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Chemical Analysis and Sediment Toxicity Bioassays to Assess the Contamination of the River Lambro (Northern Italy)

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Abstract. This study consisted of a 10-day whole sediment toxicity test with the chironomid *Chironomus riparius* and a 28-day sediment toxicity test with the oligochaete *Tubifex tubifex* at seven sites to assess the quality of the River Lambro (Italy), one of the most contaminated rivers of the Po Basin. Endpoints measured were survival and growth for chironomids and cocoon deposition and development of young worms for tubificid oligochaetes. Responses were evaluated in relation to the occurence of organic micropollutants (PCBs, DDT, HCB, and HCH) representative of the industrial and agricultural contamination of the area. Though survival of the organisms remained unaffected, sublethal effects were observed at all sites. The sediment sampled at the farthest upstream site differed from the control only in the number of cocoons deposited by the worms. Both test species in the next three sediments, where concentrations of PCB and DDT were in the range 21.9–39.5 ng g^{-1} DW and 0.6–1.3 ng g^{-1} DW, respectively, experienced greater toxicity in terms of growth and reproduction. Contamination was particularly high in the site closest to Milan, where the river receives untreated urban and industrial discharges. Levels of total PCBs and total DDT here were up to two orders of magnitude higher than those found at the other sampling locations, and chironomid growth and the reproductive endpoints of tubificids were significantly lower than in the control and the other sites. The test results for the next two stations showed improvement relative to that of Milan, although contamination was still evident. Sublethal effects were in agreement with the measured concentrations of the principal persistent organic pollutants and should be included as part of environmental monitoring efforts as a basis for assessing the recovery of the river.

The development of ecotoxicological studies has coincided with the increasing awareness that sediments act as a major pollutant reservoir and that there are a number of sites around the world where contaminated sediments represent a significant problem. Toxicity testing has proved extremely useful in environmental and chemical hazard assessments, because it can be done relatively quickly and inexpensively compared with chemical analysis. Early toxicity testing of contaminated sediments focused primarily on acute toxicity, but short-term exposure, which measures effects on survival, can generally be used only to identify high levels of contaminated sediments (Burton and Scott 1992; Sibley *et al.* 2001). Recent studies have shown that selected sensitive sublethal endpoints (*e.g.*, growth, reproduction, etc.) may provide better estimates of the responses of benthic organisms to contaminants under laboratory (Sibley *et al.* 1997) and field (Kemble *et al.* 1994) conditions. Suitable test species for sediment toxicity tests are the oligochaete *Tubifex tubifex* (Reynoldson *et al.* 1991) and the larvae of the dipteran *Chironomus riparius:* these are commonly used to evaluate the toxicity of freshwater sediments both in Europe and in the United States, because they have a wide sensitivity to contaminants (US EPA 1994). Due to the differences in their ecology (*e.g.*, feeding behavior) and sensitivity, these species are used in bioassays of different durations. In tests with these two organisms, the toxicity of sediments is assessed by measuring the growth of surviving larval insects in a subchronic test, and reproductive endpoints of tubificid worms are used to assess contamination in a long-term test.

The present study is part of a national program to assess the state of the River Lambro (Northern Italy) environment to identify the most compromise areas forecasting its restoration. All the water resources of the basin are exposed to a high level of anthropogenic pressure: about 17 million inhabitant equivalents (not including breeding rearing) live along the river, and more than 53% of its water entering the River Po is industrial waste. The River Lambro has significant adverse effects on the River Po, and for this reason represents one of the two hot spots of its basin. Ecotoxicological information on the river are generally scanty and focused on the area where River Lambro enters River Po; in our study the whole course of the River Lambro was assessed using sediment toxicity tests with *C. riparius* and *T. tubifex.* River sediments were also analyzed for the main organic micropollutants (polychlorobiphenyls, PCBs; dichloro-diphenyl trichloroethane, ΣDDT; hexachlorocyclohexane, HCH; hexachlorobenzene, HCB) representative of both industrial and agricultural contamination, for assigning causality in relation to the toxicity observed (*e.g.*, concentration-response relationship).

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Materials and Methods

Study Area

The River Lambro is one of the most important tributaries of the River Po, the largest watercourse in Italy. The Lambro rises in the prealpine region of Lombardy (northern Italy) at 1456 m ASL and drains an area of 900 km^2 with a course of about 130 km, collecting a large quantity of contaminants along its course. Most of the industrial and civil discharges in the northern area of the Lambro as far as the town of Monza (6,221 factories out of the total present in this area; Consorzio Alto Lambro personal communication) are collected and treated before entering the river between stations 4 and 5 (Figure 1). There are some other treatment plants along the course of the river, but the urban discharge of the city of Milan (nearly 3 million inhabitant equivalents) and the runoff of many other industrial and agricultural concerns are not yet being treated. About 100,000 m^3 day^{-1} of urban waste water from the agglomeration is discharged directly into the River Lambro at Milan, and a further 1,000,000 m^3 day^{-1} of untreated sewage flows into the river through its main tributary, the River Lambro Meridionale. This tributary originates in Milan from an urban channel, which receives water from the River Olona and from the urban sewage network, and flows parallel to the Lambro, joining it at S. Angelo Lodigiano; other channels starting from the city meet the river at Melegnano.

Sediment Collection

Seven sites on the bank, chosen to typify the quality of the sediments of the entire course of the River Lambro, were sampled in summer 2001 (Figure 1). Bottom sediments were collected with a steel spade from the top 5–7-cm layer of sediment in depositional areas along the banks. Sediments were collected over 2 days in stable flow conditions.

Sediment Extraction and Analysis

In the laboratory the sediments were wet-sieved at 250 μm to remove indigenous fauna (Day *et al.* 1995) and then stored at 4°C. A small amount of sediment (10–15 g WW) was freeze-dried for organic matter and micropollutant analysis. Organic matter was determined as loss on ignition (LOI) at 550°C (Bengtsson and Enell 1986).

Dried sediments (3 g) were extracted for 12 h in a Soxhlet apparatus using a mixture (1:1) of *n*-hexane and acetone (200 ml). The extracts were concentrated to 2 ml by rotary evaporation and then digested with 3 ml of H_2SO_4 for 24 h to remove all the organic matter. The digested samples were than concentrated (2 ml) and cleaned by columns (0.5 ID, 4 cm long) made of silica (Florisil, Sigma) with a thin top layer of Na_2SO_4 to catch residual water and copper to keep sulfur compounds. The copper was left in HCl (18%) before use. After 24 h it was rinsed with dechlorinated water until pH 7 was reached, then rinsed with acetone and hexane. The column was eluted with 25 ml hexane, and the purified extract was concentrated to 2 ml. Samples (1 μl) were injected into a Carlo Erba 8000 Mega Series Gas Chromatograph equipped with a ^{63}Ni electron capture detector (ECD). The on-column system was used for injection. A fused silica capillary column (HP-5 crosslinked 5% PH ME Siloxane, 60 m × 0.25 mm ID) with a film thickness of 0.25 μm was used. After a minute at 100°C, the temperature program runs to 180°C at 20°C min^{-1}, then from 180°C to 200°C at 1.5°C min^{-1}, from 200°C to 270°C at 3°C min^{-1}, and at the end remains at 270°C for 20 min. The carrier gas was helium at 1 ml min^{-1}, and the auxiliary gas was nitrogen at 40 ml min^{-1}. The detector temperature was fixed at 280°C. Analysis was performed for

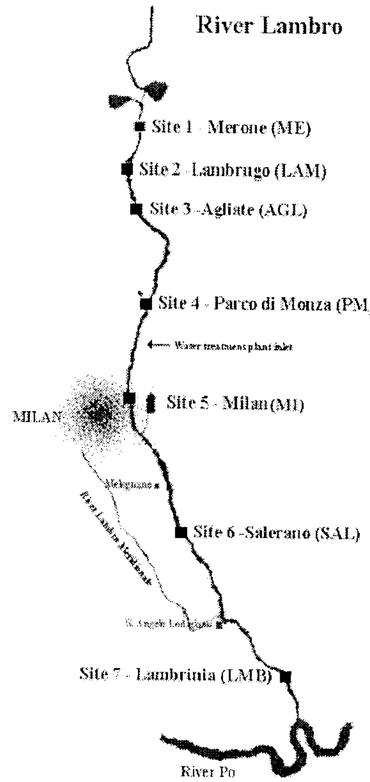

Fig. 1. Sampling sites along the River Lambro. The abbreviations identifying the sampling sites are given in brackets

PCBs, ΣDDT, HCH, and HCB. Aroclor 1260 (Alltech) containing known quantities of 23 PCB congeners was used as reference standard for PCBs; a prepared standard mixture of pure DDT, HCH, and HCB was used as reference standard for their determination.

The external standard method was used for quantification. Peaks were automatically integrated using the Chrom-Card 1.16 software (Fisons Instruments). Analyses were performed in duplicate; the detection limits were 0.1 ng g^{-1} for PCBs and 0.05 ng g^1 for DDT, HCH, and HCB.

Test Organisms

The test organisms, *T. tubifex* and *C. riparius*, were obtained from culture vessels maintained at the University of Milan. The cultures of *T. tubifex* were initially started from 100 adults collected in Lake Suviana (Italy). The worms were kept in the dark at 21 ± 1°C in glass containers (diameter = 10.5 cm, height = 6 cm) half filled with sterilized sand and dechlorinated tap water (hardness: 320 mg L^{-1} $CaCO_3$). Every week the sand was washed and the water completely renewed. The worms were then fed with frozen spinach thawed at room temperature immediately before use and placed beneath the sand. New cocoons were removed from the sand every week and put into other containers to start new cultures.

The chironomids used came initially from the cultures maintained at ECT Oekotoxikologie GmbH (Flörsheim, Germany). They were 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^{-1} $CaCO_3$) 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.

28-Day T. tubifex *Bioassay*

T. tubifex toxicity tests were performed according to the methods by Reynoldson *et al.* (1991). Four sexually mature worms at their first reproductive event were added to 250-ml glass beakers filled with 70 g wet sediment (approximately 50% water content) mixed with 80 mg dry powdered Tetramin fish food and 150 ml of an Italian commercial mineral water (pH = 7.8, HCO_3 = 56.5 mg L^{-1}). Before the addition of the worms, the contents of the beakers were left to settle for 24 h. Five replicate beakers were prepared for each site, including the control. Tests were performed in the dark for 28 days at 21 ± 1°C; the overlying water was kept aerated. Every 2 days water was added to the beakers, if required, to compensate for evaporation. pH and dissolved oxygen were measured in all the beakers at days 0 and 28. At the end of the test the contents of each beaker were sieved through a 250-μm mesh. The surviving adults were counted immediately; cocoons and young worms were preserved in 70% alcohol and then counted under the dissecting microscope.

10-Day C. riparius *Bioassay*

Tests were performed according to the guidelines proposed by Day *et al.* (1994) with minor modifications. One day before the addition of first-instar larvae, 250-ml glass beakers were filled with 70 g wet sediment and 200 ml commercial mineral water. Three and one-half milliliters (3.5 ml) of a 4 g L^{-1} water suspension of fish food, corresponding to 14 mg DW 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 replicate beakers were prepared for each site, including the control. 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 animals were fed with 3.5 ml Tetramin suspension, and the water lost to evaporation was added. pH and dissolved oxygen were measured in all the beakers before and at the end of the test. At the end of the test the number of surviving chironomids and their wet weights were determined. 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 (Blockwell *et al.* 1996; Watts and Pascoe 1996).

Sediment collected in the unpolluted Lake Monate (northern Italy) was used as a control (Bettinetti *et al.* 2002) for all tests.

Data Analysis

One-way ANOVA was performed (α level = 0.05) followed by the post hoc Tukey test for unequal sample sizes (Spjotvoll and Stoline test) to evaluate differences between the responses of each endpoint at the sites. 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).

Results and Discussion

Table 1 shows the level of organic micropollutants found in the surface sediment samples from the seven collection sites of the River Lambro. These data indicate that anthropogenic activities, such as urbanization, industry, and agricultural practices have affected the quality of the ecosystem.

In general, contaminant concentrations increased from site 1 to site 5 and then decreased downstream, although concentrations here did not reach values comparable to those before site 5. Milan was the most contaminated site, with levels of total PCBs and total DDT up to two orders of magnitude higher than those found in the other sampling points. HCB also increases just before Milan. This is not surprising, because the organic pollutants entering the upper part of the River Lambro are not all degraded by the treatment plant upstream of Milan, where they are discharged into the river. Only total HCH reached its maximum value at the mouth of the river, probably because of the highly developed agriculture in this area: HCHs, especially lindane (γ-HCH), are still used, notwithstanding their restriction by law.

Although some of the chlorinated pesticides found in the sediments have been banned and are no longer in use, they persist in the sediments, representing a danger for the benthic fauna. This is apparent if we look at the concentrations of total DDT in sediments; however, an indication that it is no longer used is given by the low ratio *pp′* DDT/*pp′* DDD (lower than 1), generally considered to be an index of pollution age (Galassi *et al.* 1993). *pp′* DDE was the main metabolite recovered in the sediments in all the sites, with the exception of site 6 where the concentration of *pp′* DDT was higher than the other metabolites. Organic matter was lower than 5.1% except at Milan, where it reached 13.2% (Table 1).

The concentration trend shows significant evidence of toxic risk, particularly at the level of the city of Milan and farther downstream. As far as we know, no concentrations of organic micropollutants as high as those found at the Milan site and downstream from it have been reported in other Italian rivers (Table 2). Even the total DDT concentration in the sediments of the River Toce, the major tributary of Lake Maggiore (northern Italy) recently seriously contaminated with DDT by a company producing the pesticide, was lower in the summer of 2001 (115.6 ng g^{-1} DW) compared to the concentrations found in Milan.

Moreover, the PCB level at Milan was higher than those measured in other European rivers, with the sole exception of the Laborec River (Kocan *et al.* 2001), where the presence of a PCB manufacturer has resulted in sediment concentrations of 3900 ng g^{-1} DW. If we do not consider the values recorded at Milan and downstream, the PCB and DDT concentrations found in the northern River Lambro are within the range of or even lower than the levels observed in various Italian and European rivers (Table 2).

Because the river Lambro collects large amounts of many pollutants, including heavy metals, a complete chemical characterization is almost impossible. As a consequence, toxicity tests, currently widely used for monitoring contaminated areas (Reynoldson 1994; Martinez-Madrid *et al.* 1999a, 1999b; Cheam *et al.* 2000; Lyytikainen *et al.* 2001) are the only means of monitoring the level and the threat of contamination in the River Lambro.

During the *C. riparius* test, pH did not change by more than 1 unit in most of the test beakers (range 6.8–7.8) and changed even less in the control beakers (range 6.8–7.1). At the end of the test, dissolved oxygen in the water was over 68% in the controls and at sites 1, 2, 6, and 7. It was about 55–60% at sites 3 and 4, whereas at site 5 (Parco Lambro) it dropped to 30%.

No chironomid mortality significantly different from the

Table 1. %LOI and concentrations of contaminants (ng g^{-1} DW) in the 250-μm fraction of the river Lambro sediments

Sites	%LOI	Total PCB $\bar{x} \pm$ SD	Total HCH	HCB	*pp′*DDT	*pp′*DDE	*pp′*DDD	*op′*DDT	*op′*DDE	Total DDT $\bar{x} \pm$ SD
1 (ME)	2.4	10.0 ± 0.05	0.5	0.2	0.6	1.0	0.04	0.5	ND	2.1 ± 0.2
2 (LAM)	1.2	21.9 ± 0.05	0.6	1.3	0.8	2.2	0.2	0.4	ND	3.5 ± 0.05
3 (AGL)	4.5	16.1 ± 2.6	1.2	n.d.	0.4	1.0	0.03	0.1	ND	1.5 ± 0.5
4 (PM)	5.1	39.5 ± 0.06	1.3	0.5	1.0	1.5	0.1	0.4	ND	2.9 ± 0.4
5 (MI)	13.2	3053.8 ± 0.1	1.3	16.4	63.3	102.2	1.5	ND	ND	167.0 ± 0.2
6 (SAL)	2.0	563.0 ± 0.04	18.4	6.3	19.2	15.4	1.2	ND	0.4	35.7 ± 0.2
7 (LMB)	1.8	255.7 ± 0.04	27.6	10.8	7.0	11.1	1.1	2.0	0.4	21.2 ± 0.08

Data are expressed as mean values; for Total PCB and Total DDT the standard deviation is also indicated. The numbers in the first column correspond to the site locations shown in Figure 1; site abbreviations are reported in the text.
ND = not detectable.

Table 2. Total PCB and total DDT in sediments of different rivers in Italy and Europe (ng g^{-1} DW)

Rivers	Total PCB	Total DDT	Reference
Grote Gete (Belgium)	23.0		De Cooman *et al.* 1998
Sein (France)	400–2,200		Chevreuil *et al.* 1998
Hollandse IJssel (The Netherlands)	15.7–214	21.1	M. Wagelmans personal communication
Adige (Italy)	3–154		Pavoni *et al.* 1987
Po (Italy)	4.5–70.7	3.9–18.7	Camusso *et al.* 2002
Magra (Italy)	58.2	3.4	Bettinetti *et al.* 2003
Toce (Italy)	92.8	115.6	Bettinetti *et al.* 2003
Ondava (Slovakia)	52		Kocan *et al.* 2001
Laborec (Slovakia)	52–3,900		Kocan *et al.* 2001
Ebro (Spain)	5.3–1,771.8	0.4–51.8	Fernandez *et al.* 1999
Guadalquivir (Spain)	12.2	18.2	Hernandez *et al.* 1992
Jarama (Spain)	266–1,730	107–969	Fernandez *et al.* 2000
Nervion (Spain)	3–458		Martinez-Madrid *et al.* 1999b

control was recorded at any of the sites. In control beakers the range of mortality was 0–10%. The wet weight of larvae followed an opposite trend to that of contamination (Figure 2). The post hoc comparison ($p < 0.05$) showed that at the first three sites the chironomids had a weight similar to the control, and the minimum average weight was recorded at site 5, a significant reduction compared to all the other sites. Weights recorded at sites 4, 6, and 7 were similar and slightly higher than those at site 5.

In the *T. tubifex* tests, pH did not change by more than 1 unit in all the test beakers (range 6.8–7.8). Dissolved oxygen in water was over 75% in controls and at sites 1, 2, and 3. It was about 50% at sites 4, 6, and 7, whereas at site 5 (Parco Lambro) it dropped to 26%.

The survival of adult worms was not affected after 4 weeks of exposure to the different sediments. The number of cocoons laid in the control sediment was significantly higher than that found in all the other sediments (Figure 3A). The number of cocoons decreased going from site 1 to Milan, where it reached its minimum value; a small increase was observed in the following two sites. The post hoc comparison ($p < 0.05$) showed that four groups of situations can be identified along the course of the river: site 1, which was different from the control; sites 2, 3, and 4 were similar, as were sites 6 and 7. Site 5 was the most contaminated.

The trend of the number of young worms was in agreement with that of the concentrations of organic micropollutants and similar to that of the number of cocoons, with the exception of site 3. In this station the average number of young worms appeared to be higher than the average at sites 2 and 4 (Figure 3B), whereas the number of cocoons was nearly the same. Post hoc comparison ($p < 0.05$) showed that site 1 and the control were significantly different from sites 2, 3, and 4, which were similar to each other. Site 5 presented the lowest number of young worms and was different from all the other sites. Sites 6 and 7 had a similar number of young worms. In greater detail, the results of bioassays and chemical analysis show that the situation of contamination upstream of the city of Milan is different from that downstream of the city. In the northern part as far as the town of Monza, all the toxicity data indicate that the contamination of the river sediments worsens with closer proximity to Milan; however, in this stretch of the river concentrations of PCB can be regarded as low according to the standard classes described by Chevreuil *et al.* (1998). Moreover, DDT values are in the lower part of the range of levels observed along the first part of the Ebro River, which is considered a background area (Fernandez *et al.* 1999). This could be due to the fact that here most of the industrial discharge does not reach the river directly, as most of the sewage in the area is collected and treated in a treatment plant which discharges downstream of Monza (Figure 1). The growth of chironomids, similar to the control organisms, did not appear to be affected, but more apparent effects were recorded on the reproductive endpoints of the sludgeworms.

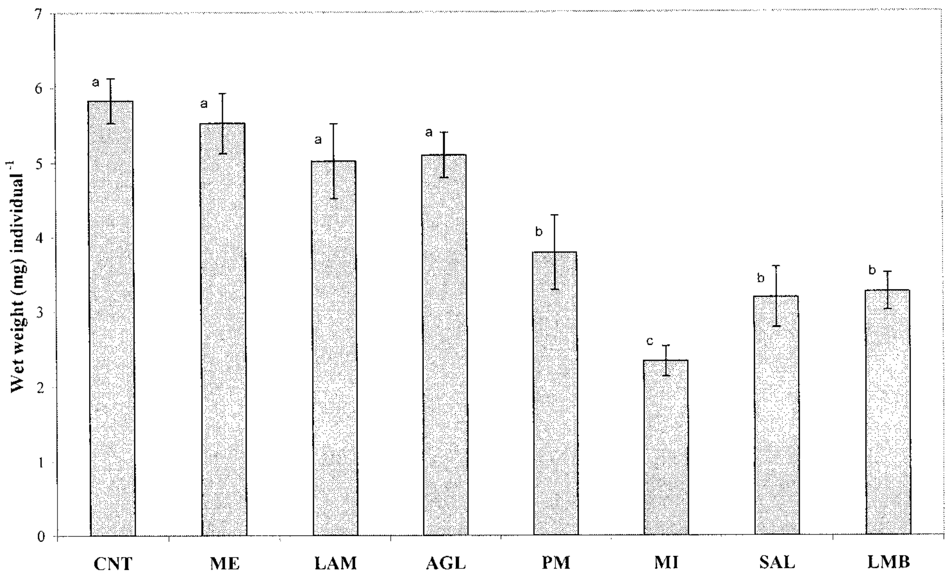

Fig. 2. Average wet weight ($\pm$ SD) of chironomid larvae after 10 days of exposure. CNT is the sediment control. For the other site abbreviations see Figure 1. Statistically different (ANOVA; Tukey test, $p < 0.05$) observations are marked with different letters

Because the tubificid bioassay lasts 28 days as opposed to the 10 days of the chironomid test, longer-term toxic effects can be expected on benthic organisms in these stations.

The worst situation was found near Milan, which has a profound and direct impact on the river. The low responses to the toxicity tests performed here can be ascribed to the high concentrations of organic micropollutants in the sediment. It is also probable that many other contaminants, such as heavy metals (Pettine *et al.* 1996), nonylphenols (Valsecchi *et al.* 2001), and other known and unknown compounds (Galassi *et al.* 1988), are present in this part of the river, which receives untreated industrial and urban sewage, and the adverse effects could be the result of the cocktail of contaminants in the sediments. In this area the river flows more slowly, allowing the deposition of suspended matter and of the persistent organic contaminants not retained by the upstream treatment plant, which are added to the urban discharges of the city of Milan. The deposition of fine particles increases the percentage of organic matter in sediments and excludes the possibility that the organisms suffered from starvation during the experiments, a point further supported by the good development of organisms observed in control sediments with a lower LOI percentage (3.8%). Low oxygen levels (4.5 mg L^{-1}) were recorded at Parco Lambro site during the tests, but *T. tubifex* is an euroxybiont species and the normal development of embryos requires dissolved oxygen values ranging around 2.5–7 mg L^{-1} (Brandt 1978; Poddubnaya 1980). Even *C. riparius* can stand very low concentrations of oxygen (Sansoni 1988; Cranston 1995), an adaptation that is enhanced by the presence of hemoglobin.

The two stations below Milan, though still contaminated, were found to be in better condition than the Milan site. The concentrations of PCB and DDT remained high at site 6 and decreased slightly at the last site, where there may have been a dilution effect from irrigation channels flowing to the river. This is a largely agricultural area, highly contaminated with herbicides (Barra *et al.* 1999), and also shows an increase of HCH and HCB concentrations.

Our results are in agreement with a previous work (Martinez-Madrid *et al.* 1999a) in which the toxicity assessment of an industrial area of Bilbao (Spain) based on sediment chronic bioassays with *T. tubifex* was performed. A drastic reduction in the number of cocoons and of young worms was observed when the sediment concentration of PCBs and γ-HCH was 458 ng g^{-1} DW and 0.75 ng g^{-1} DW, respectively (Martinez-Madrid *et al.* 1999b), similar to those recorded in the last two sites of River Lambro. However high concentrations of PAHs, naphthalene, and heavy metals were also recorded (Martinez-Madrid *et al.* 1999b), and it is not possible to find a direct relation between concentration of a single toxicant and the organism responses. The different bioavailability of contaminants stored in sediments has to be considered, and the evaluation of the toxicity of the single substances in both cases seems impossible.

Conclusions

The River Lambro flows through one of the most industrialized and densely populated areas of northern Italy, and because of its particularly high contribution to the River Po, represents one of the most critical areas of the whole Po watershed (Pettine *et al.* 1996; Camusso *et al.* 2002). Our investigation revealed the occurence of relatively high concentrations of PCBs and other organic micropollutants in sediments in spite of their prohibition by law. These compounds may represent a real threat for benthic organisms as well as for organisms higher in the food chain, because these bioaccumulative substances, besides being toxic, may exert hormone-like effects on many wildlife species, causing malformations in reproductive organs and altered sex differentiation and immune functions, which result in changes in population levels (Colborn *et al.* 1993). As much information as possible should therefore be collected on the presence of these compounds in sediments, on their mechanisms, and on the effects of the specific exposure of benthic organisms to them. It should also be remembered that once contaminated, sediments represent a further source of contamination because

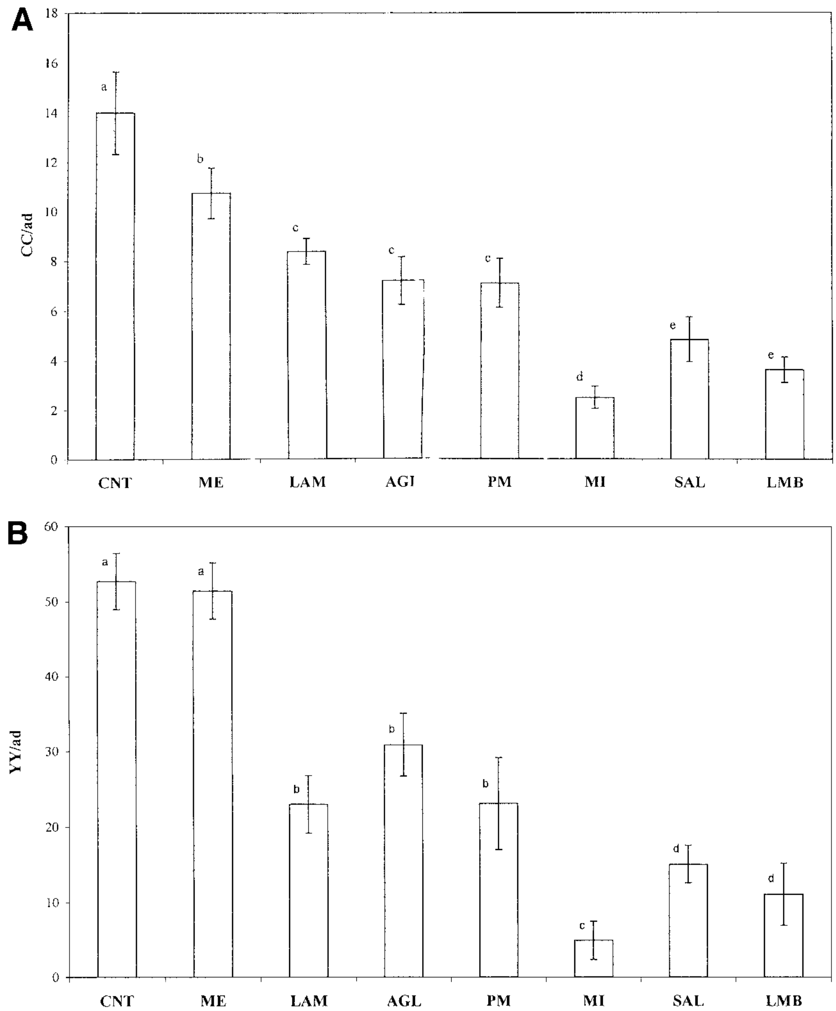

Fig. 3. Number of cocoons/Ad (± SD) (A) and of young worms/Ad (± SD) (B) after 28 days of exposure. CNT is the sediment control. For the other site abbreviations see Figure 1. Statistically different (ANOVA; Tukey test, $p < 0.05$) observations are marked with different letters

they store toxic substances, which can be subsequently released into the environment by flood events and other disturbances (Stigliani *et al.* 1991).

In general, the tests with both *C. riparius* and *T. tubifex* showed that almost all of the River Lambro sediments sampled caused toxic stress, affecting both their growth and their reproduction. The use of sublethal parameters produced good information on the toxicity of the sediments, as did the application of subchronic tests; long-term toxicity tests proved to be particularly useful in characterizing the potential toxicity of sediment, giving a more precise indication of the trend of contamination in the river. In contrast, no acute toxic effects emerged from the 10-day survival test with *C. riparius* or from evaluation of the surviving adult worms.

To date, the only research performed on the pollution of the river has been restricted to studies on heavy metals and other inorganic compounds and on the analysis of organic pollutants, limited to the confluence of the Lambro with the Po (Guzzella and Mingazzini 1994; Guzzella and Gronda 1995; Pettine *et al.* 1996; Renoldi *et al.* 1997). We hope that the results of the present study will contribute to an understanding of the sources of persistent organic pollutants along the course of the river and provide the first data that will lead to its restoration.

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