = 0.05$ ). A Pearson correlation analysis was also performed for the BMWP.

## 2.7. Statistical analysis

WQI and BMWP values are presented as the mean values of each study site for the four monitoring campaigns. Mean values were also calculated for each study season taking into account the values of all the studied sites. Significant differences between sites and seasons were detected with a bivariate analyses of variance (ANOVA), followed by Student-Neuman-Keuls multiple comparison tests (if the data were normally distributed as well as homoscedastic), or Kruskal-Wallis test for nonparametric data, both  $p < 0.05$ , using SigmaPlot version 11.0.

## 3. Results

### 3.1. Water quality index

For the Chalma-Tembembe River, mean values of WQI fluctuated from 52 to 74 (**Figure 2**), from slightly polluted to acceptable for human consumption; however, no significant differences between study sites ( $p > 0.05$ ) were observed. In Apatlaco River, the study sites Las Truchas and El Texcal achieved excellence in water quality with mean values of 96 and 84, respectively (**Figure 2**) and were significantly different from other study sites ( $p < 0.05$ ). The

other study sites showed mean WQI values from 57 to 60 with no statistical differences ( $p > 0.05$ ) and water quality fluctuated from polluted to mildly polluted for human consumption. Additionally, the WQI values decreased during the dry season ( $p < 0.05$ ), where August-September differed from February-March and June seasons; and December was statistically different from February-March and June seasons (**Figure 2**).

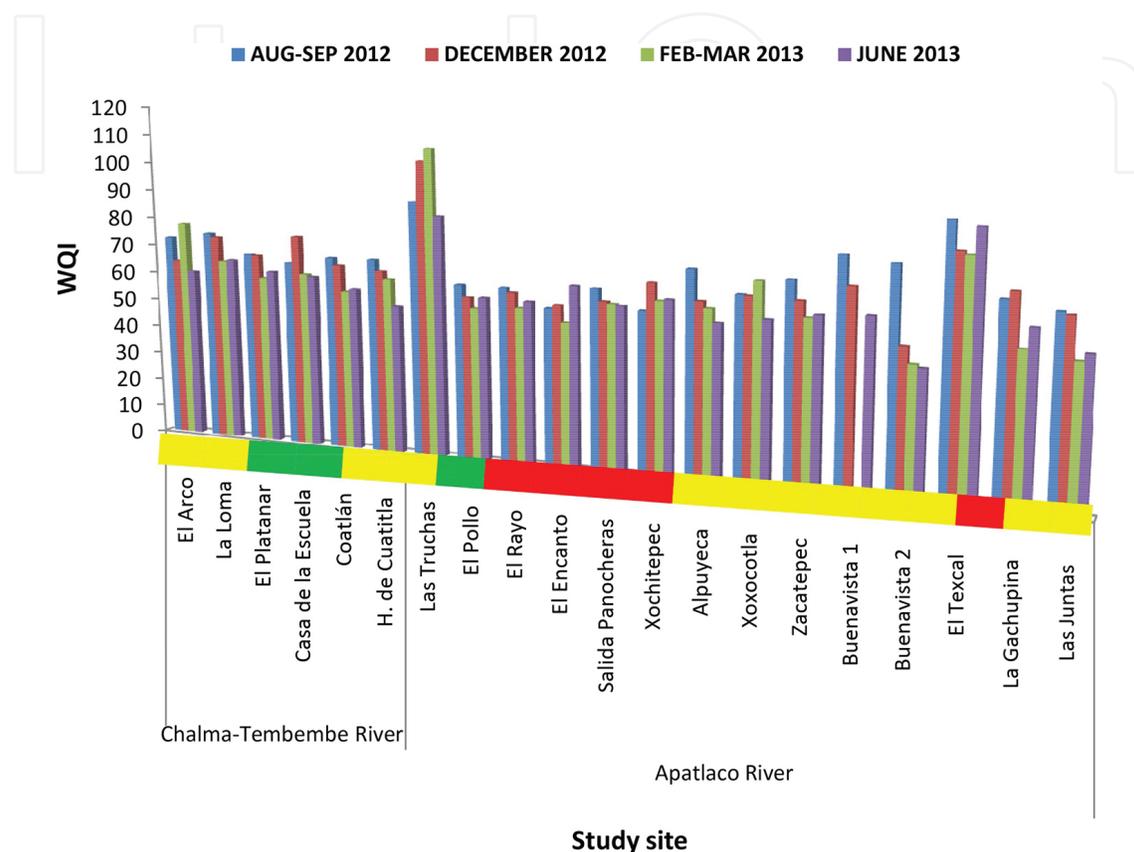


Figure 2. Water quality index.

### 3.2. Physicochemical quality and bioindication values

The factor analysis showed a total of 60.32% of explained variance for the first two axes. The parameters that showed a significant correlation, either positive or negative, in the first two axes of the factor analysis and which were considered as qualifying variables for this study,  $C_{i2}$ , were  $\text{NO}_3$ ,  $\text{NO}_2$ ,  $\text{NO}_3$ , TN, TP,  $\text{BOD}_{5r}$ , DO, DO saturation (%),  $\text{SO}_4^{2-}$ , color, alkalinity, chlorides and conductivity (**Table 1**). Using these variables to assess the physicochemical quality, we found that five study sites of the Chalma-Tembembe River obtained a  $Pcq$ , corresponding to classes 6–7 and one site in classes 7–8. The Apatlaco River sites were distributed across a broad spectrum of physicochemical quality classes: one site in classes 0–1, another in classes 2–3, four sites in classes 3–4, six sites in classes 4–5, one in classes 6–7 and finally one in classes 9–10. A total of 66 taxa were taxonomically determined, distributed in 5 phyla, 7 classes, 21 orders and 63 families: Oligochaeta, Hirudinea and Turbellaria were identified only to class level. “Las Truchas” was the site that reached the maximum taxa (35).

The bioindication values for each aquatic macroinvertebrates family (obtaining the fifth percentile of the abundance class distributions along the *Pcq* intervals) are shown in **Table 2**.

Variable	F1 (37.24%)	F2 (23.08%)
Nitrates (mg/L)	<b>0.592</b>	0.207
Nitrites (mg/L)	<b>0.567</b>	0.195
Ammonium (mg/L)	0.297	<b>0.738</b>
Total N (mg/L)	<b>0.606</b>	0.600
Total P (mg/L)	<b>0.818</b>	0.392
Sulfates (mg/L)	<b>0.750</b>	-0.525
Color (Pt-Co)	<b>0.605</b>	0.472
Alkalinity (mg/L)	<b>0.756</b>	-0.203
Chlorides (mg/L)	<b>0.906</b>	0.166
BOD (mg/L)	0.516	<b>0.599</b>
DO (mg/L)	-0.152	<b>-0.777</b>
Conductivity ( $\mu$ S/cm)	<b>0.775</b>	-0.522
% DO	0.024	<b>-0.868</b>
% Explained variance	37.24	23.08
% Accumulated variance	37.24	60.32

Numbers in bold represent significant variables from the factor analyses.

**Table 1.** Variables used for the calculation of *Pcq* and their correlations with the first two factors F1 and F2 (explained variance).

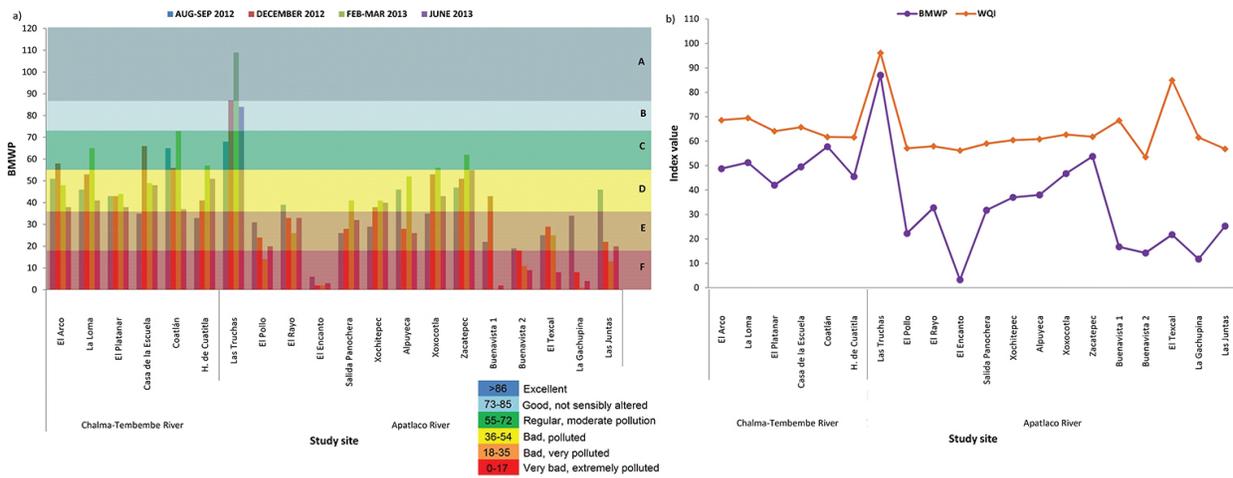
### 3.3. BMWP index and BMWP water quality classes

The BMWP values assessed with the calibrated bioindication values fluctuated from 2 to 109, but showed no statistical differences ( $p > 0.05$ ) between seasons. The study sites “Las Truchas” obtained the highest BMWP values ( $p < 0.05$ ) during the four study periods. It was selected as the reference site and their scores were averaged. The BMWP scores above this median value will correspond to the “Excellent” quality category, from which range values for all water quality classes were assigned (**Figure 3a**). Seasonal fluctuations showed that this site fluctuate from “Regular” in August, “Good” in June to “Excellent” in December and February. The study site “El Encanto” had the lowest BMWP index value during its four study periods, from 2 to 6 points and thus qualifying as “Very bad, extremely polluted”; this was followed by La Gachupina, Buenavista 1, Buenavista 2, El Texcal and El Pollo, with values between 2 and 43 points and thus reaching the “Bad, polluted” and “Bad, very polluted” categories. The remaining sites qualified as “Regular, medium pollution” (**Figure 3a**). A comparison of BMWP and WQI scores for each study site is shown in **Figure 3b**.

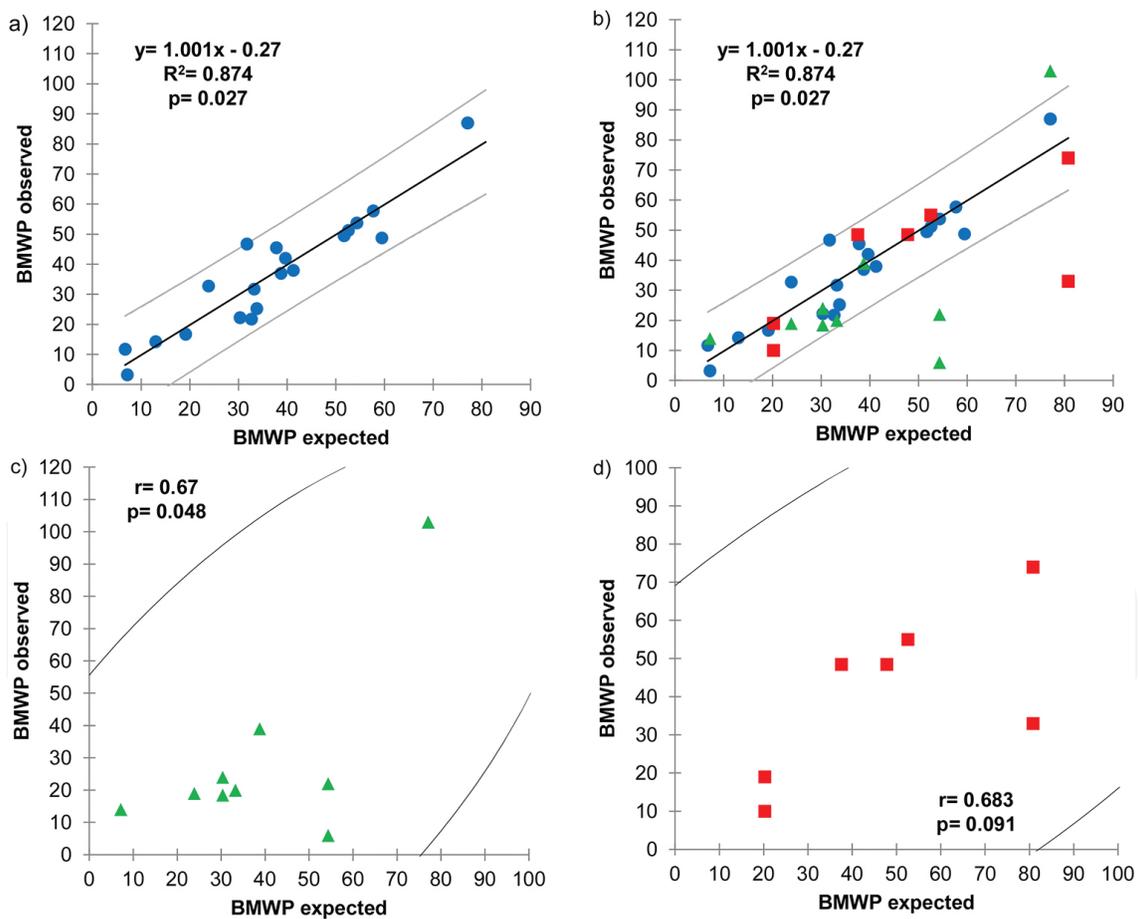
Taxon		Bioindication value
Cordulegastridae	Lepidostomatidae	10
Heptageniidae	Perlidae	
Caenidae	Scathophagidae	8
Gyrinidae		
Aeshnidae	Philopotamidae	7
Blaberidae	Pseudothelphusidae	
Cambaridae	Ptilodactylidae	
Helicopsychidae	Saldidae	
Hydrobiosidae	Scirtidae	
Naucoridae		
Corbiculidae	Hydrobiidae	5
Dixidae	Hydroptilidae	
Dryopidae	Leptophlebiidae	
Ephydriidae	Polycentropodidae	
Glossosomatidae	Pyralidae	
Gordiidae	Thiaridae	
Ancylidae	Leptohyphidae	4
Asellidae	Physidae	
Calopterygidae	Planorbidae	
Gomphidae	Psychodidae	
Hirudinea	Sphaeriidae	
Hyallolelidae	Stratiomyidae	
Hydropsychidae	Turbellaria	
Corydalidae	Elmidae	3
Belostomatidae	Libellulidae	2
Coenagrionidae	Tabanidae	
Hebridae	Tipulidae	
Staphylinidae		
Baetidae	Muscidae	1
Chironomidae	Nepidae	
Corixidae	Notonectidae	
Culicidae	Oligochaeta	
Dytiscidae	Simuliidae	
Hydrophilidae	Syrphidae	
Lestidae		

**Table 2.** Calibrated bioindication values for the aquatic invertebrates of the Apatlaco and Chalma-Tembembe Rivers.

The multiple linear regression equation of the quality test for predicting the BMWP scores is presented below (with  $r = 0.935$ ). The adjusted line of  $BMWP_{observed}$  vs  $BMWP_{expected}$  indices attained  $R^2$  values of 0.874 (**Figure 4a**); furthermore, all points related to the study sites were distributed within the confidence interval ( $\alpha = 0.05$ ). The Nash and Sutcliffe efficiency model was of 0.87, demonstrating a well-fitted model:



**Figure 3.** Scores of the BMWP. A: Excellent; B: Good, not sensible affected; C: Regular, moderate pollution; D: Bad, polluted; E: Bad, very polluted and F: Bad, extremely polluted. (a) Scores by period and study sites. (b) Scores of the BMWP and WQI.

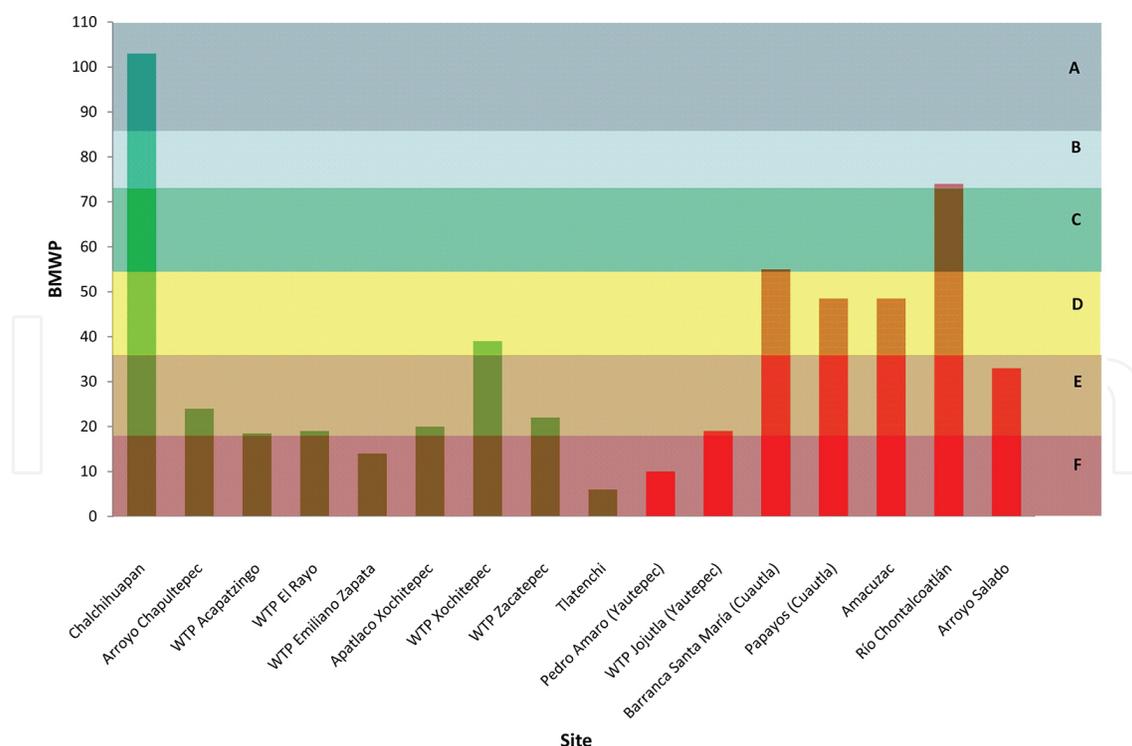


**Figure 4.** Validation model. (a) BMWP observed vs expected. (b) BMWP observed vs expected including validation data. (c) BMWP observed and expected values for the index validation. (d) BMWP observed and expected values for the regional extrapolation sites.

$$\text{BMWP}_{\text{expected}} = [138.854 - 7.69 \text{ Nitrates} + 123.51 \text{ Nitrites} - 0.18 \text{ Ammonia} + 2.17 \text{ Total N} - 33.11 \text{ Total P} + 0.80 \text{ Sulfates} - 1.18 \text{ Color} - 0.14 \text{ Alkalinity} + 0.28 \text{ Chlorides} - 1.85 \text{ BOD}_5 - 24.96 \text{ DO} - 0.18 \text{ Conductivity} + 2.19 \% \text{DO}]. \quad (3)$$

### 3.4. Index validation and regional extrapolation

The BMWP values for their validation and regional extrapolation (**Figure 5**) span the whole range of water quality classes: from “Very bad, extremely polluted” (Tlatenchi) to “Excellent” (Arriba Chalchihuapan). The observed and expected BMWP values for the nine index validation sites in the Apatlaco River and the seven regional in the neighboring river subbasins (Amacuzac, Cuautla and Yautepac) were calculated using the previously BMWP scores calibrated and the derived multiple linear regression model. Four sites of the BMWP values lay outside the confidence limits ( $\alpha = 0.05$ ) for the BMWP linear regression models (**Figure 4b**). Pearson’s correlations were calculated ( $\alpha = 0.05$ ) for the BMWP observed and expected values for the index validation and regional extrapolation sites (**Figures 4c and d**). As in the previous analysis, acceptable ( $p = 0.048$ ) values were calculated for the index validation, whereas the regional extrapolation showed weaker correlation ( $p = 0.091$ ). Three out sites of the nine independent sites were outliers for the calculated multiple linear regressions BMWP model (**Figures 4c and d**).



**Figure 5.** Scores of the BMWP for the validation and range extension study sites. A: Excellent; B: Good, not sensible affected; C: Regular, moderate pollution; D: Bad, polluted; E: Bad, very polluted and F: Bad, extremely polluted.

The index validation, adding nine independent sites, validated the regression model as a satisfactory indicator for river water quality in the Apatlaco River for the BMWP index ( $r = 0.67$ ,  $p = 0.048$ ). For the combined nine sites, there was a positive significant correlation for the BMWP index (**Figure 4a and b**).

## 4. Discussion

### 4.1. Water quality index

In Latin America, WQIs have been used to compare rivers in a country-wide dimension, the effect of a city discharge and also for a spatial and historical water quality assessment [13]. However, in Apatlaco and Chalma-Tembembe rivers, the WQI scores do not detect significant differences neither in the spatial nor in the temporal dimensions. In consequence, in this study, the WQI do not allow the detection of the most impaired portions of the rivers.

### 4.2. The BMWP index

Nowadays, the BMWP index is widely used in various countries of Europe (UK, Spain, Portugal, Turkey, Poland, among others) [8]. This index, also, has been used in some countries of Latin America [5, 14]. However, the procedure for calibrating the scores of the BMWP index has not been detailed. In the present study, we follow several steps for calibrating the BMWP values. The first step included the calculation of the minimum tolerance scores for each macroinvertebrate family from the study area. The *Pcq* index was used to obtain the bioindication values for each macroinvertebrate family. The factor analysis displayed the major explanatory variables, which are related with organic matter (N, P, BOD, DO, among others). These variables highlight the role of the different human activities that impinge on river water quality in the study area and can affect the abundance and distribution of aquatic macroinvertebrates families. Due to the particular conditions of each basin or biogeographical region, the relative importance of the physicochemical variables will vary, between study areas (basins), because of unique geological nature, land uses, as well as contamination and disturbance histories. Among changes in land use, those related to agricultural activities can affect several parameters of water quality ( $\text{NO}_3$ ,  $\text{NO}_2$ ,  $\text{NH}_3$ , TN, TP, among others), mainly because runoff contributes with the addition of agrochemicals into river, modifying water quality [33]. However, urban wastewater discharges can exert major impairments in the water quality due to their high load of organic matter that can deplete the DO and increase the  $\text{DBO}_5$  [34]. The data set from the studied rivers included a wide spectrum of conditions: from those with low impact (clean) to those very affected (highly polluted), giving rise to a wide range of the *Pcq* scores and water quality, data very valuable for the calibration scores of the BMWP index.

### 4.3. The BMWP index and BMWP water quality classes

While the WQI scores showed small differences among study sites, the BMWP index showed a wider variation, making evident that the latter has a higher level of sensitivity, as it was able

to register fine and important differences between study sites, which were not evidenced by the WQI (Figure 5). The sensitivity of the BMWP index is related with the procedure to assign the values for the water quality classes. For this step, the reference sites are indispensable. The BMWP index was able to detect the pristine condition of “Las Truchas,” the clean river reference site, located inside an undisturbed oak forest, showing the highest richness score of this study site. Furthermore, this site showed the presence of the family Perlidae, a bioindicator of excellent water quality. Lakew and Moog [30] stated that a reference site must meet both abiotic and biotic requirements; the same authors consider a reference site as the least impaired site characterized by selected physical, chemical and biological characteristics. Las Truchas site reached the higher *Pcq* and WQI scores, the less impairment of adjacent land use and also meet the biological requirements of the higher richness, as well as the higher scores of the BMWP index. In consequence, the site “Las Truchas” meets the requirements of a reference site. The BMWP scores obtained in this study also showed a wide seasonal fluctuation, particularly for Las Truchas site. The BMWP fluctuation provided for Las Truchas showed scores from 69 to 110, these data conformed the set of values for obtaining the median value that correspond to the “Excellent” quality category, from which range values for all water quality classes were assigned. The method used for the allocation of scores to each water quality class was proposed by Pond and McMurray [21]. These procedure displayed a suitable assignation of the water quality categories and consequently reducing the probability of commit errors (Type I and II) [35] for the categorization of the study sites.

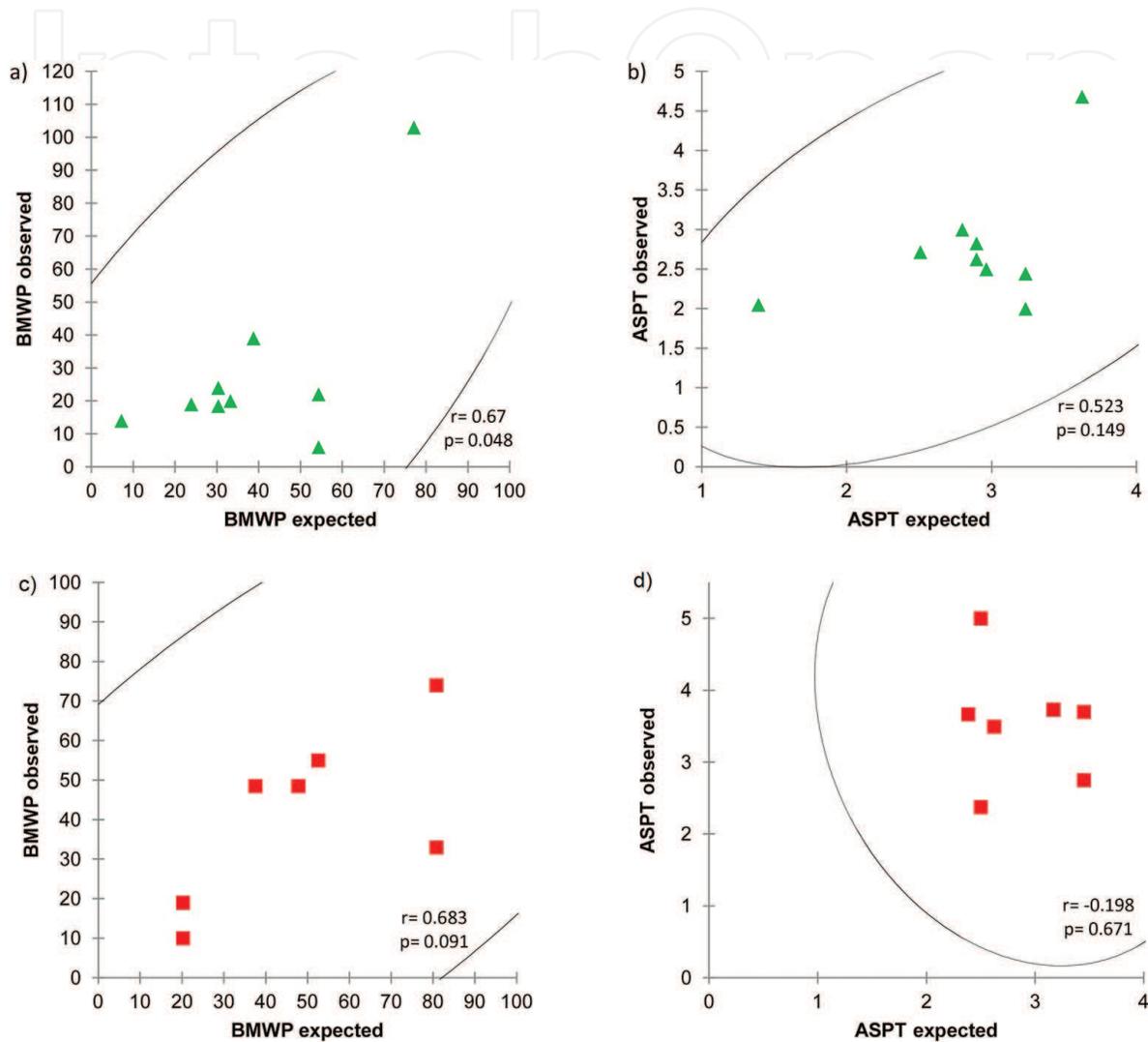
The bioindication values of the aquatic macroinvertebrate families do not always match completely from one country to another, which can be due to the variations in taxonomic tolerances of each basin and biogeographic region and to the method of assigning bioindication values, which in most cases is unknown, generating some uncertainty in the scores assigned to each family. However, there are families of aquatic macroinvertebrates characteristic of very healthy environments, as is the case of the Perlidae with a score of 10; or in extremely hostile conditions, the midges and lumbriculids with score values of 1 [14]. In the present study, the wide distribution of families in different intervals of *Pcq*, coupled with the abundance classes, allowed us to generate a bioindication value that is neither overestimated nor underestimated, as was evidenced by the degree of adjustment of the models described above.

Our results show that the BMWP index has a good discriminating capacity; nevertheless, doubtless, any index has to be adjusted, as demonstrated here, to the particular ecological conditions of each region in order to generate a powerful and representative biomonitoring tool.

#### 4.4. Index validation and regional extrapolation

A third step in our procedure included the statistical index validation process, which produced multiple linear regression models for the BMWP index with good results in general. The obtained  $R^2$  value in this study for the BMWP ( $R^2 = 0.874$ ) indicates that the model included a great proportion of the variance.

For the index validation, we included a procedure with the addition of nine independent sites, validating the regression model as a satisfactory indicator for river water quality in the Apatlaco River for the BMWP index ( $r = 0.67$ ,  $p = 0.048$ ). For the combined nine sites, there was a positive significant correlation. Three out sites of the nine independent sites were outliers for the calculated multiple linear regressions (**Figure 6a and b**).



**Figure 6.** Validation and range extension data correlation. (a) Validation BMWP observed vs expected. (b) Validation ASP observed vs expected. (c) Extension BMWP observed vs expected. (d) Extension ASP observed vs expected.

The fourth step in our procedure included the range extrapolation analysis, where the multiple linear regression model was extended to study sites from Amacuzac, Cuautla and Yautepec subbasins for BMWP index, in this case we obtained lower correlation and  $p$  values ( $r = 0.683$ ,  $p = 0.091$ ). The scores accurately reflect the human impacts on the aquatic macroinvertebrates assemblage, with scores from “Bad, polluted” to “Very Bad, Extremely polluted.” There were one outlier out of a total of seven sites for the BMWP, thus indicating that the BMWP regression model seemed to represent a more satisfactory geographical extension in this case (**Figure 6c and d**).

Therefore, the calibrated BMWP scores and the proposed water quality classes of this study can be used as a tool for the biomonitoring of water quality in the Apatlaco and Chalma-Tembembe rivers and even the subbasins Cuautla, Yautepec and Amacuzac. Furthermore, our ranges for water quality class showed also a good fit for the qualification of study sites and the spectrum of the land use conditions [36]. The studied rivers showed that a great portion of the rivers Apatlaco and Chalma-Tembembe (nine study sites), with agriculture as the main land use, is qualified as: bad polluted to regular, moderated polluted, while another great portion of the Apatlaco River mainly located in urban zones is qualified as bad, very polluted to very bad. The BMWP calibrated and the water quality assignments resulted to be suitable to assess water categories in the studied rivers. Our results make evident that Apatlaco River needs urgently a management and recovery plan.

## 5. Conclusions

The procedure followed in this study, included four steps, resulted to be efficient, reliable, repeatable and suitable for the development of a robust index to assess the water quality for rivers in the Neotropical region of México.

The tolerance values of the BMWP index developed in this study and their respective water quality classes can be applicable without modification to the adjacent river subbasins of the Apatlaco River, such as the subbasins Cuautla, Yautepec and Amacuzac.

The aquatic invertebrates and the BMWP index calibrated proved to be excellent indicators of water quality, being very sensitivity to differentiate the degree of pollution, different land uses and degrees of perturbation and thus, assign a water quality class that is also strongly related with the land use of their surrounding area.

These results make evident that the BMWP index calibrated is a suitable tool for the biomonitoring in the Neotropical region, where changes in land use have exerted strong impacts on aquatic resources and where the assessment of ecological conditions in freshwater ecosystems is urgently needed in a relatively simple, effective, reliable, fast and economical way. The procedure followed in this study to calibrate the BMWP is recommended and can be extended to other Neotropical rivers.

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