6. ECOTOXICITY TESTS AS *EFFECT-BASED* MONITORING TOOLS FOR AN IMPLEMENTATION OF WFD

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An ecotoxicological approach to assess the environmental quality of freshwater basins: a possible implementation of the Water Framework Directive?

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Abstract

Within the context of the WFD the need to identify new monitoring tools in support to the traditional chemical monitoring has emerged. Chemical characterization by itself does not provide specific biological information about potential hazards to organisms; in particular when facing cocktails of contaminants. Ecotoxicity tests can represent a useful tool in support to the chemical analysis. In the present work the value as effect-based tool of ecotoxicity tests for monitoring freshwater and sediments quality of the south-western basin of Lake Como was evaluated, assessing the potential risk of micropollutants in water and sediment samples. Results obtained from *D. magna* toxicity tests showed a temporal variation of toxic response in relation to the variability of pollutant loads characteristics of urban rivers. Sediments ecotoxicity results showed the spatial variability of the sediments micropollutants contamination confirmed by chemical analysis of some priority pollutants (DDT and PCB).
Present results confirmed the usefulness of ecotoxicity tests as effect-based monitoring tools able to identify potential risk to freshwater ecosystems associated to micropollutants in water and sediments.

Keywords Sediment ecotoxicity tests; Freshwater ecotoxicity tests; Biomonitoring; Effect based tools; Micropollutants

1. Introduction

The Water Framework Directive (WFD 2000/60/EC) is the European Union directive to protect the water environment which commits EU member states to achieve "a good qualitative and quantitative status" of all water bodies by 2015, prescribing steps to reach the common goal rather than adopting a more traditional limit value approach.

Therefore, at present a lot of work is still in the direction of the monitoring of aquatic environment since the achievement of the ecological status requested by the law implies a complete outline of the present situation as a starting point for future improvement measures. Screening the ecosystem quality situations through effective monitoring programs is requested, possibly in a cost-efficient way. Compared to past times, a new era in environmental risk assessment is growing with the aim of an integrated and holistic approach which takes into consideration the ecological effects.

Even if bioassays are not explicitly prescribed by the WFD to assess the quality of the aquatic environment, a potential for the incorporation of biological effect measures seems possible and recommendable. For the conservation of the environment is certainly important to detect effects caused by chemical substances at an early stage before damaging the resident community; therefore ecotoxicological evaluations can be thought as a valid tool to indicate hazards of toxicants, which can be present as complex mixture in the environment, in unknown or simply not analyzed components. Within the WFD chemical analyses are indeed prescribed for listed priority substances (33, recently implemented by other 12 (Annex II 2013/39/EC) in water in order to ensure a high level of protection against risks. The WFD requires member states to include this representative group of substances in monitoring programmes, but as far as we know only 75% of the priority substances can be reliably monitored in water with acceptable uncertainty when applying existing standardised methods (Ademollo *et al.,* 2012). At present, a lack of information on the concentrations of the priority pollutants requested by the Directive exists, with some sporadic exceptions (Loos *et al*., 2009; Slobodnik *et al.*, 2012; Roots and Roose 2013).

Alternative methods are under consideration for the goals of the WFD. At an international level the implementation of bioassays is still under development and inter-calibration processes have to be carried out. Ecotoxicity tests are one of the main tools in ecotoxicological assessment which can be used for setting standards for the protection of the environment (Wagner and Løkke, 1999); in the case of the freshwater environment the main standard organism for the water column evaluation is the Crustacean *Daphnia magna* In water, among the possible contaminants, organic micropollutants are generally widespread and present at concentrations that rarely cause acute toxic effects posing, in any case, a threat on long-term periods. Therefore there exists a shift from acute to chronic effects; although a standard guideline for *Daphnia magna* long-term effects exists (OECD 2008), in order to shorten times and even costs, a possible way for monitoring is to carry out more practical and feasible acute toxicity tests on concentrated water samples. Water can be extracted through SPE technique, using LiChrolut cartridges (Daignault *et al*., 1988) and the concentrated extract fraction can be ecotoxicologically evaluated at concentrations higher than those in the original samples through short-time tests.

The contamination of aquatic ecosystems concerns also sediments; hydrophobic contaminants in particular can be several orders of magnitude higher than in the overlying water, and can be available for biota, thus causing a risk to benthic organisms. While the essential role of sediments of water ecosystems is widely recognized, sediment quality role in assessing ecological quality under the WFD has been introduced only recently (2013/39/EC). At present, weight-of-evidence approaches, such as the sediment quality triad (Chapman, 2005; Bettinetti *et al*., 2012), are widely accepted to assess the ecological risk of sediment-bound contaminants, but the setting of environmental quality standards as done for surface water is still at the beginning. Over the years research has demonstrated that sediments exceeding sediment quality guidelines do not always result in toxic effects on benthic organisms, and sometimes even the opposite has been observed, probably for the different bioavailability of contaminants. In addition to chemical analysis and in situ benthic community assessment, ecotoxicity testing with single species could be therefore particularly useful.

In the present work we report the results obtained during a master plan to characterize the ecological quality of the Southern-West branch of the Como basin, Lake Como, Northern Italy, one of the deepest lake in Europe. We propose the application of an ecotoxicological approach as a "switch on/off strategy to evaluate the contamination", taking into account that this method allows to select samples where the effects are ascribable to a cocktail of contaminants. The use of the presented approach in other freshwater ecosystem is discussed for a more reliable water quality evaluation within the Water Framework Directive.

2. Research Methodology

2.1 Study area

Lake Como is the deepest (maximum depth = 425 m) southern-alpine Italian lake. It has three branches of approximately equal length; the south-westward basin is closed in its south part by the city of Como (around 85000 inhabitants), in an area particularly impacted by industrial, agricultural and urban activities. This basin is supplied by the water of two main tributary: River Breggia and River Cosia. River Cosia grows out of Monte Bollettone (IT) in its final part River Cosia passes through the city being canalized and mainly underground; in its last portion it receives waters of the sewage treatment plant Comodepur SpA. River Breggia grows out of Monte Generoso (IT) after 2.5 km the river enters in Switzerland where receives waters of the sewage treatment plant. In the last portion of its trait river Breggia comes back in Italy and flows into the lake. Both rivers are typically urban with a high anthropogenic impact.

2.2 Water sampling, solid phase extraction and concentration

A total of 7 water samples were collected from both two main inlets (surface waters) using a bucket lowered from bridges and from the lake at a depth of 45 m in correspondence of the water supply for potable uses of the city (Figure 1) using a Niskin bottle. River Cosia was sampled in its uncovered stretch before the contribution

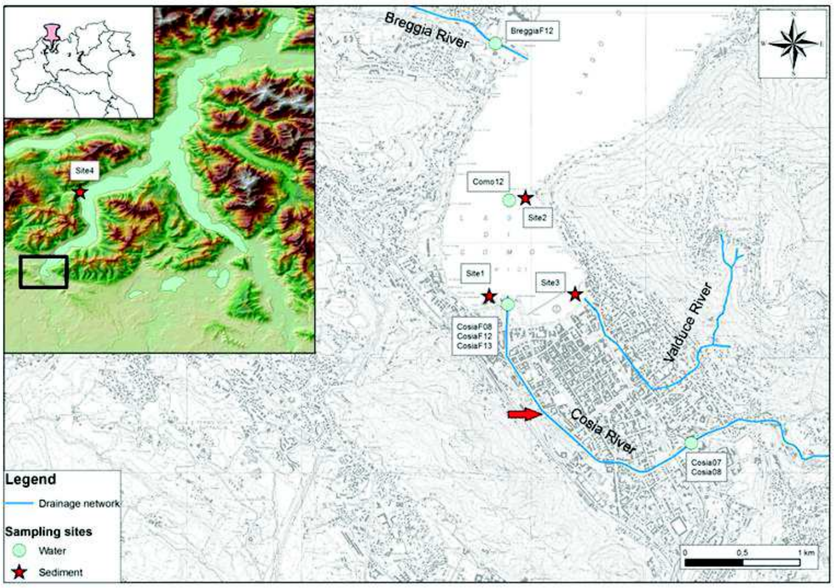

Figure 1 Map of Lake Como and map of the samples site. The light blue bullet show different water sample points with corresponding year. Red stars show different sediment sample site. The red arrow indicates the entrance of the River Cosia water in the wastewater treatment plant.

of the sewage treatment plant in 2007 (Cosia 07) and 2008 (Cosia 08) and at its estuary in 2008 (CosiaF 08), 2012 (CosiaF 12) and in 2013 (CosiaF13). River Breggia was sampled at its estuary in 2012 (Breggia 12) and Lake Como was sampled in 2012 (Lake 12). Water samples were filtered on 0.45 µm cellulose acetate filters and passed through LiChrolut EN_cartridges (500 mg, Merck, Darmstadt, Germany) for organic micropollutant extraction.

The cartridges were previously washed with 10 ml of methanol, 10 ml of acetone and 100 ml of MilliQ water and the water sample concentration was performed using a vacuum pump at a flow rate of 8ml min^{-1}. Samples of 4 l were passed through the cartridges, which were eluted with 10 ml of acetone. The solvent was evaporated to a final volume of 20 µl and the concentrated extract was added to 100 ml of *Daphnia magna* medium to obtain a concentration factor (CF) between 2.5 and 80X.

2.3 Acute tests with Daphnia magna

D. magna acute tests were performed according to the OECD guideline 202 (2004); the validity criteria were fulfilled, since dissolved oxygen concentration at the end of the test was always above 2 mg l-1 and no daphnids were immobilized in the controls was not greater than 10%.

The test medium was the same commercial mineral water (pH 7.8, hardness 240 mg $CaCO_3$ l^{-1}) used as culture medium.

Parthenogenetic daphnids of around 24 hours were exposed to different concentrated water extracts dissolved in water in four test replicates of 25 ml each. For each replicate, five individuals were used and daphnid immobilization was observed after 48 h. Control tests, one with the water medium only and one with the medium added with acetone (0.25 ml l^{-1}), were also performed.

A blank test was carried out passing 5 ml of acetone through a cartridge and concentrating the extract to 20 µl. The concentrated eluate was added to 100 ml of water medium as in the case of water extracts. No toxic effects were observed for this blank.

2.4 Sediment sampling

Sediment samples were collected with a steel grab in the southern part of the south-westward basin of Lake Como: three (Sites 1, 2 and 3, Figure 1) in the Como city basin and one (Site 4) in the northern part of the same basin outside the influence of the city at a depth of 10 m (Figure 1). Site 1 was sampled next to the mouth of river Cosia at the depth of 15 meters, sediments of Site 2 were collected in correspondence of the water intake for potable uses at the depth of 40 m, Site 3 in the harbour of the city at 10 m depth. All these samples were collected with a grab in summer 2013. Sediments were stored at 4 ± 1°C in the dark and sieved to 250 µm prior the test which started within three weeks from collection.

2.5 Sediment analyses

Sediments were characterized in terms of some priority pollutants. Polychlorinated biphenyls (28, 52, 101, 118, 138, 153, 180) and dichlorodiphenyltrichloroethane and its derivates (p,p'-DDE, o,p'-DDE, p,p'-DDD, o,p'-DDD,p,p'-DDT, o,p'-DDT). For POP determination, extraction of freeze-dried homogenized sediments (1 g) was performed in glass microfibre thimbles (19 mm internal diameter x 90 mm external length, Whatman, England) for 2 h with 60 mL of n-hexane (Carlo Erba, Italy, pesticide analysis grade) using a modified Soxhlet apparatus (Velp Scientifica – ECO 6 thermoreactor). Chlorinated compounds were recovered by several n-hexane washings, and extracts were concentrated down to ca. 2 mL and passed through a Florisil column (4 cm · 0.7 cm) with Cu powder (0.1 g) on the top. Cu powder was previously activated by HCl (18%, Carlo Erba, Italy) and washed with water, acetone and n-hexane. The Florisil column was eluted with 25 mL of n-hexane-dichloromethane (Carlo Erba, Italy, pesticide analysis grade) using a 85:15 (v/v) mixture, and the eluate was concentrated to exactly 0.5 mL. The purified extracts were analysed by gas- hromatography (GC Carlo Erba, Top 8000) coupled with a 63Ni electron capture detector (Carlo Erba ECD 80) using an on-column injection system (volume injected: 1 µL). The column was a WCOT fused silica CP-Sil-8 CB (50 m x 0.25 mm I.D., film thickness 0.25 µm, Varian, USA). The temperature program used was 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 the carrier gas (1 mL min^{-1}) and nitrogen as the auxiliary gas (30 mL min^{-1}). Sample quantification was performed using external reference standards of pp'DDT, pp'DDE and pp'DDD (Pestanal, Sigma–Aldrich, Germany) in iso-octane (Carlo Erba, Italy, pesticide analysis grade). AROCLOR 1260 (Alltech, IL, USA) with the addition of PCB 28, 52 and 118 was used for PCB quantification. The sum of the analysed PCBs corresponds to about 97% by weight of the reference standard. The detection limit for each organochlorine compound was 0.1 ng g^{-1} d.w. The recovery efficiency was tested on a reference sediment previously used in an inter-calibration exercise (CIPAIS, 2003), and was within 80–100% for the three DDT homologues and HCB and around 90% for each PCB congener. Every batch of 5 core slices was analysed in association with the reference sediment. POP data are presented as concentrations (expressed as ng) on dry weight of sediments.

2.6 Chironomus riparius culture and bioassay

The test organism *Chironomus riparius*, 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 almost completely renewed at that time. The first larval instars for use in experiment were obtained by transferring egg ropes from the culture to glass vassels containing sring eater. The time required to hatch the first instar larvae was about 3 days at 21 ± 1 °C. The animals were then transferred randomly to each test beaker with a glass pipette putting them below the water surface to avoid tapping them in the surface film.

Tests were performed according to the OECD 218 guideline (2004). One day before the addition of first-instar larvae, 250-ml glass beakers were filled with 70 g of wet sediment (water content about 50%) and 200 ml of dechlorinated water; 3.5 ml of a 4 g/L water suspension of fish food, corresponding to 14 mg d.w. Tetramin, was put in each beaker.

The contents of the beakers, covered with a plastic Petri dish with a hole for aeration, were allowed to settle in the dark 21 ± 1 °C for 24 h. Six replicated beakers were prepared for each site sediment, including the control. At the start of the test, the overlying water of each beaker was gently aerated for 2 h and then 10 first-instar larvae, chosen at random, were transferred to each beaker. Tests were performed under 16 : 8-h light: dark photoperiod for 28 days. Every 3 days the animals were fed with 3.5 ml Tetramin suspension and the water lost to evaporation was added. Temperature, pH, and dissolved oxygen were measured in all the beakers before and at the end of the tests, when ammonium was also recorded (ammoniac kit test). After firs 10 days three replicates for each site were analysed: total survival was noted and the individual larval wet weight of each larva recorded. Animals were placed in clean tap water for 6 h to purge the gut (Brooke *et al*., 1996), then they were dried on a filter paper immediately after removal. Their wet weights were recorded using a sensitive, nondestructive technique with an analytical balance (Blockwell *et al*.,1996; Watts and Pascoe, 1996).

Final larval weights were considered because they represent an integration of physiological responses which are known to be affected by the environment and by pollutant stress (Kosalwat and Knight, 1987). The remaining replicas were performed under the conditions described above till end of the test. The total number of fully emerged male and female midges was recorded daily. The maximum test duration was 28 days and if midges emerged earlier, the test was ended 5 days after the last adult emerged in the control.

2.7 Data analysis

The Effect Concentration at 48 hours *Daphnia magna* test (48 h EC50) was estimated by probit analysis using the EPA Probit analysis program. Concentration factors were used as test concentrations.

One-way ANOVA was performed (α level = 0.05) followed by the post hoc Dunnett's test to evaluate differences between the responses of each endpoint at the sites and the control using STATISTICA 8 package (StatSoft). Data were log-transformed or transformed by square root when the assumptions of ANOVA (homogeneity of variances and independence of variance from the mean) were not attained (Sokal and Rohlf, 1981).

3. Results and discussion

3.1 Contamination history of the south-western Como branch

Aquatic ecosystems are often impacted by pollution, originating from municipal and industrial wastewater effluents, airborne deposition as well as runoff from urban and agricultural areas.

Lake Como and its south-western branch in particular where the major city is located and no river outlet is present, have been interested for several decades by water quality problems. The lake has experienced intense eutrophication during the 1960s, which has only been partially solved in more recent years. At present its trophic status is meso-eutrophic on the whole (Salmaso *et al*., 2006) but particularly high phosphorus concentrations are still present in the Como branch, which is a "closed" basin. Moreover the lake and in particular its west-southern branch has a long history of chemical contamination due to the several industrial and agricultural activities within the basin. Some previous studies mainly focused on the evaluation of the water lake quality as an alternative supply for drinking uses, have shown a mutagenic activity in the '80-'90 (Galassi *et al*., 1989; Guzzella and Sora, 1998; Guzzella *et al*., 1999). Contaminants such as hexachlorebenzene, benzofenone, DDT, PCB, α and γ–HCH, were quantified in 1997 (Guzzella *et al*., 2000) in two sites of the Como basin and within these studies, the River Cosia was identified as an important source of mutagenic contamination, being therefore the main contamination source of mutagens to the lake (Guzzella *et al.,* 1999). Information on contamination of sediments go back to the '90s. The analyses of the main organic micropollutants in sediments collected in 1991 nearby the site where water is collected for drinking uses (Figure 1, Site 2), revealed a contamination by PCB higher than other large lakes in northern Italy in that period (Lake Maggiore and Lake Garda) (Provini *et al*., 1995); the same has been confirmed by a further study (Chiaudani and Premazzi, 1993) on PCB; as concern DDT, concentrations in the Como basin were particularly high during the period of its use in the '70s, then were around 15 ng g^{-1} d.w. (Galassi *et al*., 1993) in 1991 and 54 ng g^{-1} d.w. in 2009 (Bettinetti R., unpublished data).

Heavy metals, analytically determined in 1992 in sediments, confirmed a considerable contamination of the Como Basin, particularly of copper and zinc (Chiaudani and Premazzi, 1993). Recent research on mercury pollution, by analyzing sediments showed a high mercury enrichment (1 mg kg^{-1} d.w. in recent years above background values (0.1 mg kg^{-1} d.w.) (Ponti *et al*., 2012).

3.2 Ecotoxicity of water samples

The current quality status of the lake ecosystem from a ecotoxicological point of view is undoubtedly lacking and dated, but allows the recognition of the Como basin as a critical area. As concern the water quality evaluation, the exposure to the whole water samples, never affected the mobility of *D. magna* excluding direct acute toxicity. Severe effects can be expected on a long-term exposure (Fent *et al*., 2006) even due to interactions of micropollutants (Neff, 1984; Luckenbach and Epel, 2005; Pomati *et al*., 2008): pre-concentration by SPE allowed the simulation of environmental increasing exposure to a mixture of organic micropollutants to be tested. EC_{50} *Daphnia magna* acute test reported in Figure 2 points out similar responses of both rivers samples. A lower value was estimated for Lake Como water but the variability of the EC_{50} value covers statistical differences. River Cosia increased its quality through time: the most recent value of 39.9X, even considering its variability range is significantly higher than the effect concentration measured since 2007 (Figure 2). A temporal (between CosiaF sampling sites) and a spatial (among all the sampling sites) variability of toxic responses was therefore observed (Figure 2). As already observed in other studies (Musolff *et al*., 2009), time and space play important roles in the pattern of contamination: sampling time in particular may be responsible for the variability of the responses due to rainfalls or dry periods and different industrial activity. Unfortunately the flow rate of Cosia river is not routinely monitored for logistic problems, since it flows covered and the access is difficult; therefore a 15-day average rainfall before sampling was considered for its estimation. In 2012 and 2013 a relationship between the average rainfall (Figure 3) and the ecotoxicity of Cosia river water could be found, indicating a "dilution effect" of rain water. However no evident relationship was observed in 2007 and 2008 since the EC_{50s} were similar, independently from the rainfalls (Figure 3). Considering that the major impact of this river is represented by industrial activities and wastewater treatment plant effluent (Figure 1) as for other urban rivers (Daughton and Ternes,

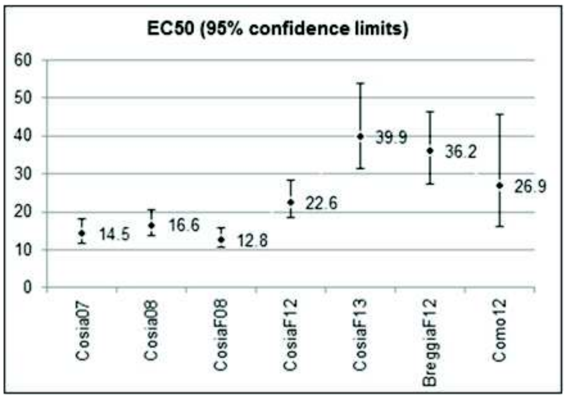

Figure 2 *Daphnia magna* acute EC_{50} values (95% confidence limits) at each sample site. ECs are referred to the concentration factors.

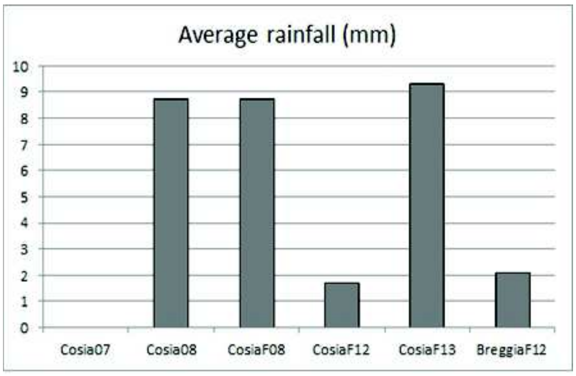

Figure 3 Average rainfall (mm) observed in the 15 days preceding the sampling.

1999) the lower production by industries due to the global financial crisis of the recent years (Figure 4) may be responsible of the water quality increase. In the case of the Breggia river the water quality seems better than the one of Cosia river, despite the low flow rate of the sampling date (0.99 m^3/sec -www.hydrodaten.admin. ch/de). Using the hazard characterisation for surface waters based on concentration factors (CF) causing toxicity to *Daphnia magna* (Galassi *et al*., 2004), all samples collected in 2012 should be considered with a low hazard for aquatic life, while a medium hazard for River Cosia was observed in 2007 and 2008. As the proposed procedure help in the discrimination between water samples according to the toxicity of the extractable toxic substances, it seems reasonable to use threshold CFs on *D. magna* for screening purposes to assess water quality as an effect based tool.

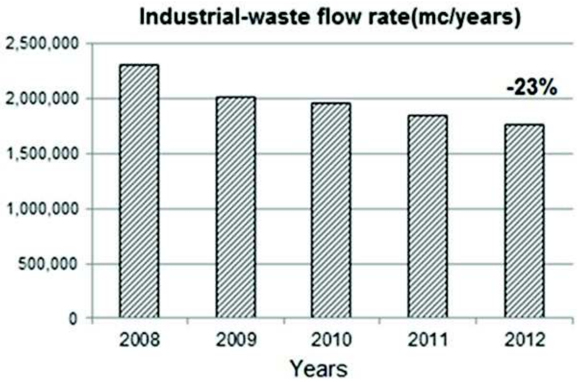

Figure 4 Average annual industrial waste flow rate. (Data from Comodepur S.p.a.)

3.3 Ecotoxicity of sediments

The WFD directs Member States to develop sediment quality standards with the clear scope for consideration of sediment quality as an integral part of water basin management. The recent Environmental Quality Standards Directive 2013/39/EU which amends the Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy, marks an important step in the possible use of sediments and biota as matrices for chemical-status assessment; the Directive indicates EQS for some priority contaminants in biota but not yet for sediments. As for water, the ecotoxicological evaluation of the quality of sediments seems therefore particularly appropriate.

The first instar larvae of *Chironomus riparius* used in this study showed acceptable e constant sensitivity to $K_2Cr_2O_7$ toxicity: the 96h LC_{50} to larvae was 14.1 mg l^{-1} (12.7-15.5), being lower than the sensitivity range indicated by RIZA (1996) for second instar larvae. The LOI of the sediments, reported in Table 1, are quite similar, comprised the reconstructed control (12.3 %). From the beginning to the end of the test, pH differences among beakers were minimal (around 0.2 pH units) and the oxygen saturation and ammonia values were constant and within the ranges reported by the guideline OECD 208 (2008).

	Site 1	Site 2	Site 3	Site 4
LOI %	13.7	12.1	14	14.1
pp'DDE	1.2	3.7	16.5	2.5
op'DDD	1.8	2.4	14.1	4.8
pp'DDD	0.6	1	7.4	1.2
op'DDT	0.1	0.3	1.4	0.4
pp'DDT	0.2	0.6	1.8	0.5
PCB tot	20.9	30.8	194.5	56.3

Table 1 Concentration (ng/g d.w.) of PCB and DDT in sediment samples. Organic matter content expressed as % of LOI.

The 10-day exposure test showed no significant differences in survival chironomid between sites and CNT with the exception of Site 3 (Dunnet post-hoc test $p< 0.05$), which was significantly lower than control (Figure 5). This difference was even more evident ($p<0.001$) in terms of growth assessed as dry weight of each larva; an impact was also observed on organisms grown in the sediment of Site 1, which were significantly smaller than those of CNT. The analysis of weights allowed to point out spatial differences in the contamination in the Como basin.

The second test lasted 25 days, five days after the last adult emerged in CNT as recommended by OECD 218 (2004). The significantly lowest emergence ratio ER was observed at Site 3 ($p<0.001$) confirming the results of the ten day exposure; even if ER of the other sites were lower than CNT, non significant differences existed. No valuable informations were obtained by sex determination of adults, showing no

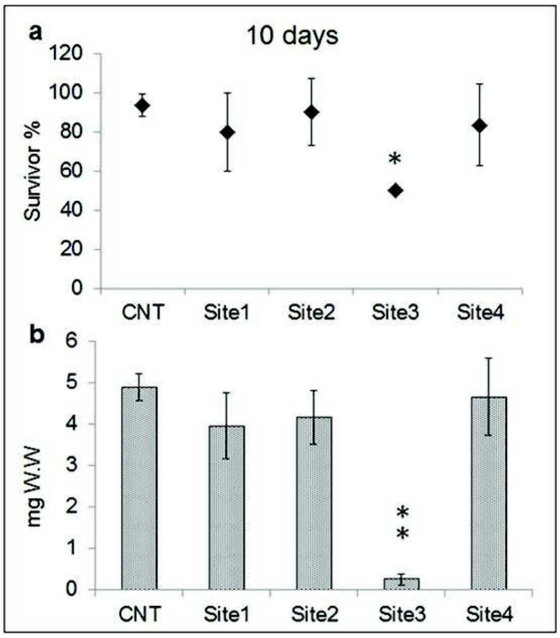

Figure 5 Panel a: percentage of chironimid larvae survived in control (CNT) and at each site (± SD) after 10 days of exposure. Panel b: average wet weight (W.W. ± SD) of chironomid larvae after 10 days of exposure. Significant differences (ANOVA one-way, Dunnet post-hoc test, p <0.05*; p<0.005**) with control are reported.

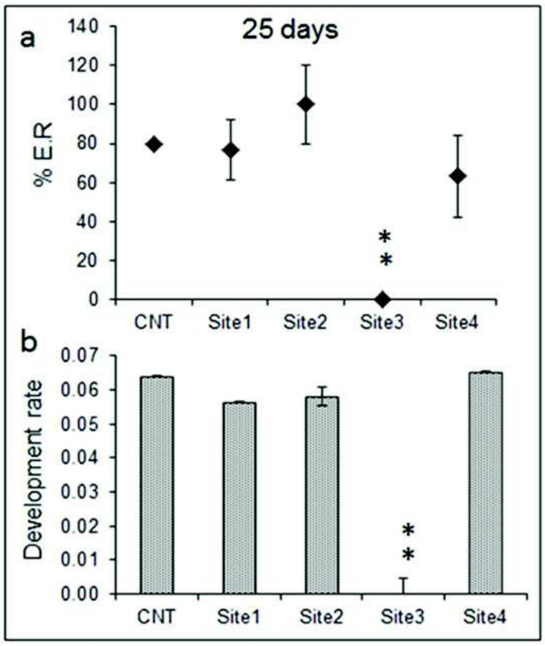

Figure 6 Panel a: Emergence Ratio (ER - ±SD) of chironimid midges for each site and control (CNT). Panel b: Development Rate (± SD). Significant differences (ANOVA one-way, Dunnet post-hoc test, p <0.05*; p<0.005**) with control are reported.

significant differences among sites in number of male/females.

The analytical characterization of DDT and of PCB as representative indicators of the priority substances to be analysed, showed a clear correspondence between the particularly high contamination for these two classes of organic micropollutants at Site 3 and the responses of the test. No evident relationship between the contaminant concentrations of the other sites and the results of the tests were observed, indicating that the concentrations of PCB and DDT have evident negative relationship with the survivor/growth of benthic organisms only at Site 3. Sediment-toxicity tests conducted with the midge *Chironomus dilutus* (10 day exposures) have showed a significant correlation between ecotoxicity and PCB (Kemble *et al*., 2013). In the case of Lake Maggiore (Northern Italy) a DDT LOEC of 80.5 ng g^{-1} d.w. was defined for chironomids growth (Bettinetti *et al*., 2005). In the case of Lake Como in any of the sites this level was never exceeded (Table 1). In absence of quality standards for sediments, Sediment Quality Guidelines (SQGs) developed in the last 20 years, may be therefore used as comparison levels. MacDonald *et al*. (2000) developed a consensus-based sediment threshold concentration for total PCBs (TEC) of 40 ng/g at which effects are unlikely to occur. Sediment collected at Site 1 and Site 2 didn’t exceed the reported threshold for PCB; sediments collected at Site 3 and Site 4 exceeded it and a significant effect of chironomid emergence was observed only at site 3 where concentrations were around 5 times the threshold. In the present work the observed spatial variability of sediment contamination could be related to the presence of different input pollution sources, being the most contaminated site (Site 3, Figure 1) located in the port area of the city of Como. Though Como is a medium-size city (about 84,876 inhabitants), it is a popular tourist destination and naval activity on the lake plays an important role in connecting the city with most of the attraction along the shores, particularly in summer time. The port area (Figure 1, Site 2) is separated from the rest of the lake by a dike perturbing the natural renewal of water and sediment; .moreover Site 3 is next to the mouth of a small urban stream (river Valduce; Figure 1) which flows covered next to the major city hospital. Even no water quality data are available for this river, during high intensity rainfall events its flow rate can become considerable, collecting the surficial runoff water and probably hospital discharges. The river Valduce therefore could become a major point source of micropollutants for the water and sediments of this area.

4. Conclusions

Chemical analysis is a fundamental step in the assessment of surface water status according to WFD. However, even analysing all the priority contaminants effects on resident organisms cannot be assumed. The overall findings of the present work represent an indication of the usefulness of ecotoxcitiy tests in toxicity characterization and hazard ranking of recipient water receiving urban runoff and wastewater. Moreover, although the WFD does not specifically deal with the quality of sediments, their monitoring can play a role when assessing impacts on environmental quality, since together with biota, they are a preferred matrix of accumulation of most hydrophobic contaminants.

Commento al lavoro

Dr. Stefano Polesello CNR IRSA, Brugherio

L'articolo presenta un'interessante applicazione di test ecotossicologici alla caratterizzazione spazio-temporale dello stato di qualità di un corpo idrico. Il tentativo di utilizzare un semplice test ecotossicologico per il monitoraggio estensivo di un corpo idrico e per lo studio del trend evolutivo temporale dello stato di qualità chimica si inquadra a pieno titolo nell'indirizzo dato dalla Commissione Europea, tramite i suoi gruppi di esperti, di introdurre metodi di monitoraggio basati sulla misura integrata degli effetti. Questo tema è infatti prioritario per l'implementazione futura della Direttiva Quadro sulle Acque (WFD) poiché permetterebbe di ottenere un miglioramento della protezione ambientale a fronte di una riduzione dei costi di monitoraggio. Per questo motivo la Commissione Europea ha prodotto una apposita Linea Guida sul monitoraggio "effect-based" in corso di pubblicazione.

L'articolo sottoposto da Ponti *et al.* dimostra che anche l'uso di un semplice test ecotossicologico, come il test acuto con *Daphnia magna* su estratto di acqua, permette di seguire l'evoluzione del carico di inquinanti in un cirpo idrico; è dimostrata inoltre una buona correlazione tra la contaminazione chimica dei sedimenti e i traits vitali della specie *Chironomus riparius*. Il consiglio è di ampliare ad altri siti e/o corpi idrici questo approccio, cercando di verificare, se i dati fossero disponibili e come questo approccio si correli allo stato di qualità definito dagli enti preposti secondo le procedure WFD in vigore.

Time and place of sampling are important in the assessment of micropollutants in the aquatic environment because concentrations are influenced by source variability, dilution, retardation, removal processes (Buerge et al., 2006; Boyd et al., 2004; Jonkers et al., 2009). So far, there is a lack of long-term monitoring of micropollutants in the aquatic environment (Sacher et al. 2008) and especially in urban areas, monitoring programs with sampling over the year are essential to overcome heterogeneity and seasonality of micropollutants in the wastewater, surface water and groundwater.

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