



Is meltwater from Alpine glaciers a secondary DDT source for lakes?

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ARTICLE INFO

Article history:

Received 20 May 2008

Received in revised form 8 July 2008

Accepted 13 August 2008

Available online 20 September 2008

Keywords:

DDTs

PCBs

Glaciers

Zebra mussel

Fish

ABSTRACT

A sharp increase in 2005 of *pp'*DDT and its metabolites was observed in mussels and fish from lakes Como and Iseo, the main glacier-fed southern Alpine lakes. DDTs in zebra mussels (*Dreissena polymorpha*) were more than 150 times higher than levels in 2003, and concentrations in pelagic fish ($0.12 \text{ mg kg}^{-1} \text{ w.w.}$) exceeded the Italian safety threshold for human consumption ($0.05 \text{ mg kg}^{-1} \text{ w.w.}$). The histological examination of the ovaries revealed many mussels with oocyte degeneration throughout the studied period. Prior to being banned in Italy in 1978, DDT was used in large amounts for fruit-tree treatment from the 1950s to the 1970s in valleys just below the glaciers. Since glacier volume was increasing at that time and then continuously retreated, meltwater should be the main cause of the pollution peak recently observed in biota of downstream lakes. PCBs did not peak in biota tissues to a comparable extent probably because local sources were not as important as for DDTs.

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1. Introduction

Persistent Organic Pollutants (POPs) have reached northern latitudes and high-altitude environments from volatilization in warm source areas and condensation–re-deposition in cold sites, and the consequence of this long-range transport, known as “cold condensation”, is the ubiquitous presence of micropollutants, including remote cold areas where no direct anthropic impact exists (Wania and Mackay, 1993; Galassi et al., 1997). Unlike latitudinal atmospheric long-range transport, altitudinal transport to high mountains can occur over relatively short distances from potential source regions, as is the case for the European Alps, which circumscribe densely populated regions. POPs can be transported to glaciers by snow, a well-known carrier of pollutants (Lei and Wania, 2004), from agricultural and industrialized areas of northern Italy and are eventually stored in the ice. During the snowmelt, contaminants can reach surface waters and may be rapidly assimilated in biological tissues, thus being bioaccumulated and/or biomagnified in food chains (Campbell et al., 2000; Kallenborn, 2006). Annual cycles for POPs are expected since the prevailing processes of deposition in winter and volatilization in summer occur at the watershed scale (Galassi et al., 2006). However, glaciers cannot be considered long-term stable systems because they are subject to volume fluctuations determined by temperature trends.

According to a recent report of the intergovernmental panel on climate change (IPCC, 2007), there is a worldwide decline in glacier mass due to global warming. Many mountain areas have lost significant portions of their glaciers during the last two decades. Mid-latitude mountain ranges such as the Himalayas, Rocky Mountains, Cascade Range and Southern Andes, as well as isolated tropical summits such as Mount Kilimanjaro in Africa, have shown some of the largest proportionate glacial losses (IPCC, 2007).

After the advance episode between 1965 and 1985, the Italian Alpine glaciers have been showing a worrying retreat since 1980 (Rovelli, 2008). It was estimated that the Adamello Glacier, the largest Italian glacier with an area of 18 Km^2 , had a terminus retreat of about 2000 m since the beginning of the nineteenth century and an increased altitude of the same terminus from 1780 m to the actual 2580 m a.s.l. (Ranzi and Taschner, 2005). Although Alpine glaciers represent only a small percentage of the world's total ice mass, they can be considered representative of a more generalized situation occurring in the cold areas of the planet due to climate changes. Moreover, the quick recycling of meltwater should produce serious effects due to micropollutant release, as reported for some freshwater ecosystems (Blais et al., 1998; Blais et al., 2001; Meyer and Wania, 2008). Chlorinated pesticides which entered the environment in huge amounts due to agricultural use between 1965 and the mid-1980s in northern Italy (Galassi and Provini, 1981), might have been stored in glaciers during their volumetric growth (Rovelli, 2008) and then released into glacier-fed lakes during the recent retreat.

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Considering that the maximum volume reduction of the glaciers was observed in the summer of 2003, when the highest temperatures were recorded in Central Europe (Jankowski et al., 2006), huge amounts of contaminants were expected to enter these environments with the summer snowmelt.

Moreover, a complete water overturn was observed in 2005–2006 in the Italian oligomictic southern Alpine lakes. This is a rare event, made easier by the increase of the hypolimnium temperature in recent years in these deep lakes (CIP AIS, 2006) as a consequence of climate warming. POPs stored in the hypolimnium might have been released and subsequently bioaccumulated in biota living in the surface waters.

The zebra mussel, *Dreissena polymorpha*, a filter-feeding littoral mussel used in previous studies to biomonitor POP contamination of Italian lakes (Binelli and Provini, 2003; Mantecca et al., 2008; Riva et al., 2008), and the pelagic landlocked shad, *Alosa fallax lacustris*, were collected from Como and Iseo lakes in 2005, 2006 and 2007 to determine whether climate warming had influenced contamination levels in the biota. The results of the present work were compared to those of previous studies carried out on the same two lakes (Riva et al., 2008), and to data obtained from Lake Maggiore (CIP AIS, 2007), another southern Alpine lake where DDTs have been monitored using zebra mussels since 1996. Histopathological analyses were also performed on the reproductive organs of the zebra mussels, previously reported as representing the main targets of many POPs (Mantecca et al., 2003).

2. Materials and methods

2.1. Study area

Lake Como ($V = 22.5 \text{ km}^3$, maximum depth = 425 m) and Lake Iseo ($V = 7.6 \text{ km}^3$, maximum depth = 251 m) (Fig. 1) are two sub-alpine oligomictic lakes lying in mountainous areas glaciated during the Pleistocene. Lake Como has three branches of approximately equal length; one stretches northward, another south-westward to the city of Como, and the third south-eastward beyond Lecco, with Bellagio promontory marking the bifurcation between the latter two branches. The lake is mainly fed by the Bernina, Ortles-

Cevedale and Disgrazia glaciers through the Adda River, which enters the northern part and issues into the Lecco branch, and also by the waters of numerous other rivers and mountain streams, including the River Mera. Lake Iseo has a more consistent shape and is mainly fed by the Oglio River, which flows down from the Val Camonica valley after receiving a great amount of water from the upstream Adamello glacier.

Both lakes experienced intense eutrophication during the 1960s, which has only been partially solved. Lake Como currently shows conditions between mesotrophy and eutrophy (Salmaso et al., 2006) with particularly high phosphorus concentrations in the Como branch, which can be considered a “closed” basin since it has no outlets. Lake Iseo is still undergoing a progressive deterioration in water quality, mainly due to eutrophication (Garibaldi et al., 2003).

2.2. Sampling of mussels and histological analysis

Adult specimens of *D. polymorpha* (shell length >15 mm) were collected between March 2005 and April 2006 from Lake Iseo (Fig. 1). At each sampling, 100 mussels were collected at a depth of about 2–3 m and a sub-sample of 50 organisms was fixed in Bouin's fluid. Once in the laboratory, the visceral sacks of about 30 randomly selected mussels were isolated and processed for histological analysis according to standard procedures. A total of 293 mussels were histologically examined. The visceral sacks were cut in 7 μm transverse sections at the proximal, central and distal levels and about 10 serial sections from each portion were placed on microscope slides and stained with haematoxylin–eosin. Slides were observed with a Leica DMRA2 light microscope and images were taken with a Leica DC300F digital camera. All mussels were observed and analyzed microscopically for the presence of diseases mainly in the gonads. Severity levels of ovarian pathologies were determined by screening each slide and allocating a score of 0 to 3, according to Mantecca et al. (2008). The score depended mainly on the cytological state of the germinative epithelium, with the lowest value representing the normal condition and the highest indicating the most altered state. An Ovarian Degeneration Index (ODI) was calculated as the mean of the cytological score of all

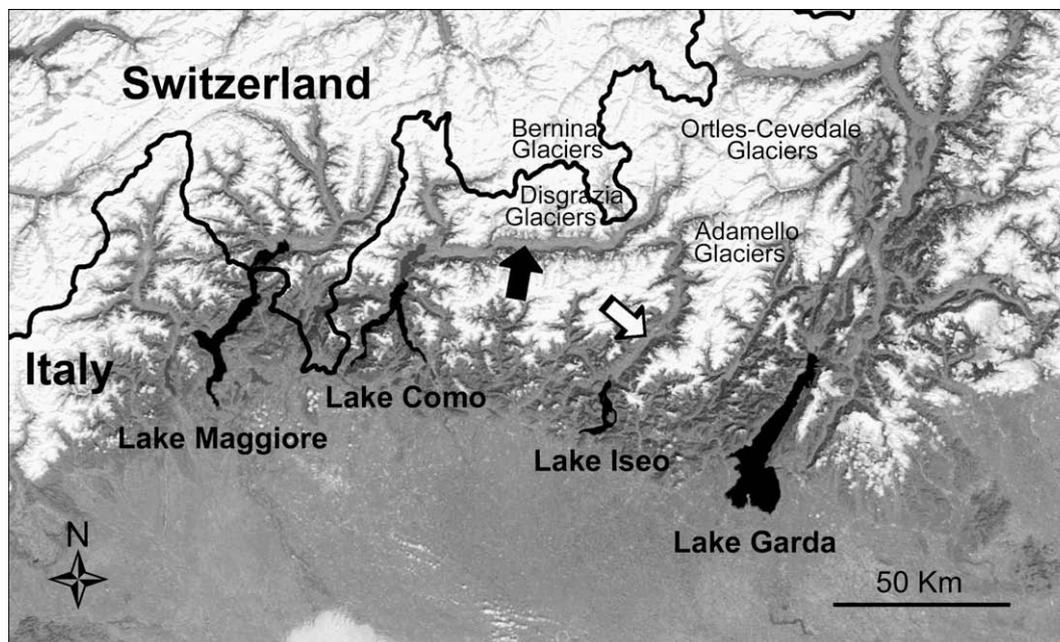


Fig. 1. Study area (black arrow: Adda river valley; white arrow: Oglio river valley).

specimens for each sampling date to determine the general conditions of the organ.

2.3. Sampling of mussels and fish and chemical analysis

A sub-sample of about 50 specimens of *D. polymorpha* collected from March to November 2005 in Lake Iseo (Fig. 1) was used for the analytical characterization. Other samples from Lake Iseo were collected in May 2006 and September 2007; samples from Lake Como were collected in March 2005, in September 2006 and in September 2007. Adult specimens (1–2 years) of *A. fallax lacustris* were collected using fyke nets in May 2005 and 2006 from the Como branch, and in May 2007 from Lake Iseo and both of the Lake Como branches.

All the samples were stored frozen at -20°C in the laboratory and then freeze-dried (Freeze dryer Mod. 24, Edwards, USA).

A pool of 25–58 freeze-dried molluscs equal to 0.2 and 0.7 g d.w. and a sub-sample (1 g) of a pool of freeze-dried fish muscle of 5–6 specimens of both sexes from each sampling site were homogenized and extracted with an acetone-hexane (pesticide grade, Carlo Erba, Italy) 1:1 (v/v) mixture in glass microfibre thimbles (19-mm internal diameter \times 90-mm external length, Whatman, England) using a modified Soxhlet apparatus (Velp Scientifica – ECO 6 thermoreactor). The gravimetric determination of lipids was performed after solvent evaporation. Lipids were suspended in 2 mL of *n*-hexane and destroyed with H_2SO_4 (98%, Carlo Erba, Italy). Chlorinated compounds were then recovered by several *n*-hexane washings. Hexane extracts were concentrated to about 2 mL and cleaned-up on a Florisil column (4×0.7 cm). The purified extracts (1 μL) of each sample were introduced by on-column injection into a gas chromatograph (Carlo Erba TOP 8000) equipped with a capillary column (WCOT fused silica CP-Sil 8CB, Varian USA, 50 m \times 0.25 mm, film thickness 0.25 μm). A Carlo Erba ECD 80 (ThermoQuest Italia) was used as an electron capture detector heated at 320°C .

A reference standard mixture of *pp'* and *op'* DDT, DDD and DDE was prepared by dissolving 10 mg of pure compounds (Pestanal, Sigma–Aldrich, Germany) in iso-octane and diluting the solution to a final concentration of $10 \mu\text{g L}^{-1}$. Aroclor 1260 (Alltech, IL, USA) was used as a reference standard for polychlorinated biphenyl (PCB) quantification.

Good laboratory practices were tested on the standard reference materials BCR-598 and BCR-349 (Community Bureau of Reference-BCR Brussels) for DDT and PCB residues, respectively, with tests analysing samples in triplicate. The percentages of recovery of *pp'*DDE was 107.5 ($\pm 4\%$), of *pp'*DDD 106.2 ($\pm 4\%$), of *pp'*DDT 106.2 ($\pm 3\%$) and of PCB within the range 91.3 ($\pm 1.1\%$) and 102.2 ($\pm 1.6\%$). The detection limit for single DDT and PCB homologues was 0.005 mg kg^{-1} lipids.

3. Results and discussion

A sharp increase of DDT homologue concentrations was recorded for zebra mussels collected in 2005 compared to 1996 and 2003 (Riva et al., 2008) from both Lake Como and Lake Iseo (Table 1), but not from Lake Maggiore (CIP AIS, 2007). The concentrations recently measured in mussels from Lake Como and Lake Iseo were even higher than those measured in Lake Maggiore during 1996 when a DDT production plant was discharging its wastes into the lake (Riva et al., 2008). The increase in DDT concentrations cannot be explained by the complete overturn occurring in 2005 in Lake Como (GLLC, 2006) and Lake Iseo (Salmaso et al., 2007) because the same event occurred in 2006 in Lake Maggiore and did not cause any concentration peaks (Table 1). Therefore, it is reasonable to think that the causes of the increase in concentrations

Table 1

POPs compounds (mg kg^{-1} lipids) in *Dreissena polymorpha*. In brackets standard deviation of five analysed samples is reported

	ΣDDTs			PCBs	
	Lake Iseo	Lake Como	Lake Maggiore	Lake Iseo	Lake Como
1996	0.10*	0.11*	1.1–3.0**	1.07*	0.32*
2003	0.15*	0.20*	0.7–1.4**	1.81*	1.20*
2005	28.86 (15.07)	28.30	1.1–1.9**	1.16 (0.55)	1.05
2006	6.61	–	0.7–1.0**	0.81	–
2007	11.18	6.11	–	0.70	0.42

Literature data:

* Riva et al. (2008);

** CIP AIS (2007).

observed in Como and Iseo lakes in 2005 must be due to factors other than the overturn of the lakes.

As a matter of fact, the other substantial difference between the lakes is that Como and Iseo lakes are mainly fed by glaciers, whereas meltwater input from glaciers is quite irrelevant for Lake Maggiore. This is why we focused our attention on glaciers as a possible secondary pollution source for these lakes.

As DDT formulations were used in large amounts for fruit-tree pest control in Valtellina (Adda River Valley) and Val Camonica (Oglio River Valley) (Fig. 1) from the 1950s to the end of the 1970s (ISTAT, 1963–1980) before their ban in 1978 (D.M. 11-10-1978), it is very likely that DDT and its metabolites were trapped in glaciers during this period of agricultural use and then released in recent years as a consequence of the glacier retreat. In fact, the Disgrazia, Bernina, and Ortles-Cevedale glaciers in the basin of Lake Como and the Adamello group of glaciers in the basin of Lake Iseo (Fig. 1) increased their volume between 1965 and 1980, corresponding to the period of use of DDT applications for pest control, and then started to retreat until reaching a volume in 2005 that was lower than that measured in 1965 (Rovelli, 2008).

The pollutants released by melting ice might be retained by the complex system of natural and artificial high-altitude basins, but an abrupt input of pollutants could have occurred after the summer of 2003 when a prolonged period of high temperatures caused the melting of a big layer of ice (in the Lombard Alps 3–4 m at 2800 m a.s.l.; Smiraglia and Diolaiuti, 2008), and large volumes of water were released from Alpine basins into the lakes with the effect of counterbalancing the drought caused by the scarcity of rain.

We therefore think that the peak levels of *pp'*DDT and its metabolites in the biota of the two main glacier-fed southern Alpine lakes is due to climatic conditions that caused the release of pollutants previously trapped in the ice.

The pollutant input occurred as a consequence of the recent abrupt ice melting and even caused a change in metabolite levels (Fig. 2): *pp'*DDE prevailed in tissues of *D. polymorpha* in 1996, whereas the main compound in 2005 was *pp'*DDD. The parent compound gradually decreased from 1996 to 2007, when *pp'*DDE again became the main component. The occurrence of *pp'*DDD is usually attributed to the degradation of the parent compound in anoxic conditions (Aislabie et al., 1997), which should have occurred in the permafrost and/or littoral lake sediments. This metabolite was never found above the detection limit in the glacial stream fed by the glacier meltwater (Villa et al., 2006). These secondary pollution sources should also be responsible for the changes of DDT homologue distribution observed in 2005. The histopathological evaluation of ovaries identified many mussels with oocyte degeneration throughout the 2005–2006 period (Fig. 3). As these specimens were about 2–3 years old, they could have been exposed to the peak of DDT homologue concentrations probably occurred in the summer of 2003. Despite the constant presence of high numbers of mussels with degenerating oocytes during

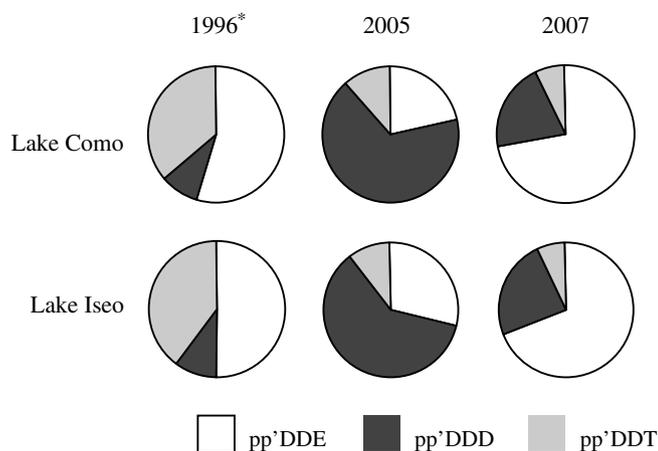


Fig. 2. DDTs distribution (%) in *Dreissena polymorpha* tissues in Lake Como and Lake Iseo (Literature data: ^{*}Binelli and Provini, 2003).

the entire 2005–2006 period, the most affected fields were observed in the late-winter/early-spring months. This is in good agreement with previous studies that identified the developing ovary as the main affected developmental stage (Mantecca et al., 2003; Binelli et al., 2004). The largest ODI differences between 2001/2002 and 2005/2006 were found in April when the ODI measured in 2006 was 14 times higher than that of 2002. We need to emphasize the high level of contamination by DDTs in zebra mussels living in Lake Iseo during 2005, which was around 700 times higher than that measured in 1996 (Riva et al., 2008) in a fairly unpolluted area of Lake Garda (Fig. 1) and up to 15 times higher than those that caused serious effects on oocytes in mussels from Lake Maggiore (Binelli et al., 2001; Mantecca et al., 2003). The endocrine-disrupting properties of DDTs (Keith, 1997; Turusov et al., 2002) would suggest that these compounds are the main cause of the pathologies observed, but it is difficult to discriminate between isomers and metabolites, whose percentages changes from year to year and whose effects might be different. It is well-known that *pp'*DDE is an antiandrogenic compound while *op'*DDT was found to determine pseudo-estrogenic effects on several organisms (Kelce et al., 1995).

Organochlorine compound concentrations were also measured in the muscle of a commercial fish (Table 2). Landlocked shad (*Alosa fallax lacustris*) collected from Lake Como during 2005, when

Table 2

POPs compounds in *Alosa fallax lacustris* (mg kg⁻¹ lipids) in Lake Como and for the year 2007 also in Lake Iseo

	Basin/lake	Lipids (%)	<i>pp'</i> DDE	<i>pp'</i> DDD	<i>pp'</i> DDT	ΣDDTs	PCBs
1985 [*]	Como branch	–	–	–	–	1.97	2.33
2005 ^{**}	Como branch	2.17	4.43	0.72	0.12	5.27	1.65
2006	Como branch	8.43	0.51	0.26	0.24	1.01	1.05
2007	Como branch	11.49	0.58	0.13	0.13	0.84	1.24
	Lecco branch	12.97	0.44	0.08	0.09	0.61	0.56
	Lake Iseo	12.54	0.43	0.07	0.07	0.57	2.00

Literature data:

^{*} Cantoni et al. (1985);

^{**} Volta et al. (2006) for DDTs.

DDT residue concentrations peaked in zebra mussel tissues, showed DDT levels of 0.12 mg kg⁻¹ (w.w.) and by far exceeded the Italian limit for human consumption (0.05 mg kg⁻¹ w.w. for fish with a lipid content lower than 10%; O.M. 18-7-1990). However, DDT compound concentrations in fish were lower than concentrations in mussels, which occupy a lower trophic level because they are filter-feeding organisms (Tables 1 and 2). The phytoplankton biomass, which is very abundant in these two mesotrophic/eutrophic lakes in late summer (Buzzi, 2002; Garibaldi et al., 2003) when most of the meltwater enters the lakes, should have adsorbed most of the DDT homologues dissolved in the photic layer of the pelagic environment, where *A. fallax lacustris* lives, transporting them into the deep sediments (Larsson et al., 1992). As the hypolimnium is separated from the rest of the water column due to thermal stratification, the pollutants could not be recycled. This non-equilibrium condition for POPs has already been observed in eutrophic environments (Dachs et al., 2000). Conversely, particles settled in the littoral area, where zebra mussels live, could be re-suspended by coastal streams, allowing a longer period of time for mussels to bioaccumulate pollutants from both coastal water and re-suspended particles.

PCB concentrations in zebra mussel tissues did not show a dramatic increase similar to that of DDT in 2005. After a moderate rise in 2003 and 2005, PCB concentrations decreased again to levels comparable to those measured in 1996 (Table 1). Moreover, PCB concentrations were higher in fish (Table 2) compared to mussels (Table 1), as expected for compounds whose concentration is rather homogeneous in littoral and pelagic environments. These findings indicate that input of PCB due to glacier melting was lower than that of DDT homologues. Actually, PCBs were used in Italy only in closed systems and, as in other parts of the world, they en-

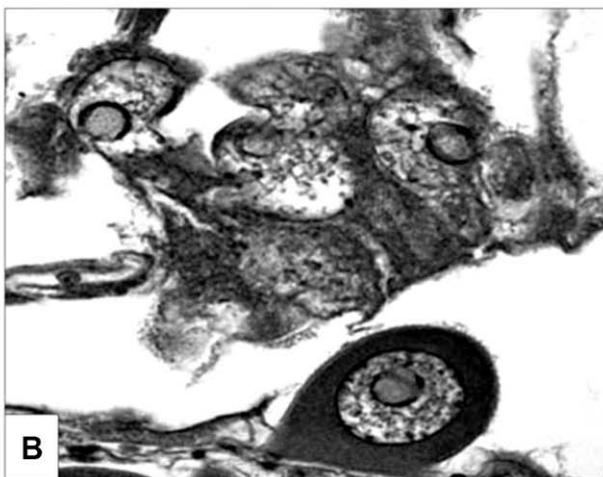
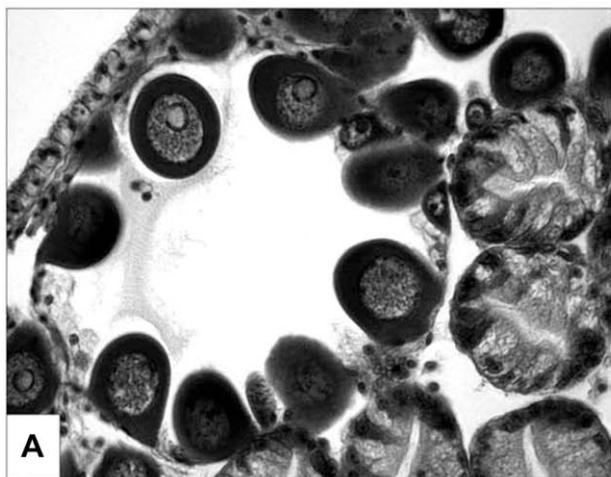


Fig. 3. Control (A) and degenerating (B) oocytes of *Dreissena polymorpha* collected in Lake Iseo in 2001 and 2006, respectively.

tered the environment accidentally or as a consequence of release from polluted soils and landfills, or from waste discharged by treatment plants (Desai et al., 2007). Since tourism and agriculture are the prevailing activities in the Alpine and sub-alpine area, it is very likely that local sources of PCB pollution were not as significant as those of DDT, which was used for fruit-tree pest control in an area very close to the glaciers' zone.

4. Conclusions

In the light of the results of this study, there should be concern for aquatic life and human health in Italy and many other regions of the world in which high-altitude glaciers are present near areas where pesticides were used in large amounts during previous decades. Moreover, since high-mountain glacial lakes and artificial basins might work as sinks for pollutants released as a consequence of glacier melting, these environments should be considered potential secondary pollution sources for POPs. Most of the pollutants are probably still stored in the sediments of high-altitude natural and artificial basins and can reach other water bodies as a result of floods caused by heavy rains or maintenance operations on artificial basins used to produce hydroelectric power.

Further studies are therefore needed to assess the consequences of the glaciers' retreats in regard to the release of several classes of micropollutants trapped in the ice, which in the case of Lake Como and Lake Iseo are a concern due to their potential impact on aquatic life and human health.

Acknowledgements

The authors thank Prof. Derek Muir and Prof. Ing. Marino Gatto for their kind help in reviewing the manuscript and for valuable suggestions. Thanks are also due to Dr. Carlo Romanò (Como Province, Fishery Office) for providing fish specimens.

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