J. Limnol., 2016; 75(s2): 107-119
DOI: 10.4081/jlimnol.2016.1232

ORIGINAL ARTICLE

Long-term persistence of sedimentary copper contamination in Lake Orta: potential environmental risks 20 years after liming

Davide A.L. VIGNATI,[1*§] Roberta BETTINETTI,[2] Aldo MARCHETTO[3]

[1]National Research Council, Water Research Institute, UOS Brugherio, Via del Mulino 19, 20861 Brugherio (MB); [2]Department of Theoretical and Applied Sciences, Insubria University, Via Valleggio 11, 22100 Como; [3]National Research Council, Institute of Ecosystem Study, L. Tonolli 50, 28922 Verbania- Pallanza, Italy.
*Corresponding author: david-anselmo.vignati@univ-lorraine.fr
[§]Current address: CNRS and Université de Lorraine, Laboratoire Interdisciplinaire des Environnements Continentaux UMR7360, Metz, F-57070, France.

ABSTRACT

Lake Orta, northern Italy, has suffered from severe copper pollution and human-induced acidification between the 1920s and the 1990s because of discharges from a rayon factory and electroplating industries located in its drainage basin. Following liming operations in the late 1980s, the chemical quality of the water column has been restored and signs of, still ongoing, biological recovery observed. Two sediment cores (07/1A and 07/1B) were collected close to the main historical Cu discharge (in the southern part of the lake) and one (07/2A) in the central part of the lake. Cores 07/1A and 07/2A were analyzed for Cu content, Loss on Ignition (LOI) and water content. Diatom profiles (cores 07/1B and 07/2A) were used to confirm sediment dating and to identify alterations in the sediment sequence because, among other taxonomic groups, diatom assemblages were strongly affected by lake pollution and recovery. Copper concentrations in the uppermost layers of sediment cores (2007 AD) were above 1,000 mg kg^{-1} and around 500 mg kg^{-1} in cores 07/1A and 07/2A, respectively. These values were lower than the corresponding maximum ones of approx. 3500 and 4500 mg kg^{-1} recorded at the end of the 1950s at both locations, but remained 10 to 40-fold higher than the pre-industrial levels of about 40 mg kg^{-1} measured in both cores. Diatom profiles revealed a continuous sedimentation pattern in core 07/1A, but highlighted interruptions in sediment deposition in core 07/2A. Copper levels in 2007 were still comparable to or higher than concentrations reported to cause adverse effect on the survival, growth and reproduction of sediment-ingesting organisms. A critical comparison of measured concentrations with relevant ecotoxicological values suggests that particular attention should be given to understand the effects of dietary ingestion of sedimentary Cu which, unlike in previous ecotoxicological studies, may now represent the main route of exposure to Cu for sediment-ingesting benthic organisms.

Key words: Lake Orta; copper contamination; fossil diatoms; paleoecotoxicology; legacy pollution.

Received: April 2015. Accepted: October 2015.

INTRODUCTION

Lake bottom sediments are faithful temporal archives of events and processes that occur in the overlying water column and in the drainage basin. In particular, sediments can show the footprints of past chemical discharges long after the corresponding inputs to the overlying water column have been stopped or reduced to environmentally acceptable levels (Guilizzoni *et al.*, 2012). Because many organisms live in or are in contact with the bottom, sediment may act as an important route of exposure to aquatic organisms. Copper is an essential trace element whose geochemical cycle has been severely disrupted by human uses since Roman times (Hong *et al.*, 1996) and, even nowadays, often occurs in sediments at levels high enough to alter organisms' health or ecosystem functioning (Gardham *et al.*, 2015).

In Italy, a dramatic case of copper contamination has been well documented starting from the early 20th century (Bonacina and Baudo, 2001). Lake Orta, a deep southern Alpine lake, has been heavily polluted by copper and ammonium sulfates through a direct input from an industrial rayon factory manufacturing since 1926. The increase in the concentrations of heavy metals led to a rapid disappearance of many water life forms, and dramatic change in the species composition of planktonic communities including *e.g.*, cladocerans (Manca and Comoli, 1995) and diatom assemblages (Ruggiu *et al.*, 1998). A treatment plant for copper recovery was adopted by the factory in 1958. However, this improvement was counterbalanced by a number of electrogalvanic activities that, over the same period, developed in the lake's watershed with direct discharges into its basin. Moreover, lake water had a low natural buffering capacity due to the watershed geology: mainly gneiss, micaschists and granites. As a consequence, starting from the 1960s, the biochemical oxidation of ammonium acidified the lake water down to the lowest pH value of 3.8 units that was reached in 1985. To recover the lake from acidification, a liming intervention was carried out in 1989/90 by spreading a suspension of finely pow-

pagepress

dered natural limestone on the water surface. Post-liming investigations showed a recovery of the main chemical characteristics of the water column (mainly pH and ammonium) associated with a slower restoration of the trophic web. Nonetheless, the vulnerability and the instability of the lake were pointed out by the last survey including chemical and biological information carried out in 2001 (Bonacina, 2001). For example, nowadays the zooplankton community is more structured and diversified than in the past, but it seems that still remains substantially different from the original one (Piscia *et al.*, 2016).

The bottom environment was also strongly affected by the anthropogenic discharges and sediments quickly became highly enriched in copper after the opening of the rayon factory. Despite several interventions to reduce Cu releases from the rayon factory (Corbella *et al.*, 1958), other authors have documented the persistence of sediment contamination in the 1980s (Provini *et al.*, 1987) and in the late 1990s (Baudo and Beltrami, 2001). Ecotoxicological studies confirmed the adverse effect of sediment contamination on various target organisms including invertebrates, plants, and bacteria (Guzzella *et al.*, 1996; Rossi and Beltrami, 1998; Burton *et al.*, 2001). Yet, all ecotoxicological evaluations focused on risk to pelagic or epibenthic organisms without considering possible effects to sediment-ingesting organisms such as chironomids, oligochaetes or tubificids. Using spiked artificial sediments, Roman *et al.* (2007) showed that copper concentrations causing a 50% reduction in survival or growth of *Chironomus riparius*, *Lumbriculus variegatus* and *Tubifex tubifex* ranged between 211 and 327 mg kg^{-1} dry weight (d.w.) for survival and 126 and 150 mg kg^{-1} d.w. for growth. Pasteris *et al.* (2003) determined values between 81 and 107 mg kg^{-1} d.w. for the number of youngs produced by individuals of *T. tubifex* exposed for 28 days to a Cu-spiked reconstructed sediments. In the case of spiked natural sediments, Méndez-Fernández et al. (2013) reported similar results for growth and survival of *T. tubifex*, while Cairns et al. (1984) observed effects on the survival of *C. tentans* only at concentrations between 685 and 3100 mg kg^{-1}. Copper concentrations measured in Lake Orta sediments (see Baudo and Beltrami, 2001 for a review) are often in the range reported to be potentially toxic to sediment-dwelling organisms; also when compared with toxic concentrations in spiked natural sediments. Although the actual Cu toxicity will depend on sediments characteristics (Simpson, 2005; Campana *et al.*, 2013), a comparison of the recent state of Cu contamination in Lake Orta with available information on potentially toxic sediment concentrations would be instructive.

Here, we present a detailed chronology of Cu contamination and diatom profiles from the pre-industrial period until 2007 at 2 sites of Lake Orta located at a different distance from known pollution sources. Diatom profiles, compared with the known history of lake phytoplankton and with a previous study (Ruggiu *et al.*, 1998), were used to confirm sediment dating and to identify the presence of irregularities in sediment deposition. Specifically, the present work will examine if sediment contamination decreased over the decade 1997–2007, if liming has left a clear mark on Cu sediment concentrations and if Cu concentrations are similar in the southern and central part of the basin. Furthermore, data on the most recent levels of Cu in bed sediments will be assessed for the potential of residual Cu contamination to cause toxicity to sediment-dwelling organism.

METHODS

Three sediment cores were collected in October 2007 with a gravity corer (inner diameter 6.3 cm) in Lake Orta. Cores 07/1A (52.5 cm in length) and its parallel core 07/1B (46.5 cm) were sampled in the southern basin of the lake at a depth of 31 m in an undisturbed area (45° 46’ 35” N, 8° 24’ 56” E) not far from the rayon factory (Fig. 1). Core 07/2A (61.5 cm) was collected in the deepest part (>100 m) of the central part of the basin (45° 48’ 44” N, 8° 23’ 35” E;

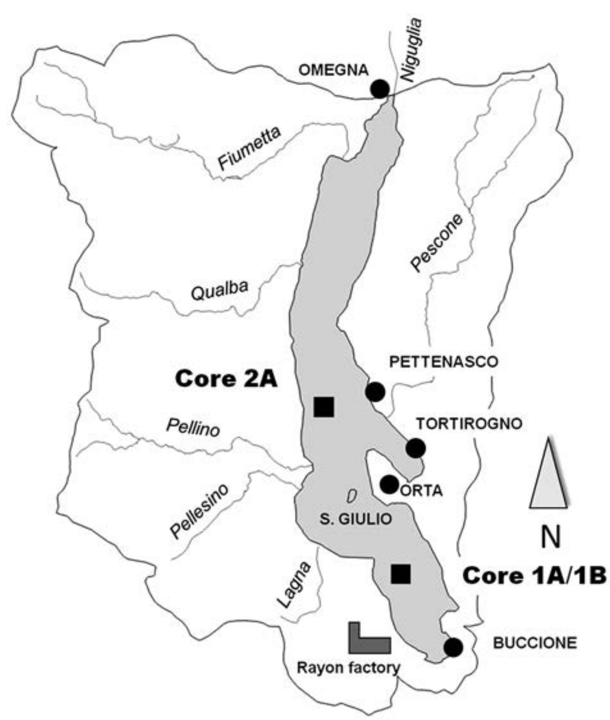

Fig. 1. Map of Lake Orta with indication of the sampling points (black squares) for this study and of the position of the rayon factory at the south end of the lake. Major towns of interest (Omegna, Pettenasco, Orta, Tortirogno, and Buccione) are also shown as black circles. Among tributaries, note the position of river Lagna whose basin is characterized by the presence of metal-releasing activites (*i.e.* electroplating industries).

Fig. 1). All cores were stored in the dark at 4° C. Once opened longitudinally, cores were visually inspected and, because they comprised mainly clastic and homogeneous materials, they were subsampled into 1 cm slices. Radiometric dating was performed on cores 07/1A and 07/2A by P. Appleby, Liverpool University, Environmental Radioactivity Laboratory, through the analyses of ^{210}Pb and ^{137}Cs radionuclides by direct gamma assay using Ortec HPGe GWL series well-type coaxial low-background intrinsic germanium detectors (Appleby *et al.*, 1986; Piscia *et al.*, 2012). Core 07/1B was only used for diatom analysis and was dated after core-to-core stratigraphic correlation. An evident change in sediment color at the depth of 5.5 cm was correlated with a very similar change in core 07/1A at the depth of 4 cm.

In core 07/1A, total Cu content was measured every cm in the first upper 6 cm and then at 10, 20, 40, and 51.5 cm. In core 07/2A, total Cu content was measured in all sections of the uppermost 20 cm, every 5 cm from 25 to 50 cm and at 60.5 cm corresponding to the core bottom. About 50 mg dry mass of each section were weighed into Teflon bombs and mineralized by microwave assisted digestion (Milestone, MLS 1200) with 3 mL of concentrated nitric acid (65% v/v, VWR, Suprapur) and 1 mL of ultrapure water (milliQ, Bernstein, Nanopure). Procedural blanks and reference material S7 from the AQUACON project *CHECK* (certified Cu content 100±2 mg kg^{-1}) were included in each digestion run. After each digestion, Teflon bombs were cleaned three times with 50% v/v analytical grade nitric acid while heating in the microwave oven for 5 minutes at 500 W.

The digested solutions were transferred to pre-conditioned (24 h in 10% v/v HNO_3 followed by three rinses in ultrapure water) polypropylene bottles and diluted to 25 mL with ultrapure water. Total copper concentration was measured by graphite furnace Atomic Absorption Spectroscopy using a Perkin Elmer AAnalyst600 with Zeeman background correction. Following manufacturer instructions, 5 µL of a matrix modifier (Pd+$Mg(NO_3)_2$) were injected into the graphite tube along with a 20 µL aliquot of each sample. On each sediment sample analyzed for Cu, water content and organic matter content as LOI (Loss on Ignition; Dean, 1974) were analyzed in duplicate by weight loss after ignition at 60°C for two days and 550°C for six h.

Diatoms were prepared from cores 07/1B and 07/2A using standard H_2O_2-HCl digestion (Renberg, 1990) and mounted in Naphrax. On each slide, a minimum of 250 diatom valves were enumerated by light microscopy under phase contrast at 1000x (Zeiss Axioplan).

RESULTS

Quality control

Blank values for microwave assisted digestions ranged from below detection limit (0.2 µg L^{-1}) to 7.4 µg L^{-1}; the latter figure indicating some residual contamination after the digestion of samples with high Cu content. With one exception (blank signal equal to 8.4% of the sample signal), blank values were below 5% of sample signals (Supplementary Tab. 1). Analytical results (in µg L^{-1}) were corrected for the corresponding blank values before conversion to mass-based concentrations (mg kg^{-1}). Concentrations for the reference material S7 ranged from 86.34 to 123.8 mg kg^{-1} d.w. with a mean recovery (n=9) of 94±12 % (Supplementary Tab. 2).

Copper pollution, loss on ignition and water content

A detailed chronology of core 07/1A has been described by Piscia *et al.* (2012) who report accumulation rates between 0.0078 g cm^{-2} yr^{-1} (from 5.5 cm to 3.5 cm) and 0.0144 g cm^{-2} yr^{-1} at 1.5 cm. In the same core, mean (volumetric) sedimentation rates increased from 0.041 cm year^{-1} at 4.5 cm to 0.083 cm year^{-1} at 0.5 cm. A sedimentation rate of 0.033 cm year^{-1} is reported at 5.5 cm. Using the Constant Rates of Supply (CRS) ^{210}Pb dating model, Piscia *et al.* (2012) propose the following radiometric dates for core ORTA 07/1A: 2001 (0.5 cm), 1989 (1.5 cm), 1975 (2.5 cm), 1957 (3.5 cm), 1935 (4.5 cm), and 1909 (5.5 cm). Extrapolating the sedimentation rate of 0.033 cm year^{-1} to the core bottom dates the deepest section of the core at around 500 AD.

Copper concentrations were low and stable (from 34 to 52 mg kg^{-1} d.w.) between the core bottom and 10.5 cm (*ca* 1750 AD). Contamination by Cu started in 1927 (being first visible at 5.5 cm), increased with time and peaked at 3.5 cm before declining again, albeit to levels about 30-fold higher than those observed at the core bottom (Fig. 2a). Water content decreased from 89% w.w. at the top of the core to 69% w.w. at 25.5 cm and remained around 70% w.w. down to the core bottom. Percent LOI content followed a similar trend (Supplementary Tab. 3), with values decreasing from 25% d.w. at the top of the core to about 12% d.w. at 25.5 cm and then stabilizing around 15% d.w. in the lowermost part of the core. For core 07/1A, percent LOI and water content were significantly correlated (Spearman R, $P < 0.01$, n=10) and Cu concentrations also correlated with the two variables ($P < 0.05$). No regression could be established between Cu concentrations and the other variables over the entire length of the core. However, Cu and percent LOI increased proportionally between 5.5 cm (1909 AD) and 3.5 cm (1957 AD); *i.e.* the period between the entry into service of the rayon factory and the installation of a wastewater treatment plant to treat its effluent in 1958.

In core 07/2A, sediment accumulation rate ranged from a maximum of 0.27 g cm^{-2} y^{-1} to a minimum of 0.029 g cm^{-2} y^{-1} in the upper 13 cm-section of core ORTA 07/2A (Piscia *et al.*, 2016). In the deepest parts of the core

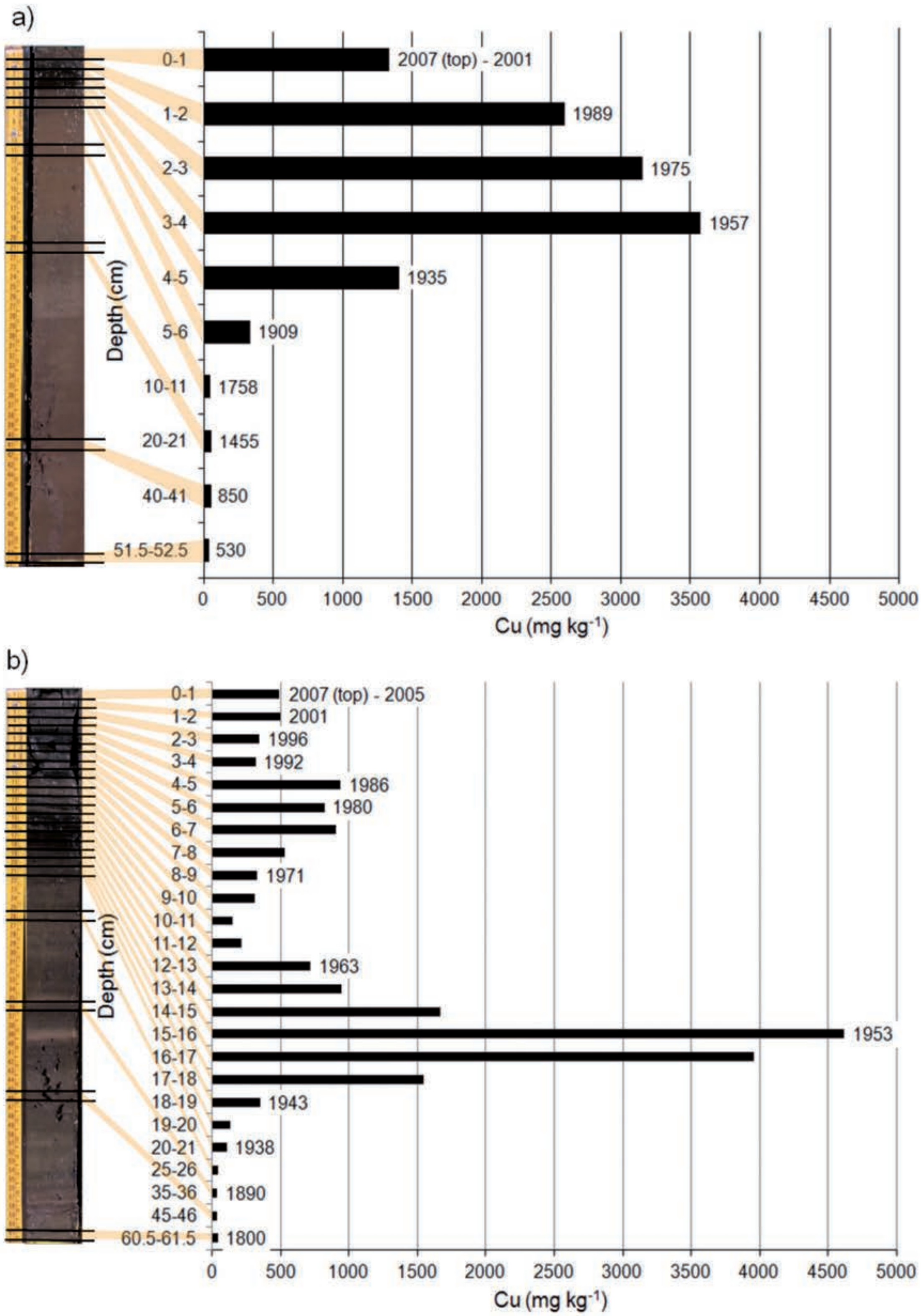

Fig. 2. Concentration profiles (mg kg^{-1}) of total copper in sediment cores collected in 2007 in: a) the southern part of lake Orta (core ORTA 07/1A); and b) the central part of lake ORTA (core ORTA 07/2A). Labels on the right-hand side of the bars indicate the estimated age (calendar years) of the corresponding sediment section. Pictures of the corresponding cores are shown on the right of each graph. Note that, in core ORTA 07/2A, the sedimentary record has been altered at a depth of about 14 cm (see text for details).

(13-61.5 cm), sedimentation rate was around 0.29 cm y^{-1} and unsupported ^{210}Pb concentration declined exponentially with depth. Extrapolating the sedimentation rate of 0.29 cm yr^{-1} to the core bottom, the base of the core should represent *circa* 1800 AD. ^{210}Pb dates calculated by CRS model place 1986 at depth of 4.5 cm and 1963 at 12–13 cm, in good agreement with results of ^{137}Cs record (Piscia *et al.*, 2016). Based on the available detailed chronology (Fig. 2b), deposition rates ranged between 0.17 and 0.25 cm $year^{-1}$ between 1980 and 2007 and were somewhat higher (0.27-0.50 cm $year^{-1}$) between 1800 and 1980 (Supplementary Tab. 4).

Copper concentrations in the deep sections of core ORTA 07/2A ranged between 31 and 43 mg kg^{-1} d.w. and were comparable to the levels observed in core ORTA 07/1A for sections not impacted by anthropogenic activity. A moderate increase of Cu concentration (up to about 350 mg kg^{-1} d.w.) occurred between 21 and 18 cm; while between 18 and 16 cm Cu levels raised abruptly to a maximum of about 4600 mg kg^{-1} d.w. Starting from the depth of 15 cm to the top of the core, the temporal evolution of Cu contamination did not follow a uniform trend suggesting that sedimentation was not homogeneous and material of different origin was reworked in this core. Copper concentrations decreased from 4,600 mg kg^{-1} at 15 cm to 150 mg kg^{-1} at 10 cm, but increased again to about 950 mg kg^{-1} between 10 and 4 cm. In the topmost 4 cm of the core, Cu concentrations fluctuated between 300 and 500 mg kg^{-1}.

Water content and percent LOI also did not show a regular profile along core ORTA 07/2A (Supplementary Tab. 3). Water content increased from ca*.* 65% in the uppermost 4 cm to a maximum of ca*.* 80% between 4 and 8 cm. Then it decreased again to 60% between 8 and 14 cm section, and increased to 80% between 14 and 21 cm. Finally, in the deepest section (>21 cm) it was around 70%, with two relative minima (at 25-26 and 60.5-61.5 cm). LOI was relatively high (ca*.* 20% of the dry weight) between 4 and 8 cm and between 14 and 18 cm, but decreased to ca*.* 15% between 8 and 14 cm. In core 07/2A, Cu concentrations and percent LOI were significantly correlated (Spearman R, $P<0.01$, $n=25$).

Diatom assemblages

In core ORTA 07/1B, diatom profiles (Fig. 3a) fit well with a previously published core profile (Ruggiu *et al.*, 1998) and with the known effects of water pollution on the diatom communites of Lake Orta, indicating regular sediment accumulation. In particular, in the lower part of the core, diatom assemblages were dominated by small centrics (*Cyclotella comensis, Discostella stelligera* and *Handmannia bodanica*) typical of deep oligotrophic lakes with stable stratification (Fig. 3a).

After the beginning of pollution, small centric diatoms declined and the diatom community was dominated by *Achnanthidium minutissimum* and *Fragilaria nanoides* (identified as *Synedra acus* by Tonolli 1961, *Synedra tenera* by Ruggiu *et al.*, 1998) (Fig. 3a). The former is a metal-tolerant taxon, generally reported as benthic (Potapova and Hamilton, 2007) and circumneutral (van Dam *et al.*, 1994). In the recent period, the core profile showed an increase in the relative abundance of *Asterionella formosa*, *C. comensis* and *Fragilaria crotonensis*.

In core ORTA 2007/2A (collected in a deeper area with steeper slopes), the section dominated by *A. minutissimum* and *F. nanoides* (between 4 and 22 cm) and representing the period of lake pollution, is split into two parts by another section (8-14 cm) which contains a large proportion of *C. comensis* (Fig 3b). It seems reasonable that the latter section represents older sediment material carried towards the deepest part of lake bottom by a slump.

DISCUSSION

Temporal evolution of copper contamination

In the southern part of Lake Orta (core 07/1A), Cu sediment concentrations follow well the known pollution history of the lake. The first increase in Cu level at 5.5 cm is dated at 1927 AD, in excellent agreement with the beginning of the activity of the rayon factory on the southern shore of the lake. The peak concentration (about 3500 mg kg^{-1}) at 3.5 cm corresponds to 1958 AD, just before the introduction of water treatment processes at the factory (Baudo and Beltrami, 2001). From 1958 to 2007, Cu concentrations declined because of the water treatment plant and its subsequent improvement in 1981 (Baudo and Beltrami, 2001). The decrease in Cu concentrations has apparently been faster between 1.5 and 0.5 cm than between 3.5 and 1.5 cm. The halving of Cu concentration between 1989 (1.5 cm) and 2001-2007 (0.5 cm) may reflect, at least partly, the effect of liming operations that were carried out in 1989 as it is explained below.

Calderoni *et al.* (1993) report that about 85% of the $CaCO_3$ added during liming operations (*i.e.*, 12,580 tons out of 14,800) eventually dissolved in the water column. Considering the solubility of $CaCO_3$ at a pH of 6.5 (value after liming), precipitation of part of the remaining 15% of the added $CaCO_3$ (*i.e.*, 2220 tons) could have been enough to contribute to reducing Cu levels in contaminated bottom sediments. Our calculations (see supporting information for the details) estimate that 1,477 tons of $CaCO_3$ could have precipitated following liming operations. Assuming a Cu adsorption capacity of 0.015 kg of copper per ton of $CaCO_3$ (Aziz *et al*., 2008), the precipitated $CaCO_3$ could potentially have adsorbed a total of 22.15 kg of copper. This figure must be compared with a dissolved concentration of about 40 µg L^{-1} Cu in the water column during the late 1980s (Camusso *et al.*, 1989); resulting in a stock of dissolved Cu around 51.5 kg for the entire lake.

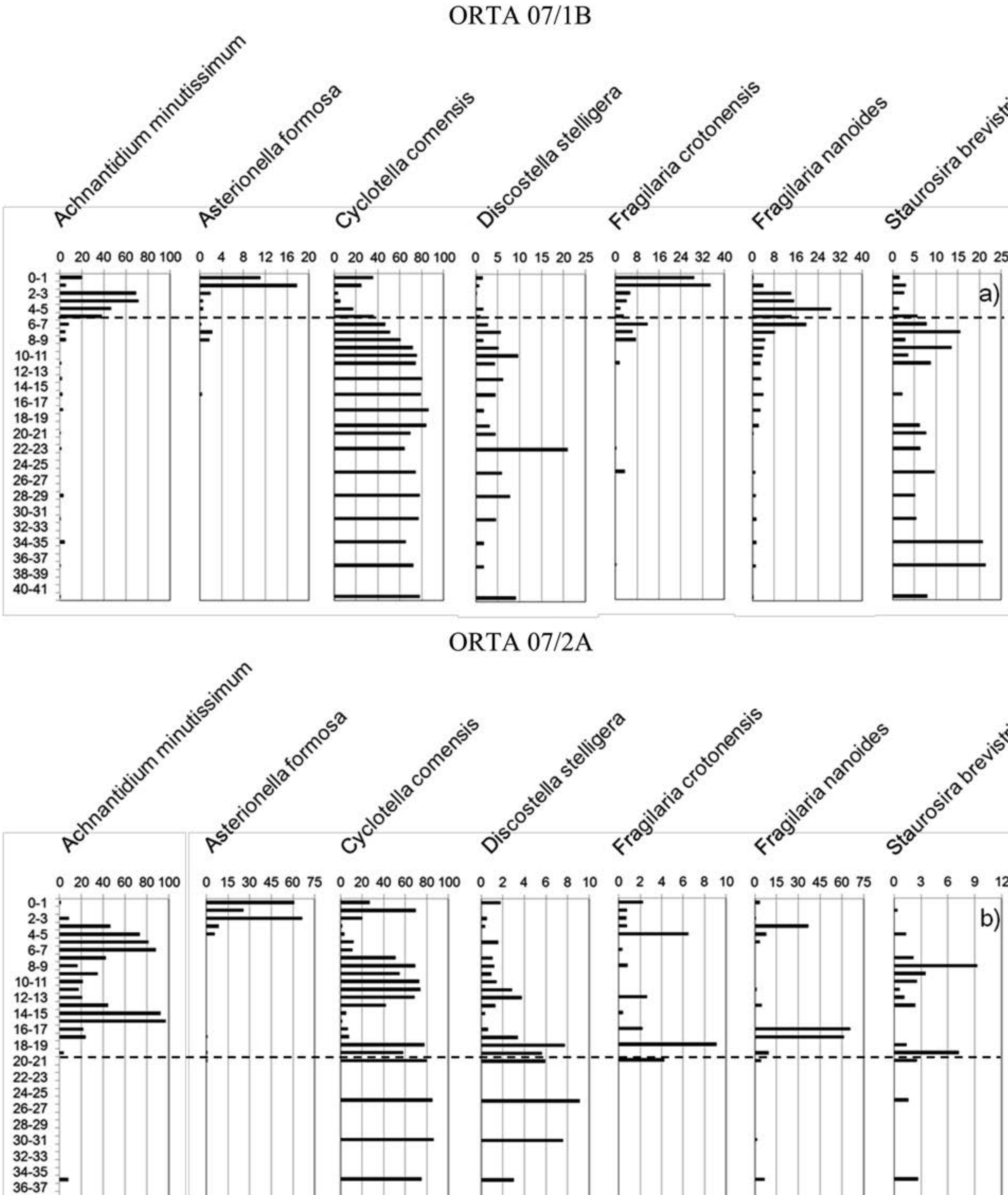

Fig. 3. Profiles of diatom percent abundances along cores ORTA 07/1B (a) and ORTA 07/2A (b). Abrupt decrease in the abundance of *Cyclotella comensis* and increase of the metal tolerant *Achnanthidium minutissimum* are observed after the onset of Cu pollution in the second half of the 1920s (dashed horizontal lines). Note that, in core ORTA 07/2A, the sedimentary record has been altered at a depth of about 14 cm (see text for details).

In practice, the precipitating $CaCO_3$ had the potential to adsorb about half of the dissolved Cu stock present in Lake Orta in pre-liming conditions. The other factor to consider is the contribution of the settling $CaCO_3$ particles to the total sedimentation flux in Lake Orta. Considering a lake area of 18.2 km^2 (and neglecting that liming was performed only on 2/3 of the lake surface; Camusso *et al.*, 1989), we obtain: 1477 tons of precipitated $CaCO_3$/18.2×10^{-6} m^2 (surface of Lake Orta)=81.15 g m^2 of precipitated material. This figure is comparable with the annual particulate flux of 243 g m^{-2} year^{-1} reported by Camusso *et al.* (1989). These calculations support the hypothesis that deposition of $CaCO_3$ with relatively low Cu content could have contributed to decrease Cu levels in sediments after liming. In favour of the same hypothesis, Baudo and Beltrami (2001) report that the Ca content of the upper 2-cm of sediment grab samples collected in 1996 (post-liming) showed an increased content of Ca compared with 1985 (pre-liming) samples.

After liming, the extremely slow sedimentation rate in this part of the lake results in contaminated sediments being covered by unpolluted material at a very slow pace and, hence, persisting contamination. We notice that results from core 07/1A do not agree with the post-liming increase in Cu concentration reported by Baudo and Beltrami (2001) in the southern part of Lake Orta. This discrepancy may partly arise from methodological issues. Baudo and Beltrami (2001) could not observe Cu enrichment when comparing pre-liming (1985) Ponar grab samples (collecting the top 10-15 cm of sediments) with post-liming (1992) grab samples or 10-cm core sections. An increase in sedimentary Cu levels between pre- (1985) and post-liming period was observed for sediment samples collected in 1996 when only the topmost 2-cm of grab samples were retained for analysis (Baudo and Beltrami, 2001). According to the results of the present study, a 10-cm thick sediment section in the southern part of Lake Orta could cover a period of over 200 years, thus representing a mix of polluted and unpolluted sediments (Fig. 2a). In such situation, the Cu content of homogenised grab or core samples collected in 1985 and 1992 could have been partially lowered by the presence of material not impacted by anthropogenic Cu pollution, thus leading to the observation of Cu enrichment in the 1985 *vs* 1996 comparison. On the other hand, Baudo and Beltrami (2001) also examined the distribution of Cu contamination in sediments over the whole Lake Orta and reported completely different Cu patterns for the two periods. Most notably, the highest Cu concentrations were recorded in the northern part of the lake in 1985 and in the southern part in 1996. Finally, Guilizzoni et al. (2001) reported variable mean sedimentation rates in the southern part of the basin. All considered, while the use of homogenised sediment samples can be (partly) useful for ecotoxicological purposes considering that biota can penetrate to different depth into the sediments, the study of Cu levels should be performed with the best possible resolution to follow the evolution of contamination.

While the exact age of the bottom sediment slice (51.5-52.5 cm) of core 07/1A cannot be established with certainty, Cu levels measured between 51.5 and 10 cm are similar and likely correspond to the background concentration of the sediment. Levels of Cu at the core bottom are comparable with those measured in old (1100-1300 AD) and recent (2006 AD) sediments in the nearby Lake Maggiore (Vignati and Guilizzoni, 2011), further supporting the hypothesis that background Cu levels in Lake Orta are around 30-50 mg kg^{-1} d.w.

The pollution history of the lake is also faithfully reflected in the diatom assemblages present in core 07/1B. After the beginning of pollution, the rapid decline of *C. comensis*, typical of oligotrophic lakes, was accompanied by the increase of *A. minutissimum* (Fig. 3a) which is a metal-tolerant taxon. In Lake Orta, this taxon is epilithic (van Dam and Mertens, 1990) and epiphytic (Cattaneo, 1992), but it was and is, together with other less important congeneric species, a constant component of the plankton even when water pH was around 4 units (Ruttner, 1959; Vollenweider, 1963, with a taxonomic confirmation by F. Hustedt and by A. Cleve-Euler). In the top section of core 07/1B, the increase in the abundances of *A. formosa*, *C. comensis* and *F. crotenensis* indicates the recovery of Lake Orta towards its original, pre-pollution conditions. While there are no systematic phytoplankton studies in Lake Orta before 1926, the diatom community in the pre-pollution sections of the core is very similar to the one found at that time in neighboring, deep lakes Maggiore (Marchetto *et al.,* 2004), Como and Iseo (Marchetto and Bettinetti, 1995). The species in the upper part of core 07/1B are those typical of present phytoplankton assemblages in similar lakes, such as Lake Maggiore (Marchetto *et al.,* 2004), and may reasonably represent present diatom assemblages in Lake Orta.

In the central and deeper part of the lake, sedimentation is faster than in the southern part and the 60 cm core ORTA 07/2A covers a period of approximately 200 years from 1800 AD to 2007 (Fig. 2b). Copper levels remained between 30 and 40 mg kg^{-1} up to section 25-26 cm, corresponding to ca. 1920. Copper levels started to increase from section 20-21cm (1938 AD) as a consequence of anthropogenic inputs in the southern part of the lake. Peak values of over 4500 mg kg^{-1} were observed in the early 1950s, in good agreement with the known anthropogenic sources of Cu and with the period of maximum contamination in the core 07/1A. After this absolute maximum at the depth of 15.5 cm, Cu concentrations rapidly decreased to about 150 mg kg^{-1} (at 10.5 cm) in the time span of about 15 years (Fig. 2b). While this decrease is compatible with the introduction of effluent treatment at the rayon factory in 1958, the sharp decrease in Cu concentration between 15–16 cm (about 4600 mg kg^{-1}) and 10-11 cm (about 150 mg kg^{-1}) may also reflect sudden inputs of eroded soil material following the floods that interested the lake basin in 1963 and 1965 (Guilizzoni *et al.,* 2001). Two independent proxies support this interpretation: first, in the diatom profile, these sections are rich in *C. comensis* (a species typical of the period before pollution, Fig. 3b) and this pattern is not shared with core 07/1B, nor with previous cores (Ruggiu *et al.,* 1998). Second, a minimum of LOI percentage occurs in this part of the core (Supplementary Tab. 3), suggesting the input of mineral material carried by the floods. Furthermore, the ranges of copper and organic matter percentage in sediments collected in the littoral zone of Lake Orta in 1969 were 210-237 mg kg^{-1} and 1.1-4.9%, respectively (Chiaudani, 1969). Otherwise stated, slumps bringing inputs of littoral sediments to the lake bottom had the potential to dilute contaminated sediments deposited under normal conditions.

Because of the higher sedimentation rate, Cu contamination in this part of the lake has decreased faster than in the southern part, but Cu levels remained around 500 mg kg^{-1} d.w. in 2007; *i.e.* 10-15-fold above background. Furthermore, at the end of the 1950s, several galvanic industries entered into service in the basin of the River Lagna (Fig. 1) and could contribute to Cu discharge in the central part of the lake. Copper input from these activities seems to have offset the water treatment improvement in the rayon factory considering that Cu levels in section 13-14 cm (1960 AD) and 4-5 cm (1986 AD) are comparable. Liming operations have not left a mark of increased Cu deposition in this part of the lake either, in agreement with Baudo and Beltrami (2001) who did not observe post-liming increases in Cu concentrations in the central part. All considerations made with regard to core 07/1A are valid, but the higher sedimentation rates in the central part of the basin make the comparison of homogenized grab samples and top sediment sections of cores less problematic. Finally, Cu levels at the top of core ORTA 07/2A for 2007 were higher than those measured for the 1990s indicating that further monitoring of dated sediment cores is needed to verify the most recent trends. Indeed, results for cores 07/1A and 07/2A compare well with previous studies, suggesting that they are representative of the recent evolution of the basin between the late 1990s and 2007.

In 1985, Baudo *et al.* (1989) collected 57 grab sediment samples covering approximately 30-40 years and concluded that concentrations (observed range 1.88-3598 mg kg^{-1} d.w.) were decreasing compared with earlier studies. Between 1994 and 1997, Beltrami *et al.* (1999) collected cores at several locations including Buccione and Tortirogno (Fig. 1). In the Buccione core (25 cm long), Cu levels ranged from 71 mg kg^{-1} d.w. at the core bottom to 3433 mg kg^{-1} d.w. at 6-8 cm and 865 mg kg^{-1} d.w. at the top of the core. In absolute terms, minimum, maximum and surface values are comparable with those measured in core 07/1A, suggesting that decrease in Cu concentration in the southern part of Lake Orta slowed down or stopped between 1997 and 2007. Cu levels at the bottom of the Tortirogno core were around 30-40 mg kg^{-1} d.w., indicating that sediments between 20 and 12 cm depth were unaffected by Cu anthropogenic pollution. In the same core, maximum and top (1997) Cu concentrations were about 1400 and 1000 mg kg^{-1} d.w., respectively. In this case, a direct comparison with core ORTA 07/2A is not straightforward. In another core collected in the zone of Pettenasco (Fig. 1) at a depth of 120 m (Guilizzoni *et al.*, 2001), maximum and surface (1992 AD) Cu levels were over 3000 mg kg^{-1} d.w. and around 1000 mg kg^{-1} d.w., respectively. The latter value agrees well with section 4-5 cm of core ORTA07/2A, the section being dated at 1986. In general, the sedimentation pattern appears somehow disturbed in the central part of the lake. However, decrease in Cu levels seems to have slowed down also in this zone between 1997 and 2007. Overall, legacy contamination persists; especially in the southern part of the lake because the low input rate of clean material leaves old, contaminated sediments exposed at the interface with the water column.

Differences observed between cores 07/1A and 07/2A mostly seem to originate from disturbances in the sedimentation pattern in the central part of the lake (core 07/2A). It is noteworthy that changes in the Cu profiles of the two cores are in good agreement during the contamination phase, but not after the contamination peak of the late 1950s. During the contamination phase, the increase in Cu sedimentary concentration is slightly delayed in core 07/2A (between 1938 and 1943; Fig. 2b) compared with core 07/1A (between 1909 and 1935; Fig. 2a) and may simply reflect the distance of the sites from the main contamination sources at that time. Peak contamination values were reached in the mid 1950s at both sites and were even higher at 07/2A possibly because finer sediments were trapped in the central basin. As already mentioned, the sedimentary record of core 07/2A was then perturbed either by the occurrence of slumps bringing littoral sediments with low Cu content to the lake bottom or by inputs of uncontaminated material following the historical floods of 1965-1963 (Guilizzoni *et al.*, 2001). In the meanwhile, the reduced input of Cu from the rayon factory and the opening of other Cu-discharging activities in the basin of the river Lagna (Fig. 1) may have changed the pattern of Cu deposition in Lake Orta. In this scenario, most Cu discharges would have preferentially reached the central part of the basin and increased Cu concentrations in core 07/2A between the end of the 1960s and 1986 (Fig. 2b). At the opposite, after the peak Cu levels at the end of the 1950s, sediments in the southern part of the lake began to be slowly covered by material with low Cu levels resulting in a continuous, albeit slow, decrease in Cu concentration in core 07/1A. The abrupt decrease between 1986 and 1992-1996 could be due to the deposition of $CaCO_3$ following the liming period as previously explained for core 07/1A. Baudo and Beltrami (2001) actually report an increased Ca content between pre- (1985) and post-liming (1996) sediment material; which could be a proxy for increased carbonate content in sediments. In the case of a major constituent such as calcium, the methodological issues associated with the use of homogenized sediment samples are likely less important than for Cu.

At present, we are unable to ascribe the persistence of Cu contamination in bottom sediments of Lake Orta to the presence of Cu inputs from its tributaries or to early diagenetic processes at the sediment-water interface. The average Cu content for suspended particulate matter (SPM) collected in Lake Orta during the period 2012-2013 was 1180±570 mg kg^{-1} d.w., comparable with the levels of 1250±750 mg kg^{-1} measured for the neighbouring Lake Maggiore (Casati, 2013). In the case of Lake Maggiore, Cu concentrations in bed sediments were around 60 mg kg^{-1} d.w. during the period 2002-2006 (Vignati and Guilizzoni, 2011) and in the range 60-100 mg kg^{-1} d.w. in the uppermost section of sediment cores collected in 2008 (CIPAIS, 2009). Considering the similar Cu concentrations in SPM of lakes Maggiore and Orta and the low dissolved Cu concentrations (about 2 µg L^{-1}) in Lake Orta (Casati, 2013), inputs from tributaries would rather contribute to a decrease in Cu contamination in Lake Orta sediments. The persistence of elevated Cu concentrations in sediments of Lake Orta over the period 1997-2007 is therefore likely caused by early diagenetic processes at the sediment-water interface and, especially in core 07/1A, very low sedimentation rates. This issue would however benefit from direct investigation in the future.

Ecotoxicological implications of copper contamination

While the most recent trends of Cu geochronology require further study, 2007 copper levels in Lake Orta sediments remained comparable to, or higher than, toxic threshold determined in various studies using both artificial and natural Cu-spiked sediments (Tab. 1). Using artificial spiked sediments, Roman *et al.* (2007) established thresholds of 50% mortality (LC50, lethal concentrations killing 50% of exposed organisms) at values between 150 and 327 mg kg^{-1} d.w. for different sediment-dwelling organisms (Tab. 1), with corresponding No Observed Effect Concentration (NOEC) always below 180 mg kg^{-1} d.w. Lower Effective Concentrations (EC50) and NOEC values were obtained when growth was considered as an endpoint. However, Cairns *et al*. (1984) reported EC50 above 2,000 mg kg^{-1} d.w. for the survival of *C. tentans* in spiked natural sediments (Tab. 1), cautioning about the direct ap-

Tab. 1. Compilation of ecotoxicological data for No Observed Effect Concentrations (NOEC), Lowest Observable Effect Concentrations (LOEC), half maximal Lethal Concentrations (LC50) or Effective Concentrations (EC50) for tests with Cu-spiked sediments. All values are in mg Cu kg^{-1} sediment d.w.

Species	Endpoint	NOEC	LOEC	LC50 or EC50	Matrix
Tubifex tubifex	Survival (28 d)	138	158	327	A
	Survival (14d)	n.a.	n.a.	375±148.7	B
	Survival (28d)	n.a.	109–(>802)	n.a.	D
	Growth (28 d)	78.3	102	126	A
	Growth(28d)	n.a.	n.a.	288±22	B
	Reproduction (28d)	n.a.	n.a.	183±12.7	B
	Reproduction (28d)	n.a.	69–(>802)	n.a.	D
	Reproduction (28d)	n.a.	92–129	81–107	E
Hyalella azteca	Survival (14 d)	100	180	316	A
	Survival (10 d)	n.a.	n.a.	1078 (922–1259)	C
	Growth (28 d)	53.2	95,4	194	A
Chironomus riparius	Survival (14d)	<180	180	320	A
	Growth (28d)	89.2	188	150	A
Chironomus tentans	Survival (10 d)	n.a.	n.a.	2296 (1690–3119)	C
	Survival (10 d)	n.a.	n.a.	857 (685–1073)	C
Lumbriculus variegatus	Survival	114	140	211	A
Gammarus pulex	Survival (35d)	94.7	176	151	A
	Growth (35d)	94.7	176	148	A
Gammarus lacustris	Survival (10 d)	n.a.	n.a.	964 (777–1196)	C

A, spiked artificial sediments (Roman et al.*, 2007); B, spiked natural sediment (Méndez-Fernández* et al.*, 2013); C, spiked natural sediments (Cairns* et al.*, 1984); D, spiked natural sediments (Vecchi* et al.*, 1999); E, reconstructed spiked sediment (Pasteris* et al.*, 2003); n.a., not available from the original study* plicability to real field situation of results obtained using formulated sediments.

Indeed, actual toxicity is controlled by the specific copper-binding properties of sediments rather than by the sedimentary Cu concentration alone. Simpson (2005) calculated an LC50 for the amphipod *Melita plumulosa* exposed to copper equal to 163 mg kg^{-1} d.w. for a sediment with low complexing capacity (K_d=1×10^3 L kg^{-1}), 825 d.w. mg kg^{-1} d.w. for intermediate complexing capacity (K_d=1×10^4 L kg^{-1}) and 1400 mg kg^{-1} d.w. for highly complexing sediments (K_d=1×10^5 L kg^{-1}). In the past, Cu occurred mainly in the dissolved phase (Camusso *et al.,* 1989) and aqueous exposure controlled toxic effects for organisms. At present, dissolved Cu concentrations have decreased below 2 µg L^{-1} (Casati, 2013) so that the importance of dietary uptake for benthic organisms from sediment ingestion, and hence the importance of residual Cu contamination in sediments, is likely higher than in the past. However, no recent data on Cu distribution between pore water and sediment solids in Lake Orta are available to confirm this hypothesis. The lithology of cores 07/1A and 07/2A excluded the massive presence of sandy sediments with low complexing capacity and copper partition coefficients between 1×10^4 and 1×10^5 L kg^{-1} should be the most realistic. This hypothesis compares favourably with the K_d values between 3.1×10^4 L kg^{-1} and 6.1×10^4 L kg^{-1} reported for Cu in the water column of Lake Orta (depth 140 m, northern basin) during the period 1985-1987 (Camusso *et al.*, 1989). Caution is clearly needed when comparing the post-liming situation in sediments deposited after 1988 and the pre-liming conditions examined in Camusso *et al*. (1989). Indeed, Casati (2013) reported even higher K_d values for Cu partitioning in the water column (an average of 9.3 × 10^5 L kg^{-1}) for the period 2012-2013. On the other hand, Baudo and Beltrami (2001) examined Cu concentrations in sediment cores and associated pore waters collected in 1992. Using the reported concentrations values, we could estimate a K_d for Cu between 0.4 and 1 × 10^3 L kg^{-1}. We notice that, in the case of these partition coefficients for sediments, dissolved concentrations were obtained analyzing pore waters recovered by centrifugation instead of filtration as in the case of samples collected in the water column. This methodological difference may also be the cause of the lower K_d of Cu between sediments and pore waters compared with those between suspended particulate matter and the water column.

Considering all this information, copper concentration expected to cause 50% mortality in benthic organisms can be estimated between 800 and 1400 mg kg^{-1} d.w. based on the findings of Simpson (2005). With a different approach, Campana *et al.* (2013) determined a safe Cu concentrations of 5.5 mg Cu g^{-1} organic carbon for the sediment fraction <63 µm. We estimated organic carbon (OC) content in our sediment samples by multiplying LOI by 0.4 (Supplementary Tab. 3) according to the observations of Lami et al. (2000) who observed OC to represent between 35 and 45% of LOI. In 2007, normalized Cu concentrations in surface (0-5 cm) Lake Orta sediments were between 13 and 36 mg Cu g^{-1} OC in core 07/1A and 4.5-9.5 mg Cu g^{-1} OC in core 07/2A. The corresponding values for sediments with background Cu levels were between 0.05 and 1 mg Cu g^{-1} OC (Supplementary Tab. 3). Thus, the potential for chronic toxicity may exist, but the actual Cu concentrations not posing risks to organisms dwelling in sediments of Lake Orta should be verified with site-specific studies.

The toxic potential of Lake Orta sediments has actually been documented by various studies published between the late 1990s and early 2000s. Sediments collected at three stations in the southern, central and northern parts of the lake in 1994-1997 were tested for phytotoxicity using commercially available seeds (*Cucumis sativus*, *Lactuca sativa*, and *Lepidium sativum*) (Beltrami *et al.*, 1999). The germination index (GI) of the three plant species was determined after 72 h of incubation on dried sediment material (2-cm thick sediment sections) covered with filter paper and re-wetted with deionised water. A statistically significant reduction of the GI was observed for sediment sections containing over 1000 mg kg^{-1} d.w. of Cu, but also in less contaminated sediments. Sediment toxicity was also tested *in situ* by caging individuals of *Daphnia obtusa* and *Echinogammarus stammeri* in contact with the sediments at 10 m depth for 48 h (Rossi and Beltrami, 1998). Organisms were brought back to the laboratory, maintained and counted till their death. The mortality of organisms exposed to sediments for 48 hours was significantly higher than controls, although year-to-year variations were observed. Burton Jr. *et al.* (2001) assessed sediment contamination using 6 toxicity assays on sections of 20-cm long sediment cores collected in 1994, four years after liming, at Omegna, at station 45 (central part of the lake and comparable to ORTA 07/2A) and Buccione (comparable to ORTA 07/1A) (Fig. 1). The top sediment layers (1994) were less toxic to *D. magna* (85% survival) and *Hyalella azteca* (85% survival) than intermediate layers. Complete mortality was observed between 4 and 20 cm for sediments collected in the central part of the lake. At the community level, Baudo *et al.* (2001) examined the benthos of Lake Orta in 51 stations distributed throughout the lake in 1996. While sediments hosted a variety of organisms, Baudo *et al.* (2001) observed that the chironomid community probably remained the same present before liming and that numerical abundances of macrofauna often remained lower than those typical of unpolluted freshwater environments. On the other hand, no clear correlation could be found between the level of sediment contamination and the presence/absence of the three most represented taxa: *Tubifex tubifex*, *Chironomus thummi*, and *Procladius* sp. The possibility of adaptation of benthic organisms to metals does indeed make sense (Ponti *et al.,* 2010; Fischer *et al.,* 2013; Piscia *et al.,* 2015), considering that individuals of *Tubifex tubifex* were reported to be present in Lake Orta sediments even during the 1980s (Nocentini *et al.*, 2001). However, Nocentini *et al.* (2001) surmised that contamination of water and sediments could still influence the colonisation of Lake Orta by macrobenthic organisms in 1993-1994.

Extrapolation of previous ecotoxicological knowledge to the present situation is not straightforward because of the changing exposure scenarios linked to the limnological evolution of Lake Orta. Despite chemical recovery, Lake Orta remains a vulnerable ecosystem with regard to populations of pelagic invertebrates (Ponti *et al.*, 2010; Piscia *et al.*, 2015), but no analogous information is available for benthic organisms. In particular, knowledge of Cu bioavailability from the dietary pathway is the most prominently missing piece of the puzzle to understand whether Cu contamination in Lake Orta sediments remains of concern or not. Past ecotoxicological studies do not provide direct information on the contribution of dietary Cu to toxicity because only epibenthic (*H. azteca*) or pelagic (*D. obtusa*) organisms were tested (Burton *et al.,* 2001; Rossi and Beltrami, 1998). Although laboratory tests may not be able to fully catch long-term toxic effects on long-lived organism in field conditions, Besser *et al.* (2015) report a significant relationship between percentage amphipod survival in standard tests and invertebrate taxa richness in the Vermont copper belt. However, findings from laboratory tests may not directly apply to real field situations (Ferrari et al., 2014). Studies on the bioavailability and toxicity of copper contained in sediments of Lake Orta should therefore use a combination of conventional and *in-situ* approaches.

CONCLUSIONS

Sediments of Lake Orta have faithfully recorded the onset of Cu contamination in the 1920s, as well as the events leading to a progressive decrease of Cu loading to the lake, both in the southern and in the central part of the basin. In the southern part of Lake Orta, Cu concentrations have been regularly decreasing since the peak levels of 3500 mg kg^{-1} d.w. measured in the 1950s. The extremely low sedimentation rates in this part of the lake do however prevent a fast recovery of sediments from past contamination and Cu levels were still above 1000 mg kg^{-1} d.w in 2007. In the central part of the lake, Cu concentrations also peaked at about 4500 mg kg^{-1} d.w. in the late 1950s and had decreased to 500 mg kg^{-1} d.w. in 2007. The decrease of Cu concentration in the central basin of Lake Orta could have been facilitated by the occurrence of turbidites which, in this zone, is documented by the changes in the composition of the fossil diatom communities. Liming operations performed at the end of the 1980s could also have contributed to lower Cu concentration in sediments. About 15% of the $CaCO_3$ added for liming (about 1500 tons) could actually have precipitated and contributed to reduce Cu concentrations in bed sediments.

Despite an improved situation compared with the preliming period, Cu levels remain high enough to be of potential concern in terms of ecotoxicity to sediment-dwelling organisms in both the southern and central basin of Lake Orta. Particular attention should be given to understand the effects of dietary ingestion of Cu from sediments which, unlike in previous ecotoxicological studies, may now represent the main route of Cu exposure for sediment-ingesting benthic organisms. Information is also needed on present Cu behaviour (*e.g.*, partitioning between sediments and associated pore waters) as a function of sediment characteristics and on the actual bioavailability of Cu in order to rule out subtle and lasting effect on benthic organisms and communities. Other *in situ* phenomena, *e.g.* the occurrence of slumps causing massive mixing of sediments with different ages and degrees of contamination, cannot be modelled or predicted. Periodic monitoring of sediments therefore remains necessary to follow the progress of Lake Orta recovery after the liming operations carried out at the end of the 1980s. Considering the sedimentation rates in Lake Orta, the use of sediment cores with appropriate dating should be preferred to grab samples for following the future evolution of Cu contamination.

ACKNOWLEDGMENTS

We thank Laura Puppieni (CNR-ISE) for her help with literature research, Ivan Grof (CNR-IRSA and University of Insubria) for help with Cu analyses and Piero Guilizzoni and Andrea Lami for their assistance in core sampling and preparation. Credit also to two anonymous reviewers whose remarks helped to greatly improve the original submission.

REFERENCES

Appleby PG, Nolan PJ, Gifford DW, Godfrey MJ, Oldfield F, Anderson NJ, Battarbee RW, 1986. ^{210}Pb dating by low background gamma counting, p. 21-27. In: H. Löffler (ed.), Paleolimnology IV. Developments in hydrobiology. Springer, Dordrecht.

Aziz HA, Adlan MN, Ariffin KS, 2008. Heavy metals (Cd, Pb, Zn, Ni, Cu and Cr(III)) removal from water in Malaysia: post treatment by high quality limestone. Bioresource Technol. 99:1578-1583.

Baudo R, Amantini L, Bo F, Cenci R, Hannaert P, Lattanzio A, Marengo G, Muntau H, 1989. Spatial distribution patterns of metals in the surface sediments of Lake Orta (Italy). Sci. Tot. Environ. 87/88:117-128.

Baudo R, Beltrami M, 2001. Chemical composition of Lake Orta sediments. J. Limnol. 60:213-236.

Baudo R, Occhipinti A, Nocentini AM, Sabolla M, 2001. Benthos of Lake Orta in the year 1996. J. Limnol. 60:241-248.

Beltrami M, Rossi D, Baudo R, 1999. Phytotoxicity assessment of Lake Orta sediments. Aquat. Ecosyst. Health 2:391-401.

Besser JM, Brumbaugh WG, Ingersoll CG, 2015. Characterizing toxicity of metal-contaminated sediments from mining areas. Appl. Geochem. 57:73-84.

Bonacina C, 2001. Lake Orta: the undermining of an ecosystem. J. Limnol. 60:53-59.

Bonacina C, Baudo R, 2001. Lake Orta: a case study. Part 1. J. Limnol. 60:1-166.

Burton Jr. AG, Baudo R, Beltrami M, Rowland C, 2001. Assessing sediment contamination using six toxicity assays. J. Limnol. 60:263-267.

Cairns MA, Nebeker AV, Gakstatter JH, Griffis WL, 1984. Toxicity of copper-spiked sediments to freshwater invertebrates. Environ. Toxicol. Chem. 3:435-445.

Calderoni A, de Bernardi R, Mosello R, 1993. Lago d'Orta ecosystem recovery by liming. In: S.E. Jørgensen (ed.), Management of lake acidification. International Lake Environment Committee. Guidelines of lake management 5:105-144.

Campana O, Blasco J, Simpson SL, 2013. Demonstrating the appropriateness of developing sediment quality guidelines based on sediment geochemical properties. Environ. Sci. Technol. 47:7483-7489.

Camusso M, Tartari G, Cappelletti E, 1989. Seasonal trends of copper sedimentation in lake Orta (Italy). Sci. Tot. Environ. 87/88:59-75.

Casati F, 2013. [I metalli in tracce nelle acque del Lago d'Orta: fase disciolta e particellata]. [Master Thesis in Italian]. Insubria University.

Cattaneo A, 1992. The anthropogenically stressed periphyton of Lake Orta, Italy. Hydrobiologia 234:111-121.

Chiaudani G, 1969. [Contenuti normali ed accumuli di rame in *Phragmites communis* L. come risposta a quelli nei sedimenti di sei laghi italiani].[Article in Italian]. Mem. Ist. Ital. Idrobiol. 25:81-95.

CIPAIS, 2009. [Indagini su DDT e sostanze pericolose nell'ecosistema del Lago Maggiore. Programma 2008-2012].[Report in Italian].Commissione Internazionale per la protezione delle acque italo-svizzere: 89 pp.

Corbella C, Tonolli V, Tonolli L, 1958. [I sedimenti del Lago d'Orta testimoni di una disastrosa polluzione cuproammoniacale].[Article in Italian]. Mem. Ist. Ital. Idrobiol. 10:9-50.

Dean WE Jr, 1974. Determination of carbonate and organic matter in calcareous sediments and sedimentary rocks by loss on ignition: comparison with other methods. J. Sed. Petrol. 44:242-248.

Ferrari BJD, Vignati DAL, Dominik J, 2014. Bioaccumulation kinetics and effects of sediment-bound contaminants on chironomids in deep waters: new insights using a low-disturbance in situ system. Environ. Technol. 35:456-469.

Fischer BB, Pomati F, Eggen RIL, 2013. The toxicity of chemical pollutants in dynamic natural systems: The challenge of integrating environmental factors and biological complexity. Sci. Tot. Environ. 449:253-259.

Gardham S, Chariton A, Hose G, 2015. Direct and indirect effects of copper-contaminated sediments on the functions of model freshwater ecosystems. Ecotoxicology 24:61-70.

Guilizzoni P, Lami A, Marchetto A, Appleby PG, Alvisi F, 2001. Fourteen years of palaeolimnological research of a past industrial polluted lake (L. Orta, Northern Italy): an overview. J. Limnol. 60:249-262.

Guilizzoni P, Levine SN, Manca M, Marchetto A, Lami A, Ambrosetti W, Brauer A, Gerli S, Carrara EA, Rolla A, Guzzella L, Vignati DAL, 2012. Ecological effects of multiple stressors on a deep lake (Lago Maggiore, Italy) integrating neo and palaeolimnological approaches. J. Limnol. 71:1-22.

Guzzella L, Bartone C, Ross P, Tartari G, Muntau H, 1996. Toxicity identification evaluation of Lake Orta (Northern Italy) sediments using the microtox system. Ecotox. Environ. Safe. 35:231-235.

Hong S, Candelone JP, Patterson CC, Boutron CF, 1996. History of ancient copper smelting pollution during Roman and Medieval times recorded in Greenland ice. Science 272:246-249.

Lami A, Marchetto A, Lo Bianco R, Appleby PG, Guilizzoni P, 2000. The last ca 2000 years palaeolimnology of Lake Candia (N. Italy): inorganic geochemistry, fossil pigments and temperature time-series analyses. J. Limnol. 59:31-46.

Manca M, Comoli P, 1995. Temporal variations of fossil Cladocera in the sediments of Lake Orta (N. Italy) over the last 400 years. J. Palolimnol. 14:113-122.

Marchetto A, Bettinetti R, 1995. Reconstruction of the phosphorus history of two deep, subalpine Italian lakes from sedimentary diatoms, compared with long-term chemical measurements. Mem. Ist. Ital. Idrobiol. 53:27-38.

Marchetto A, Lami A, Musazzi S, Massaferro J, Langone L, Guilizzoni P, 2004. Lake Maggiore (N. Italy) trophic history: fossil diatoms, plant pigments, and chironomids, and comparison with long-term limnological data. Quat. Internat. 113:97-110.

Méndez-Fernández L, Martínez-Madrid M, Rodriguez P, 2013. Toxicity and critical body residues of Cd, Cu and Cr in the aquatic oligochaete *Tubifex tubifex* (Müller) based on lethal and sublethal effects. Ecotoxicology 22:1445-1460.

Nocentini AM, Boggero A, De Margaritis G, Gianatti M, 2001. First phase of macroinvertebrate repopulation of Lake Orta (Buccione Basin) after liming. J. Limnol. 60:110-126.

Pasteris A, Vecchi M, Reynoldson TB, Bonomi G, 2003. Toxicity of copper-spiked sediments to *Tubifex tubifex* (Oligochaeta, Tubificidae): a comparison of the 28-day reproductive bioassay with a 6-month cohort experiment. Aquat. Tox. 65:253-265.

Piscia R, Colombini M, Ponti B, Bettinetti R, Monticelli D, Rossi V, Manca M, 2015. Lifetime Response of Contemporary Versus Resurrected Daphnia galeata Sars (Crustacea, Cladocera) to Cu(II) Chronic Exposure. B. Environ. Contam. Tox. 94:46-51.

Piscia R, Guilizzoni P, Fontaneto D, Vignati DAL, Appleby PG, Manca M, 2012. Dynamics of rotifer and cladoceran resting stages during copper pollution and recovery in a subalpine lake. Ann. Limnol.-Int. J. Lim. 48:151-160.

Piscia R, Tabozzi S, Bettinetti R, Nevalainen L, Manca M, 2016. Unexpected increases in rotifer resting abundances during the period of contamination of Lake Orta. J. Limnol. 75(s2):76-85.

Ponti B, Piscia R, Bettinetti R, Manca M, 2010. Long-term adaptation of Daphnia to toxic environment in Lake Orta: the effects of short-term exposure to copper and acidification. J. Limnol. 69:217-224.

Potapova M, Hamilton PB, 2007. Morphological and ecological variation within the *Achnanthidium minutissimum* (bacillariophyceae) species complex. J. Phycol. 43:561-575.
Provini A, Premazzi G, Gaggino GF, 1987. [Valutazione del rischio potenziale da metalli pesanti nei laghi mediante l'analisi dei sedimenti].[Article in Italian]. Ing. Amb. 16:68-76.
Renberg I, 1990. A procedure for preparing large sets of diatom slides from sediment cores. J. Paleolimnol. 4:87-90.
Roman YE, De Schamphelaere KAC, Nguyen LTH, Janssen CR, 2007. Chronic toxicity of copper to five benthic invertebrates in laboratory-formulated sediment: sensitivity comparison and preliminary risk assessment. Sci. Tot. Environ. 387:128-140.
Rossi D, Beltrami M, 1998. Sediment ecological risk assessment: in situ and laboratory toxicity testing of lake Orta sediments. Chemosphere 37:2885-2894.
Ruggiu D, Lugliè A, Cattaneo A, Panzani P, 1998. Paleoecological evidence for diatom response to metal pollution in Lake Orta (N. Italy). J Paleolimnol 20:333-345
Ruttner F, 1959. [Einige Beobachtungen über das Phytoplankton norditalienischer Seen].[Article in German]. Mem. Ist. Ital. Idrobiol. 11:73-111.
Simpson SL, 2005. Exposure-effect model for calculating copper effect concentrations in sediments with varying copper binding properties: a synthesis. Environ. Sci. Technol. 39:7089-7096.
Tonolli L, 1961. [La polluzione cuprica del Lago d'Orta. Comportamento di alcune popolazioni di Diatomee].[Article in Italian]. Verh. Intern. Ver. Limnol. 14:900-904.
van Dam H, Mertens A, 1990. A comparison of recent epilithic diatom assemblages from the industrially acidified and copper polluted Lake Orta (northern Italy) with old literature data. Diatom Res. 5:1-13.
van Dam H, Mertens A, Sinkeldama J, 1994. Coded checklist and ecological indicator values of freshwater diatoms from The Netherlands. Aquat. Ecol. 28:117-133.
Vecchi M, Reynoldson TB, Pasteris A, Bonomi G, 1999. Toxicity of copper-spiked sediments to *Tubifex tubifex* (oligochaeta, tubificidae): comparison of the 28-day reproductive bioassay with an early-life-stage bioassay. Environ. Toxicol. Chem. 18:1173-1179.
Vignati DAL, Guilizzoni P, 2011. [Metalli nel Lago Maggiore: livelli naturali e antropici].[Article in Italian]. Acqua Aria 1:22-27.
Vollenweider RA, 1963. [Studi sulla situazione attuale del regime chimico e biologico del Lago d'Orta].[Article in Italian]. Mem. Ist. Ital. Idrobiol. 16:21-125.