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Biological, Chemical, and Ecotoxicological Assessments Using Benthos Provide Different and Complementary Measures of Lake Ecological Status

Roberta Bettinetti [1,2,*], Silvia Zaupa [2], Diego Fontaneto [2] and Angela Boggero [2]

[1] Department of Human Sciences and Innovation for the Territory, University of Insubria, DiSUIT, Via Valleggio 11, 22100 Como, Italy

[2] National Research Council, Water Research Institute (CNR-IRSA), Corso Tonolli 50, 28922 Verbania, Italy; silvia.zaupa@irsa.cnr.it (S.Z.); diego.fontaneto@irsa.cnr.it (D.F.); angela.boggero@irsa.cnr.it (A.B.)

* Correspondence: roberta.bettinetti@uninsubria.it

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Abstract: The Water Framework Directive (WFD) aims to monitor continental water bodies in Europe to achieve good ecological status. Indexes based on biological quality elements (BQEs), ecotoxicological tests, and chemical characterizations are commonly used with standardized protocols to assess sediment quality and the associated risks. Here, we compare the results of quality assessment of benthic macroinvertebrates as BQEs as required by the WFD with the results of ecotoxicological tests and assessment of selected persistent organic pollutants (POPs) in sediments of the same eight water bodies in Italy. The aim was to verify if the assessment of quality through macroinvertebrates through POPs analyses and ecotoxicological tools can yield comparable, overlapping, or complementary results. We used the Benthic Quality Index (BQIES) for macroinvertebrates (two different applications), legacy POPs (dichloro-diphenyl-trichloroethane and metabolites (DDTs) and polychlorinated-biphenyls (PCBs)), and the emergence ratio (ER) and development rate (DR) for ecotoxicology. The results showed that the two indices within each approach were highly correlated, but between approaches, each result can lead to a completely different scenario, with rather different results of the assessment of ecosystem quality. The most striking result was that very few significant correlations existed between sediment quality assessment through macroinvertebrates and the risk assessment through analyses of micropollutants and ecotoxicological tests. The highest absolute r-value (0.81) was for the correlation between the $BQIES_{bottom}$ index and PCBs for micropollutants, whereas all other pairwise comparisons between indices had r-values ranging between 0.07 and 0.53. Our analysis calls for a caveat in the blind application of one or only a few indices of water/sediment quality, as the results of a single index may not represent the complexity of a freshwater ecosystem.

Keywords: biological quality element; chemical analysis; *Chironomus riparius*; DDTs; legacy contaminants; PCBs; POP; standard ecotoxicological tests; Water Framework Directive

1. Introduction

The Water Framework Directive (WFD—Directive 2000/60/EC) of the European Community has the aim of achieving a good qualitative ecological status for all types of water bodies in Europe. The importance of this piece of legislation is that water is considered as an exhaustible resource to be protected, emphasizing the role of aquatic ecology in management decisions. Several implementations of monitoring and assessment methods across Europe have been developed [1–4]. The evaluation of water quality within the WFD requires an integrated approach based on the use of specific biological metrics and on analyses of chemical compounds and hydro-morphological conditions [5].

Regarding biological indices, the composition and characteristics of the living communities of European waters are used as a primary focus to assess the quality of lakes and rivers as a whole. Several biological metrics using biological quality elements (BQEs) were set up by different European countries to address different risks [1,6–9], trying to harmonize classification systems across Europe. Regarding lakes, in Italy, the Benthic Quality Index (BQIES) was developed and implemented considering eutrophication as the main pressure [10,11], because of its importance in the national territory, given that nearly 41% of the Italian lakes are eutrophic [12]. The BQIES is based on detailed taxonomical identification on macroinvertebrates, mostly at the species level [13].

Regarding the chemical assessment of lake sediments within the WFD, this has to be performed with legally binding Environmental Quality Standards (EQSs) for selected chemical pollutants, known as priority substances, of EU-wide concern [14]. These include persistent organic pollutants (POPs) such as dichloro-diphenyl-trichloroethane and metabolites (DDTs) and polychlorinated-biphenyls (PCBs). A well-known chemical constrain can be the fact that not all potentially dangerous substances can be assessed, such as PCBs for which there are no EQSs yet. Moreover, chemical analyses of these substances are not always straightforward considering that contaminants are often present in the environment in mixtures [15].

An additional environmental test that is not yet included in the WFD involves the use of laboratory bioassays that involve a direct combination of chemistry (i.e., pollution) and biology (i.e., effect on living organisms). Laboratory ecotoxicology bioassays could evaluate the potential risk of toxic substances in the benthic environment, even without identifying the substances themselves [16], bypassing the impediment of the current implementation of the WFD, which does not include all potentially dangerous substances. These bioassays involve direct toxicity assays of environmental samples that are transferred to the laboratory and analyzed for toxicity against selected organisms [17]. Such samples contain the combination of the different pollutants present *in situ* and enable factors such as the bioavailability of contaminants and their interactions (synergistic and antagonistic) to be simultaneously studied (see [18] for more details).

The aim of the present paper was to compare the results of quality assessment of lakes obtained using different methods. Analyses of macroinvertebrates and analyses of organic pollutants according to the Italian standards of the WFD were compared with the results of security assessments obtained through ecotoxicological bioassays in the laboratory using sampled sediments according to standard guidelines with larvae of the chironomid midge *Chironomus riparius*. The purpose of the present study was to assess whether the three approaches (biological, chemical, ecotoxicological) provide similar and overlapping results, or whether each of them will provide different results for different facets of water quality and security.

2. Materials and Methods

2.1. Study Area

In all, eight natural and exploited lakes were selected as representative of the following aspects.

- Different weather and climate conditions, belonging to the Alpine (AL) and the Mediterranean (ME) Ecoregions covering the typical Italian climate conditions.
- Different types according to the Italian classification system (WFD requests) (Table 1 [19]): six of the eight lakes belong to the Alps and are in North-Western Italy (Piedmont region), the other two lakes belong to the insular Italy (Sardinia region) (Figure S1); the analyzed lakes cover five groups according to the national classification system in relation to their abiotic characteristics of altitude, surface area, mean depth, and catchment geo-lithology.
- Different origin, with five lakes being natural (mainly of glacial origin) and three representing the results of an artificial impoundment (reservoirs-Table S1). Also, different water uses are represented: the alpine reservoir is used as hydro-power generation plant, and the Mediterranean reservoirs as drinking water supplies.

- Different trophic level covering a gradient from ultra-oligotrophic to eutrophic conditions.

Thus, given their differences, any consistent pattern when comparing the lakes can provide supported information for reliable inference on the comparison between approaches in water quality and security assessment.

2.2. Sampling Methodology

We followed the Italian national protocol for monitoring macroinvertebrates for the application of BQIES [20]. In detail, water bodies were sampled in spring and autumn along transects connecting littoral, sub-littoral, and bottom areas of each lake. For micropollutants and ecotoxicological analyses, only the bottom area, where pollutants accumulate, was sampled and considered for subsequent analyses.

For biological analysis, an area of 675 cm^2 of sediment was sampled with a grab. The collected samples were sieved in the field through a 250-µm mesh net, fixed with 5% formalin, and bottled. Samples for micropollutants and ecotoxicological analyses were collected through the same grab. In this case, all samples were brought to the laboratory for subsequent analyses and immediately frozen for preservation.

2.3. Biological Assessment

In the lab, samples were sorted under a stereomicroscope, and specimens were separated into main groups, identified to the species level, when possible, and counted. The identification manuals were those in use at national and international level [21–23]. The Benthic Quality Index (BQIES) [24] was applied to each sampling station to evaluate the lake ecological status. Spatial (littoral, sub-littoral, and bottom) and temporal (spring and autumn) replicated samples were averaged to obtain a mean annual value representing the whole lake ecological status classification according to WFD requests. The BQIES is calculated based on the Shannon Diversity Index (SDI) corrected for the sensitivity values (indicator weights) attributed to each species; it can take values between 0, indicating low biological quality of the lake, and 1, indicating high quality near to the reference conditions [24]. Values of BQIES were averaged for the whole lake assessment ($BQIES_{all}$), or used as a separate value for the bottom samples only ($BQIES_{bottom}$) in different analyses, to allow a more direct comparison with micropollutants and ecotoxicological indices.

2.4. POPs Assessment

For POP determination, extraction was performed from 1 g of the freeze-dried and homogenized sediments in glass microfiber thimbles (19 mm internal diameter × 90 mm external length, Whatman, Maidstone, England) for 2 h with 60 mL of n-hexane (Carlo Erba, Cornaredo, Italy, pesticide analysis grade) using a modified Soxhlet apparatus (Velp Scientifica - ECO 6 thermoreactor).

Each organochlorine compound was recovered by several n-hexane washings and the extracts were concentrated down to approximately 2 mL and passed through a Florisil column (4 cm × 0.7 cm) with HCl-activated Cu powder (0.1 g) on the top. The Florisil column was eluted with 25 mL of n-hexane-dichloromethane (Carlo Erba, Cornaredo, Italy, pesticide analysis grade) using an 85:15 (v/v) mixture, and the eluate was concentrated to exactly 0.5 mL. The purified extracts were analyzed by gas-chromatography (GC Carlo Erba, Top 8000) coupled with a 63Ni electron capture detector - ECD 80 (Carlo Erba, Cornaredo, Italy), heated at 320 °C, using an on-column injection system (volume injected: 1 µL). The column was a Wall Coated Open Tubular Column fused silica CP-Sil-8 CB (50 m × 0.25 mm, film thickness: 0.25 µm, Varian, Harbor city, CA, USA). The temperature program used was as follows: from 60 °C to 180 °C at 20 °C·min^{-1}, followed by a run from 180 °C to 200 °C at 1.5 °C·min^{-1}; a further run was implemented from 200 °C to 270 °C at 3 °C·min^{-1}, followed by a final isothermal maintenance at 270 °C for 20 min, with helium as carrier gas (1 mL·min^{-1}) and nitrogen as auxiliary gas (30 mL·min^{-1}).

Quantification of DDT included 1,1-dichloro-2,2-bis (p-chlorophenyl) ethylene (pp'DDE), 1,1-dichloro-2,2-bis (p-chlorophenyl) ethane (pp'DDD), and 1,1,1-trichloro-2,2-bis (p-chlorophenyl) ethane (pp'DDT) as congeners of DDT and was performed using the external reference standards pp'DDE, pp'DDD, and pp'DDT (Pestanal, Sigma-Aldrich, Germany) in iso-octane (Carlo Erba, Italy, pesticide analysis grade). Quantification of 21 PCBs was performed on PCB 28 + 31, PCB 52, PCB 95, PCB 101, PCB 149 + 118, PCB 153, PCB 138, PCB 170, PCB 174, PCB 177; PCB 180; PCB 183, PCB 187, PCB 194, PCB 201, PCB 203, PCB 195, PCB 206, PCB 209.

The detection and quantification limits of the method varied from 0.05 to 0.1 $ng \cdot g^{-1}$ d.w. (dry weight) for DDTs and from 0.1 to 0.5 $ng \cdot g^{-1}$ d.w. for PCBs, depending on the organochlorine compound. The recovery efficiency was tested on a reference sediment previously used in an intercalibration exercise [25], and it was found to be within 80–100% for the DDTs and approximately 90% for each PCB congener. POPs data are presented as concentration per dry weight of the sediments ($ng \cdot g^{-1}$ d.w.). For the assessment, we used two summary metrics: total concentration of the analyzed DDT and total concentration of the analyzed PCB.

2.5. Ecotoxicological Assessment

The test organism *Chironomus riparius* Meigen 1804, of a strain maintained in the lab of the University of Insubria, was bred at 21 ± 1 °C under daily photoperiod in 40 L aquaria with control sediment (3 cm deep) as substrate. An 8 cm-deep column of dechlorinated tap water (hardness: 320 mg/L $CaCO_3$) was maintained over the sediment. The cultures were fed weekly with 1 g TetraMin fish food per tank, and the water was almost completely renewed every 2 months.

Bioassays were performed according to guideline 218 of the Organization for Economic Cooperation and Development (OECD) [18]. One day before the addition of first-instar larvae, 250 mL glass beakers were filled with collected sediments that were previously sieved (500 µm); 3.5 mL of a 4 $g \cdot L^{-1}$ water suspension of fish food, corresponding to 14 mg d.w. TetraMin, was put into each beaker. The contents of the beakers were allowed to settle in the dark at 21 ± 1 °C for 24 h. Five replicated beakers were prepared for each site, including the control (prepared following [18]). At the start of the test, 10 first-instar larvae chosen at random were transferred to each beaker. Tests were performed under a 16:8 h light:dark photoperiod for 10 days, with constant aeration. Every 3 days, the larvae were fed with 3.5 mL TetraMin suspension, and the evaporated water was added. Dissolved oxygen, pH and NH^{4+} were measured in all the beakers at t_0 (beginning of the test) and at the end of the test (t_{end}) to verify standardized test conditions (Table S1). Every day all emerged adults were counted. The exposure lasted at maximum 28 days.

The bioassay measured the total number of animals that emerged and their sex. More animals are expected to emerge in samples with lower concentration of pollutants. The sum of midges emerged per vessel was determined and divided by the number of larvae introduced (emergence ratio (ER)) and the mean time span between the introduction of larvae (day 0 of the test) and the emergence of the experimental cohort of midges (development rate (DR)) was calculated. ANOVA (ANalysis Of Variance) and Dunnet *post hoc* test [26] were used to assess differences between treatments and control.

2.6. Statistical Analyses

The main question was to assess whether and to what extent biological indices, micropollutant indices, and ecotoxicological indices correlated between each other. To address this question, we compared the biological indices for macroinvertebrates (BQIES) applied both to the entire lake and only to the bottom area with the results of organic micropollutants (EQSs) for both total DDT and total PCB, and from DR and ER bioassays on *Chironomus riparius*. BQIES values for each lake were obtained by averaging the values for the different depths and different sampling seasons in the case of whole lake assessment ($BQIES_{all}$); BQIES values were also obtained only for bottom areas for assessment at maximum depth ($BQIES_{bottom}$); for micropollutants and ecotoxicological assessment, we used the data

from the bottom samples. We performed the comparison using simple Spearman's rank correlation tests [26].

3. Results

3.1. Biological Assessment

Macroinvertebrates were represented by 12,483 individuals, divided in 136 taxa at the level of species, genus, or family depending on the phylum (Annelida, Arthropoda, Mollusca, and Plathyhelminthes) due to the presence of young-of-the-year organisms (see SM1 associated with [13]).

Oligochaetes and chironomids represented the most abundant groups, whose relative abundances together constituted from 46% to 100% of each macroinvertebrate lake sampling station (Figure 1).

The BQIES index provided a whole lake assessment of the ecological status with values varying between 0.52 in Lake Mergozzo and 0.22 in Lake Sirio (Table 1). Five lakes out of eight revealed values below the 0.4-threshold fixed by Italian regulations to separate good and moderate status. The BQIES applied only to the bottom samples showed values in the range between 0.004 (Lake Viverone) and 0.439 (Morasco reservoir); only the latter had good ecological status even in the deepest areas, whereas the remaining lakes showed moderate (Posada reservoir) or bad bottom ecological status (the other six lakes).

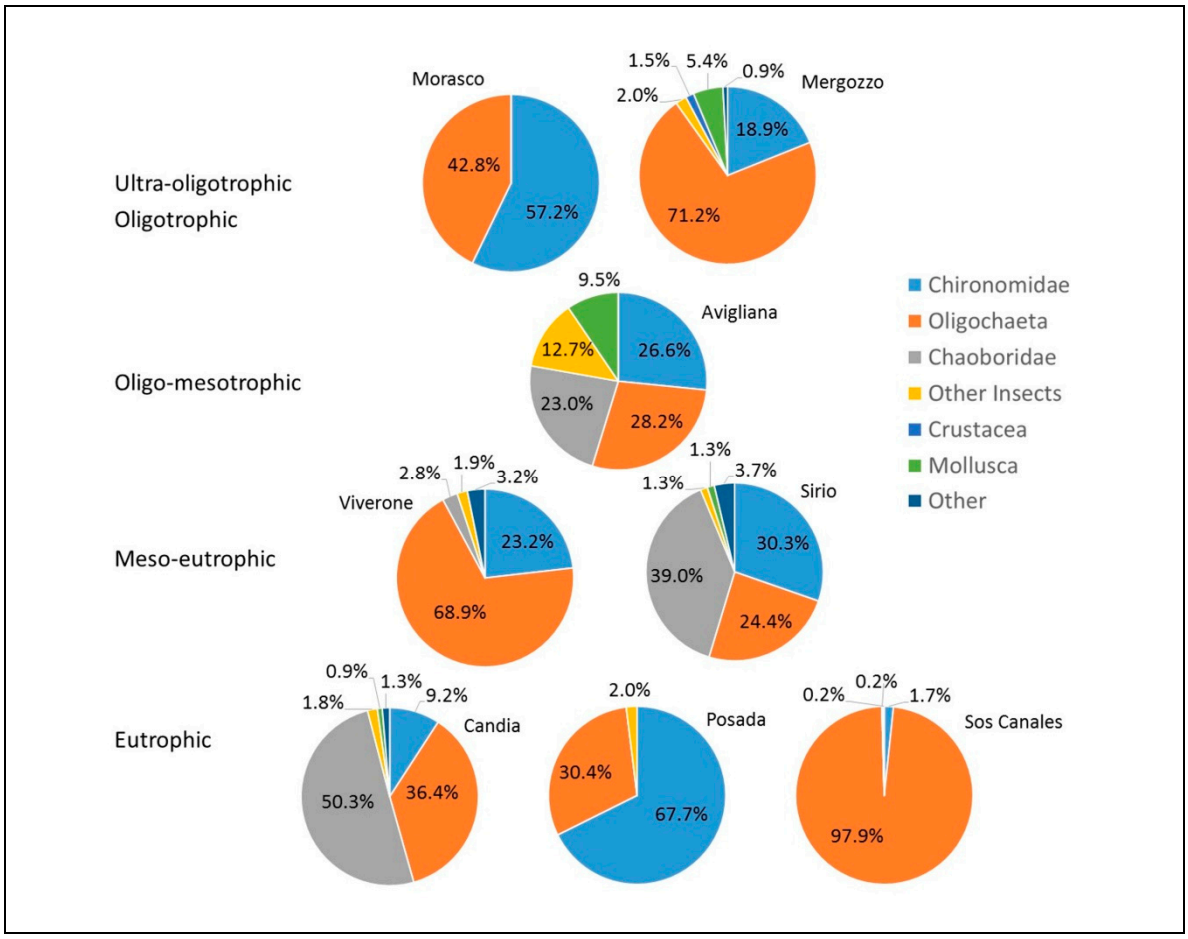


Figure 1. Annual mean macroinvertebrate assemblages (expressed as percentages) in each study lake. Trophic conditions of each lake are highlighted.

3.2. Micropollutants Assessment

Total DDT concentrations ranged between 0.7 ng·g^{-1} d.w. in Morasco reservoir and 62.8 ng·g^{-1} d.w. in Lake Sirio; total PCB concentrations ranged between 2.2 ng·g^{-1} d.w. in Morasco reservoir and 61.5 in Lake Sirio (Table 1).

Table 1. Summary of each metric assessing water quality in the eight analysed lakes. The Benthic Quality Index for whole-lake assessment ($BQIES_{all}$) and for bottom lake assessment ($BQIES_{bottom}$) are adimensional numbers; DDTs and PCBs are expressed as ng·g^{-1} d.w.; DR refers to the development ratio of chironomids; ER refers to the emergence ratio of chironomids. DDTs refers to dichloro-diphenyl-trichloroethane and metabolites, PCBs refers to polychlorinated-biphenyls; NA: not available.

Lake Name	$BQIES_{all}$	$BQIES_{bottom}$	DDTs	PCBs	DR	ER
Avigliana piccolo	0.26	0.01	7.27	19.4	0.051	55
Candia	0.42	0.21	14.73	29.8	NA	NA
Sirio	0.22	0.01	62.84	61.5	0.052	50
Viverone	0.26	0.00	25.44	38.1	0.051	70
Mergozzo	0.52	0.14	40.15	31.9	0.041	53
Morasco	0.46	0.44	0.75	2.2	0.053	51
Posada	0.37	0.37	10.36	2.7	0.049	70
Sos Canales	0.33	0.20	8.10	6.9	NA	NA

3.3. Ecotoxicological Assessment

Dissolved oxygen, pH andNH^{4+} in water remained quite constant during the expositions (Table S2). All the values were within the range of acceptability of the OECD method. Only in some cases, a decrease of pH was observed with values <6. The sediments of Lake Candia and Sos Canales reservoir were very acidic at the end of the exposure. Oxygen was always higher than 60%. Controls were within the OECD guidelines. Controls showed always absence of NH^{4+} both at the beginning and at the end of the test. Lakes Candia and Mergozzo, and Sos Canales and Posada reservoirs had a final NH^{4+} concentration of 7.74 mg·L^{-1}, while the other lakes had always NH^{4+} concentrations below 0.8 mg·L^{-1}.

Chironomids started to emerge after 17 days of exposure. Emergence in controls was within the validity criterion of OECD guidelines (ER > 70%); a minor ER in lake sediments (with the exception of Posada reservoir) occurred (Figure S2), and a significant difference (ANOVA with Dunnett *post hoc* test: $p < 0.05$) was found between all lakes and the controls. In the case of Lake Candia and Sos Canales reservoir no comparison was possible because chironomids died, probably due to the acidity of the medium. DR was significantly higher in Morasco reservoir, while Posada reservoir and Lake Mergozzo showed a minor DR indicating a specific toxicity for both lakes.

3.4. Comparison Between Assessments

The most striking result was that very few significant correlations existed between sediment quality assessment through macroinvertebrates, and risk assessment through micropollutants, and ecotoxicological tests (Figure 2). The highest absolute r-value (0.81) was between the $BQIES_{bottom}$ index and PCBs for micropollutants, whereas all other pairwise comparisons between indices had r-values ranging between 0.07 and 0.53.

Correlation values between indices within the same approach were high for micropollutants: DDTs and PCBs had a significant r-value of 0.88 (Figure 2). $BQIES_{all}$ (whole-lake assessment) and $BQIES_{bottom}$ (only bottom assessment) revealed a not significant but still high correlation (0.68; Figure 2). The correlation of DR and ER within ecotoxicological tests had an absolute r-value of 0.54 and was not significant (Figure 2).

Within each approach, our results confirm that using only one index, either DDTs or PCBs for micropollutants, and either $BQIES_{all}$ or $BQIES_{bottom}$ for macroinvertebrates, could be considered a

reliable choice, because the two indices are significantly correlated. The same cannot be stated for DR and ER from ecotoxicological bioassays, potentially also due to the failure in two out of eight cases.

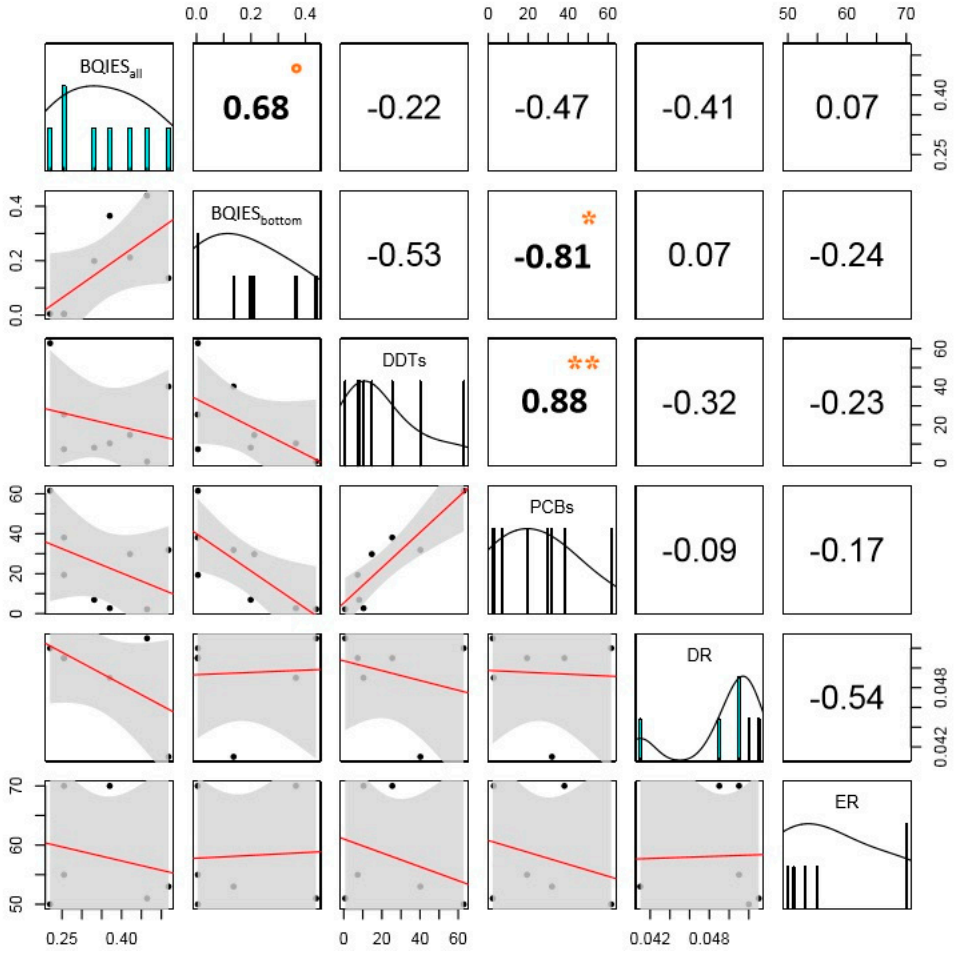


Figure 2. Pairwise correlations between the six analysed indices of water quality and security: BQIES$_{all}$ and BQIES$_{bottom}$ for ecological assessment, DDTs and PCBs for micropollutants, and DR and ER for ecotoxicological assessment. The cells in the diagonal show the histogram of distribution and the density plot for each index (see Table 1 for raw data); the cells below the diagonal show the scatterplots and the estimated linear correlation (colored line) with 95% confidence interval (grey areas) for each pairwise correlation; the cells above the diagonal report Spearman's r value of each pairwise correlation, with bold values indicating significant correlations and asterisks according to the *p*-value significance level (° for $p < 0.10$, * for $p < 0.05$, ** for $p < 0.01$). DR refers to the development ratio of chironomids; ER refers to the emergence ratio of chironomids. DDTs refers to dichloro-diphenyl-trichloroethane and metabolites, PCBs refers to polychlorinated-biphenyls 4.

4. Discussion

The main message of our study is that biological, chemical, and ecotoxicological approaches provide complementary and non-redundant information for a holistic assessment of ecological quality in lakes, when considering sediments as a model system. This seems a rather obvious result: different indices based on completely different elements should not provide the same answer to the question of quality and security assessment. Yet, what could be considered obvious may pass unnoticed to decision-makers and public administrators, given that sediment quality assessment is evaluated using only one or few indices simultaneously [7], and that water quality and risk assessment become pivotal in matching water demand and supply (domestic, industry, and agriculture supplies, but also for recreational, aesthetic, and nature conservation purposes).

Differences between biological, chemical, and ecotoxicological approaches to assess sediment quality and security are indeed expected [27]. For example, even if PCBs and DDTs are known to affect the test species (*Chironomus riparius*) we used for the ecotoxicological approach, the effects seen on the species may be also due to other contaminants acting in synergy. Nowadays, no reference values exist to predict the effects of contaminants in sediments on organisms [28].

An implementation of this aspect comes from the Environmental Quality Standards (EQS) approach for water, sediment, and biota, developed and implemented under the WFD. EQS are set for annual average concentrations (AA-EQS) and/or maximum admissible concentrations (MAC-EQS). In 2013, a new European Directive, 2013/39/EC, amended the Directives 2000/60/EC and 2008/105/EC regarding priority substances in the field of water policy, adding newly identified substances and revising the EQS of some existing substances. Following this new approach, a characterization with indexes of sediment quality seems still achievable.

Usually, a threshold effect concentration (TEC) has been used. Such TEC for total PCBs when no effects are observed is 40 $ng \cdot g^{-1}$ d.w.; in our case, this concentration was never exceeded with the exception of Lake Sirio, where the total PCBs concentration was 60.55 $ng \cdot g^{-1}$ d.w. (Table 1). In a study on Lake Maggiore contamination [29], the lowest observed effect concentration (LOEC) for total DDT in sediments on chironomids was 80.5 $ng \cdot g^{-1}$, and no samples taken during this research showed similar concentrations. It seems that PCBs (60.55 $ng \cdot g^{-1}$) had some significant ER effects only on Lake Sirio sediments. A relationship was reported between low ER and DR in Lake Como sediments when DDTs and PCBs were high (55.2 $ng \cdot g^{-1}$ d.w.; 194.5 $ng \cdot g^{-1}$ d.w., respectively) [15]. Ecotoxicological tests on sediments from Lake Como with lower contamination of these substances did not show comparable negative effects.

Regarding what has been said so far, it is useful to underline that contaminants can be present in mixtures in sediments, and two categories of them can be considered only an indication of pollution, since no information on all the present contaminants can be assessed. Even a mitigation effect due to cocktails of unknown contaminants cannot be excluded. This is the reason why the ecotoxicological approach can be an interesting and useful tool to characterize the chemical quality of a matrix, better than single analyses. However, negative effects can be therefore due to contaminants other than those analyzed by water conditions (pH and NH^{4+}). Previous work [30] identified pH effects on the survival of eggs and first instar larvae of *Chironomus riparius*, showing the important role of pH when it is lower than 4. observed A lower survival of chironomids was observed in a river where pH decreased to 4.5 [31]. Moreover, studying the effects of ammonium, it was found that 8.0 $mg \cdot L^{-1}$ could be a critical concentration for the survival of chironomids [32]. The negative effects found for the sediments of Sos Canales reservoir and Lake Candia, and in part even in Lake Mergozzo, could be therefore linked to a cocktail of different substances other than DDTs and PCBs.

The effects at the community level as seen with the application of the BQIES are even more complex and not so easily connected with the mere presence of organic pollutants. Chironomids and oligochaetes respond to a wide variety of stressors [33–35]. This is precisely the reason for the use of Biological Quality Elements (BQEs) in the Water Framework Directive (WFD) [1,7,9,36,37]. What was expected from our results was that assessing water quality in the deepest area only and across different depths may provide rather different inferences regarding the ecological quality of a water body as a whole (Table 1). The whole-lake index invariably revealed a scenario of equal, higher, or much higher quality than the index at the bottom of the lake (Table 1). Such discrepancy within the same method revealed that bottom areas, undergoing prolonged periods of anoxia or hypoxia because of eutrophication, frequently showed a highly reduced aquatic life. This is the reason why the BQIES was developed to allow for the separation of the ecological classification of individual sampling stations or individual water layers (epilimnion, hypolimnion, benthic area). In this way, the Environmental Agencies could direct mitigation actions in specific lake areas, notwithstanding that the whole-lake assessment reveals a non-critical situation. One could also speculate that the BQIES, developed to address trophic impacts [10,11], seemed not to be fully reliable to provide a comprehensive idea of the

ecological status of a lake if considered alone, and if stressors other than eutrophication are present. Contaminants or other pressures are always possible and prevalent as anthropogenic impact [35,36]; thus, additional indices should be considered to obtain a holistic and potentially reliable view of the ecological status of a lake, mainly when water is used as supply.

5. Conclusions

Overall, our results provide empirical evidence to support the idea that risk assessment cannot be easily and reliably performed using one or only a few indices to produce a single number to be used by politicians, managers, and stakeholders to base their decisions on environmental issues. We thus confirm the suggestion of the WFD that biological, chemical, and ecotoxicological approaches should be used together to provide a synergistic and holistic assessment of ecological quality and water security in lakes. Ecotoxicological and chemical indexes (such as EQS) should be improved in order to obtain more useful information when making a risk assessment evaluation, which should include ecological aspects of the lakes' environments.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4441/12/4/1140/s1, Figure S1. Regions and areas of concern (grey) for the present study, Figure S2. Emergence Rate (%) and Development Ratio of chironomids in the ecotoxicological approach, Table S1. Additional information on sampling localities. Latitude and longitude are in WGS84 system, Table S2. pH, dissolved oxygen (%), and NH^{4+} ($mg{\cdot}L^{-1}$) in water at the start (T_0) and end (T_{end}) of the ecotoxicological tests with lake sediments belonging to different lakes.

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