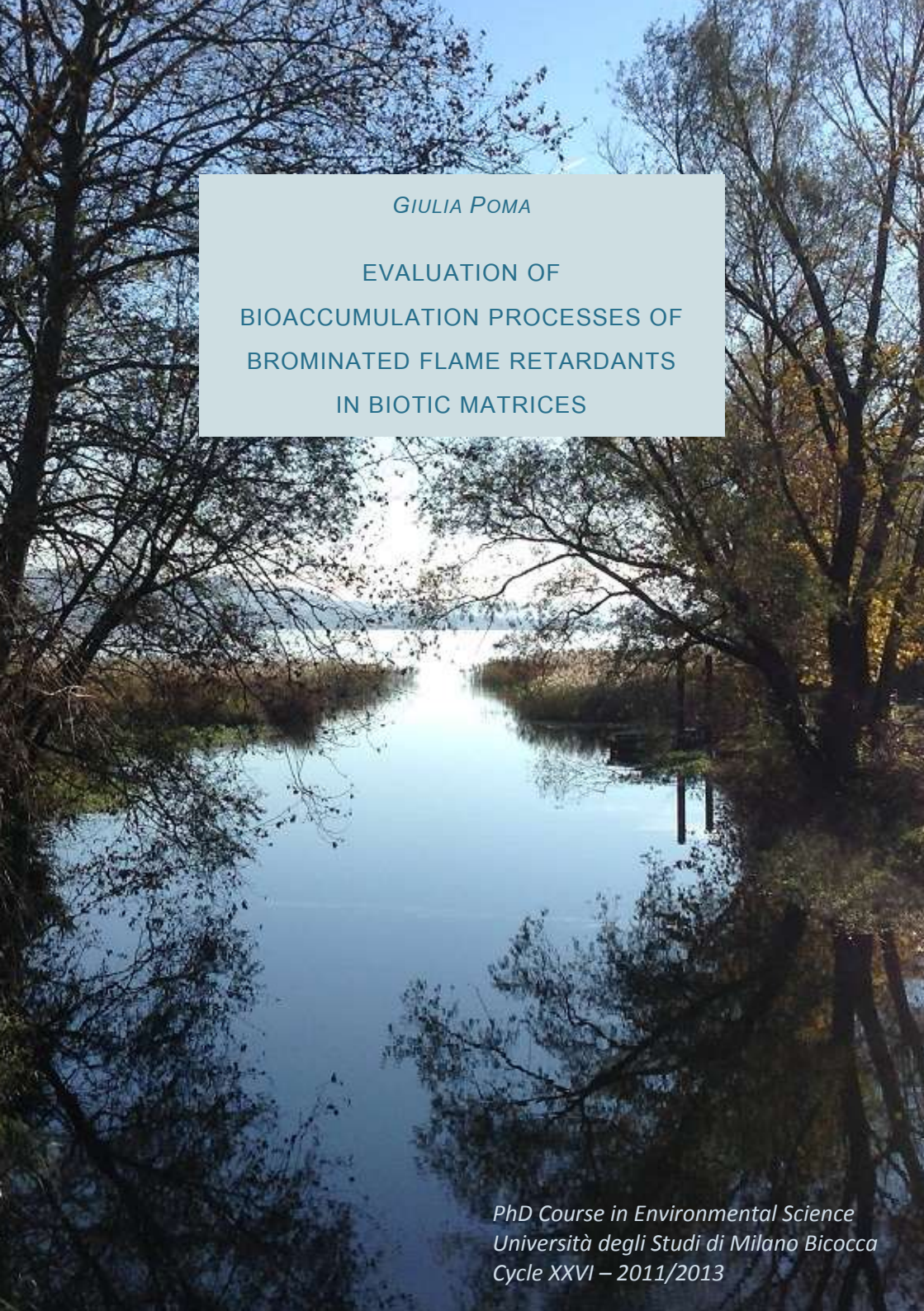

GIULIA POMA

EVALUATION OF BIOACCUMULATION PROCESSES OF BROMINATED FLAME RETARDANTS IN BIOTIC MATRICES

PhD Course in Environmental Science
Università degli Studi di Milano Bicocca
Cycle XXVI – 2011/2013

Ho avuto dei maestri duri, cinici, spietati,
altri buoni e comprensivi.
Entrambi mi hanno formato,
dei secondi conservo un buon ricordo.

Mauro Corona
(Finché il cuculo canta, 1999)

UNIVERSITÀ DEGLI STUDI DI MILANO-BICOCCA

Facoltà di Scienze Matematiche, Fisiche e Naturali

PhD Course in Environmental Science

EVALUATION OF BIOACCUMULATION PROCESSES OF BROMINATED FLAME RETARDANTS IN BIOTIC MATRICES

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Cycle XXVI

2011 – 2013

UNIVERSITÀ DEGLI STUDI
DI MILANO - BICOCCA

CNR-IRSA
Istituto di Ricerca sulle Acque
Consiglio Nazionale delle Ricerche

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Cover image: River Bardello flowing in the Lake Maggiore.
Photo by Giulia Poma

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CHAPTER I

GENERAL INTRODUCTION

1.1 Brominated Flame Retardants (BFRs)

The environment is continuously impacted by foreign organic chemicals (xenobiotics) released by urban communities and industries (van der Oost et al., 2003). In the 20th century and more recently, many thousands of organic trace pollutants have been produced and, in part, released into the environment. Since the last decades, humankind has become aware of the potential long-term adverse effects of these chemicals in general, and their potential risks for aquatic and terrestrial ecosystems in particular. The ultimate sink for many of these contaminants is the aquatic environment, either due to direct discharges or to hydrologic and atmospheric processes (Stegeman and Hahn, 1994). When released into the environment, substances will be subjected to transport and transformation processes. These processes (together with emission patterns, environmental parameters, and physicochemical properties) will govern their distribution and concentration in environmental compartments such as water, air, soil, sediment and biota (ECETOC, 1993). The partition behavior of the hydrophobic chemicals in these compartments is mainly determined by organic carbon contents; the more hydrophobic a compound, the greater the partitioning to these phases (Meador et al., 1995). Persistent hydrophobic chemicals may bioaccumulate in aquatic organisms through different mechanisms: via the direct uptake from water by gills or skin (bioconcentration), via uptake of suspended particles, and via the consumption of contaminated food (biomagnification). However, for lipophilic chemicals (e.g. POPs) bioconcentration is considered to be of less importance

for most fish when compared to dietary uptake (Borgå et al., 2004).

Among the different classes of organic pollutants released into the environment, the flame retardants (FRs) are recently causing great concern between scientists. Fire, in fact, has been a major cause of property damage and death throughout recorded history and to the present day (Alaee et al, 2003). During the past several decades, modern technology has responded to this challenge by introducing heat resistant chemicals to reduce the chances of ignition and burning of a wide range of textiles, plastics, building materials, and electronic equipment used in commerce and in residential homes. The brominated flame retardants (BFRs) are currently the largest market group due to their low cost and high performance efficiency. Typical uses are in polyurethane foam, plastics used in electric and electronic equipment, printed circuit boards, expanded and extruded plastic (such as Styrofoam), textile back-coating in furniture, various textiles used in public environments (curtains, furniture coverings, carpets), rubber for coating wire, etc. (de Wit et al., 2010).

The most widely produced brominated flame retardants are the additive polybrominated diphenyl ethers (PBDEs), and hexabromocyclododecane (HBCD). Because they do not react with the material, they may migrate out of the product and be released into the environment. Consequently, environmental concerns relating to BFRs are recently growing due to their environmental persistence, bioaccumulative properties and potential toxicity (Vastag, 2008). Moreover, in 2002, a concise review of studies demonstrating the endocrine disrupting (ED) potency of BFRs was written (Legler and Brouwer, 2003).

The main physicochemical characteristics, uses and widespread of the BFRs considered in this work of thesis are reported below.

1.1.1 PBDEs and BDE-209

Polybrominated diphenylethers (PBDEs) are being produced since the early 1970s as additive flame retardants in most type of polymers applied to computer monitors, TV sets, computer cases, wire and cable insulation (Wu et al., 2008). Their physicochemical properties are listed in Table I-1.

The PBDEs potentially involve 209 different congeners, varying in both number and position of bromination (Fig. I-1). However, there appear to be many fewer actual PBDE congeners in the commercial mixtures than the theoretical number possible, largely because many of the congeners lack stability and tend to debrominate (Birnbaum and Staskal, 2004). There are three technical PBDE products that have been in use as additive flame retardants, known as Penta-BDE, Octa-BDE and Deca-BDE. Penta-BDE contains primarily tetra- (BDE-47), penta- (BDE-99, -100) and hexa-BDE (BDE-153) congeners, Octa-BDE contains primarily a hepta-BDE (BDE-183) plus octa- (BDE- 197) and Deca-BDE consists primarily of the fully brominated BDE-209 (La Guardia et al., 2007). Due to their growing environmental and human health concern, strict bans have been imposed in Europe in 2004 on the worldwide use of Penta- and Octa-BDE formulations (Directive EEC, 2003), and the US manufacturers of these commercial mixtures voluntarily stopped their production in the same year (La Guardia et al. 2007). Moreover, in August 2010 the Stockholm Convention included tetra-, penta-, hexa- and hepta-BDEs (covering many of the major congeners of Penta-BDE and Octa-BDE technical formulations) in the Persistent Organic Pollutant list (Ashton et al., 2009). Deca-BDE have been banned throughout Europe in electrical and electronical equipment in July 2008 (European Court of Justice 2008), because of concern about a possible formation of more toxic oxidation and/or debromination residuals. In North America, a phase-out of Deca-BDE is

expected by 2013 (Hermanson et al. 2010), and in May 2013 Norway prepared a draft dossier nominating commercial Deca-BDE for potential inclusion in the Stockholm Convention on POPs.

Decabromodiphenyl ether (BDE-209 or deca-BDE) is the fully brominated congener (Fig. I-1) used as a flame retardant in the commercial mixture Deca-BDE.

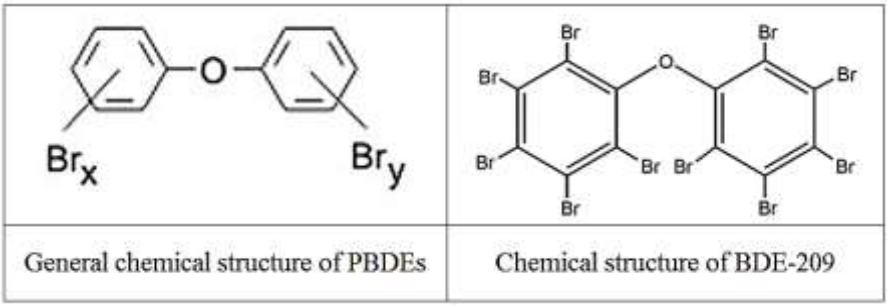

Fig. I-1 Chemical structure of PBDEs and BDE-209

Table I-1 Physicochemical properties of PBDEs (Alcock et al., 1999)

Molecular formula	Molecular weight	Melting point (°C)	Vapour pressure (Pa) (25 °C)	Water solubility (µg/L) (25 °C)	Octanol-water coefficient (Log K_{ow})
$C_{12}H_{(10-x)}Br_xO$	248.97 – 959.04				
Tri-BDE			$1.6\ 10^{-3} – 2.7\ 10^{-3}$		
Tetra-BDE		79 - 82	$2.5\ 10^{-4} – 3.3\ 10^{-4}$	10.9	5.9 – 6.2
Penta-BDE		92	$2.9\ 10^{-5} – 7.3\ 10^{-5}$	2.4	6.5 – 7.0
Esa-BDE			$4.2\ 10^{-6} – 9.5\ 10^{-6}$	1 - 4	
Epta/Octa-BDE		200	$4.4\ 10^{-8}$	$2.2\ 10^{-4}$	8.4 – 8.9
Deca-BDE		290-306	$5.8\ 10^{-11}$	<0.1	10

BDE-209 was initially thought to represent a low threat to biota due to its high hydrophobicity and high molecular size. However, several recent studies demonstrated that this compound is bioavailable and can be transformed into more

bioaccumulable and toxic PBDEs (Kierkegaard et al., 1999; Stapleton et al., 2006). Moreover, BDE-209 accumulation in sediments has recently become a matter of concern, as this compartment represents large environmental reservoirs and could therefore be a potential threat to biota in the long-term exposure (Ross et al., 2009). The main concern regarding BDE-209 is its potential for degradation and particularly biotrasformation *via* debromination, a process by which bromine atoms are sequentially removed or cleaved from an organic compound, resulting in smaller and lower brominated molecules which are slightly more water soluble. These lower brominated congeners have the potential to be more persistent and more bioaccumulative than their larger parent chemical (Stapleton, 2006). In several studies, in fact, fish fed with food spiked with BDE-209 were found to accumulate lower brominated congeners (Kierkegaard et al., 1999; Stapleton et al., 2006). Fish have widely variable capacities to assimilate and metabolize PBDEs via debromination processes, both in terms of efficiency and metabolite profiles. In fact, congeners with 3-10 bromine atoms were all found to accumulate in fish with variable assimilation efficiencies (Tomy et al., 2004).

1.1.2 HBCD

Hexabromocyclododecane (HBCD) is a nonaromatic, brominated cyclic alkane FRs (Fig. I-2). It is the principal flame retardant in extruded (XEPS) and expanded (EPS) polystyrene foams used as thermal insulation in the building industry (data from American Chemistry Council). Secondary uses of HBCD include residential and commercial furniture textiles and wall coverings (de Wit, 2002).

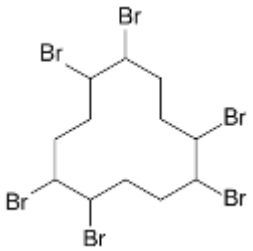

Fig. I-2 Chemical structure of HBCD

The physicochemical properties of HBCD (Table I-2) are similar to those of BDEs and other persistent organic pollutants (de Wit, 2002). It is a lipophilic compound considered bioavailable and bioaccumulative based on studies of fish and fish eating animals (Birnbaum and Staskal, 2004).

Table I-2 Physicochemical properties of HBCD (U.S. EPA, 2010)

Molecular formula	Molecular weight	Melting point (°C)	Vapour pressure (Pa) (21 °C)	Water solubility (µg/L) (20 °C)	Octanol-water coefficient (Log K_{ow})
$C_{12}H_{18}Br_6$	641.7	190	$6*10^{-5}$	66	5.2

Despite these properties and its widespread use, there is a little knowledge about the fate and the environmental levels of HBCD. It usually adsorbs strongly to suspended matter and sediment in aquatic environments and to soils. Food-chain studies have shown that HBCD is bioaccumulative and can be transferred from sediments, via invertebrates and predatory fish, to fish-eating top predators, such as birds and seals (Morris, 2004). It has been suggested that HBCD may disrupt the thyroid hormone system and it mainly targets biotransformation processes in the liver, affecting key metabolic pathways (including the metabolism of lipids and sex hormones). At the

sixth meeting of the Conference of the Parties of the Stockholm Convention (May 2013), the decision was taken to list Hexabromocyclododecane (HBCD) in the list of POP substances. The listing allows an exemption for the production and use of HBCD in expanded polystyrene (EPS) and extruded polystyrene (XPS) in buildings. The exemption will be valid until 2019 (BSEF, 2013). HBCD was also included in the "San Antonio Statement on Brominated and Chlorinated Flame Retardants" signed in September 2010 by 245 scientist from 22 countries (DiGangi et al., 2010).

The reduction in the use of PBDEs and HBCD has opened the way for the introduction of "novel" BFRs (nBFRs) in place of the banned formulations (Betts 2008), indicating those BFRs that are new in the market or newly/recently observed in the environment in respect to PBDEs and HBCD (Covaci et al., 2011). Consequently, consumption and production of these nBFRs will keep rising, and increasing environmental levels of these chemicals are expected in the near future (Wu et al., 2011). Important representatives of this group are decabromodiphenyl ethane (DBDPE), 1,2-bis(2,4,6-tribromophenoxy) ethane (BTBPE), hexabromobenzene (HBB), and pentabromoethyl-benzene (PBEB).

1.1.3 Novel BFRs

Decabromodiphenyl ethane (DBDPE) was introduced in the mid-1980s and became commercially important as an alternative to the Deca-BDE formulation in the early 1990s (Arias, 2001). DBDPE has the same applications as Deca-BDE, being an additive to different polymeric materials (Covaci et al., 2011). Europe does not produce DBDPE, but imports in 2001 were estimated to be between 1000 and 5000 tons, primarily to Germany (Arias, 2001). Based on its structural resemblance to

BDE-209, the physicochemical properties of DBDPE (Table I-3) and deca-BDE are similar, but the inclusion of the ethane bridge between the aromatic rings makes it slightly more hydrophobic than BDE-209 (Fig. I-3). It also introduces more conformational flexibility in the molecule (Dungey and Akintoye, 2007) and reduces its potential for producing dioxins or furans under pyrolysis conditions (Pettigrew et al., 1992).

1,2-bis(2,4,6-tribromophenoxy) ethane (BTBPE) (Fig. I-3) is an additive flame retardant produced since the mid-1970s and is being used from 2005 as a replacement for Octa-BDE technical formulation (Renner, 2004). BTBPE is marketed for use in ABS, HIPS, thermoplastics, thermoset resins, polycarbonate and coatings (WHO, 1997). The total annual production of BTBPE is estimated to be approximately 5000 tons (WHO, 1997). In the EU, BTBPE is listed as a low production volume (LPV) chemical (ESIS, 2010), while worldwide production/usage was estimated to be 16,710 tons in 2001 (Verreault et al., 2007). Physicochemical properties (Table I-3) show that BTBPE is a non-volatile chemical with Log K_{oa}>9.5, classified as a single hopper with low LRAT potential (Wania and Dugani, 2003). Studies on environmental fate of BTBPE suggest that this chemical have a high potential of biomagnification in the aquatic food webs, while no metabolites were detected. Biochemical results indicate that BTBPE is not a potent thyroid axis disruptor (Tomy et al, 2007).

Hexabromobenzene (HBB) (Fig. I-3) was widely used in Japan as an additive flame retardant to paper, woods, textiles, electronic and plastic goods, but at present it is used at lower volumes (350 tons in 2001) (Watanabe and Sakai, 2003). HBB is not reported by EU industry as a currently produced chemical (ESIS, 2010). Physicochemical properties of HBB are listed in Table I-3. Tittlemier (Tittlemier et al., 2002) predicted HBB to be primarily distributed in soils (>98%) and sediments and the

release into the environment would result in localized distributions. However, HBB was analyzed in pooled herring gull egg samples from the Great Lakes of North America in 2004.

Pentabromoethylbenzene (PBEB) (Fig. I-3) is an additive flame retardant mostly used in thermoset polyester resins (circuit boards, textiles, adhesives, wire and cable coatings, polyurethane foam) (Hoh et al., 2005). PBEB is classified as a LPV chemical in the EU (ESIS, 2010) and is included in the OSPAR list of chemicals, being ranked as persistent, liable to bioaccumulate and toxic, but not currently produced (OSPAR, 2001). PBEB physicochemical properties are reported in Table I-3.

Studies of dietary absorption efficiency in rainbow trout showed that PBEB had a whole-body half-life of 38 days (Harju et al., 2008; 2009).

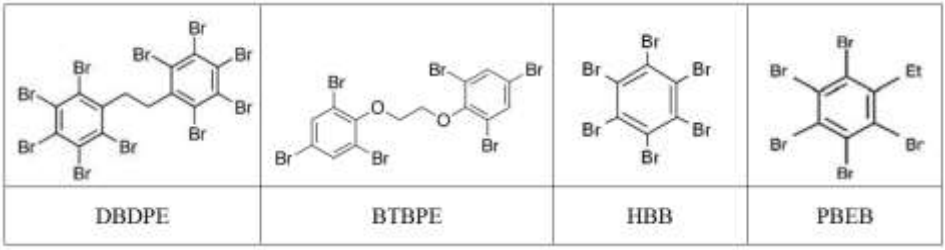

Fig. I-3 Chemical structure of the considered novel BFRs

Table I-3 Physicochemical properties of novel BFRs (Covaci et al., 2011)

Molecular formula	Molecular weight	Melting point (°C)	Vapour pressure (Pa) (25 °C)	Water solubility (g/L) (25 °C)	Octanol-water coefficient (Log K_{ow})
DBDPE $C_{14}H_4Br_{10}$	971.2	334-337	$6*10^{-15}$	$2.1*10^{-7}$	11.1
BTBPE $C_{14}H_8Br_6O_2$	687.6	na	$3.88*10^{-10}$	$1.9*10^{-5}$	7.8
HBB C_6Br_6	551.5	327	$1.14*10^{-4}$	$7.7*10^{-4}$	6.1
PBEB $C_8H_5Br_5$	500.7	138	$3.2*10^{-4}$	$3.05*10^{-4}$	6.4

1.2 Thesis objectives

In Italy, previous studies (Guzzella et al., 2008, CIPAIS 2010, 2011) have shown that some BFRs (PBDEs) were measured at high concentrations in the Varese province due to the presence of a great number of textile and plastic industries, and particularly in the sediments of Lake Maggiore, where those facilities wastewaters are finally collected mainly through two lake tributaries (Bardello and Boesio). For these reasons, the present thesis has the aim to evaluate the presence, and the potential bioaccumulation and biomagnification processes of six different classes of BFRs (PBDEs, HBCD, DBDPE, BTBPE, HBB and PBEB) in the Lake Maggiore ecosystem, with particular regard to zebra mussels (*Dreissena polymorpha*), zooplankton, one littoral fish species (common roach - *Rutilus rutilus*), and two different pelagic species (twaite shad – *Alosa agone* and European whitefish – *Coregonus lavaretus*).
The target organisms were selected considering their characteristics and role within the Lake Maggiore ecosystem. In particular, mollusks are widely used as sentinel organisms for monitoring chemical contaminants in water because they, being filter feeders, can process large amounts of water, bioaccumulating contaminants (Wu et al., 2012). Between

mollusks, *Dreissena polymorpha* was chosen having appropriate characteristics such as wide distribution, continuous availability throughout the year, firm site attachment capability by the bissus, and ease of sampling (Binelli et al. 2001). A few fish species have specialized teeth and jaws that are strong enough to break the shells of mollusks and some of them do eat zebra mussels. Among them, the roach (*Rutilus rutilus*) is a major predator of zebra mussels in Lake Maggiore and the most abundant fish species in the littoral areas (Volta and Jepsen 2008; Volta et al. 2013). Zooplankton accumulate organochlorine compounds (OCs) both from water and from food, and may do so much more rapidly than fish (Borgå et al., 2005). Moreover, zooplankton are expected to respond much faster than their predators to fluctuations of pollutants occurring in the water column (Bettinetti et al., 2010). These attributes suggest that this component of the pelagic food web may be used as an early warning tool of a possible contamination (Bettinetti et al., 2012). The shad and whitefish are potentially zooplanktivorous species and are often used as bioindicators in bioaccumulation studies (Volta et al., 2009; Bettinetti et al., 2010; Infantino et al., 2013), being considered as key species in large and deep subalpine lakes (Volta et al., 2011).

Finally, the study has also considered the BFR contamination in the lake sediments with the aim of characterizing in detail the possible presence of temporal trends and/or identifying potential sources of contamination. Moreover, it is plausible that the BFR uptake by benthic organisms, followed by fish predation, might be a significant source of bioaccumulation.

1.3 Thesis structure

The results of the present doctoral study are summarized in three papers dealing with the presence and potential bioaccumulation processes in different matrices from Lake Maggiore ecosystem. In particular:

- In **Chapter II**, nBFRs, PBDEs, and HBCD have been investigated in the sediments from Lake Maggiore and its tributaries with the aim of characterizing in detail the possible presence of temporal trends and/or identifying potential sources of contamination (*Manuscript submitted to Environmental Monitoring and Assessment*).
- In **Chapter III**, the spatial distribution and accumulation of nBFRs, PBDEs, and HBCD in the biota have been investigated in the littoral zone of Lake Maggiore, prompt to accumulate pollutants transported through tributaries, using zebra mussel (*Dreissena polymorpha*) and roach (*Rutilus rutilus*) as bioindicators (*Manuscript accepted by Environmental Science and Pollution Research with minor revisions*).
- The **Chapter IV** has finally the aim to evaluate whether or not nBFRs and HBCD can bioaccumulate in a pelagic food web of Lake Maggiore. PBDEs were also included in the study to estimate the lake current contamination following their production ban. With this purpose, the trophic level-adjusted BMFs (BMF_{TL}) and the Trophic Magnification Factors (TMFs) of BFRs were calculated and compared to each other. Moreover, to evaluate the structure and dynamics of the considered food web, the trophic role of fish was evaluated using the Stable Isotope Analysis (SIA) approach (*Manuscript accepted by Science of the Total Environment*).

1.4 REFERENCES

Alaee, M., Arias, P., Sjödin, A., Bergman, Å., 2003. An overview of commercially used brominated flame retardants, their application, their use pattern in different countries/regions and possible modes of release. Environment International 29(6), 683-689.

Albemarle. Albemarle to Expand Production of Popular SAYTEX® 8010 Flame Retardant. Newsletter September 17, 2007. http://www.albemarle.com/Products_and_services/Polymer_additives/?news=text&releaseID=1052248, Accessed July 2010.

Alcock, R.E., Sweetman, A., Jones, K.C., 1999. Assessment of Organic Contaminant Fate in Waste Water Treatment Plants I: Selected Compounds and Physiochemical Properties. Chemosphere 38(10), 2247-2262.

Arias, P.A., 2001. Brominated flame retardants - An overview. The Second International Workshop on Brominated Flame Retardants (BFR 2001) May 14–16 Stockholm 17-19.

Ashton, M., Kantai, T., Kohlerm P.M., Roemer-Mahler, A., Templeton, J., 2009. Summary of the fourth conference of the parties to the Stockholm convention on persistent organic pollutants. International Institute for Sustainable Development.

Bettinetti, R., Galassi, S., Guzzella, L., Quadroni, S., Volta, P., 2010. The role of zooplankton in DDT biomagnification in a pelagic food web of Lake Maggiore (Northern Italy). Environmental Science and Pollution Research 17(9), 1508-1518.

Bettinetti, R., Quadroni, S., Manca, M., Piscia, R., Volta, P., Guzzella, L., Roscioli, C., Galassi, S., 2012. Seasonal fluctuations of DDTs and PCBs in zooplankton and fish of Lake Maggiore (Northern Italy). Chemosphere, 88(3), 344-51.

Betts, K., 2008. New flame retardants detected in indoor and outdoor environments. Environmental Science & Technology 42(18), 6778-6778.

Binelli, A., Galassi, S., Provini, A., 2001. Factors affecting the use of dreissena polymorpha as a bioindicator: the PCB pollution in Lake Como (N. Italy). Water, Air, & Soil Pollution 125(1), 19-32.

Birnbaum, L.S., Staskal, D.F., 2004. Brominated flame retardants: Cause for concern? Environmental Health Perspectives, 112(1), 9-17.

Borgå, K., Fisk, A.T., Hoekstra, P.F., Muir, D.C.G., 2004. Biological and chemical importance in the bioaccumulation and trophic transfer of persistent organochlorine contaminants in arctic marine food webs. Environmental Toxicology and Chemistry 23(10), 2367-2385.

Borgå, K., Fisk, A.T., Hargrave, B., Hoekstra, P.F., Swackhamer, D., Muir, D.C.G., 2005. Bioaccumulation factors for PCBs revisited. Environmental Science & Technology 39(12), 4523-4532.

BSEF Bromine Science Environmental Forum 2013. http://www.bsef.com/regulation/international-regulations/what-are-the-united-nations-conventions

CIPAIS Commissione Internazionale per la Protezione delle Acque Italo-Svizzere, 2010. Indagini sul DDT e sostanze pericolose nell'ecosistema del Lago Maggiore. Rapporto annuale 2010 (in It.).

CIPAIS Commissione Internazionale per la Protezione delle Acque Italo-Svizzere, 2011. Indagini sul DDT e sostanze pericolose nell'ecosistema del Lago Maggiore. Rapporto annuale 2011 (in It.)

Covaci, A., Harrad, S., Abdallah, M.A.-E. Ali, N., Law, R.J., Herze, D., de Wit, C.A., 2011. Novel brominated flame retardants: a review of their analysis, environmental fate and behaviour. Environment International 37(2), 532-556.

de Wit, C.A., 2002. An overview of brominated flame retardants in the environment. Chemosphere 46(5), 583-624.

de Wit, C.A., Herze, D., Vorkamp, K., 2010. Brominated flame retardants in the Arctic environment – trends and new candidates. Science of the Total Environment 408(15), 2885-2918.

DiGangi, J., Blum, A., Bergman, A., de Wit, C.A., Lucas, D., Mortimer, D., Schecter, M., Shaw, S.D., Webster, T.F., 2010. San

Antonio Statement on brominated and chlorinated flame retardants. Environmental Health Perspectives, 118(12), 516-518.

Directive 2003/11/EC of the European Parliament and of the Council of 6 February 2003. Official Journal of the European Union, 2003, L42/45e46.

Dungey, S.M., Akintoye, L., 2007. Environmental risk evaluation report: 1,1′-(Ethane-1,2-diyl) bis[penta-bromobenzene]. Bristol, UK Environment Agency.

ESIS. European Chemical Substance Information System. European Commission, Joint Research Centre, European Chemicals Bureau; 2010. http://ecb.jrc.ec. europa.eu/esis/

European Court of Justice. Cases C-14/06 and C-295/06, Judgment of the Court, 1 April 2008, Directive 2002/95/EC and Commission Decision 2005/717/EC; 2008. http://curia.europa.eu - accessed July 2010.

Guzzella, L., Roscioli, R., Binelli, A., 2008. Contamination by polybrominated diphenyl ethers of sediments from the Lake Maggiore Basin (Italy and Switzerland) Chemosphere 73(10), 1684-1691.

Harju, M., Heimstad, E.S., Herzke, D., Sandanger, T., Posner, S., Wania, F., 2009. Emerging "new" brominated flame retardants in flame retarded products and the environment. Report 2462. Oslo, Norway: Norwegian Pollution Control Authority. 113 pp.

Harju, M., Heimstad, E.S. Herzke, D., Sandanger, T., Posner, S., Wania, F., 2008. Current state of knowledge and monitoring requirements - Emerging "new" brominated flame retardants in flame retarded products and the environment (TA-2462/2008). 114 pp.

Hermanson, M.H., Isaksson, E., Forsström, S., Teixeira, C., Muir, D.C.G., Pohjola, V.A., Van de Wal, R.S.V., 2010. Deposition history of brominated flame retardant compounds in an ice core from Holtedahlfonna, Svalbard, Norway. Environmental Science & Technology 44(19), 7405-7410.

Hoh, E., Zhu, L., Hites, R.A., 2005. Novel flame retardants, 1,2-Bis(2,4,6- tribromophenoxy)ethane and 2,3,4,5,6-pentabromoethyl

benzene, in United States' environmental samples. Environmental Science & Technology 39(11), 2472-2477.

http://www.epa.gov/oppt/existingchemicals/pubs/actionplans/RIN2070-AZ10_HBCD%20action%20plan_Final_2010-08-09.pdf

Infantino, A., Morselli, M., Di Guardo, A., 2013. Integration of a dynamic organism model into the DynA Model: Development and application to the case of DDT in Lake Maggiore, Italy. Science of the Total Environment 454-455, 358-365.

Kierkegaard, A., Balk L, Tjarnlund, U., de Wit, C.A., Jansson, B., 1999. Dietary uptake and biological effects of decabromodiphenyl ether in rainbow trout (*Oncorhynchus mykiss*). Environmental Science & Technology 33(10), 1612-1617.

La Guardia, M.J., Hale, R.C., Harvey, E., 2007. Evidence of debromination of decabromodiphenyl ether (BDE-209) in biota from a wastewater receiving stream. Environmental Science & Technology 41(19), 6663-6670.

Legler, J., Brower, A., 2003. Are brominated flame retardants endocrine disruptors? Environment International 29(6), 879-885.

Meador, J. P., Stein, J. E., Reichert, W. L., Varanasi, U., 1995. Bioaccumulation of polycyclic aromatic hydrocarbons by marine organisms. Reviews of Environmental Contamination and Toxicology 143, 79-165.

Morris, S., et al., 2004. Distribution and Fate of HBCD and TBBPA Flame Retardants in North Sea Estuaries and Aquatic Food Webs. Environmental Science & Technology 38, 5497-5504.

OSPAR. OSPAR List of Chemicals for Priority Action. http://www.ospar.org/content/content.asp?menu=00120000000074_000000_0000002001 (Update 2007).

Pettigrew, F.A., Landry, S.D., Reed, J.S., 1992. Proceedings of the 5th Flame Retard. '92 Conference. New York. Elsevier Applied Sciences, 156-167.

Renner, R., 2004. In US, flame retardants will be voluntarily phased out. Environmental Science & Technology, 38(1), 14A-14A.

Ross, P.S., Couillard, C.M., Ikonomou, M.G., Johannessen, S.C., Lebeuf, M., Macdonald, R.W., Tomy, G.T., 2009. Large and growing environmental reservoirs of Deca-BDE present an emerging health risk for fish and marine mammals. Marine Pollution Bulletin, 58, 7-10.

Stegeman, J.J., Hahn, M.E., 1994. Biochemistry and molecular biology of monooxigenases: current perspectives on forms, functions and regulation of cytochrome P450 in aquatic species. In: Malins, D.C., Ostrander, G.K. (Eds.), "Aquatic Toxicology: Molecular, Biochemical and Cellular Perspectives", Lewis Publishers, Boca Raton, FL, pp. 87-206.

Stapleton, H.M., 2006. Assessing DecaBDE debromination in the environment. Epha Environment Network, 1-9.

Stapleton, H.M., Brazil, B., Holbrook, R.D., Mitchelmore, C.L., Benedict, R., Konstantinov, A., Potter, D., 2006. In vivo and in vitro debromination of decabromodiphenyl ether (BDE 209) by juvenile rainbow trout and common carp. Environmental Science & Technology 40, 4653-4658.

Tittlemier, S.A., Halldorson, T., Stern, G.A., Tomy, G.T., 2002. Vapor pressures, aqueous solubilities, and Henry's law constants of some brominated flame retardants. Environmental Toxicology and Chemistry 21, 1804-1810.

Tomy, G.T., Palace, V.P., Pleskach, K., Ismail, N., Oswald, T., Danell, R.T., et al., 2007. Dietary exposure of juvenile rainbow trout (*Oncorhynchus mykiss*) to 1, 2-bis(2, 4, 6-tribromophenoxy) ethane: bioaccumulation parameters, biochemical effects, and metabolism. Environmental Science & Technology 41(14), 4913-4918.

Tomy, G.T., Palace, V.P., Halldorson, T., Braekevelt, E., Danell, R.,Wautier, K., Evans, B., Brinkworth, L., Fisk, A.T., 2004. Bioaccumulation, biotransformation and biochemical effects of brominated diphenyl ethers in juvenile lake trout (*Salvelinus*

namaycush). Environmental Science and Technology 38(5), 1496-1504.

U.S. EPA 2010 US. EPA 2008. Tox review of Deca-BDE. EPA/635/R-07/008F.

van der Oost, R., Beyer, J., Vermeulen, N.P.E., 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environmental Toxicology and Pharmacology 13, 57-149.

Vastag, B., 2008. EPA feels heat over flame retardant. Nature 452, 513.

Verreault, J., Gebbink, W.A., Gauthier, L.T., Gabrielsen, G.W., Letcher, R.J., 2007. Brominated flame retardants in Glaucous Gulls from the Norwegian Arctic: more than just an issue of polybrominated diphenyl ethers. Environmental Science & Technology 41, 4925-4931.

Volta, P., Jepsen, N., 2008. The recent invasion of roach (Pisces: Cyprinidae) in a large South-Alpine Lake. Journal of Limnology 67(2), 163-170.

Volta, P., Tremolada, P., Neri, M.C., Giussani, G., Galassi, S., 2009. Age-dependent bioaccumulation of organochlorine compounds in fish and their selective biotransformation in top predators from Lake Maggiore (Italy). Water Air & Soil Pollution 197(1-4), 193-209.

Volta, P., Oggioni, A., Bettinetti, R., Jeppesen, E., 2011. Assessing lake typologies and indicator fish species for Italian natural lakes using past fish richness and assemblages. Hydrobiologia 671, 227-240.

Volta, P., Grimaldi, E., Calderoni, A., Polli, B., 2013. Andamento della pesca professionale sul Lago Maggiore nel periodo 1979-2011. Commissione italo-svizzera per la Pesca (CISPP) 20pp (in It) (in press)

Wania, F., Dugani, C.B., 2003. Assessing the long-range transport potential of polybrominated diphenyl ethers: a comparison of four multimedia models. Environmental Toxicology and Chemistry 22(6), 1252-1261.

Watanabe, I., Sakai, S., 2003. Environmental release and behavior of brominated flame retardants. Environment International 29, 665-682.

WHO World Health Organization, Geneva, 1997. Flame retardants: a general introduction, 192. Environmental health criteria.

Wu, J.P., Guan, Y.T., Zhang, Y., Luo, X.J., Zhi, H., Chen, S.J., Mai, B.X., 2011. Several current-use, non-PBDE brominated flame retardants are highly bioaccumulative: evidence from field determined bioaccumulation factors. Environment International 37 (1), 210-215.

Wu, J.P., Zhang, Y., Luo, X.J., et al., 2012. A review of polybrominated diphenyl ethers and alternative brominated flame retardants in wildlife from China: Levels, trends, and bioaccumulation characteristics. Journal of Environmental Sciences (China) 24(2), 183-194.

CHAPTER II

PBDE, HBCD AND NOVEL BROMINATED FLAME RETARDANT CONTAMINATION IN SEDIMENTS FROM LAKE MAGGIORE (NORTHERN ITALY)

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ABSTRACT

The reduction in the use of polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane (HBCD) has opened the way for the introduction of "novel" BFRs (nBFRs) in place of the banned formulations. Important representatives of this group are decabromodiphenyl ethane (DBDPE), 1,2-bis(2,4,6-tribromophenoxy) ethane (BTBPE), hexabromobenzene (HBB), and pentabromoethylbenzene (PBEB). In this study, the contamination due to novel BRFs has been investigated, for the first time in Italy, in the sediments from Lake Maggiore basin with the aim of characterizing in detail the possible presence of temporal trends and/or identifying potential sources of contamination. The study also considered the PBDE and HBCD lake sediment present contamination. The analytical results showed that Lake Maggiore and its tributary sediments had weak concentrations of PBEB, HBB, and BTBPE, but they had a non negligible contamination with HBCD (up to 23.7 ng/g d.w.). The determination of PBDEs in sediments showed that BDE-209 was the predominant congener (up to 217 ng/g d.w. and 28 ng/g d.w. in river and lake sediments respectively). DBDPE was detected in the sediments with relevant

concentrations (up to 280 ng/g d.w in the River Boesio sediments). The positive correlation of DBDPE with BDE-209 confirmed the wide and important use of this compound in the Lake Maggiore basin and the hypothesis that this compound will soon become one of the most important nBFRs used in Northern Italy. The contamination of Lake Maggiore sediments due to PBDEs and nBFRs were comparable to other worldwide situations.

Keywords: Novel Brominated Flame Retardants; HBCD; PBDEs; Lake Maggiore; sediments

2.1 INTRODUCTION

Among the different classes of organic pollutants released into the environment, the flame retardant compounds (FRs) are recently causing great concern amongst scientists. The brominated flame retardants (BFRs), including PBDEs and HBCD, are currently the largest market group because of their low cost and high performance efficiency. Their global market demand, in fact, greatly continues to grow: from 145,000 tons in 1990 (BSEF 2000) to 411,000 tons in 2007 (BSEF 2013, personal communication). PBDEs are applied in polyurethane foam, electrical and electronic equipment, plastic, furniture textiles, and other materials (de Wit 2002; de Wit et al. 2010). They were produced in commercial mixtures at three different levels of bromination, known as Penta-BDE, Octa-BDE, and Deca-BDE. However, due to their growing environmental and human health concern, the Penta-BDE and Octa-BDE mixtures were banned in Europe in 2004, and the US manufacturers of these commercial mixtures voluntarily stopped their production in the same year (La Guardia et al. 2006). Further, in 2009 the main components of the Penta-BDE and Octa-BDE mixtures were included in the list of persistent organic pollutants (POPs) (UNEP, 2010). Deca-BDE have been banned throughout Europe in electrical and electronical equipment in July 2008 (European Court of Justice 2008), because of concern about a possible formation of more toxic oxidation and/or debromination residuals. Hexabromocyclododecane (HBCD) is the principal flame retardant used in extruded (XEPS) and expanded (EPS) polystyrene foams as thermal insulation in the building industry. The physicochemical properties of HBCD are similar to those of PBDEs and other POPs (de Wit 2002), and recently it has been detected in environmental and biota samples (Abdallah and Harrad 2011). For these reasons, at the sixth meeting of the Conference of the Parties of the Stockholm Convention (May

2013), HBCD was included in the list of POP substances. The listing allows an exemption, valid until 2019, for the production and use of HBCD in expanded polystyrene (EPS) and extruded polystyrene (XPS) in buildings (BSEF, 2013).

The reduction in the use of PBDEs and HBCD has consequently opened the way for the introduction of "novel" BFRs (nBFRs) in place of the banned formulations (Betts 2008). Important representatives of this group are decabromodiphenyl ethane (DBDPE), 1,2-bis(2,4,6-tribromophenoxy) ethane (BTBPE), hexabromobenzene (HBB), and pentabromoethylbenzene (PBEB). DBDPE was introduced in the mid-1980s and became commercially widely used as an alternative to the Deca-BDE formulation in the early 1990s (Arias 2001), being an additive to different polymeric materials (Covaci et al. 2011). Europe does not produce DBDPE, but imports, primarily to Germany, with a quantity between 1000 and 5000 tons in 2001 (Arias 2001). The inclusion of the ethane bridge between the aromatic rings makes DBDPE slightly more hydrophobic than BDE-209. BTBPE has been produced since the mid-1970s and from 2005 it is being used as a replacement for Octa-BDE (Renner 2004). It is marketed for use in ABS, HIPS, thermoplastics, thermoset resins, polycarbonate and coatings (WHO 1997). HBB was widely used in Japan as an additive flame retardant to paper, woods, textiles, electronic and plastic goods, but, at present, it is used at lower volumes (350 tons in 2001) (Watanabe and Sakai 2003). This compound has been found recently in different environmental samples, including herring gull tissues and egg samples from the Great Lakes (Gauthier et al. 2007; 2009), glaucous gulls in the Norwegian arctic (Verreault et al. 2007), air samples in Toronto, Canada (Gouteux et al. 2008), and human blood samples in Tianjin, China (Zhu et al. 2009). PBEB is mostly used in thermoset polyester resins (circuit boards, textiles, adhesives, wire and cable coatings, polyurethane foam)

(Hoh et al. 2005), and it is included in the OSPAR (Oslo/Paris Convention for the protection of the marine environment of the North-East Atlantic) list of chemicals, being ranked as persistent, bioaccumulative and toxic compound, but not currently produced (OSPAR, 2007). PBEB have appeared recently in herring gull eggs and glaucous gull tissues (Gauthier et al. 2007; Verreault et al. 2007), and in Chicago air samples (Hoh et al. 2005).

In Italy, previous studies (Guzzella et al. 2008) have shown high concentrations of PBDEs in the sediments of Boesio and Bardello rivers, located in the Lake Maggiore basin and flowing in the Varese province, where a great number of textile and plastic industries are located. For this reason and for the first time in Italy, in the present investigation, the novel BFR contamination has been investigated, in the sediments from Lake Maggiore and three main tributaries with the aim of characterizing in detail the possible presence of temporal trends and/or identifying potential sources of contamination. The study also considered the PBDE and HBCD lake sediment present contamination. The Lake Maggiore contamination due to the presence of BFRs has been studied by analyzing sediment cores collected in the lake and grab samples taken at the mouth of three main tributaries: Bardello, Boesio and Toce. Moreover, investigating the sediment contamination is crucial in environmental studies because it is plausible that the contaminant uptake by benthic organisms, followed by fish predation, might be a significant source of bioaccumulation.

2.2 MATERIALS AND METHODS

2.2.1 Lake Maggiore sampling stations

Lake Maggiore is the second-largest Italian lake and its BFR contamination was studied in this work by analyzing six sediment cores (Fig. II-1). Sampling stations were selected in order to cover mostly the Central/Southern part of the lake (corresponding to the inflow of Boesio and Bardello, and to the stations LM_55, 27, 28), which represents the area in which the particulate matter transported by tributaries is mainly settled, and Pallanza Bay (stations LM_16, 17, 51). Lake sediments were collected by CNR-ISE in March 2011 with a gravity corer (i.d. = 6 cm). The sediment cores were then opened in the laboratory, photographed, lithologically described, cut into slices, and finally frozen at -18 °C, pending analysis. Only superficial slices were analyzed (Table II-1), whereas the core LM_28 was divided into 11 slices according to a sedimentation rate (about 0.25 cm $year^{-1}$), in order to reconstruct the BFR distribution since the 1970s (Table II-1). The LM_28 core chronology was derived by core correlation based on magnetic susceptibility, biomarkers and geochemical proxies profile with respect to a core dated by Marchetto et al. (2004). In Marchetto et al. (2004), a detailed chronology based on radiometric technique (^{137}Cs and ^{210}Pb), as well as a lithostratigraphical description of sand/clay successions and a planktonic diatom profiles, is described. They estabilished clear marker for the 1963 and 1989 algal transitions, i.e. the documented shift in water samples from *Cyclotella comensis* to *Stephanodiscus* spp and vice versa, and the episodic appearance of *Tabellaria flocculosa.*

Table II-1 Section depth (cm) and relative time dating of Lake Maggiore sediment cores

	LM_16	LM_17	LM_51	LM_55	LM_27
Section (cm)	0-3.6	0-4.5	0-3.6	0-1.5	0-3
Period	9/05-3/11	3/05-3/11	9/05-3/11	9/04-3/11	6/04-3/11

	LM_28										
Section (cm)	0-0.5	0.5-1	1-1.5	1.5-2.5	2.5-3.5	3.5-4.5	4.5-5.5	5.5-6.5	6.5-7.5	7.5-8.5	8.5-9.5
Period	12/08-3/11	10/06-12/08	8/04-10/06	3/00-8/04	10/95-3/00	6/91-10/95	1/87-6/91	9/82-1/87	4/78-9/82	11/73-4/78	7/69-11/73

Station	North Latitude	East Longitude	Depth (m)
LM_16	45°55'06"	8°31'86"	118
LM_17	45°55'45"	8°31'93"	123
LM_51	45°53'75"	8°31'30"	59
LM_55	45°56'29"	8°37'04"	366
LM_27	45°48'60"	8°34'50"	85
LM_28	45°44'55"	8°35'11"	39
Bardello	45°50'46"	8°37'52"	-
Boesio	45°54'20"	8°37'13"	-
Toce	45°56'50"	8°29'23"	-

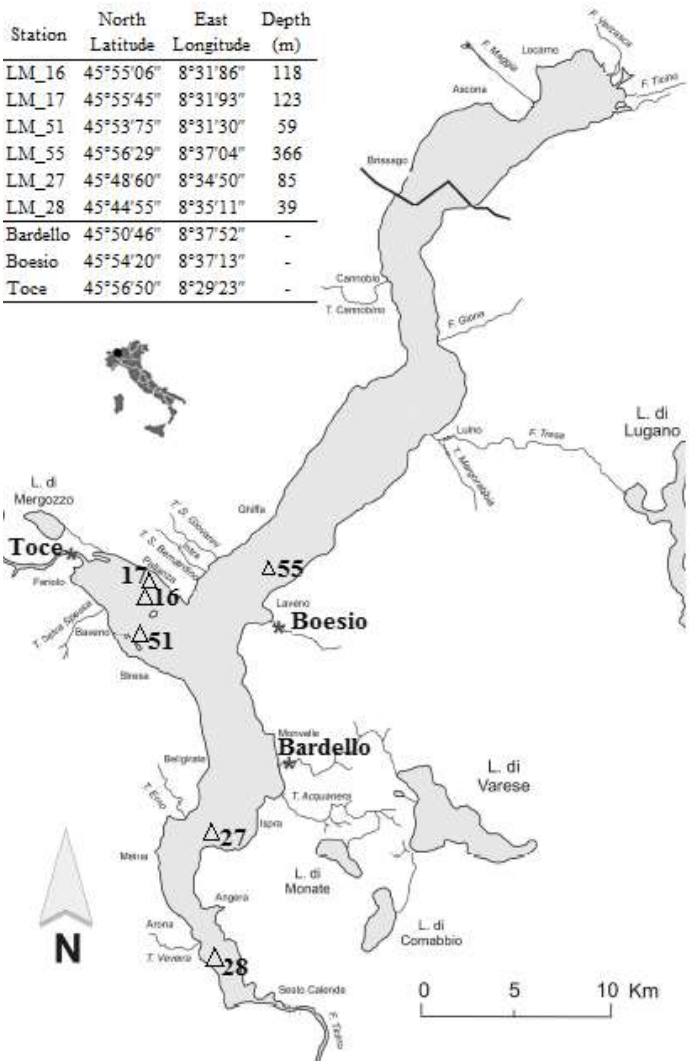

Fig. II-1 Location and geographical coordinates of sediment core sampling stations in the Lake Maggiore (Δ) and of river sampling stations (*)

In addition, three lake tributaries (Toce, Bardello and Boesio) were studied by collecting grab samples at each river mouth. Toce was selected for analysis being the unique river flowing directly in Pallanza Bay, while Bardello and Boesio were considered because of their position in a highly populated and industrialized area. Sediments were taken by CNR-IRSA from March 2011 to July 2012 every three months. For each river, 10 different sub-samples were collected and mixed in order to obtain a representative sediment sample. Organic carbon content of sediments was determined on 0.5-1 g of dry weight by back-titration after oxidation with potassium dichromate in the presence of sulphuric acid (Walkley and Black 1934).

2.2.2 Sample preparation and analytical procedure

Sample preparation can be summarized as follow: after lyophilisation, sediment samples were sieved collecting the fine fraction (< 63 µm). Before analysis, a variable amount of sieved sample (from 1 to 2 g) was spiked with a recovery standard containing the labeled compounds [$^{13}C_{12}$]γHBCD, [$^{13}C_{12}$]BDE-209, [$^{13}C_{12}$]BDE-28, -47, -99, -153, -154, -183 (Wellington Labs, Canada), and then extracted in a hot Soxhlet apparatus (Buchi - Flawil, Switzerland) using a *n*-hexane/acetone mixture (3:1 *v/v*) for 25 cycles. The extracts were concentrated to 1 mL by Turbovap (Zymark - Hopkinton, USA) on a gentle nitrogen stream. The clean-up procedure was performed using a multi-layer column (1.5 x 20 cm) packed (bottom to top) with 1.5 g of acidified silica gel 30% *w/w* sulphuric acid (Sigma-Aldrich, Germany), and 1.5 g of Florisil® (100-200 mesh, Sigma-Aldrich, Germany). The column was pre-washed with 15 mL of *n*-hexane/dichloromethane (*n*-hexane/DCM) 1:1 *v/v*, and the elution was performed collecting 40 mL of the same solvent. 1 mL of toluene was added to the extract, concentrated by

Turbovap on a gentle nitrogen stream, and then reconstituted to 100 µL using toluene.
GC analysis for BFR compounds were performed using a Thermo Electron TraceGC 2000 coupled with a PolarisQ Ion Trap (ThermoElectron - Austin, Texas) mass spectrometer and equipped with a PTV injector and an AS 3000 auto sampler. The system was managed by ThermoFinnigan Xcalibur software version 1.4.1. Separation of PBDE congeners (BDE-28, 47, 100, 99, 153, 154, 183) was achieved using a Agilent DB-5MS-UI capillary column, 50 m x 0.25 mm i.d. x 0.25 µm film thickness (Agilent, Palo Alto, California, USA) in the following conditions: carrier gas helium at 1.2 mL/min; injection pressure of 120 kPa; transfer pressure of 240 kPa; injector temperature starting at 70 °C and maintained for 1.2 min, then ramped to 280 °C (held 1.2 min) at 14 °C/s; initial oven temperature set at 70 °C (held 1 min), then ramped to 220 °C at 30 °C/min (held 1 min) and finally to 290 °C at 4 °C/min (held 20 min). Samples were analyzed using tandem mass spectrometry under the following instrumental conditions: EI mode with standard electron energy of 70 eV; the transfer line was maintained at 280 °C, the damping gas at 1 mL/min, and the ion source at 260 °C. Separation of PBDE congeners (BDE-179, 188, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209) and HBCD, PBEB, HBB, BTBPE was achieved using a Restek RXi-1MS capillary column, 12 m x 0.20 mm i.d. x 0.33 µm film thickness (Restek U.S., Bellefonte, Pennsylvania, USA) in the following conditions: carrier gas helium at 1.1 mL/min; injection pressure of 70 kPa; transfer pressure of 110 kPa; injector temperature starting at 70 °C and maintained for 0.2 min, then ramped to 300 °C (held 1.5 min) at 8 °C/s; initial oven temperature set at 100 °C (held 1 min), then ramped to 220 °C at 40 °C/min (held 0.1 min) and finally to 300 °C at 15 °C/min (held 14 min). Samples were analyzed using tandem mass

spectrometry under the following instrumental conditions: EI mode with standard electron energy of 70 eV; the transfer line was maintained at 300 °C, the damping gas at 1.5 mL/min, and the ion source at 250 °C.
Quantitative analysis was performed using external standard method (purchased from Wellington Labs, Canada). DBDPE measurements were undertaken using a TraceGC Ultra equipped with a cold on-column injector and an ECD-40 detector (ThermoElectron, Austin, Texas) using a Restek RTX-5 capillary column (15 m x 0.53 mm i.d. x 0.1 µm film thickness; Restek, Bellefonte, USA).
The use of a different analytical method and of a shorter column for DBDPE allows the analysis of this more unstable compound. Injections (0.5 µL) were performed using a TriPlus autosampler (Thermo Electron) and carried out in the following analytical conditions: carrier gas helium at 6.0 mL/min; starting temperature of 100 °C (held 0.5 min) after which it was ramped to 280 °C at 15 °C/min (held 8 min). Quantitative analysis was obtained by comparing results with external standard (purchased from Wellington Labs, Canada).

2.2.3 Quality Assurance (QA) and Quality Control (QC)

For PBDE (BDE-28, 47, 99, 153, 154, 209) sediment analysis, the method performance was evaluated using the BROC-2 candidate CRM (Candidate Reference Material) for sediments purchased from RIVO (Netherlands Institute of Fisheries Research). All values found were within the certified range of reference concentration (± 30%). The mean recoveries of the spiked standards was 53% ± 19 for [$^{13}C_{12}$]BDE-28, 65% ± 22 for [$^{13}C_{12}$]BDE-47, 61% ± 27 for [$^{13}C_{12}$]BDE-99, 62% ± 18 for [$^{13}C_{12}$]BDE-153, 55% ± 23 for [$^{13}C_{12}$]BDE-154, 67% ± 35 for [$^{13}C_{12}$]BDE-209, and 83% ± 9 for [$^{13}C_{12}$]γHBCD. The analytical results obtained were corrected considering the recoveries, and

the sample analysis was repeated if its mean recovery was below 30%. The results were obtained using the external standard method with four calibration points (a R^2>0.9900 was considered): PBDE concentrations of tri to nona-congeners ranged from 5 to 100 µg/L; BTBPE concentrations ranged from 25 to 500 µg/L; BDE-179 concentrations ranged from 10 to 200 µg/L; BDE-188, HBB, PBEB, HBCD, DBDPE concentrations ranged from 25 to 500 µg/L; BDE-209 concentrations ranged from 12 to 240 µg/L.
Using a signal-to-noise ratio of 3:1, the limits of detection (LODs) were estimated as 0.01 ng/g dry weight for each compound in sediment samples. Blank concentrations were below LOD levels for all BFR compounds.

2.3 RESULTS AND DISCUSSION

2.3.1 Concentration and distribution of BFRs in sediments

The BFR concentrations in the river sediments and in the lake sediment layers settled between 2004/2005 and 2011 are summed up in Table II-2. Concentrations in river and lake sediments on organic carbon content are listed in Table II-3. In all river sediments, the concentrations of PBEB and HBB were very low and in most cases close to LOD. The contamination due to BTBPE was limited, never exceeding 2.3 ng/g d.w., as measured in the sediments of Boesio collected in March 2012. HBCD was detected in the river sediments with concentrations ranging from 2.6 to 23.7 ng/g d.w. The presence of similar HBCD contamination levels in the sediments of the three rivers (Table II-2) leads us to hypothesize that there are no industrial point emissions of contamination in the lake basin. On the contrary, the sediment analysis pointed out an important contamination due to DBDPE, ranging from 3.4 to 280 ng/g d.w. in the Toce and Boesio sediments respectively. It was supposed that the high concentrations of DBDPE in October 2011 and

March 2012 in the River Boesio could be due to a very recent source of contamination caused by the peculiar Northern Italian meteorological conditions. The occurrence of heavy rains in summer 2011, in fact, could have caused an additional input of contaminated suspended particle matter transported by this river. As it was observed for the river sediments, the concentrations of PBEB, HBB, HBCD and BTBPE in the lake sediment samples were very low and in a few cases close to or below LOD values. For HBCD and BTBPE in particular, since we presume no industrial point emissions are present in this region, the slight higher concentrations in the Southern stations (LM_27 and 28) might be attributed to the "focusing" phenomenon (Baudo et al. 1989), i.e. the preferential transport of the lighter and smaller organic carbon enriched particles (rich in organic compounds) to this area, where they settle because of the sharply reduced depth. This behaviour is due to the shape of the lake bottom and to the prevailing water movement towards the lake outlet; because of this, sorting of the sediment by size occurs (Baudo et al. 1989). Confirming this hypothesis, concentrations of HBCD and BTBPE, normalized on organic carbon content, did not show the same behaviour. Differently from PBEB, HBB, HBCD, and BTBPE, the contamination due to DBDPE in the lake sediment samples ranged from 7.2 ng/g d.w. in the station LM_17 (located in Pallanza Bay) to 31.7 ng/g d.w. in the station LM_28 (the Southernmost of the lake). As regard DBDPE, sediments collected in the stations LM_55, LM_27, and LM_28 showed a generally higher contamination than those located in Pallanza Bay, being affected by the contaminant input from Bardello and Boesio rivers.

Table II-2 BFR concentration values (ng/g d.w.) in river (A) and in lake (B) sediments; LOD=limit of detection

	March 2011			July 2011		October 2011			March 2012			July 2012	
A	Bardello	Boesio	Toce	Bardello	Toce	Bardello	Boesio	Toce	Bardello	Boesio	Toce	Bardello	Toce
%OC	3.1	3.9	4.2	3.5	1.3	3.2	7.8	1.5	3.2	4.9	2	2.9	0.6
PBEB	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.2
HBB	0.21	0.22	<LOD	<LOD	0.10	<LOD	<LOD	0.09	<LOD	<LOD	0.05	0.07	0.06
HBCD	11.63	23.70	<LOD	<LOD	3.5	15.13	12.60	9.62	3.76	7.86	7.50	2.63	2.76
BTBPE	0.44	<LOD	<LOD	<LOD	<LOD	0.53	0.895	<0,01	<LOD	2.26	<LOD	<LOD	<LOD
DBDPE	21.40	12.50	28.80	58.40	<LOD	42.20	251.00	15.13	19.50	280.00	10.25	14.00	3.35
Σ hepta-decaBDE	47.28	43.79	14.25	143.34	2.23	146.52	46.91	21.61	219.34	94.56	15.15	46.18	4.52

B	LM_16	LM_17	LM_51	LM_55	LM_27	LM_28
% OC	2.4	2.5	3.6	5.2	4.3	6.7
PBEB	<LOD	<LOD	<LOD	0.02	<LOD	0.03
HBB	<LOD	<LOD	0.02	<LOD	0.03	0.03
HBCD	4.73	4.40	2.60	4.10	5.11	2.78
BTBPE	<LOD	0.18	0.22	0.69	0.77	1.78
DBDPE	19.70	7.16	16.37	24.40	31.70	31.57
Σ hepta-decaBDE	10.35	7.05	8.28	28.04	14.69	17.57

Table II-3 BFR concentration values (ng/mg O.C.) in river (A) and lake (B) sediments; LOD=limit of detection

	March 2011			July 2011		October 2011			March 2012			July 2012	
A	Bardello	Boesio	Toce	Bardello	Toce	Bardello	Boesio	Toce	Bardello	Boesio	Toce	Bardello	Toce
PBEB	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.0333
HBB	0.0068	0.0056	<LOD	<LOD	0.0075	<LOD	<LOD	0.0057	<LOD	<LOD	0.0024	0.0024	0.0100
HBCD	<LOD	0.6077	<LOD	<LOD	0.2692	<LOD	0.1615	0.6413	<LOD	<LOD	0.3750	<LOD	0.4600
BTBPE	0.0140	<LOD	<LOD	<LOD	<LOD	0.0166	0.0115	<LOD	<LOD	0.0461	<LOD	<LOD	<LOD
DBDPE	0.6903	0.3205	0.6857	1.6686	<LOD	1.3188	3.2179	1.0087	0.6094	5.7143	0.5125	0.4828	0.5583
Σ hepta-decaBDE	1.5251	1.1228	0.0012	4.0953	0.1714	4.5787	0.6010	1.4407	6.8543	1.9299	0.7573	1.5924	0.7530

B	LM_16	LM_17	LM_51	LM_55	LM_27	LM_28
PBEB	<LOD	<LOD	<LOD	0.0004	<LOD	0.0004
HBB	<LOD	<LOD	0.0005	<LOD	0.0007	0.0004
HBCD	0.1954	0.1732	0.0718	0.0796	0.1200	0.0001
BTBPE	<LOD	0.0071	0.0061	0.0134	0.0181	0.0265
DBDPE	0.8140	0.2819	0.4522	0.4738	0.7441	0.4690
Σ hepta-decaBDE	0.4276	0.2776	0.2288	0.5444	0.3448	0.2610

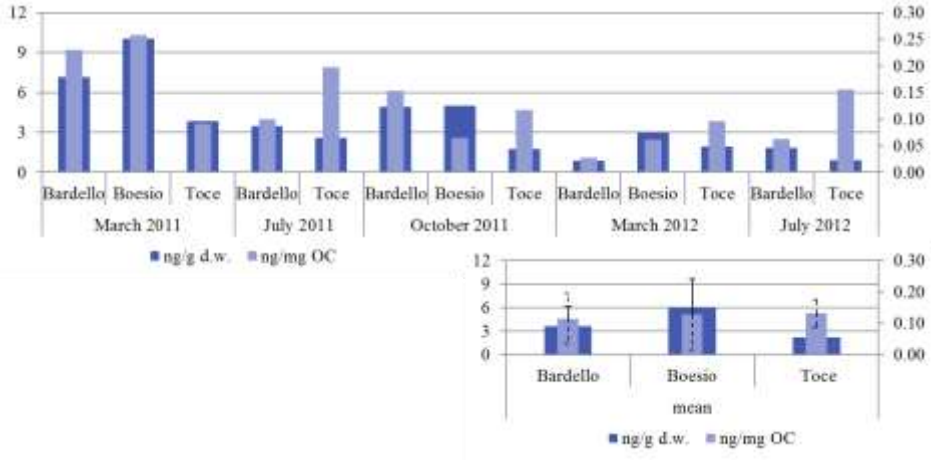

Fig. II-2 Tri- to hepta-BDE concentrations (ng/g d.w. and ng/mg OC) in river sediments

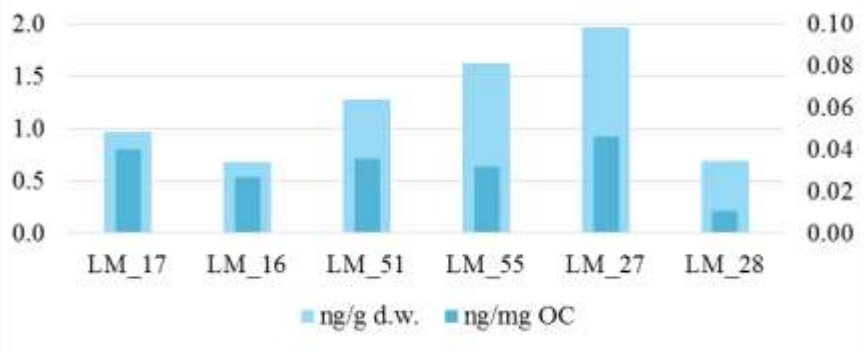

Fig. II-3 Tri- to hepta-BDE concentrations (ng/g d.w. and ng/mg OC) in lake sediments

Regarding the ΣBDE analysis, and considering tri- to hepta-BDE congeners (BDE-28, 47, 99, 100, 153, 154, 183), the concentrations measured in the river sediments confirmed that

Bardello and Boesio are the most polluted rivers (Fig. II-2). The ΣBDE concentrations in the lake sediments ranged from 0.68 ng/g d.w. in station LM_16 to 2.0 ng/g d.w. in station LM_27 (Fig. II-3). In particular, because of the contribution of contaminated sediments transported by Boesio and Bardello, the lake sediments at station LM_27 and LM_55, collected in the nearby of the two rivers, were the most contaminated samples.
Finally, the concentrations of Σhepta- to deca-BDE (BDE-188, 179, 202, 201, 204, 197, 198, 199, 200, 203, 196, 205, 194, 206, 207, 208, 209) in river and lake sediments are shown in Table II-4 and Table II-5 respectively. In particular, the hepta- and octa-BDE contamination was very low and close to LOD. On the contrary, BDE-209 was the predominant congener in all the sediments, representing the 97-99 % of the considered ΣBDEs. Nona-BDE congeners were detected in all sediments, showing a slight increasing trend from the Central to the Southern stations. Moreover, the percentage distribution of BDE-206, 207, 208 and 209 observed in the river and lake sediments was similar to the one of Deca-BDE commercial mixture, indicating that nona-BDE contamination in sediments could likely derive from technical formulation impurities and not from BDE-209 debromination.

Table II-4 Single hepta- to deca-BDE congener concentrations (ng/g d.w.) in river sediments; LOD=limit of detection

	March 2011			July 2011		October 2011			March 2012			July 2012	
	Bardello	Boesio	Toce	Bardello	Toce	Bardello	Boesio	Toce	Bardello	Boesio	Toce	Bardello	Toce
BDE-188	<LOD	0.03	<LOD	<LOD	<LOD	<LOD	<LOD	0.03	<LOD	<LOD	0.03	<LOD	0.06
BDE-179	<LOD	0.02	0.028	<LOD	0.05	<LOD	<LOD	0.06	0.021	<LOD	0.04	0.06	0.47
BDE-202	0.04	<LOD	<LOD	0.037	<LOD	0.037	<LOD	<LOD	<LOD	<LOD	0.04	<LOD	<LOD
BDE-201	0.043	0.04	0.021	<LOD	<LOD	0.021	<LOD	0.03	<LOD	0.05	0.05	0.053	<LOD
BDE-204-197	0.05	<LOD	<LOD	<LOD	0.06	<LOD	<LOD	<0,01	<LOD	<LOD	0.1	0.103	0.16
BDE-198-199-200-203	<LOD	0.133	<0,01	<0,01	0.06	<0,01	<LOD	0.06	<LOD	<LOD	0.13	0.054	0.12
BDE-196	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.09	0.07	<LOD	<LOD	0.13	<LOD	0.11
BDE-205	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.03	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
BDE-194	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
BDE-208	0.14	0.075	<LOD	0.17	0.04	0.135	0.619	0.06	0.246	0.492	0.15	0.253	0.03
BDE-207	0.281	0.141	<LOD	0.54	0.04	0.188	1.058	0.18	0.59	1.032	0.24	0.361	0.06
BDE-206	0.823	0.55	<LOD	1.69	0.2	0.736	3.808	0.43	0.78	4.989	0.55	0.796	0.19
BDE-209	45.9	42.8	14.2	140.9	1.77	145.4	41.3	20.7	217.7	88	13.7	44.5	3.31

Table II-5 Single hepta- to deca-BDE congener concentrations (ng/g d.w.) in lake sediments; LOD=limit of detection

	LM_16	LM_17	LM_51	LM_55	LM_27	LM_28
BDE-188	<LOD	<LOD	<LOD	0.02	0.02	0.05
BDE-179	<LOD	<LOD	<LOD	<LOD	<LOD	0.04
BTBPE	<LOD	0.18	0.22	0.69	0.77	1.78
BDE-202	0.02	<LOD	0.03	<LOD	0.02	0.05
BDE-201	0.30	<LOD	<LOD	0.06	0.03	0.05
BDE-204_-197	<LOD	<LOD	0.03	0.05	0.05	0.09
BDE-198_-199_-200_-203	<LOD	<LOD	<LOD	<LOD	0.07	0.01
BDE-196	<LOD	<LOD	<LOD	<LOD	0.04	0.01
BDE-205	<LOD	<LOD	<LOD	<LOD	<LOD	0.01
BDE-194	<LOD	<LOD	<LOD	<LOD	<LOD	0.01
BDE-208	0.02	0.03	<LOD	<LOD	0.05	0.06
BDE-207	0.03	0.06	0.03	0.04	0.07	0.16
BDE-206	0.08	0.07	0.10	0.08	0.14	0.24
BDE-209	9.90	6.90	8.10	27.80	14.20	16.80

2.3.2 Temporal trends of BFR contamination in lake sediments

The temporal trends of BFR contamination in the lake sediments were measured in the core LM_28 considering a period of about 40 years, from 1969 to 2011 (Table II-6).

Table II-6 BFR values and single hepta- to deca-BDE congener concentrations (ng/g d.w.) in LM_28 lake sediments; LOD=limit of detection

	LM_28											
	3/11-12/08	12/08-10/06	10/06-8/04	8/04-3/00	3/00-10/95	10/95-6/91	6/91-1/87	1/87-9/82	9/82-4/78	4/78-11/73	11/73-7/69
PBEB	0.02	0.06	<LOD	0.17	0.92	0.11	0.03	0.05	<LOD	<LOD	0.02
HBB	0.08	<LOD	<LOD	<LOD	0.15	0.16	<LOD	<LOD	<LOD	<LOD	<LOD
HBCD	3.44	0.77	4.12	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
BTBPE	0.35	2.90	2.10	1.41	4.23	0.27	1.52	0.29	2.90	<LOD	<LOD
DBDPE	37.10	28.70	28.90	16.23	10.30	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
BDE-188	0.02	0.07	0.06	0.03	0.06	<LOD	<LOD	<LOD	0.04	<LOD	<LOD
BDE-179	0.03	0.10	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
BDE-202	0.02	0.12	<LOD	0.02	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
BDE-201	0.07	0.09	<LOD	0.06	0.15	<LOD	0.03	<LOD	<LOD	<LOD	<LOD
BDE-204_-197	0.09	<LOD	0.17	0.18	0.26	0.03	0.54	0.03	<LOD	<LOD	<LOD
BDE-198_-199_-200_-203	<LOD	<LOD	<LOD	<LOD	<LOD	0.02	<LOD	<LOD	<LOD	<LOD	<LOD
BDE-196	<LOD	<LOD	<LOD	0.12	<LOD	<LOD	0.29	<LOD	<LOD	<LOD	<LOD
BDE-205	<LOD	<LOD	<LOD	<LOD	<LOD	0.02	<LOD	<LOD	<LOD	<LOD	<LOD
BDE-194	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
BDE-208	0.08	0.04	0.06	0.10	0.16	<LOD	0.09	0.05	<LOD	<LOD	<LOD
BDE-207	0.16	0.24	0.09	0.23	0.22	0.03	0.24	0.15	0.07	0.04	<LOD
BDE-206	0.26	0.26	0.19	0.25	0.39	0.02	0.37	0.09	0.12	0.15	<LOD
BDE-209	16.10	15.00	19.30	15.20	25.00	14.05	19.00	12.90	11.70	10.80	2.20

As regard the contamination due to PBEB and HBB, the concentrations were very low and in most cases close to LOD, but the BTBPE concentrations ranged from 0.27 ng/g d.w. in 1991-1995 to 4.2 ng/g d.w. in the period 1995-2000. HBCD was detected only in the three recent sediment layers (from 2004 to 2011), with concentration ranged from 0.7 to 4.1 ng/g d.w., suggesting a recent use of HBCD in the lake basin. Considering Σtri- to hepta-BDE contamination in the LM_28 core samples, the concentrations ranged from <LOD in the deepest layers (from 1969 to 1991) to 3.1 ng/g d.w. in the most recent one. The Fig. II-4 showed an increasing trend of tri- to hepta-BDE from the beginning of the '90s up to now, confirmed also by the data normalized on organic carbon content.

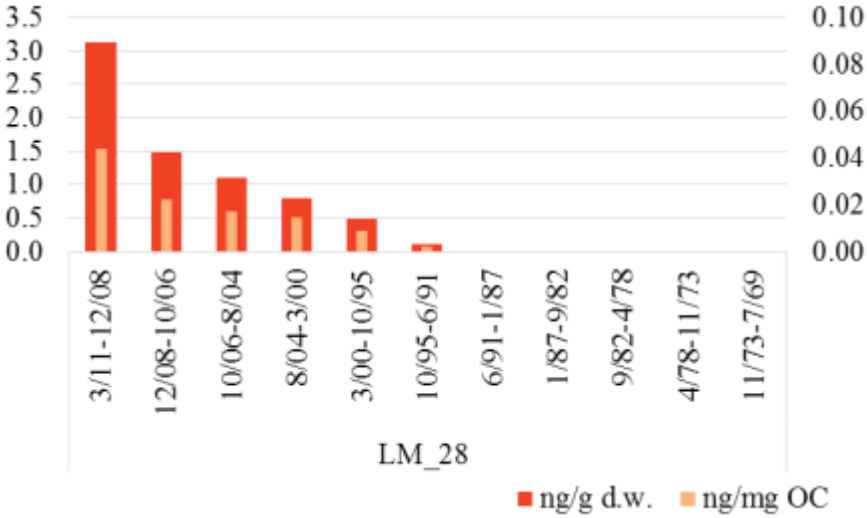

Fig. II-4 Tri- to hepta-BDE concentrations (ng/g d.w. and ng/mg O.C.) in the LM_28 lake sediments

Despite the ban of these substances in 2004 (Directive EEC 2003), the presence of these congeners could likely be due to the disposal of materials containing these compounds. Regarding the Σhepta to deca-BDE contamination, BDE-209 was the predominant congener also in the LM_28 sediments, representing the 97-99 % of the considered BDEs and also in this case, the comparison between the percentage distribution of nona/deca BDE congeners confirmed that nona-BDE could derive from the use of Deca-BDE technical formulation rather than BDE-209 debromination. Besides, DBDPE contamination in the sediment core LM_28 showed a very evident increasing trend from 1995 up to now (37.1 ng/g d.w.) (Fig. II-5) probably correlated to its mass production as an alternative to the Deca-BDE formulation since 1990s (Arias 2001).

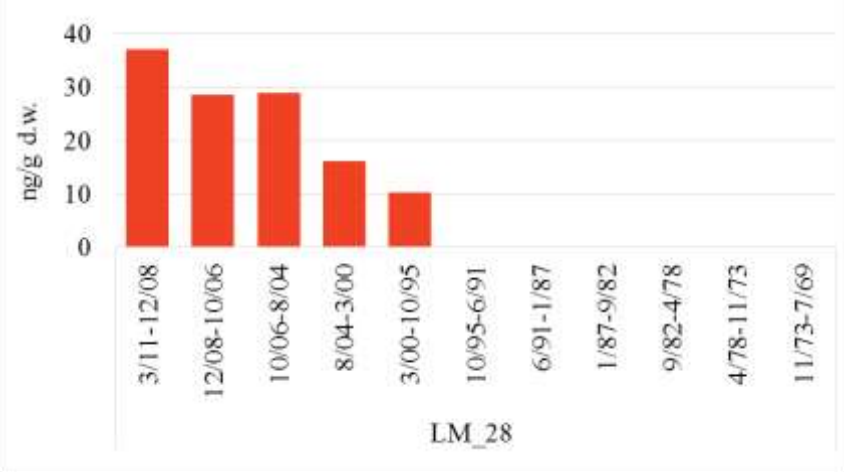

Fig. II-5 DBDPE contamination (ng/g d.w.) in the LM_28 lake sediments

Our data trends regarding the Lake Maggiore contamination due to BDE-209 and DBDPE are very similar to those reported by Wei et al. (2012) who calculated the concentration temporal trend of these substances in sediment cores collected from water bodies located close to BFR manufacturing industries in the Eastern and Southern Arkansas. In particular, they evidenced the onset of BDE-209 in sediments from the early 1970s, with a maximum BDE-209 concentration approximately dated to 1996-1999 period, while the concentration of DBDPE have been increasing since 2000 with the highest concentrations in the surface sediments (Wei et al. 2012).

2.3.3 Considerations on BFR composition in sediments

The mean relative contributions of the different BDE congeners to the total ΣBDE concentration were determined in the lake and river sediments (Fig. II-6). The dominance of BDE-209 in the total ΣBDEs is evident both in river and lake sediment samples, with an average contribution of 84.3% and 88.6% respectively, showing a clear contribution to the lake contamination due to the use of Deca-BDE technical mixture in the basin. Next to BDE-209, BDE-99 and -47 are the most abundant congeners, with a very similar percentage of 3.5 % and 2.9% in the tributaries, and 3.1 % and 2.9 % in the lake sediments respectively. Focusing only on these penta-BDE congeners, their average contributions to the sediments contamination are similar to the one of Bromkal 70-5DE technical formulation (Sjödin et al. 1998), confirming the hypothesis that also technical Penta-BDE formulation had an important role in the Lake Maggiore basin. Regarding the spatial distribution of the different congeners (Fig. II-6), the sediments collected in Pallanza Bay have a similar fingerprint, consistent with the contribution of BDE-47, 99 and 100, while a different profile, less enriched in penta-BDE congeners, was observed for LM_55 and LM_28 sediments. The highest content in organic

carbon of these last sediments may explain a possible faster biodegradation of penta-BDE congeners compared to BDE-209.

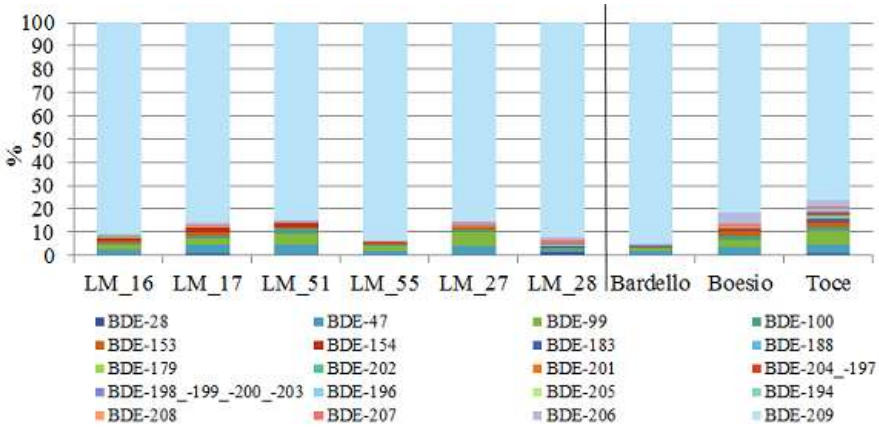

Fig. II-6 Percentual contribution of different congeners to total PBDEs in the lake and river sediments

In order to explore the possible relationship between the main contaminants measured in the Lake Maggiore basin, the correlations between HBCD, DBDPE, and nine BDE congeners were calculated using a Spearman correlation in R statistical software (version 2.12.1) (Table II-7). The results showed a significant correlation among BDE-208, -207, -206, and -209, confirming that these congeners, composing the Deca-BDE technical formulation, are strongly related. The correlation was highly significant also between BDE-100 and BDE-99 or BDE-153 ($r=0.760$, $p<0.01$, and $r=0.467$, $p<0.05$ respectively), suggesting a common contamination source. Similarly, a statistically significant correlation was evident for BDE-209 and DBDPE ($r=0.596$, $p<0.05$), implying a potential similar usage of these compounds as flame retardants in the lake basin. On the

contrary, the correlations between BDE-209 and the other congeners were very low and not statistically significant (p>0.05), pointing out that the presence of tri- to hepta-congeners in sediments derived likely from the use of different commercial PBDE formulations in the lake basin.

Table II-7 Correlations coefficients among HBCD, DBDPE, and PBDEs using the Spearman method

	HBCD	DBDPE	BDE-47	BDE-99	BDE-100	BDE-153	BDE-154	BDE-208	BDE-207	BDE-206	BDE-209
HBCD	1.000										
DBDPE	0.144	1.000									
BDE-47	0.118	-0.002	1.000								
BDE-99	0.062	-0.123	0.374	1.000							
BDE-100	0.036	-0.042	0.368	0.760**	1.000						
BDE-153	0.077	0.291	-0.130	0.361	0.467*	1.000					
BDE-154	0.490	-0.110	-0.370	0.101	0.200	0.381	1.000				
BDE-208	0.359	0.335	0.164	-0.106	-0.079	0.005	0.039	1.000			
BDE-207	0.355	0.391	0.111	-0.183	-0.105	-0.038	-0.007	0.977**	1.000		
BDE-206	0.356	0.391	0.233	-0.015	0.081	0.011	0.099	0.943**	0.939**	1.000	
BDE-209	0.296	0.596**	0.515*	-0.047	-0.005	-0.078	-0.205	0.6869**	0.726**	0.740**	1.000

* p-value < 0.05

** p-value < 0.01

2.3.4 Comparison of different BFRs in sediments

In the Fig. II-7 the concentrations of different BFRs in the river and lake sediments are compared. Results showed that no significant difference was observed between the average concentrations of PBEB, HBB, HBCD, BTBPE, and ΣBDEs (from tri- to hepta-BDE) in both cases. On the contrary, the levels of contamination due to DBDPE and ΣBDEs (from hepta- to deca-BDE) in river and lake sediments are quite different. For these compounds, in fact, we supposed that the lake contamination might be due to the important input of contaminated sediments through Boesio and Bardello rivers. Moreover, the contamination due to DBDPE was slightly greater than the one due to Σhepta-deca BDE (even though not statistically significant), and it has been hypothesized that it might be because DBDPE is recently becoming commercially more important than the Deca-BDE technical formulation (Arias 2001). To verify the hypothesis of the possible extended use of DBDPE in Italy, the ratio between the concentrations of DBDPE and BDE-209 was calculated for recent (2004-2011) sediments collected in the Lake Maggiore and the three rivers. The results showed that the ratio is generally greater than 1 in 52% of samples (90% and 30% of the lake and river samples respectively), ranging from 1.0 to 6.1, depending on the sample. Our considerations are consistent with the study conducted by Guerra et al. (2010) regarding the ratio between DBDPE and BDE-209 in the sediments of the Llobregat river basin in Spain, confirming therefore the wide and recent important use of DBDPE in Italy.

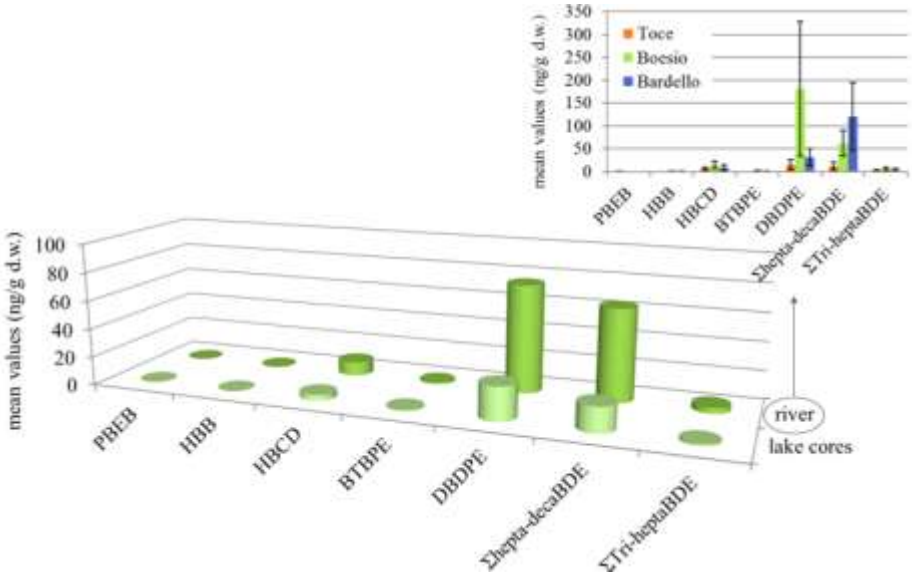

Fig. II-7 Mean BFR contamination in river and lake sediments

2.3.5 Comparison with other studies

The results of the contamination due to PBDE, HBCD, and nBFRs in sediments from Lake Maggiore basin were compared with those from other contaminated and uncontaminated regions. In sediment samples collected in Japan in 1982, Watanabe and Sakai (2003) detected HBB ranging from <0.9 to 4.3 ng/g d.w.. Guerra et al. (2010) investigated the occurrence of emerging BFRs, including PBEB and HBB, in sediments samples from Llobregat River basin (Spain) and the contents of PBEB and HBB ranged from 3 to 10 ng/g d.w. and from 0.4 to 2.4 ng/g d.w., respectively. Wu et al. (2010) determined the average concentrations of HBB (8672 ng/g w.w.) and PBEB (132 ng/g w.w.) in the sediments collected from an e-waste recycling site in South China. HBB and PBEB concentrations detected in the present study are lower than those previously

reported, suggesting that the sediments collected in the Lake Maggiore basin are poorly polluted for these compounds. The contamination due to HBCD was investigated in European sediments, showing very different concentration levels. For example, Harrad et al. (2009) determined the presence of HBCD in sediments from English lakes ranging from 0.88 ng/g d.w in Wake Valley Pond to 4.8 ng/g d.w. in Edgbaston Pool, very similar to the concentrations found in the sediments from Lake Maggiore. The English lakes were hypothesized to be not directly impacted by point emissions of HBCD production industries, such as the case of Lake Maggiore sediments. Regarding DBDPE, Guerra et al. (2010) investigated its presence in sediments from Llobregat river basin finding concentrations ranged from 4.8 to 24 ng/g d.w. In comparison with these data, the DBDPE contamination in the Lake Maggiore appears to be moderately high.

Considering the PBDE sediment contamination, it is evident that the main publications are focused on the study of the main congener composition, highlighting the presence of BDE-209 as the main congener affected the superficial sediments (Gereke et al. 2003; Voorspoels et at. 2003; Sawal et al. 2004; Söderström et al., 2004; Cai et al. 2012). Klosterhaus et al. (2012) recently published a study regarding the presence of BFRs in San Francisco Bay sediments considering PBDEs (BDE-28, 47, 99, 100, 153, 154, 206, 207, 208, and 209), HBCD, PBEB, BTBPE, HBB, and DBDPE. ΣBDEs ranged from 2 to 8 ng/g d.w. and BDE-209 was the dominant congener. PBDE concentrations in San Francisco Bay were typically two times lower than those in Lake Maggiore, confirming the relevant pollution of this Italian site. Total HBCD concentrations in sediments ranged from 0.1 to 2 ng/g d.w., generally from two to ten times lower than HBCD concentration in Lake Maggiore sediments in this study. PBEB in San Francisco Bay sediments was detected at maximum

concentrations of 0.1 ng/g d.w., very similar to those of Lake Maggiore samples, while BTBPE levels in San Francisco Bay was detected at maximum concentration of 0.06 ng/g d.w., about 10 to 100 times lower than results from Lake Maggiore sediments here reported. HBB and DBDPE, on the contrary, were not detected in San Francisco Bay sediments.

2.4 CONCLUSIONS

NBFR contamination was measured in sediments from the Lake Maggiore basin, providing information on their levels, distribution patterns, temporal trends, and possible correlations with other contaminants. The results showed that the lake and river sediments had weak concentrations of PBEB, HBB, and BTBPE, but a not negligible contamination due to HBCD. BDE-209 was the predominant congener in all the considered samples, still highlighting the use of Deca-BDE formulation in the Lake Maggiore basin. Moreover, a limited but still detectable presence of congeners BDE-47, 99 and 100 in the sediments was observed and it might confirm the hypothesis that also technical Penta-BDE formulation had an important use in the basin. DBDPE was detected in the Lake Maggiore sediments with concentrations similar to BDE-209, showing a moderately high contamination of the lake sediments and a particularly important pollution of Boesio River, on the Lombardy coast. In addition, a positive correlation between DBDPE and BDE-209 was observed, confirming a wide and important use of DBDPE in the Lake Maggiore basin and the hypothesis that this compound will soon become one of the most important nBFRs used in the Northern Italy.

2.5 ACKNOWLEDGEMENTS

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2.6 REFERENCES

Abdallah, M.A-E., Harrad, S., 2011. Tetrabromobisphenol-A, hexabromocyclododecane and its degradation products in UK human milk: Relationship to external exposure. *Environment International* 37(2), 443-448.

Arias, P.A., 2001. Brominated flame retardants - An overview. The Second International Workshop on Brominated Flame Retardants (BFR 2001) May 14–16 Stockholm 17-19.

Ashton, M., Kantai, T., Kohler, P.M., Roemer-Mahler, A., Templeton, J., 2009. Summary of the Fourth Conference of the Parties to the Stockholm Convention on Persistent Organic Pollutants, May 4–8. http://chm.pops.int/Programmes/NewPOPs/The9newPOPs/tabid/672/language/en-US/Default.aspx. Accessed 26 July 2013.

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). Science of the Total Environment 87/88, 117-128.

Betts, K., 2008. New flame retardants detected in indoor and outdoor environments. Environmental Science & Technology 42(18), 6778.

Binelli, A., Roscioli, C., Guzzella, L., 2006. Improvements in the analysis of decabromodiphenyl ether using on-column injection and electron-capture detection. Journal of Chromatography A 1136(2), 243-247.

Cai, M.G., Hong, Q.Q., Wang, Y., Luo, X.J., Chen, S.J., Cai, M.H., Qiu, C.R., Huang, S.Y. Mai, B.X., 2012. Distribution of polybrominated diphenyl ethers and decabromodiphenylethane in surface sediments from the Bering Sea, Chukchi Sea, and Canada Basin. Deep-Sea Research II, 81-84, 95-101.

Covaci, A., Harrad, S., Abdallah, M.A-E, Ali, N., Law, R.J., Herzke, D., et al., 2011. Novel brominated flame retardants: a review of their analysis, environmental fate and behaviour. Environment International 37(2), 532-556.

de Wit, C.A., 2002. An overview of brominated flame retardants in the environment. Chemosphere 46(5), 583-624.

de Wit, C.A., Herzke, D., Vorkamp, K., 2010. Brominated flame retardants in the Arctic environment–trends and new candidates. Science of the Total Environment 408(15), 2885-2918.

Directive 2003/11/EC of the European Parliament and of the Council of 6 February 2003amending for the 24th time Council Directive 76/769/EEC relating to restrictions on the marketing and use of certain dangerous substances and preparations (pentabromodiphenyl ether and octabromodiphenyl ether). Official Journal of the European Union, L42/45, 15/02/2003.

EPA Environmental Protection Agency, 2010. http://www.epa.gov/oppt/existingchemicals/pubs/actionplans/hbcd.html. Last Access 13 June 2013

European Court of Justice. Cases C-14/06 and C-295/06, Judgement of the Court, 1 April 2008, Directive 2002/95/EC and Commission Decision 2005/717/EC. 2008.

Gauthier, L.T., Hebert, C.E., Weseloh DVC, Letcher, R.J., 2007. Current-use flame retardants in the eggs of herring gulls (*Larus argentatus*) from the Laurentian Great Lakes. Environmental Science & Technology 41(13), 4561-4567.

Gauthier, L.T., Potter, D., Hebert, C.E., Letcher, R.J., 2009. Temporal trends and spatial distribution of non-polybrominated diphenyl ether

flame retardants in the eggs of colonial populations of Great Lakes herring gulls. Environmental Science & Technology 43(2), 312-317.

Gerecke, A.C., Kohler, M., Zennegg, M., Schmid, P., Heeb, N.V., 2003. Detection of aisomer dominated HBCD (hexabromocyclododecane) in Swiss fish at levels comparable to PBDEs (polybrominated diphenyl ethers). Organohalogen Compounds 61, 155-158.

Gouteux, B., Alaee, M., Mabury, S.A., Pacepavicius, G., Muir, D.C.G., 2008. Polymeric Brominated Flame Retardants: Are They a Relevant Source of Emerging Brominated Aromatic Compounds in the Environment? Environmental Science & Technology 42(24), 9039-9044.

Guerra, P., Eljarrat, E., Barcelò, D., 2010. Analysis and occurrence of emerging brominated flame retardants in the Llobregat River basin. Journal of Hydrology 383(1-2), 39-43.

Guzzella, L., Roscioli, R., Binelli, A., 2008. Contamination by polybrominated diphenyl ethers of sediments from the Lake Maggiore Basin (Italy and Switzerland). Chemosphere 73(10), 1684-1691.

Harrad, S., Abdallah, M.A-E, Rose, N.L., Turner, S.D., Davidson, T.A., 2009. Current-Use Brominated Flame Retardants in Water, Sediment, and Fish from English Lakes. Environmental Science & Technology 43(24), 9077-9083.

Hoh, E., Zhu, L., Hites, R.A., 2005. Novel flame retardants, 1,2-Bis(2,4,6-ribromophenoxy)ethane and 2,3,4,5,6-pentabromoethyl benzene, in United States' environmental samples. Environmental Science & Technology 39(8), 2472-2477.

Klosterhaus, S.L., Stapleton, H.M., La Guardia, M.J., Greig, D.J., 2012. Brominated and chlorinated flame retardants in San Francisco Bay sediments and wildlife. Environment International 47(Oct 2012), 56-65.

La Guardia, M.J., Hale, R.C., Harvey, E., 2004. Environmental debromination of decabrominated diphenyl ether. The Third International Workshop on Brominated Flame Retardants (BFR 2004). June 6-9, Toronto, Canada.

Marchetto, A., Lami, A., Musazzi, S., Massaferro, J., Langone, L., Guilizzoni, P., 2004. Lake Maggiore (N. Italy) trophic history: fossil diatom, plant pigments and chironomids and comparison with long-term limnological data. Quaternary International 113(1), 97-110.

OSPAR Commission. OSPAR List of Chemicals for Priority Action. Update 2007. http://www.ospar.org/content/content.asp?menu=00120000000074_000000_0000002001.

Renner, R., 2004. In US, flame retardants will be voluntarily phased out. Environmental Science & Technology 38(1),14A.

Ross, P.S., Couillard, C.M., Ikonomou, M.G., et al., 2009. Large and growing environmental reservoirs of Deca-BDE present an emerging health risk for fish and marine mammals. Marine Pollution Bulletin 58(1), 7–10.

Sawal, G., Stachel, B., Lepom, P., 2004. Polybrominated diphenyl ethers in sediments from the river Elbe, Germany. Proceedings of the Third International Workshop on Brominated Flame Retardants BFR2004. Toronto, Canada, June 6–9, 2004, 151-154.

Stapleton, H.M., Brazil, B., Holbrook, R.D., Mitchelmore, C.L., Benedict, R., Konstantinov, A., Potter, D., 2006. In vivo and in vitro debromination of decabromodiphenyl ether (BDE-209) by juvenile rainbow trout and common carp. Environmental Science & Technology 40(15), 4653-4658.

Sjödin, A., Jakobsson, E., Kierkegaard, A., Marsh, G., Sellström, U., 1998. Gas chromatographic identification and quantification of polybrominated diphenyl ethers in a commercial product Bromkal 70-5DE. Journal of Chromatography A 822, 83-89.

Söderström, G., Sellström, U., de Wit, C.A., Tysklind, M., 2004. Photolytic debromination of decabromodiphenyl ether (BDE 209). Environmental Science & Technology 38, 127-132.

Tomy, G.T., Palace, V., Marvin, C., Stapleton, H.M., Covaci, A., Harrad, S., 2011. Biotransformation of HBCD in biological systems

can confound temporal-trend studies. Environmental Science & Technology 45(2), 364-365.

Verreault, J., Gebbink, W., Gauthier, L.T., Gabrielsen, G.W., Letcher, R.J., 2007. Brominated flame retardants in Glaucous Gulls from the Norwegian arctic: more than just an issue of polybrominated diphenyl ethers. Environmental Science & Technology 41(14), 4925-4931.

Voorspoels, S., Covaci, A., Schepens, P., 2003. Polybrominated diphenyl ethers in marine species from the Belgian North Sea and the Western Scheldt estuary: levels, profiles and distribution. Environmental Science & Technology 37, 4348-4357.

Walkley, A., Black, I.A., 1934. An examination of the Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Science 37(1), 29-38.

Wania, F., Dugani, C.B., 2003. Assessing the long-range transport potential of polybrominated diphenyl ethers: a comparison of four multimedia models. Environmental Toxicology and Chemistry 22(6), 1252-1261.

Watanabe, I., Sakai,S., 2003. Environmental release and behavior of brominated flame retardants. Environment International 29(6), 665-682.

Wei, H., Aziz-Schwanbeck, A.C., Zou, Y., Corcoran, M.B., Poghosyan, A., Li, A., Rockne, K.J., Christensen, E.R., Sturchio, N.C., 2012. Polybromodiphenyl Ethers and Decabromodiphenyl Ethane in aquatic sediments from Southern and Eastern Arkansas, United States. Environmental Science & Technology 46(15), 8017-8024.

WHO World Health Organization, Geneva, 1997. Flame retardants: a general introduction, 192. Environmental health criteria.

Zhu, L., Ma, B., Hites, R.A., 2009. Brominated flame retardants in serum from the general population in Northern China. Environmental Science & Technology 43(18), 6963-6968.

CHAPTER III

EVALUATION OF SPATIAL DISTRIBUTION AND ACCUMULATION OF NOVEL BROMINATED FLAME RETARDANTS, HBCD AND PBDEs IN AN ITALIAN SUBALPINE LAKE USING ZEBRA MUSSEL (*Dreissena polymorpha*)

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ABSTRACT

Because of the reduction in the use of polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane (HBCD), novel brominated flame retardants (nBFRs), including 1,2-bis(2,4,6-tribromophenoxy) ethane (BTBPE), decabromodiphenyl ethane (DBDPE), hexabromobenzene (HBB), and pentabromoethyl-benzene (PBEB), started to be marketed as alternatives to the banned formulations. In this study, the spatial distribution and accumulation of nBFRs, PBDEs, and HBCD in the biota have been investigated in the littoral compartment of a large and deep subalpine lake (Lake Maggiore, Northern Italy), using zebra mussel *Dreissena polymorpha* and roach (*Rutilus rutilus*) as bioindicators. To our knowledge, this is the first study reporting the contamination of nBFRs in the freshwater invertebrate *Dreissena polymorpha*. Contamination of zebra mussel due to

PBEB, HBB and BTBPE was low, ranging from 0.9 to 2.9 ng/g lipid weight, from 1.1 to 2.9 ng/g l.w., and from 3.5 to 9.5 ng/g l.w. respectively. PBEB and BTBPE in roach were always below the detection limit, while the contamination of HBB ranged from <LOD to 1.74 ng/g l.w., indicating a weak contamination. DBDPE was <LOD in all the considered biological samples. Finally, HBCD was detected in all organic tissues with mean concentrations up to 74.4 ng/g l.w. PBDE results, supported by PCA elaboration, suggested a possible contamination due to the congeners composing the Penta- and Deca-BDE technical formulations, which are present in the Lake Maggiore basin. The biomagnification factor (BMF) values showed that tetra- and penta-BDE biomagnified, while octa-, nona-, and deca-BDE were still bioavailable and detectable in the fish muscles, but they do not biomagnified. Considering the other BFRs, only HBCD showed a moderate biomagnification potential.

Keywords: Novel Brominated Flame Retardants (nBFRs); Polybrominated diphenyl ethers (PBDEs); Hexabromocyclododecane (HBCD); Lake Maggiore; Zebra mussel; Common roach

3.1. INTRODUCTION

Brominated Flame Retardants (BFRs) are generally added to industrial polymers used in plastics, textiles, electronic circuitry and other materials in order to prevent fires (Covaci et al. 2003). The global market demand for these substances greatly continues to grow: from 145,000 tons in 1990 (BSEF 2000) to 411,000 tons in 2007 (BSEF 2013, personal communication).
The most used BFRs are polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane (HBCD). They are both additive mixed directly into polymers, but do not react with them during the production. Due to losses during the industrial production, the use of material containing BFRs, and the disposal of products containing these substances (Hermanson et al. 2010), BFRs have been observed in environmental matrices worldwide, and their appearance in many organisms shows that they are lipophilic and can bioaccumulate in the biota and humans (de Wit 2002), similarly to other persistent organic pollutants.
One of the most widely used classes of BFRs, until 2008, were PBDEs produced in commercial mixtures at three different levels of bromination, known as Penta-BDE, Octa-BDE, and Deca-BDE. However, due to their growing environmental and human health concern, the Penta-BDE and Octa-BDE mixtures were banned in Europe in 2004, and the US manufacturers of these commercial mixtures voluntarily stopped their production in the same year (La Guardia et al. 2006). Deca-BDE have been banned throughout Europe in electrical and electronical equipment in July 2008 (European Court of Justice 2008), because of concern about a possible formation of more toxic oxidation and/or debromination residuals. In North America, a phase-out of deca-BDE is expected by 2013 (Hermanson et al. 2010). Moreover, in August 2010 the Stockholm Convention included tetra-, penta-, hexa- and heptaBDEs, covering many of

the major congeners of Penta-BDE and Octa-BDE technical formulations, in the Persistent Organic Pollutant list.

Hexabromocyclododecane (HBCD) is the principal flame retardant used in extruded (XEPS) and expanded (EPS) polystyrene foams as thermal insulation in the building industry. The physical-chemical properties of HBCD are similar to those of PBDEs and other POPs (de Wit 2002). HBCD is persistent and can bioaccumulate entering the aquatic environment through atmospheric deposition, direct discharges from wastewater treatment plants or land runoff. Recently, HBCD has been detected in environmental and biota samples (Abdallah and Harrad 2011; Hu et al. 2010) and also in Arctic food web (Tomy et al. 2008). At the sixth meeting of the Conference of the Parties of the Stockholm Convention (May 2013), the decision was taken to list Hexabromocyclododecane (HBCD) in the list of POP substances. The listing allows an exemption for the production and use of HBCD in expanded polystyrene (EPS) and extruded polystyrene (XPS) in buildings. The exemption will be valid until 2019 (BSEF, 2013).

Thus, many countries and organizations have increasingly restricted the use of HBCD.

The reduction in the use of PBDEs and HBCD has consequently opened the way for the introduction of novel BFRs (nBFRs) in place of the banned formulations (Betts, 2008), including 1,2-bis(2,4,6-tribromophenoxy) ethane (BTBPE), decabromo-diphenyl ethane (DBDPE), hexabromobenzene (HBB), and pentabromoethylbenzene (PBEB). BTBPE had been introduced in the mid-1970s and used as the alternative flame retardant to Octa-BDE commercial formulation (Covaci et al. 2011). Since the structure of BTBPE is similar to that of hexabrominated BDE congeners, their physical-chemical properties, and consequently the environmental fate and toxicity, might also be similar. The levels of BTBPE in sediment and fish samples were

reported from the Great Lakes and China (Law et al. 2006; Isobe et al. 2012). DBDPE was introduced in the mid-1980s and became commercially widely used as an alternative to the Deca-BDE technical formulation in the early 1990s (Arias 2001). It is used as an additive BFR in high impact polystyrene, acrylonitrile butadiene styrene, polypropylene and textiles (Covaci et al. 2011). With a log K_{ow} of 11, it has extremely high affinity to particles and is chemically stable (Kierkegaard et al. 2004). Therefore, DBDPE is expected to accumulate in sediments and be persistent in the environment. In fact, it was detected at levels ranging from less than one to several tens of nanograms per gram in dry weight sediments from all over the world (Klosterhaus et al. 2012; Guerra et al. 2010; Wei et al. 2012; Poma et al. submitted).

Hexabromobenzene (HBB) was widely used as an additive flame retardant to paper, woods, textiles, electronic and plastic goods, but at present it is used at lower volumes (350 tons in 2001) (Watanabe and Sakai 2003), and it is not reported by EU industry as a currently produced chemical (ESIS 2010). Pentabromoethylbenzene (PBEB) is mostly used in thermoset polyester resins (circuit boards, textiles, adhesives, wire and cable coatings, polyurethane foam) (Hoh et al. 2005). It is classified as a Low Production Volume (LPV) chemical in the EU (ESIS 2010) and it is included in the OSPAR (Oslo/Paris Convention for the protection of the marine environment of the North-East Atlantic) list of chemicals, being ranked as persistent, bioaccumulative and toxic compound, but not currently produced (OSPAR 2007). PBEB have appeared recently in herring gull eggs and glaucous gull tissues (Gauthier et al. 2007; Verreault et al. 2007), and in Chicago air samples (Hoh et al. 2005).

In this study, we investigated the spatial distribution and accumulation in the biota of PBDEs, HBCD and nBFRs in a

large and deep subalpine lake (Lake Maggiore, Northern Italy), whose catchment is intensely industrialized and populated. We focused on the littoral zone of the lake, which likely is more prompt than pelagic waters to accumulate pollutants transported through tributaries. We used zebra mussel *Dreissena polymorpha* (Pallas 1771; Binelli et al. 2008; Isobe et al. 2012), and roach (*Rutilus rutilus* Linnaeus 1758), as bioindicator species. *Dreissena polymorpha* is a good sentinel-organism since it has appropriate characteristics such as wide distribution, continuous availability throughout the year, adequate body size, firm site attachment capability by the bissus, and ease of sampling (Binelli et al. 2001). In Italy, the zebra mussel invaded some aquatic environments, reaching the Lake Maggiore in the late 1990s (Camusso et al. 2001), largely because there was an empty ecological niche and few natural predators. As a matter of facts, most fish are not able to eat zebra mussels because they cannot crush the shells. A few fish species have specialized teeth and jaws that are strong enough to break the shells of mollusks and some of them do eat zebra mussels. Among them, the roach (*Rutilus rutilus*) is a major predator of zebra mussels in Lake Maggiore and the most abundant fish species in the littoral areas (Volta and Jepsen 2008; Volta et al. 2013). In this work, the BFR contamination was measured in zebra mussel specimens and common roach, and our data gave us also the opportunity to analyze the possible transfer of BFRs from bivalve to fish, calculating a biomagnification factor (BMF) as the ratio of the BFRs concentration in the roach (ng/g l.w.) to that in its prey at the steady state (Arnot and Gobas 2006). To our knowledge, this is the first study reporting the novel BFR contamination in the freshwater invertebrate zebra mussel.

3.2. MATERIALS AND METHODS

3.2.1 Lake Maggiore sampling stations

BFR contamination of the Lake Maggiore was studied by analyzing tissues of zebra mussels collected in 8 sampling stations (selected in order to cover the major part of the lake), and the muscle of common roach sampled into Pallanza Bay. In detail, zebra mussels were collected in May and September 2011 and 2012 (in the pre- and post-reproductive period, respectively) from 8 different sampling sites ((Fig. III-1) at 5-10 m of depth by a scuba diver that explored the shoreline in accordance to environmental conditions, morphometric characteristics, and anthropic impact. About 200 mussel specimens were collected at each site. Mollusks were separated from rocks cutting off the byssus, washed with lake water, wrapped up separately on aluminum sheets, transported to the laboratory in refrigerated bags, and frozen at -20 °C pending chemical analysis. Once in laboratory, the mollusks were defrosted, the shell and byssus removed, the soft tissues were pooled for the analysis and finally freeze-dried (Freeze-dryer Edwards mod. 24) for about 24 h. The samples were then weighed, ground with an Ultra-Turrax tissue grinder (Miccra D-8, ART, Germany) and stored in dark glass bottles. The total amount of samples was divided in three rates (for different projects) and one third of the samples was finally sent to IRSA CNR for the BFR analysis. The zebra mussel morpho-physiological characteristics are reported in Table III-1.

Fig. III-1 Mussel (Δ) and fish (@) sampling stations in the Lake Maggiore

Table III-1 Water temperature measured at eight sampling stations, shell length and lipid percentage measured in zebra mussel specimens; number of samples, morphological characteristics, age, and lipid content of common roach collected from Lake Maggiore

	Water temperature (°C)				Average shell length (cm±s.d.)				Lipid % of dry weigth			
Dreissena polymorpha	2011		2012		2011		2012		2011		2012	
Sampling station	May	Sept.	May	Sept.	May	Sept.	May	Sept.	May	Sept.	May	Sept.
Brissago	15	21	14	20	1.9±0.2	1.9±0.2	2.0±0.2	1.6±0.2	16.6	9.9	14.6	9.6
Luino	14	22	19	21	1.8±0.2	1.9±0.2	1.9±0.2	1.7±0.2	18.3	8.4	15.7	10.8
Pallanza	14	21	14	20	1.7±0.2	1.7±0.2	1.7±0.2	1.5±0.2	17.3	9.8	16.6	9.8
Laveno	16	22	19	21	2.0±0.2	1.7±0.2	1.8±0.2	1.9±0.9	17.7	10.7	12.6	10.7
Baveno	16	22	16	20	1.9±0.2	2.0±0.2	1.9±0.3	1.9±0.2	11.9	8.5	11.0	10.8
Suna	19	19	14	20	2.0±0.2	1.8±0.2	1.9±0.2	1.7±0.2	15.0	10.3	13.3	9.4
Brebbia	16	21	13	22	2.0±0.2	2.0±0.2	1.7±1.2	1.7±0.2	16.4	9.9	13.8	9.7
Ranco	16	22	13	22	2.0±0.2	1.8±0.2	1.9±0.2	1.7±0.2	19.3	8.4	13.5	11.3

Rutilus rutilus	May 2011	July 2011	November 2011	February 2012	May 2012
N	10	10	10	10	10
Weight (g±s.d.)	69.6 ± 25.8	90.3 ± 11.1	95.7 ± 29.0	39.1 ± 16.0	125.0 ± 21.3
Length (cm±s.d.)	18.6 ± 2.7	19.7 ± 0.8	19.4 ± 3.7	15.4 ± 1.8	21.3 ± 2.1
Age (y±s.d.)	3.0	2.0	2.7 ± 0.4	1.9 ± 0.5	2.3 ± 0.5
Lipid % dry weight	2.4	4.4	3.6	2.7	4.2

Roach specimens were collected seasonally by CNR-ISE from May 2011 to May 2012 in the littoral area of the Borromeo Gulf using benthic multimesh survey gillnets (Nordic type) set at dusk and retrieved the following morning. After capture, fish were stored at 4 °C and subsequently their individual body length (cm) and weight (g) were measured. Age was determined by scale analysis and young individuals of 2-3 years were selected for chemical analyses. Morphometric characteristics of fish and their age are listed in Table III-1. The muscle sample for the analysis was taken from the caudal portion of the fish, and the tissues of ten fish having the same morphometric characteristics were pooled together and homogenized by a steel mixer in order to obtain a single sample. All samples were kept at −20°C until they were sent to IRSA-CNR for BFR analysis.

3.2.2 Sample preparation

Sample preparation can be summarized as follow: after lyophilisation, a variable amount of dried sample (0.1 g for mussels and 1 g for fish) was spiked with 50 µL of the recovery standard (250 µg/L containing the labeled compounds: [$^{13}C_{12}$]γHBCD, [$^{13}C_{12}$]BDE-209, and [$^{13}C_{12}$]BDE-28, -47, -99, -153, -154, -183, all purchased from Wellington Labs, Canada), and then extracted in a hot Soxhlet apparatus (Buchi, Flawil, Switzerland) using a *n*-hexane/acetone mixture (3:1 *v/v*) for 25 cycles. The tissue extracts were concentrated to 5 mL by Turbovap on a gentle nitrogen stream, and then subjected to Gel Permeation Chromatography (GPC). GPC system included a GPC Basix equipped with a GPC 1122 solvent delivery system (LCTech GmbH, Dorfen, Germany). A second phase clean-up was performed using a multi-layer column (1.5 x 20 cm) packed (bottom to top) with 1.5 g of acidified silica gel (30% *w/w* sulphuric acid, Sigma-Aldrich, Germany) and 1.5 g of Florisil® (100-200 mesh, Sigma-Aldrich, Germany). The column was

pre-washed with 15 mL of *n*-hexane/dichloromethane (*n*-hexane/DCM) 1:1 *v/v*, and the elution was performed collecting 40 mL of the same solvent. 1 mL of toluene was added to the extract, concentrated by Turbovap on a gentle nitrogen stream, and then reconstituted to 100 µL using toluene. The lipid content of zebra mussels and roach tissues (Table III-1) was determined gravimetrically after solvents were evaporated under a gentle nitrogen stream, and the extract brought to constant weight (at 105 °C).

3.2.3 Analytical procedure

GC analysis for BFR compounds was performed using a Thermo Electron TraceGC 2000 coupled with a PolarisQ Ion Trap (ThermoElectron, Austin, Texas) mass spectrometer and equipped with a PTV injector and an AS 3000 auto sampler. The system was managed by ThermoFinnigan Xcalibur software version 1.4.1. Separation of PBDE congeners (BDE-28, 47, 100, 99, 153, 154, 183) was achieved using a Agilent DB-5MS capillary column, 50 m x 0.25 mm i.d. x 0.25 µm film thickness (Agilent, Palo Alto, California, USA) in the following conditions: carrier gas helium at 1.2 mL/min; injection pressure of 120 kPa; transfer pressure of 240 kPa; injector temperature starting at 70 °C and maintained for 1.2 min, then ramped to 280 °C (held 1.2 min) at 14 °C/s; initial oven temperature set at 70 °C (held 1 min), then ramped to 220 °C at 30 °C/min (held 1 min) and finally to 290 °C at 4 °C/min (held 20 min). Samples were analyzed using tandem mass spectrometry under the following instrumental conditions: standard electron energy of 70 eV; the transfer line was maintained at 280 °C, the damping gas at 2 mL/min, and the ion source at 260 °C.
Separation of nBFR and of some PBDE congeners (BDE-179, 188, 201, 202, 206, 207, 208, 209; HBCD, PBEB, HBB, BTBPE) was achieved using a Restek RXi-1MS capillary

column, 12 m x 0.20 mm i.d. x 0.33 µm film thickness (Restek U.S., Bellefonte, Pennsylvania, USA) in the following conditions: carrier gas helium at 1.1 mL/min; injection pressure of 70 kPa; transfer pressure of 110 kPa; injector temperature starting at 70 °C and maintained for 0.2 min, then ramped to 300 °C (held 1.5 min) at 8 °C/s; initial oven temperature set at 100 °C (held 1 min), then ramped to 220 °C at 40 °C/min (held 0.1 min) and finally to 300 °C at 15 °C/min (held 14 min). Samples were analyzed using tandem mass spectrometry under the following instrumental conditions: standard electron energy of 70 eV; the transfer line was maintained at 300 °C, the damping gas at 1.5 mL/min, and the ion source at 250 °C. Quantitative analysis was performed using external standard method.
DBDPE measurements were undertaken using a TraceGC Ultra equipped with a cold on-column injector and an ECD-40 detector (ThermoElectron, Austin, Texas) using a Restek RTX-5 capillary column (15 m x 0.53 mm i.d. x 0.1 µm film thickness)(Restek, Bellefonte, USA). The use of a different analytical method and of a shorter column allows the analysis of this more unstable compound. Injections (0.5 µL) were performed using a TriPlus autosampler (Thermo Electron) and carried out in the following analytical conditions: carrier gas helium at 6.0 mL/min; starting temperature of 100 °C (held 0.5 min) after which it was ramped to 280 °C at 15 °C/min (held 8 min). Quantitative analysis was obtained by comparing results with external standard.

3.2.4 Quality Assurance (QA) and Quality Control (QC)

The validation of the analytical method for PBDEs (BDE-47, 99, 100, 153, 154) was carried out using NIST (National Institute of Standard and Technology) SRM 1947 Lake Michigan Fish Tissue. All values found were within the certified range of reference concentration (± 30%).

The mean recoveries of the spiked standards for [$^{13}C_{12}$]BDE-28, 47, 99, 153, 154, 209, [$^{13}C_{12}$]γHBCD ranged from 41 to 97 % in *D. polymorpha* specimens, and from 50 to 80 % in fish tissues. The obtained analytical results were corrected considering the recoveries, and the sample analysis was repeated if its mean recovery was below 30%. Using a signal-to-noise ratio of 3:1, the limits of detection (LODs) were estimated as 0.01 ng/g dry weight for each compound in zebra mussels and roach tissues. A procedural blank was analyzed every eight samples to check for laboratory contamination; the blank concentrations were below LOD levels for all BFR compounds.
The eventually debromination of BDE-209 in the inlet system and the column, leading to the formation of octa- and nona-BDE congeners, was monitored by the presence of labeled congeners of octa and nona-BDE deriving from internal standard [$^{13}C_{12}$]BDE-209. In case of evidence of BDE-209 debromination, the inlet liner was replaced and the column was cleaned heating at high temperature overnight.

3.2.5 Statistics

Statistical analysis was carried out using Statistica 8.0 (Principal Component Analysis – PCA) and SigmaPlot 11.0 (Analysis of Variance – ANOVA) software. PCA was performed to evaluate the relationships between the relative importance of BFRs in this study and the mussel sampling stations. One-way ANOVA was used to evaluate significant differences in BFR concentrations among *D. polymorpha* sampling stations.

3.3. RESULTS AND DISCUSSION

3.3.1 Considerations on sample characteristics

The samples were collected in different seasons to point out possible differences of BFR concentrations caused by physiological or environmental variability. For example, lipid content might significantly affect the BFR accumulation and it is strictly related to the annual life cycle of the organisms. For instance, the spawning period (in late spring for both zebra mussel and roach) is a crucial physiological moment since reproduction greatly interferes with POP bioaccumulation. Spring samples of zebra mussel clearly reflect the typical pre-spawning behaviour, with lipid values higher than 11 % both in 2011 and in 2012; late summer values are always lower than spring ones (between 8 and 11 % d.w.), showing the loss of fat due to the reproduction event. On the contrary, the lipid content of roach is rather constant during the whole period due to the typical species physiology (Table III-1).

3.3.2 Levels of nBFRs in mussels and fish tissues

BFR results are reported as lipid-based concentrations in order to allow a meaningful comparison between different biological data. NBFR concentrations in zebra mussels and roach were calculated as an average of the different periods of sampling and they are shown in Fig. III-2, while the single values are reported in Table III-2 and Table III-3.

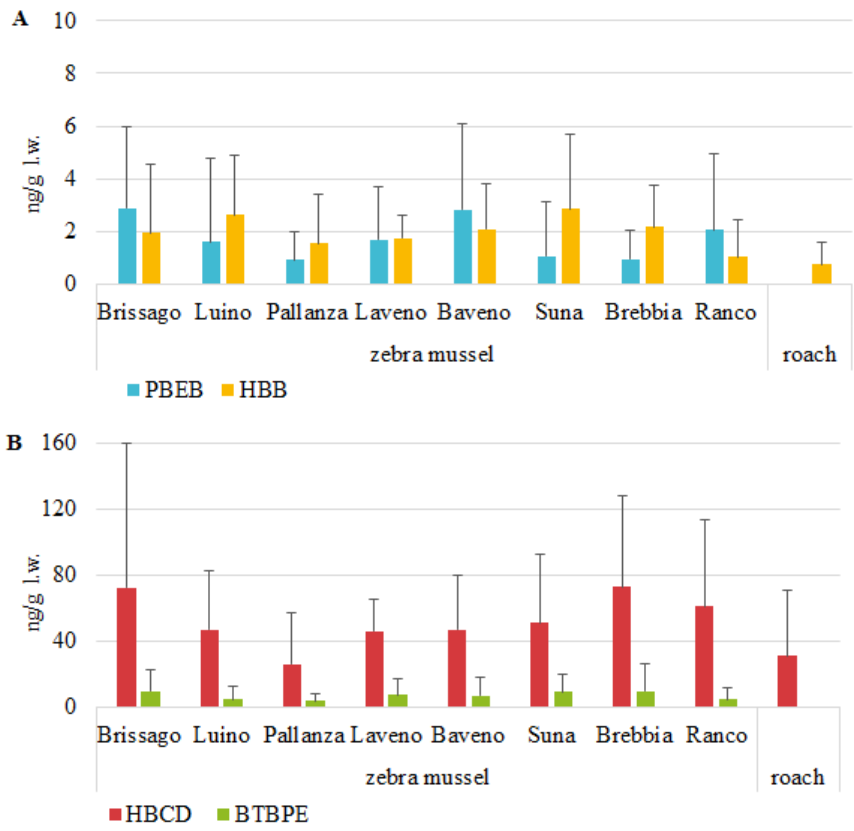

Fig. III-2 Mean concentrations (ng/g l.w.) and standard deviation of HBB and PBDE (A) and HBCD and BTBPE (B) in zebra mussels and roach tissues from Lake Maggiore

Table III-2 Concentrations of HBCD and novelBFRs (ng/g l.w.) in *D. polymorpha* specimens collected in eight different sites of Lake Maggiore from May 2011 to September 2012

May-11	Brissago	Luino	Pallanza	Laveno	Baveno	Suna	Brebbia	Ranco
PBEB	0.54	<LOD	<LOD	0.68	<LOD	0.08	0.12	0.05
HBB	0.84	0.05	0.06	1.07	0.15	0.12	0.06	0.05
HBCD	23.49	6.10	5.53	45.05	7.22	1.76	14.17	90.25
BTBPE	<LOD	0.16	0.63	1.07	0.35	<LOD	<LOD	<LOD
DBDPE	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

Sep-11	Brissago	Luino	Pallanza	Laveno	Baveno	Suna	Brebbia	Ranco
PBEB	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
HBB	<LOD	5.48	<LOD	1.04	1.52	6.67	3.11	<LOD
HBCD	<LOD	83.43	<LOD	42.69	83.14	99.52	86.26	<LOD
BTBPE	<LOD	<LOD	<LOD	2.94	1.41	8.60	<LOD	<LOD
DBDPE	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

May-12	Brissago	Luino	Pallanza	Laveno	Baveno	Suna	Brebbia	Ranco
PBEB	5.91	<LOD	1.57	1.66	5.17	<LOD	2.39	6.10
HBB	1.14	2.01	3.73	2.92	4.26	3.12	3.62	3.01
HBCD	69.44	29.29	28.43	71.55	36.75	36.73	143.23	116.73
BTBPE	28.85	15.95	10.24	21.39	23.59	23.99	34.76	15.76
DBDPE	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

Sep-12	Brissago	Luino	Pallanza	Laveno	Baveno	Suna	Brebbia	Ranco
PBEB	5.16	6.39	2.14	4.49	6.11	4.15	1.30	2.12
HBB	5.83	2.96	2.45	1.96	2.50	1.53	1.95	1.17
HBCD	196.77	68.80	69.59	25.05	60.74	66.28	47.84	37.17
BTBPE	8.60	2.22	3.27	3.92	2.69	4.10	3.10	2.21
DBDPE	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

Table III-3 BFR single concentrations (ng/g l.w.) in roach tissues

	2011			2012	
	May	July	November	February	May
BDE-28	<LOD	<LOD	<LOD	<LOD	<LOD
BDE-47	143.54	100.00	142.09	162.26	52.38
BDE-99	23.92	67.05	78.29	83.02	21.43
BDE-100	56.55	28.41	52.20	84.91	17.86
BDE-154	<LOD	<LOD	<LOD	<LOD	<LOD
BDE-153	<LOD	<LOD	<LOD	<LOD	<LOD
BDE-183	<LOD	<LOD	<LOD	<LOD	<LOD
PBEB	<LOD	<LOD	<LOD	<LOD	<LOD
HBB	1.74	<LOD	<LOD	0.42	0.11
HBCD	33.49	3.64	98.55	11.88	7.99
BTBPE	<LOD	<LOD	<LOD	<LOD	<LOD
DBDPE	<LOD	<LOD	<LOD	<LOD	<LOD
BDE-188	1.74	<LOD	<LOD	0.35	0.09
BDE-179	1.74	<LOD	<LOD	0.28	0.06
BDE-202	<LOD	<LOD	<LOD	<LOD	<LOD
BDE-201	<LOD	<LOD	<LOD	<LOD	<LOD
BDE-208	<LOD	<LOD	<LOD	<LOD	<LOD
BDE-207	<LOD	1.82	<LOD	<LOD	<LOD
BDE-206	<LOD	<LOD	<LOD	<LOD	<LOD
BDE-209	29.58	36.36	7.97	6.85	8.90

PBEB and HBB concentrations in zebra mussel samples were very similar, ranging from 0.9 to 2.9 ng/g l.w. and 1.1 to 2.9 ng/g l.w., respectively. No difference was evident between the eight sampling stations, highlighting the absence of a local

contamination source of pollution in the basin and confirming that PBEB and HBB are classified as Low Production Volume (LPV) chemicals in the EU (ESIS 2010). Besides, BTBPE concentration levels in zebra mussels ranged from 3.5 to 9.5 ng/g l.w. Very few studies have reported the concentrations of these nBFRs in freshwater mussels. For example, on a wet-weight basis, concentrations of BTBPE in zebra mussels collected from Lake Maggiore (from 0.4 to 1.0 ng/g l.w.) are similar to those observed by Law et al. (2006) (mean of 1.3 ng/g l.w.) in freshwater mussels (*Lampsilis radiata*) from Lake Winnipeg, Canada, and much lower than those observed by La Guardia et al. (2012) in the bivalve *Corbicula fluminea* (up to 153 ng/g l.w.) in Yadkin river, North Carolina. In the fish tissues, PBEB and BTBPE were always below the detection limit, while the mean contamination due to HBB was 0.8 ng/g l.w. Consequently, the low contamination due to these compounds might indicate a weak tendency to bioaccumulate in this fish species.

On the contrary, HBCD was detected in all the considered samples, with mean concentrations ranging from 25.9 to 72.4 ng/g l.w. in zebra mussel and of 31 ng/g l.w. in roach samples. In the case of zebra mussels, the differences among the sampling stations were not statistically significant ($\alpha = 0.05$; $P = 0.887$) (ANOVA test), suggesting that Lake Maggiore is not directly impacted by HBCD point emissions due to industrial production. Similar hypothesis was suggested by the authors who studied the HBCD contamination in sediments from Lake Maggiore and its tributaries (Poma et al., submitted). However, the high temporal variability among stations could have greatly affected the statistical results. In particular, no evident seasonal trend could be observed among stations, even if the HBCD concentrations were mostly higher in 2012 than in 2011. Moreover, considering the single stations (Table III-2), the HBCD concentrations in September were generally higher than

in May (particularly in Brissago, Luino, Pallanza, Baveno and Suna). Considering where these stations are located, we suggest that the meteorological conditions (with frequent precipitation events occurred in early summer) caused a more significant input of HBCD to the lake mainly deriving from the Northern area of the basin. Anyway, these are the first data about the HBCD concentrations in the Lake Maggiore basin, and it is probable that some more investigations, considering a longer temporal trend, will help to better understand the dynamics of this contamination. Differently from Lake Maggiore, a case of direct pollution due to HBCD was studied by La Guardia et al. (2012), who measured a very high contamination (HBCD up to 363.000 ng/g l.w. on wet weight basis) in freshwater mussels collected at the outfall of a textile manufacturing in North Carolina. Regarding DBDPE contamination, despite the high concentrations (up to 30 ng/g d.w.) measured in the sediments of Lake Maggiore (Poma et al. submitted), DBDPE was below the detection limit in all the considered biological samples, probably because of its high log K_{ow} value (log K_{ow} = 11), which reduced the potential bioaccumulation in organisms as mentioned by other studies (Law et al. 2006).

3.3.3 BDE congener patterns in mussels and fish tissues

Considering the sum of -hepta, -octa, -nona, and deca-BDE, the mean concentrations in zebra mussel and roach samples are shown in Table III-4 (concentrations of individual congeners are reported in Table III-5). Total BDE concentrations ranged from 88.2 to 182.8 ng/g l.w. in mussels and was equal to 21.2 ng/g l.w. in fish samples. Also in this case, results of the ANOVA test showed that the differences between the mussel sampling stations were not statistically significant ($\alpha = 0.05$; $P = 0.749$).

Table III-4 Average of the concentrations of the analyzed hepta- to deca-BDE congeners (ng/g l.w.) in the seasonal samples of zebra mussels and roach tissues from the Lake Maggiore

	Zebra mussel								Roach
	Brissago	Luino	Pallanza	Laveno	Baveno	Suna	Brebbia	Ranco	
BDE-188	3.6	2.5	1.4	10.3	1.8	3.6	4.2	23.5	0.7
BDE-179	3.6	1.4	1.7	9.7	9.0	1.3	2.0	1.6	0.7
BDE-202	1.8	1.6	0.4	1.9	0.3	2.0	1.5	1.3	<LOD
BDE-201	3.1	1.3	1.1	3.1	0.4	1.3	2.9	0.8	<LOD
BDE-208	1.0	2.0	1.7	7.6	2.4	3.3	15.0	1.4	<LOD
BDE-207	0.2	2.8	3.9	6.2	2.2	3.8	18.0	1.2	1.8
BDE-206	1.2	2.4	3.3	10.8	3.3	5.7	15.6	1.9	<LOD
BDE-209	85.5	74.2	95.9	65.7	71.2	144.7	123.7	79.8	17.9
ΣBDE	100.1	88.2	109.5	115.2	90.7	165.7	182.8	111.6	21.2

Table III-5 Concentrations of individual PBDE congeners (ng/g l.w.) in *D. polymorpha* specimens collected in eight different sites of Lake Maggiore from May 2011 to September 2012

May-11	Brissago	Luino	Pallanza	Laveno	Baveno	Suna	Brebbia	Ranco
BDE-28	0.12	0.15	0.12	0.24	0.14	0.17	<LOD	0.12
BDE-47	0.66	7.48	13.43	10.63	5.64	6.66	7.59	6.79
BDE-99	0.10	3.76	7.68	3.43	5.74	1.86	3.68	2.93
BDE-100	<LOD	0.98	1.45	1.30	1.10	0.20	1.17	0.93
BDE-154	0.12	1.02	0.65	0.39	0.67	0.13	0.06	0.21
BDE-153	<LOD	0.49	0.66	0.11	0.54	0.82	0.30	0.41
BDE-183	<LOD	0.38	<LOD	0.56	0.40	<LOD	0.73	0.52
BDE-188	1.14	0.05	0.06	0.79	0.10	<0.07	0.06	<0.05
BDE-179	<0.06	0.16	0.12	<0.06	0.20	0.08	0.06	<0.05
BDE-202	<0.06	0.22	0.29	0.15	0.35	0.32	0.30	0.10
BDE-201	0.12	0.22	0.46	0.32	0.20	0.32	0.18	0.16
BDE-208	<0.06	1.36	1.73	3.27	2.31	3.28	1.70	1.40
BDE-207	0.24	1.85	3.86	6.87	3.68	3.75	1.89	1.19
BDE-206	<0.06	2.40	3.28	20.83	5.47	5.71	3.89	1.95
BDE-209	81.93	35.29	85.43	41.10	116.56	133.69	73.60	41.96

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Sep-11	Brissago	Luino	Pallanza	Laveno	Baveno	Suna	Brebbia	Ranco
BDE-28	1.31	1.78	2.56	4.52	1.49	5.00	<LOD	4.74
BDE-47	7.93	12.97	24.09	<LOD	30.56	20.27	5.98	56.26
BDE-99	7.40	6.57	11.43	5.70	18.55	8.19	6.05	26.90
BDE-100	2.90	13.55	2.84	<LOD	6.86	<LOD	1.10	18.43
BDE-154	<LOD	1.70	<LOD	2.62	3.43	4.50	0.50	1.71
BDE-153	<LOD	<LOD	<LOD	3.82	<LOD	3.70	1.07	<LOD
BDE-183	<LOD	10.37	<LOD	4.60	1.38	13.26	<LOD	<LOD
BDE-188	5.64	2.38	<LOD	<LOD	1.05	7.92	<LOD	<LOD
BDE-179	<LOD	1.19	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
BDE-202	<LOD	1.31	<LOD	0.85	0.39	0.97	0.90	<LOD
BDE-201	<LOD	2.62	<LOD	0.95	0.28	1.74	1.71	<LOD
BDE-208	0.50	<LOD	<LOD	<LOD	0.23	<LOD	<LOD	<LOD
BDE-207	<LOD	<LOD	<LOD	<LOD	0.70	<LOD	<LOD	<LOD
BDE-206	1.21	<LOD	<LOD	<LOD	1.05	<LOD	<LOD	<LOD
BDE-209	71.50	42.67	118.42	43.64	81.97	197.10	141.42	72.40

May-12	Brissago	Luino	Pallanza	Laveno	Baveno	Suna	Brebbia	Ranco
BDE-28	1.17	0.82	1.30	0.55	1.18	1.09	0.65	1.67
BDE-47	37.57	16.85	29.86	21.51	28.55	17.60	38.81	24.83
BDE-99	12.45	1.91	2.89	14.10	10.71	10.19	14.05	0.04
BDE-100	5.22	6.06	3.49	5.55	0.91	2.55	7.97	2.01
BDE-154	2.47	2.48	3.07	4.12	4.81	2.17	0.04	0.04
BDE-153	3.41	1.44	0.03	3.08	5.11	2.25	8.11	6.47
BDE-183	5.49	0.96	0.03	0.04	0.05	0.04	0.04	0.04
BDE-188	3.67	4.28	<LOD	28.61	<LOD	1.56	7.68	45.65
BDE-179	2.22	0.78	0.48	9.98	15.79	1.24	1.88	<LOD
BDE-202	3.43	3.51	0.24	4.75	<LOD	5.85	3.62	3.72
BDE-201	8.93	1.28	1.81	10.30	<LOD	1.95	8.91	1.93
BDE-208	<LOD	2.55	<LOD	11.89	<LOD	<LOD	28.24	<LOD
BDE-207	<LOD	3.83	<LOD	5.55	<LOD	<LOD	34.03	<LOD
BDE-206	<LOD	<LOD	<LOD	3.17	<LOD	<LOD	31.14	<LOD
BDE-209	92.79	92.60	74.70	105.15	30.85	91.75	103.55	119.78

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Sep-12	Brissago	Luino	Pallanza	Laveno	Baveno	Suna	Brebbia	Ranco
BDE-28	14.79	14.26	17.96	5.89	10.60	2.55	6.60	1.93
BDE-47	55.36	35.81	49.69	47.72	57.85	40.60	58.52	33.61
BDE-99	31.80	19.41	24.08	19.63	29.02	21.83	22.47	19.65
BDE-100	10.45	8.43	16.12	7.07	14.66	6.24	6.60	7.38
BDE-154	4.33	6.76	11.22	1.77	8.98	2.49	2.27	2.83
BDE-153	8.30	9.54	14.29	2.63	11.39	1.96	1.75	1.06
BDE-183	7.48	4.63	11.22	3.81	6.39	0.74	1.96	0.04
BDE-188	3.75	3.15	2.82	1.39	4.35	1.40	4.74	1.38
BDE-179	5.07	3.45	4.59	9.35	11.15	2.66	3.99	1.58
BDE-202	0.26	1.39	0.82	<LOD	0.28	0.74	1.13	0.16
BDE-201	0.31	1.24	<LOD	0.84	0.74	<LOD	0.62	0.45
BDE-208	1.46	<LOD	<LOD	<LOD	4.63	<LOD	<LOD	<LOD
BDE-207	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
BDE-206	<LOD	<LOD	<LOD	8.41	<LOD	<LOD	11.86	<LOD
BDE-209	95.73	126.39	105.10	72.90	55.37	156.38	176.29	84.87

However, some differences between the sampling stations have been observed for the mussel samples. For instance, a high value of ΣBDEs at Brebbia station (182.8 ng/g l.w) might be related to the input of contaminants deriving from the Bardello River. Supporting this hypothesis, previous studies (Guzzella et al. 2008; Poma et al. submitted) have shown high concentrations of BFRs in Bardello and Boesio river sediments collected in the Varese province, the most heavily industrialized and anthropogenic area of the basin, where a great number of textile and plastic industries are located.

The dominant congener detected in mussel samples was BDE-209 (up to 144.7 ng/g l.w.), although it was generally considered to be non-bioavailable and resistant to any degradation (La Guardia et al. 2007). BDE-209 in mussels was measured together with some lower brominated congeners (BDE-179, -188, -201, -202). The presence of these hepta/octa-BDE congeners might be due to two different contributions. (i)

Accordingly to other previous studies (Wei et al. 2013; Kohler et al. 2008; Söderström et al. 2004), environmental debromination of BDE-209 is possible, as proven by the detection of several less brominated congeners identified in several matrices as specific products of photodegradation and/or microbiological transformation of BDE-209; mussels, as filter feeders, could hence accumulate these contaminants from the environment (Arnot and Gobas 2006). (ii) Besides, evidences of BDE-209 metabolism in some fish species was demonstrated by the presence of BDE-179, -188, -201, -202 in fish tissues by La Guardia et al. (2007), while these congeners are not present in the technical commercial BDE mixtures. Anyway, the detection of BDE-179 in zebra mussel samples, while in literature it has never been identified as product of microbial or photolytic debromination (Wei et al. 2013; Viganò et al. 2011), leads us to hypothesize that these congeners have a metabolic origin even in mussel organisms. On the contrary, concentrations of hepta/octa congeners in fish tissues were generally very low and/or close to the LOD value. These results may be explained considering that less brominated congeners might be rather bioaccumulated in fish liver, while only muscle was considered in this study (Stapleton et al., 2006).

The concentrations and the relative contribution of tri- to hepta-BDE congeners (BDE-28, -47, -99, -100, -154, -153, and -183) to the contamination of zebra mussel are shown in Fig. III-3.

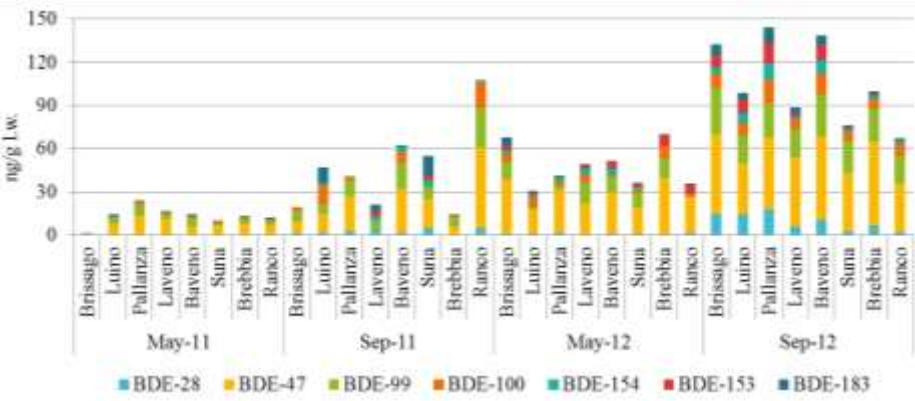

Fig. III-3 Single tri- to hepta-BDE congener concentrations (ng/g l.w.) in zebra mussels collected from eight sampling stations in Lake Maggiore from May 2011 to September 2012

No standard deviation is reported because the total amount of samples was not enough to allow replicates. Total tri- to hepta-BDE concentrations ranged from 1.0 ng/g l.w. in May 2011 to 144.6 ng/g l.w. in September 2012. Despite the Penta-BDE formulation was banned in Europe in 2004 (Directive EEC 2003), increasing concentrations of tri- to hepta-BDE congener in mussels from 2011 to 2012 have been evidenced (Fig. III-3), still highlighting the presence of a congener profile resembled the commercial Penta-BDE formulation (de Wit 2002). However, a same trend has been observed in the core sediments collected in the Lake Maggiore in 2011 (Poma et al., submitted), in which the sum of tetra/penta/hexa/hepta-BDE started to increase since the '90s, explained by the probable leaching from consumer products during use and/or after disposal in the lake basin. In addition, it is known that the biotransformation from higher- to lower-brominated PBDEs may lead to an increase of more bioaccumulative lower-brominated PBDEs such as BDE-

47 and BDE-99 (Ross et al. 2009; Gandhi et al. 2011) in the environment.

Similar to HBCD, in Fig. III-3 it is also evident that mussels collected in September are generally more contaminated than those collected in May. Several studies previously conducted on zebra mussels in Lake Maggiore showed that the spawning period (late spring) leads to a sort of "biological depuration", i.e. the loss of contaminants due to the release of gametes (Binelli et al. 2008; CIPAIS 2010; CIPAIS 2011). The peculiar meteorological situation probably caused an additional input of PBDEs, and it might explain the unusual behaviour of these mussel samples. Other studies (CIPAIS 2012; CIPAIS 2013) have reported a similar phenomenon concerning the PCB concentrations measured in zebra mussel samples collected in Lake Maggiore in 2011 and 2012.

Differently from mussel samples, only BDE-47, -99, and -100 were detected in roach tissues with concentration ranging from 52.4 to 162.3 ng/g l.w., from 21.4 to 83.0 ng/g l.w., and from 17.9 to 84.9 ng/g l.w. respectively. A possible explanation might be found in a faster debromination metabolism in fish than in mussels.

The relative percentual distribution of different PBDE congeners in mussel and fish tissues in this study was quite different. In zebra mussels the mean relative presence of BDE congeners was BDE-209 > -47 > -99 > -100, with BDE-209 representing about 50% of the total amount of PBDEs. La Guardia et al. (2012) observed a similar distribution (BDE-209 contribution from 37 to 67%) in bivalves collected downstream from a textile manufacturing outfall in North Carolina (U.S.A.). Therefore, it is likely that also the commercial Deca-BDE product is still in use in the Lake Maggiore basin, since otherwise a quick decrease of BDE-209 in mussel tissues is expected due to metabolic transformations to lower brominated

congeners (Voorspoels et al. 2003). On the contrary, the percentual distribution of PBDE congeners in fish was BDE-47 > -99 > -100 > -209, consistent with congener profile from other investigations concerning freshwater fish worldwide (Hu et al. 2010; Yu et al. 2012).

We performed the Principal Component Analysis (PCA) in order to better investigate the pattern of contamination present in each sampling site. PCA results, calculated on the whole zebra mussel dataset, are represented in Fig. III-4. In particular, results showed that the first two PCs represented 65.4% of the total variances of BFR concentrations in mollusks. Combined with the variables and cases plots, PBEB and the sum of lower-brominated BDE congeners were congregated into one group represented by the heavily polluted mussels collected from Brissago, Baveno, and Ranco stations. BDE-209 and HBB are the main compounds accumulated in mussels sampled at Suna station, while HBCD, BTBPE and the higher-brominated BDE congeners affected mainly the Brebbia station. Despite there is evidence that PBDE contamination in Lake Maggiore is strictly related with inputs of contaminated sediments through Bardello and Boesio rivers (Guzzella et al. 2008; Poma et al. submitted), the PCA plots showed that the PBDE contamination reached the majority of the lake, maybe because of its hydrology and water flows. These crucial results highlighted that, although the apparent homogeneous PBDE contamination found in the entire lake cuvette, there are several different patterns of pollution typical for each sampling site. This demonstrated that the use of *D. polymorpha* is able not only to point out the contamination present in an aquatic ecosystem, but also to differentiate the pollution fingerprint.

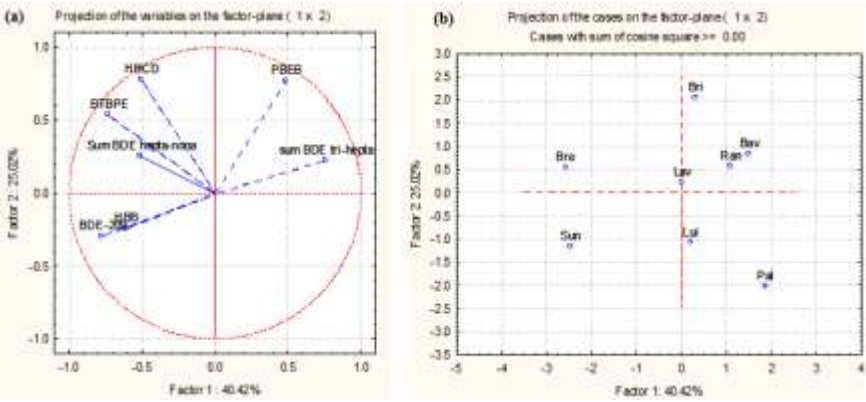

Fig. III-4 PCA diagrams showing relationships between some of the BFR variables (a) and cases (b)

3.3.4 BFR biomagnification occurrence

The BMFs of pollutants were obtained directly from the ratio of the mean lipid equivalent chemical concentrations in roach and zebra mussel, when available. BMFs were calculated using the expression

$$BMF_{LW} = C_{B(LW)}/C_{D(LW)}$$

where C_B and C_D are the chemical concentrations (ng/g l.w.) in fish and in its diet, respectively (Arnot and Gobas 2006). An average of three stations in which mussels were collected (Pallanza, Baveno and Suna) were considered in the calculation of BMF, being the closest to the fish feeding area and where roach were captured. Besides, in order to have homogeneous data for the BMF calculation, only BFR concentrations from May 2011 to May 2012 of zebra mussel and roach were considered. Our results showed that tetra- and penta-BDE are able to biomagnify with a mean BMF of 6.3, 6.8 and 23.2 for BDE- 47, -99, and -100 respectively. This is in agreement with

the hypothesis assuming that biomagnification of BDEs with six or more bromine atoms seems to be negatively correlated with the increasing number of bromine, due to their relatively high molecular weight and size, leading to inefficient dietary uptake (La Guardia et al. 2012; Burreau et al. 2004). These results are quite higher than those observed by Law et al. (2006) in Lake Winnipeg food web. Moreover, BDE-188 and -179 showed a mean BMF of 4.7 and 1.2 respectively. Concerning these octa-congeners, we suggest that it is possible that the metabolically mediated debromination in mussels and fish of higher to lower brominated congeners may increase the BMF for these compounds, and that their presence may consequently lead to an apparent increase in BMF. On the contrary, BDE-202, -201, nona-, and deca-BDE were still bioavailable in this study and therefore detected in the fish muscles, but biomagnification is unlikely to occur, having a BMF<1. Considering the other BFRs, it was calculated that only HBCD at Pallanza had a BMF>1 (BMF = 1.2), suggesting a moderate biomagnification potential.

3.4. CONCLUSIONS

In this study, contamination due to PBDEs, HBCD and several nBFRs has been investigated in Lake Maggiore, considering their spatial distribution and accumulation in zebra mussels and in roach. The low contamination due to HBB, PBEB, BTBPE and the absence of DBDPE in the considered organisms might indicate a scarce use of these compounds in the Lake Maggiore basin or a weak behaviour to biomagnificate. On the contrary, HBCD was detected in all organic tissues, but the differences in contamination among the sampling stations were not statistically significant, suggesting that Lake Maggiore is not directly impacted by industrial point emissions of HBCD.

Analytical results, supported by PCA plots, suggested that sources of contamination due to the congeners composing the

Penta- and Deca-BDE technical formulations are present in the basin, and that the PBDE contamination (considering the different congeners) reached the majority of the lake, maybe because of its hydrology and water flows. BDE-209 was the dominant BDE congener detected in mussel samples, together with some hepta/octa-BDE congeners (BDE-179, -188, -201, -202); the detection of BDE-179 in zebra mussel samples leads us to hypothesize that these congeners have a metabolic origin. On the contrary, concentrations of hepta/octa congeners in fish tissues were very low and close to the LOD value. This could be because these less brominated congeners might be rather accumulated in liver, while in this study only muscle was considered. Considering the contamination due to tri- to hepta-BDE congeners, despite the Penta-BDE formulation was banned in Europe in 2004, a general increasing trend in mussels was noted, still highlighting the presence of a congener profile resembled the commercial Penta-BDE formulation explained by the probable leaching from consumer products during use and/or after disposal. Moreover, it was noted that mussels collected in September were generally more contaminated than those collected in May. This unusual behaviour of mussels could be due to the peculiar meteorological situation (with frequent precipitation events) affected Northern Italy in 2011 and 2012, which probably caused an additional input of contaminants. Finally, results on BMFs showed that tetra- and penta-BDE biomagnified, and that octa-, nona-, and deca-BDE were still bioavailable and detected in the fish muscles, but biomagnification is difficult to assess because of the likely different metabolic transformation in mussels and fish, and the different uptake of the compounds in fish. Considering the other BFRs, only HBCD showed a moderate biomagnification potential.

3.5 ACKNOWLEDGEMENTS

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3.6 REFERENCES

Abdallah, M.A-E., Harrad, S., 2011. Tetrabromobisphenol-A, hexabromocyclododecane and its degradation products in UK human milk: Relationship to external exposure. Environment International 37(2), 443-448.

Arias, P.A., 2001. Brominated flame retardants - An overview. The Second International Workshop on Brominated Flame Retardants (BFR 2001) May 14–16 Stockholm 17-19.

Arnot, J.A., Gobas, F.A.P.C., 2006. A review of bioconcentration factor (BCF) and bioaccumulation factor (BAF) assessments for organic chemicals in aquatic organisms. Environmental Reviews 14(4), 257-297.

Betts, K., 2008. New flame retardants detected in indoor and outdoor environments. Environmental Science & Technology 42(18), 6778.

Binelli, A., Galassi, S., Provini, A., 2001. Factors affecting the use of *Dreissena polymorpha* as a bioindicator: the PCB pollution in Lake Como (N. Italy). Water, Air, & Soil Pollution 125(1), 19-32.

Binelli, A., Guzzella, L., Roscioli, C., 2008. Levels and congener profiles of polybrominated diphenyl ethers (PBDEs) in Zebra mussels (D. polymorpha) from Lake Maggiore (Italy). Environmental Pollution 153(3), 610-617.

BSEF Bromine Science Environmental Forum, 2001.

BSEF Bromine Science Environmental Forum, 2013. http://www.bsef.com/regulation/international-regulations/what-are-the-united-nations-conventions

Burreau, S., Zebühr, Y., Broman, D., Ishaq, R., 2004. Biomagnification of polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) studied in pike (*Esox lucius*), perch (*Perca fluviatilis*) and roach (*Rutilus rutilus*) from the Baltic Sea. Chemosphere 55(7), 1043-1052.

Camusso, M., Balestrini, R., Binelli, A., 2001. Use of zebra mussel (Dreissena polymorpha) to assess trace metal contamination in the largest Italian subalpine lakes. Chemosphere 44(2), 263-270.

CIPAIS Commissione Internazionale per la Protezione delle Acque Italo-Svizzere, 2010. Indagini sul DDT e sostanze pericolose nell'ecosistema del Lago Maggiore. Rapporto annuale 2010 (in It.)

CIPAIS Commissione Internazionale per la Protezione delle Acque Italo-Svizzere, 2011. Indagini sul DDT e sostanze pericolose nell'ecosistema del Lago Maggiore. Rapporto annuale 2011 (in It.)

CIPAIS Commissione Internazionale per la Protezione delle Acque Italo-Svizzere, 2012. Indagini su DDT e sostanze pericolose nell'ecosistema del Lago Maggiore. Rapporto annuale 2012 (in It.)

CIPAIS Commissione Internazionale per la Protezione delle Acque Italo-Svizzere, 2013. Indagini su DDT e sostanze pericolose nell'ecosistema del Lago Maggiore. Rapporto annuale 2013 (in It.)

Covaci, A., Harrad, S., Abdallah, M.A-E., Ali, N., Law, R.J., Herzke, D., et al., 2011. Novel brominated flame retardants: a review of their analysis, environmental fate and behaviour. Environment International 37(2), 532-556.

Covaci, A., Voorspoels, S., de Boer, J., 2003. Determination of brominated flame retardants, with emphasis on polybrominated diphenyl ethers (PBDEs) in environmental and human samples - a review. Environment International 29(6), 735-756.

de Wit, C.A., 2002. An overview of brominated flame retardants in the environment. Chemosphere 46(5), 583-624.

Directive 2003/11/EC of the European Parliament and of the Council of 6 February 2003 amending for the 24th time Council directive

76/769/EEC relating to restrictions on the marketing and use of certain dangerous substances and preparations (pentabromodiphenyl ether and octabromodiphenyl ether). Official Journal L042. 15/02/2003

ESIS European Chemical Substance Information System European Commission, Joint Research Centre, European Chemicals Bureau (2010) http://esis.jrc.ec.europa.eu/

European Court of Justice (2008) Cases C-14/06 and C-295/06, Judgement of the Court, 1 April 2008, Directive 2002/95/EC and Commission Decision 2005/717/EC.
http://curia.europa.eu/. Accessed July 2010

Gandhi, N., Bhavsar, S.P., Gewurtz, S.B. et al., 2011. Can biotransformation of BDE-209 in lake trout cause bioaccumulation of more toxic, lower-brominated PBDEs (BDE-47,-99) over the long term? Environment International 37(1), 170-177.

Gauthier, L.T., Hebert, C.E., Weseloh, D.V.C., Letcher, R.J., 2007. Current-use flame retardants in the eggs of herring gulls (*Larus argentatus*) from the Laurentian Great Lakes. Environmental Science & Technology 41(13), 4561-4567.

Guerra, P., Eljarrat, E., Barcelò, D., 2010. Analysis and occurrence of emerging brominated flame retardants in the Llobregat River basin. Journal of Hydrology 383(1-2), 39-43.

Guzzella, L., Roscioli, C., Binelli, A., 2008. Contamination by plybrominated diphenyl ethers of sediments from the Lake Maggiore Basin (Italy and Switzerland). Chemosphere 73(10), 1684-1691.

Hermanson, M.H., Isaksson, E., Forsstrom, S. et al., 2010. Deposition History of Brominated Flame Retardant Compounds in an Ice Core from Holtedahlfonna, Svalbard, Norway. Environmental Science & Technology 44(19-SI), 7405-7410.

Hoh, E., Zhu, L., Hites, R.A., 2005. Novel flame retardants, 1,2-Bis(2,4,6-ribromophenoxy)ethane and 2,3,4,5,6-pentabromoethyl benzene, in United States' environmental samples. Environmental Science & Technology 39(8), 2472-2477.

Hu, G.C., Dai, J.Y., Xu, Z.C. et al., 2010. Bioaccumulation behavior of polybrominated diphenyl ethers (PBDEs) in the freshwater food chain of Baiyangdian Lake, North China. Environment International 36(4), 309-315.

Isobe, T., Ogawa, S.P., Ramu, K., Sudaryanto, A., Tanabe, S., 2012. Geographical distribution of non-PBDE-brominated flame retardants in mussels from Asian coastal waters. Environmental Science and Pollution Research 19(8), 3107-3117.

Kierkegaard, A., Bjorklund, J., Friden, U., 2004. Identification of the flame retardant decabromodiphenyl ethane in the environment. Environmental Science & Technology 38(12), 3247-3253.

Klosterhaus, S.L., Stapleton, H.M., La Guardia, M.J., Greig, D.J., 2012. Brominated and chlorinated flame retardants in San Francisco Bay sediments and wildlife. Environment International 47, 56-65.

Kohler, M., Zennegg, M., Bogdal, C., et al., 2008. Temporal trends, congener patterns, and sources of Octa-, Nona-, and decabromodiphenyl ethers (PBDE) and hexabromocyclododecanes (HBCD) in Swiss lake sediments. Environmental Science & Technology 42(17), 6378-6384.

Hale, R.C., La Guardia, M.J., Harvey, E., et al., 2006. Brominated flame retardant concentrations and trends in abiotic media. 3rd International Workshop on Brominated Flame Retardants held in Toronto, Canada 06-09 June 2004. Chemosphere 64(2), 181-186.

La Guardia, M.J., Hale, R.C., Harvey, E., 2007. Evidence of debromination of decabromodiphenyl ether (BDE-209) in biota from a wastewater receiving stream. Environmental Science & Technology 41(19), 6663-6670.

La Guardia, M.J., Hale, R.C., Harvey, E., Matteson Mainor, T., Ciparis, S., 2012. In Situ Accumulation of HBCD, PBDEs, and Several Alternative Flame-Retardants in the Bivalve (*Corbicula fluminea*) and Gastropod (*Elimia proxima*). Environmental Science & Technology 46(11), 5798-5805.

Law, K., Halldorson, T., Danell, R,. Stern, G., Gewurtz, S., Alaee, M., 2006. Bioaccumulation and trophic transfer of some brominated flame retardants in a lake Winnipeg (Canada) food web. Environmental Toxicology and Chemistry 25(8), 2177-2186.

Marvin, C.H., Tomy, G.T., Alaee, M., MacInnis, G., 2006. Distribution of hexabromocyclododecane in Detroit River suspended sediments. Chemosphere 64(2), 268-275.

OSPAR Commission. OSPAR List of Chemicals for Priority Action. http://www.ospar.org/content/content.asp?menu=00120000000074_000000_0000002001. Update 2007

Ross, P.S., Couillard, C.M., Ikonomou, M.G., et al., 2009. Large and growing environmental reservoirs of Deca-BDE present an emerging health risk for fish and marine mammals. Marine Pollution Bulletin 58(1), 7-10.

Söderström, G., Sellström, U., de Wit, C.A., Tysklind, M., 2004. Photolytic Debromination of Decabromodiphenyl Ether (BDE 209). Environmental Science & Technology 38(1), 127-132.

Stapleton, H.M., Brazil, B., Holbrook, R.D., Mitchelmore, C.L., Benedict, R., Konstantinov, A., Potter, D., 2006. In vivo and in vitro debromination of decabromodiphenyl ether (BDE-209) by Juvenile Rainbow Trout and Common Carp. Environmental Science Technology 40, 4653-4658.

Stockholm Convention web site: http://chm.pops.int/Convention/POPsReviewCommittee/LatestMeeting/POPRC8/POPRC8Followup/HBCDRecommendation/tabid/2912/Default.aspx

Tomy, G.T., Pleskach, K., Oswald, T., et al., 2008. Enantioselective bioaccumulation of hexabromocyclododecane and congener-specific accumulation of brominated diphenyl ethers in an eastern Canadian Arctic marine food web. Environmental Science & Technology 42(10), 3634-3639.

Ueno, D., Isobe, T., Ramu, K., Tanabe, S., Alaee, M., Marvin, C., Inoue, K., Someya, T., Miyajima, T., Kodama, H., Nakata, H., 2010.

Spatial distribution of hexabromocyclododecanes (HBCDs), polybrominated diphenyl ethers (PBDEs) and organochlorines in bivalves from Japanese coastal waters. Chemosphere 78(10), 1213-1219.

Verreault, J., Gebbink, W., Gauthier, L.T., Gabrielsen, G.W., Letcher, R.J., 2007. Brominated flame retardants in Glaucous Gulls from the Norwegian arctic: more than just an issue of polybrominated diphenyl ethers. Environmental Science & Technology 41(14), 4925-4931.

Viganò, L., Roscioli, C., Guzzella, L., 2011. Decabromodiphenyl ether (BDE-209) enters the food web of the River Po and is metabolically debrominated in resident cyprinid fishes. Science of the Total Environment 409(23), 4966-4972.

Volta, P., Jepsen, N., 2008. The recent invasion of roach (Pisces: Cyprinidae) in a large South-Alpine Lake. Journal of Limnology 67(2), 163-170.

Volta, P., Grimaldi, E., Calderoni, A., Polli, B., 2013. Andamento della pesca professionale sul Lago Maggiore nel periodo 1979-2011. Commissione italo-svizzera per la Pesca (CISPP) 20pp (in It) (in press)

Voorspoels, S., Covaci, A., Schepens, P., 2003. Polybrominated diphenyl ethers in marine species from the Belgian North Sea and the Western Scheldt estuary, levels, profiles and distribution. Environmental Science & Technology 37(19), 4348-4357.

Watanabe, I., Sakai, S., 2003. Environmental release and behaviour of brominated flame retardants. Environment International 29(6), 665-682.

Wei, H., Zou, Y., Li, A., Christensen, E.R., Rockne, K.J., 2013. Photolytic debromination pathway of polybrominated diphenyl ethers in hexane by sunlight. Environmental Pollution 174, 194-200.

Wei, H., Aziz-Schwanbeck, A.C., Zou, Y., Corcoran, M.B., Poghosyan, A., Li, A., Rockne, K.J., Christensen, E.R., Sturchio, N.C., 2012. Polybromodiphenyl Ethers and Decabromodiphenyl Ethane in aquatic sediments from Southern and Eastern Arkansas,

United States. Environmental Science & Technology 46(15), 8017-8024.

Yu, Y.X., Zhang, S.H., Huang, N.B., Li, J.L., Pang, Y.P., Zhang, X.Y., Yu, Z.Q., Xu, Z.G., 2012. Polybrominated diphenyl ethers and polychlorinated biphenyls in freshwater fish from Taihu lake, China: their levels and the factors that influence biomagnification. Environmental Toxicology and Chemistry 31(3), 542-549.

CHAPTER IV

CONCENTRATIONS AND TROPHIC INTERACTIONS OF NOVEL BROMINATED FLAME RETARDANTS, HBCD, AND PBDEs IN ZOOPLANKTON AND FISH FROM LAKE MAGGIORE (NORTHERN ITALY)

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ABSTRACT

Following the release of the international regulations on PBDEs and HBCD, the aim this study is to evaluate the concentrations of novel brominated flame retardants (nBFRs), including 1,2-bis(2,4,6-tribromophenoxy) ethane (BTBPE), decabromodiphenyl ethane (DBDPE), hexabromobenzene (HBB), and pentabromoethylbenzene (PBEB), in an Italian subalpine lake, located in a populated and industrial area. The study investigated specifically the potential BFR biomagnification in a particular lake's pelagic food web, whose structure and dynamics were evaluated using the Stable Isotope Analysis. The potential BFR biomagnification was investigated by using the trophic-level adjusted BMFs and Trophic Magnification Factors (TMFs), confirming that HBCD and some PBDE congeners are able to biomagnify within food webs.

Comparing the calculated values of BMF_{TL} and TMF, a significant positive correlation was observed between the two factors, suggesting that the use of BMF_{TL} to investigate the biomagnification potential of organic chemical compounds might be an appropriate approach when a simple food web is considered.

Keywords: Brominated Flame Retardants; Lake Maggiore; pelagic food web; trophic-level adjusted BMF; Trophic Magnification Factor

4.1 INTRODUCTION

Brominated Flame Retardants (BFRs) are used in a wide range of commercial and household products, including plastics, textiles, electronics, and polyurethane foam in order to reduce their flammability (de Wit, 2002). They are widely diffused in aquatic environment, are persistent, and bioaccumulative in biota (de Jourdan et al., 2013). Polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane (HBCD) are among the most abundant BFRs detected in the environment, in wildlife, and in human tissues (Alaee et al., 2003) because they do not form chemical bonds to the matrix of the flame-retarded product and, therefore, can be easily leached into the environment (de Wit, 2002). Due to their growing environmental and human health concerns, the production and use of technical PBDE mixtures (Penta-, Octa- and Deca-BDE) have been phased-out or restricted in both Europe and North America (Cox and Efthymiou, 2003; Cox and Drys, 2003; U.S. Environmental Protection Agency, 2009). Furthermore, the main components of the technically produced Penta-BDE and Octa-BDE mixtures were recently introduced on the list of persistent organic pollutants (POPs) (UNEP, 2010). Moreover, at the sixth meeting of the Conference of the Parties of the Stockholm Convention (May 2013), HBCD was included in the list of POP substances. The listing, however, allows an exemption for the production and use of HBCD in expanded polystyrene (EPS) and extruded polystyrene (XPS) in buildings, and will be valid until 2019 (BSEF, 2013).

The reduction in the use of PBDEs and HBCD has consequently opened the way for the introduction of novel BFRs (nBFRs) taking the place of the banned formulations (Betts, 2008), including 1,2-bis(2,4,6-tribromophenoxy) ethane (BTBPE), decabromodiphenyl ethane (DBDPE), hexabromobenzene (HBB), and pentabromoethylbenzene (PBEB). BTBPE and

DBDPE are used as replacement products for Octa-BDE and Deca-BDE, respectively (Great Lakes Chemical Corporation, 2004; Gauthier et al., 2007). Both compounds have been detected in environmental samples such as air (Hoh et al., 2005; Salamova and Hites, 2011), sediments (Wu et al., 2010; Lopez et al., 2011; Poma et al., submitted), and fish (Law et al., 2006). HBB and PBEB have been detected in sediments, wildlife, and humans (Guerra et al., 2010; Gauthier et al., 2007; Verreault et al., 2007; Hoh et al., 2005). However, little quantitative data are available yet on the presence and trophic transfer of these novel BFRs in food webs, which is a crucial criterion for assessing their ecological risk (Wu et al., 2010).

Following the global phase out of PBDEs and the recent decisions on HBCD, this study aims to evaluate whether novel BFRs can bioaccumulate in a pelagic food web of a large and deep subalpine lake (Lake Maggiore, Northern Italy), whose catchment is a highly populated area with many manufacturing plants. This study also intends to estimate the presence of PBDE and HBCD in today's lake contamination. Because the novel BFRs share physicochemical properties similar to those of PBDEs, analogous environmental fate (e.g. bioaccumulation) is expected (Wu et al., 2011; Wu et al., 2012), and their behaviour in the aquatic system could be described in terms of food web structure. The Trophic Magnification Factor (TMF) currently represents one of the most conclusive kinds of evidence for the biomagnification behavior of a chemical substance in food webs (Conder et al., 2011). However, a trophic level-adjusted BMF (BMF_{TL}) could be used to explore more rigorously the variable behaviour of different BFRs directly between prey and predator (Cullon et al., 2012), and to examine individual predator-prey relationships. In this study, the values of BMF_{TL} were thus compared to those of TMF to determine if certain combinations result in greater or less accumulation than the values indicated

by the TMF. To reach this goal and to evaluate the structure and dynamics of the pelagic food web, the trophic role of fish was determined using the carbon and nitrogen Stable Isotope Analysis (SIA), as the isotopic signature of an animal reflects its assimilated diet (Coat et al., 2009).

4.2 MATERIALS AND METHODS

4.2.1 Sample collection

Pelagic zooplankton and fish were sampled from Lake Maggiore from May 2011 to January 2012 in four different seasons: late spring, summer, late autumn and winter. Zooplankton samples were collected at Ghiffa (the point of maximum depth of the lake), Baveno (in the Pallanza Bay and near the inflow of the Toce River), and Lesa (located in the Southern, shallower part of the lake basin) (Fig. IV-2). Zooplankton samples were collected using a 58 cm diameter, 450 µm mesh net hauled twice from 0 to 50 m depth. Total volume filtered for zooplankton was ca. 26 m3 of lake water. One third of the zooplankton sample was separated for the main taxa identification by CNR-ISE (CIPAIS 2012) at 40x or 100x using compound microscopy, and the dominant crustacean zooplankton taxa (Cladocera and Copepoda) are shown in Fig. IV-1.
The other zooplankton samples were filtered on a 2 µm pore glass–fibre-filters (GF/C, 4.7 cm of diameter), pooled for analysis, frozen at −20°C, and sent to CNR-IRSA for BFR analysis.

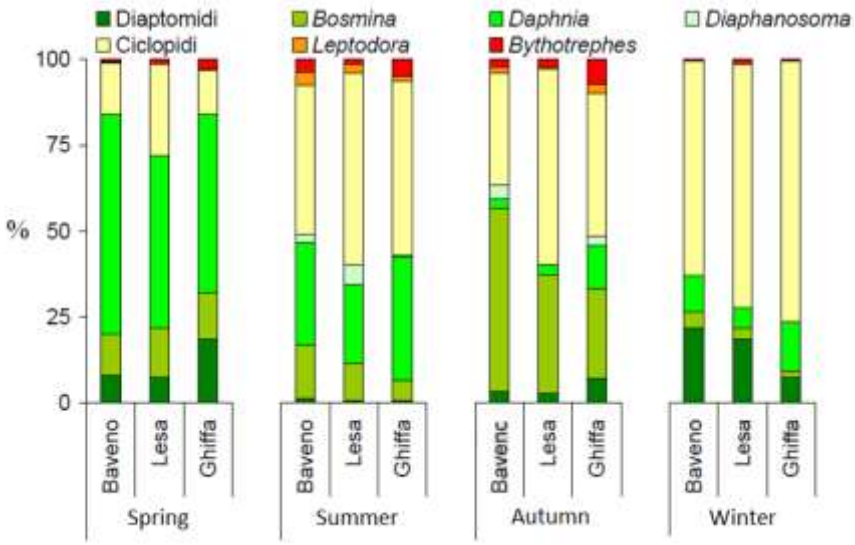

Fig. IV-1 Percentage composition of mesozooplankton biomass in Lake Maggiore from spring to winter 2011 (data from CIPAIS 2012)

Specimens of shad (*Alosa agone* - Scopoli, 1786) and whitefish (*Coregonus lavaretus* - Linneaus, 1758) were collected with pelagic gill nets by CNR-ISE in the same periods as that of zooplankton at Ghiffa station (Fig. IV-2). These two mostly zooplanktivorous fish are often used as bioindicators in bioaccumulation studies (Volta et al., 2009; Bettinetti et al., 2010; Infantino et al., 2013), being considered key species in large and deep subalpine lakes (Volta et al., 2011). All samples were collected and then segregated in order to obtain two age groups of organisms: "I" - from 1 to 3 years (young fish) - and "II" ≥ 3 years (adult fish). After capture, fish were stored at 4 °C and their individual body length (cm) and weight (g) were measured immediately. Age was determined by scale reading and the muscle sample for the analysis was taken from the fish caudal portion. Also the liver of the fish was considered for the analysis, due to its importance linked to storage, metabolism and detoxification of chemical compounds (Song et al., 2006). The muscles and the livers of about ten fish for each age class were pooled together and homogenized by a steel mixer in order to obtain single samples, and finally stored at -25 °C until they were sent to CNR-IRSA for BFR analysis. Detailed information on fish biological parameters and lipid contents are given in Table IV-1.

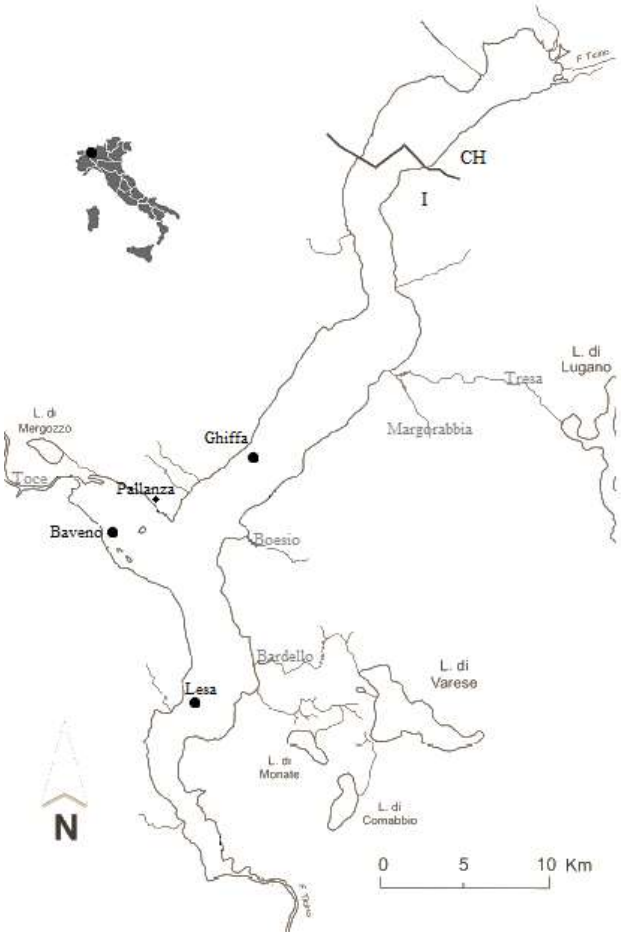

Fig. IV-2 Pelagic zooplankton and fish sampling stations in Lake Maggiore

4.2.2 Sample preparation and analytical procedure

Sample preparation can be summarized as follow: after lyophilisation, a variable amount of dried sample (0.1 g for zooplankton and 1 g for fish) was spiked with 50 µL of a recovery standard solution (250 µg/L containing the labeled compounds [$^{13}C_{12}$]γHBCD, [$^{13}C_{12}$]BDE-209, and [$^{13}C_{12}$]BDE-47, -99, -154, -183, purchased from Wellington Labs, Canada), and then extracted in a hot Soxhlet apparatus (Buchi, Flawil, Switzerland) using a n-hexane/acetone mixture (3:1 v/v) for 25 cycles. The extracts were concentrated to 5 mL by Turbovap (Zymark, Hopkinton, USA) on a gentle nitrogen stream, and then subjected to Gel Permeation Chromatography (GPC), which included a GPC Basix system equipped with a GPC 1122 solvent delivery system (LCTech GmbH, Dorfen, Germany). A second phase clean-up was performed using a multi-layer column (1.5 x 20 cm) packed (bottom to top) with 1.5 g of acidified silica gel (30% w/w sulphuric acid, Sigma-Aldrich, Germany) and 1.5 g of Florisil® (100-200 mesh, Sigma-Aldrich, Germany). The column was pre-washed with 15 mL of n-hexane/dichloromethane (n-hexane/DCM) 1:1 v/v, and the elution was performed collecting 40 mL of the same solvent. 1 mL of toluene was added to the extract, concentrated by Turbovap, and then reconstituted to 100 µL using toluene. The lipid content of zooplankton samples and fish tissues was determined gravimetrically after solvents evaporation under a gentle nitrogen stream, and the extract brought to constant weight (at 105 °C).

GC analysis for BFR compounds was performed using a Thermo Electron TraceGC 2000 coupled with a PolarisQ Ion Trap (ThermoElectron, Austin, Texas) mass spectrometer and equipped with a PTV injector and an AS 3000 auto sampler. The system was managed by ThermoFinnigan Xcalibur software version 1.4.1. PBDE and BFR identification (BDE-28, 47, 100,

99, 153, 154, 183, 179, 188, 201, 202, 206, 207, 208, 209; HBCD, PBEB, HBB, BTBPE) was achieved using a Restek RTX-1614 capillary column, 15 m x 0.25 mm i.d. x 0.10 µm film thickness (Restek U.S., Bellefonte, Pennsylvania, USA) and analyzed using tandem mass spectrometry in the following conditions: carrier gas helium at 1.2 mL/min; injection pressure of 51 kPa; transfer pressure of 102 kPa; injector temperature starting at 100 °C and maintained for 1.2 min, then ramped to 300 °C (held 1 min) at 4 °C/s; initial oven temperature set at 120 °C (held 1.2 min), then ramped to 275 °C at 15 °C/min (held 0 min) and finally to 300 °C at 5 °C/min (held 5 min). Samples were analyzed using tandem mass spectrometry under the following instrumental conditions: EI mode with standard electron energy of 70 eV; the transfer line was maintained at 300 °C, the damping gas at 2 mL/min, and the ion source at 260 °C. Quantitative analysis was performed with an external standard method. DBDPE concentrations were determined using a TraceGC Ultra equipped with a cold on-column injector and an ECD-40 detector (ThermoElectron, Austin, Texas) using a Restek RTX-5 capillary column (15 m x 0.53 mm i.d. x 0.1 µm film thickness) (Restek, Bellefonte, USA). The use of a different analytical method was used for DBDPE determination, because of the thermal instability of this compound. Sample injections (0.5 µL) were performed using a TriPlus autosampler (Thermo Electron) and carried out in the following analytical conditions: carrier gas helium at 6.0 mL/min; starting temperature of 100 °C (held 0.5 min) after which it was ramped to 280 °C at 15 °C/min (held 8 min). Quantitative analysis was obtained by comparing results with an external standard.

4.2.3 Quality Assurance (QA) and Quality Control (QC)

The validation of the analytical method for PBDEs (BDE-47, 99, 100, 153, 154) was carried out using the NIST (National Institute of Standard and Technology) SRM 1947 Lake Michigan Fish Tissue. All measured values were within the certified range of the reference concentration (± 30%).

The mean recoveries of the spiked standards for [^{13}C12]BDE-47, 99, 154, 209, [$^{13}C_{12}$]γHBCD ranged from 56 to 97 % in zooplankton samples, from 51 to 75 % in fish muscle, and from 48 to 104 % in the livers of the fish. The obtained analytical results were corrected considering the recoveries, and the sample analysis was repeated if its mean recovery was below 40%. Using a signal-to-noise ratio of 3:1, the limits of detection (LODs) were estimated for each compound as 0.1 ng/g dry weight in biological samples. A procedural blank was analyzed every eight samples to check for BFR laboratory contaminations; the blank concentrations were below LOD levels for all BFR compounds. The eventually debromination of BDE-209 in the inlet system and during the column transfer, leading to the formation of octa- and nona-BDE congeners, was monitored by the presence of labeled octa and nona-BDE congeners deriving from the debromination of the internal standard [$^{13}C_{12}$]BDE-209. In case of evidence of BDE-209 debromination, the inlet liner was replaced and the column was cleaned heating overnight at high temperature (300 °C).

4.2.4 Stable Isotope Analysis (SIA) and Trophic Level (TL) estimation

Sample of fish caudal muscle were oven-dried at 60 °C for 3 days and finely powdered. Subsamples of about 1 mg d.w. were transferred to 5x9 mm capsules and sent to the G.G. Hatch Stable Isotope Laboratory (University of Ottawa, Canada), where the isotopic composition of the organic carbon and nitrogen was determined by the analysis of CO_2 and N_2, produced by combustion on a Carlo Erba 1110 Elemental Analyser, followed by GC separation and on-line analysis by continuous-flow with a DeltaPlus Advantage isotope ratio mass spectrometer coupled with a ConFlo III. The internal standards used were ($\delta^{15}N$, $\delta^{13}C$ in ‰): C-51 Nicotiamide (0.07,-22.95), C-52 mix of ammonium sulphate + sucrose (16.58,-11.94), C-54 caffeine (-16.61,-34.46), blind standard C-55: glutamic acid (-3.98, -28.53). All $\delta^{15}N$ is reported as ‰ vs. AIR and normalized to internal standards calibrated to International standards IAEA-N1(+0.4‰), IAEA-N2(+20.3‰), USGS-40(-4.52‰) and USGS-41(47.57‰). All $\delta^{13}C$ is reported as ‰ vs. V-PDB and normalized to internal standards calibrated to International standards IAEA-CH-6(-10.4‰), NBS-22(-29.91‰), USGS-40(-26.24‰) and USGS-41(37.76‰). The analytical precision of the analysis, based on the laboratory internal standards (C-55), was usually better than 0.2 ‰ for both $\delta^{15}N$ and $\delta^{13}C$. Atmospheric N_2 and PeeDee Belemnite was used as $\delta^{15}N$ and $\delta^{13}C$ reference standard respectively and isotopic ratios (δ‰) were calculated using the following formula (1):

$$\delta^{15}N \text{ and } \delta^{13}C = [(R_{sample}/R_{std}) - 1]*1000 \qquad (1)$$

where R is $^{15}N/^{14}N$ for $\delta^{15}N$ and $^{13}C/^{12}C$ for $\delta^{13}C$.
We estimated seasonal values of TL of the sampled fish by applying the equation (2) (Post et al., 2002):

$$TL_{fish} = 2 + [(\delta^{15}N_{fish} - \delta^{15}N_{pelagic\ baseline})/\ 3.4] \qquad (2)$$

where 2 is the trophic level of the pelagic baseline (*Daphnia*); $\delta^{15}N_{pelagic\ baseline}$ is the measured $\delta^{15}N$ of *Daphnia* at any given time; 3.4 is the mean stepwise enrichment, i.e. the average increase in $\delta^{15}N$ from one TL to the next. Sources exploited by fish were assessed by comparing $\delta^{13}C$ consumer signatures to those of *Daphnia*, representative of the pelagic baseline. *Daphnia*, an appropriate proxy for detecting seasonal changes in the pelagic baseline, perfectly fit as a reference against which carbon isotopic signals of fish can be compared (e.g. Matthews and Mazumder, 2003; Visconti and Manca, 2011; Visconti et al., 2013).

The difference between $\delta^{15}N_{fish}$ and $\delta^{15}N_{pelagic\ baseline}$ is also referred to as enrichment (E). Time specific enrichment is crucial for estimating the fish TL as *Daphnia* and baseline isotopic signatures largely vary seasonally (Visconti et al., 2013). Reliability of fish from pelagic sources were assessed as follows, comparing $\delta^{13}C_{fish}$ time-specific signature to that of the pelagic baseline, assuming a maximum stepwise carbon fractionation of 1.9‰ (De Niro and Epstein, 1978):

$$T = (\delta^{15}N_{fish} - \delta^{15}N_{pelagic\ baseline})/\ 3.4 \quad (3)$$

where T is the trophic level of fish with respect to the pelagic baseline (*Daphnia*),

$$F_m = T*1.9 \qquad (4)$$

where F_m is the allowed maximum carbon fractionation for considering a fish exploiting on pelagic sources,

$$F = \delta^{13}C_{fish} - \delta^{13}C_{pelagic\ baseline} \quad (5)$$

where F is the actual fractionation of fish. Based on previous equations, the above threshold fractionation (F_m) of fish carbon isotopic signatures was taken as indicative of fish pelagic feeding ($F<F_m$).

4.2.5 Biomagnification Factor and Trophic Magnification Factor

The biomagnification factor normalized on trophic level (BMF_{TL}) was calculated using the following equation (Conder et al., 2012) (6):

$$\text{Log } BMF_{TL} = \frac{Log_{10}(\frac{Cpredator}{Cprey})}{TLpredator - TLprey} \quad (6)$$

where $C_{predator}$ and C_{prey} are lipid normalized values of chemical concentrations in the predator and in its prey, and $TL_{predator}$ and TL_{prey} are trophic levels of the predator and its prey.
The TMF was determined from the slope (m) derived by linear regression of logarithmically transformed lipid normalized chemical concentration in biota and the trophic position of the sampled biota (Borgå et al., 2012) (8):

$$\text{Log } C_b = a + mTL \quad (7)$$

where C_b is the contaminant concentration in the biota, thus

$$TMF = 10^m \quad (8)$$

The general scientific consensus is that an increase in chemical concentration with increasing trophic level (i.e. biomagnification) results in a BMF and/or TMF above 1, while

decreasing concentrations with increasing trophic position (TMF<1) indicates trophic dilution (Fisk et al., 2001; Arnot and Gobas, 2006). For the BMF_{TL} and TMF calculation, only pelagic fish were considered, being directly related to zooplankton samples.

4.3 RESULTS AND DISCUSSION

4.3.1 Considerations on the biological samples

All samples were collected in different seasons to investigate whether or not physiological or environmental variability could affect the BFR concentrations in the tissues. In particular, the fish spawning period is a crucial physiological stage since reproduction greatly interferes with the bioaccumulation of hydrophobic organic contaminants, concentrating in tissues with high lipid content. Confirming this, low lipid contents were generally observed in correspondence to the two fish spawning period, summer samples for shad I and II (6 and 16% respectively), and winter samples for whitefish I and II (10 and 13% respectively) (Table IV-1). In addition, also the lipid content of the liver of fish reflected this behaviour. On the contrary, the differences in the zooplankton lipid content in the four seasons (Table IV-2) could be attributed to changes in taxa composition of pooled samples.

Considering the fish species (Table IV-1), in the spring and summer of 2011 the shad and whitefish carbon isotopic signatures were consistent with pelagic food sources for both young and adult fish, ranging from -26‰ to -29.9‰ of $\delta^{13}C$, as previously determined by Visconti et al. (2013). On the contrary, in autumn and winter both species showed non pelagic carbon isotopic signatures, probably reflecting partial and/or complete reliability of pelagic fish on littoral food sources, as has already been demonstrated by Visconti et al. (2013). Both pelagic and littoral baseline carbon isotopic signatures vary with the seasons,

from more ^{13}C-depleted values in winter to less ^{13}C-depleted values in summer. A common seasonal pattern, however, does not imply overlap: time specific littoral carbon signatures, with respect to corresponding pelagic ones, are shifted towards less ^{13}C-depleted values (Visconti et al., 2013). These results are consistent with literature/research indicating that the two species are strictly zooplanktivorous during the main growth season (spring-summer) whilst they do not neglect feeding near the littoral when lake productivity declines or when they approach the shore for spawning (Berg and Grimaldi, 1965; Perga and Gerdeaux, 2005; Bettinetti et al., 2010; Volta et al., 2009; Visconti & Manca 2011).

In Table IV-1, the sampling period, the number of specimens (N), the lipid content (%) of fish muscle and liver, the biological parameters (weight, length and age), $\delta^{13}C$ and $\delta^{15}N$ isotope values and trophic level (TL) of pelagic fish species are listed; TL values were calculated from eq. (2), taking into account time-specific nitrogen enrichment with respect to signature of the pelagic baseline represented by *Daphnia*. Carbon fractionation of fish with respect to pelagic signature was used to assess reliability of fish from pelagic carbon sources. When the stepwise fractionation (F) exceeded the threshold limit (F_m), fish were attributed other than pelagic or mixed food sources (in Table IV-1 referred as LIT)

Table IV-1 Sampling period, number of specimens (N), lipid % of fish muscle and liver, biological parameters, $\delta^{13}C$ and $\delta^{15}N$ isotope values and trophic level (TL) of pelagic fish species, stepwise (F), threshold fractionation limit (F_m). LIT = fish exploiting on other than pelagic or mixed food sources

	Sampling period -2011	N	Muscle lipids (% d. w.)	Liver lipids (% d. w.)	Weight (g) mean ± s.d.	Length (cm) mean ± s.d.	Age (year) mean ± s.d.	$\delta^{13}C$ (‰) ± s.d	$\delta^{15}N$ (‰) ± s.d	F	F_m	TL
Shad (I)*		10	27.1	25	129.0±16.9	24.6±1.2	2.6±0.5	-28.5±0.4	9.2±0.03	4.0	4.0	**4.1**
Whitefish (I)	Spring	10	16.7	70	86.9±8.0	21.8±0.3	2.0±0.1	-29.9±0.02	9.7±0.03	2.6	4.2	**4.2**
Pelagic baseline								-32.5±0.5	2.1±0.05			
Shad (I)		10	5.9	17.3	86.8±12.0	24.4±1.0	1.7±0.5	-26.0±0.03	9.5±0.03	2.0	3.1	**3.6**
Whitefish (I)		6	13.1	26.6	163.9±8.6	27.6±0.5	1.7±0.5	-29.5±0.03	10.7±0.09	-1.5	3.8	**4.0**
Shad (II)**	Summer	9	16	33	253.1±49.5	30.6±1.8	3.9±0.6	-27.5±0	9.6±0.03	0.5	3.1	**3.7**
Whitefish (II)		10	12.5	25.9	282.5±123.2	31.2±4.2	3.9±0.6	-28.5±0.1	10.0±0.02	-0.5	3.4	**3.8**
Pelagic baseline								-28.0±0	4.0±0			
Shad (I)		9	23.8	49.1	133.7±28.8	24.9±1.5	1.9±0.8	-25.6±0.1	10.3±0.04	7.0	2.2	LIT
Whitefish (I)		10	10.8	25.4	245.9±45.1	29.5±2.3	2.5±0.7	-27.4±0.01	11.1±0.06	5.2	2.6	LIT
Shad (II)	Autumn	10	21.3	24.1	245.1±31.1	30.5±1.4	3.1±0.6	-28.1±0.2	10.4±0.1	4.5	2.3	LIT
Whitefish (II)		10	20.5	10.4	434.1±45.5	37.1±2.4	3.9±0.3	-29.1±0.3	9.9±0.10	3.5	2.0	LIT
Pelagic baseline								-32.6±0.5	6.4±0.1			
Shad (I)		6	12	22.2	58.8±16.7	20.8±2.7	2.2±0.3	-26.3±0.05	9.2±0.04	10.4	-0.4	LIT
Whitefish (I)		8	10.5	7.4	63.5±34.7	20.2±5.0	2.3±0.7	-26.9±0.1	11.1±0.07	9.9	0.6	LIT
Shad (II)	Winter	8	30	-	235.3±16.5	31.5±1.2	3.2±0.6	-29.4±0.01	10.6±0.1	7.4	0.3	LIT
Whitefish (II)		7	13.1	-	425.2±35.5	36.5±3.2	4.2±1.1	-28.7±0.04	10.5±0.2	8.1	0.3	LIT
Pelagic baseline								-36.7±0.5	10.0±0.5			

4.3.2 Concentrations of BFRs in biological samples

A summary of the considered BFR concentrations in zooplankton are reported in Table IV-2, while BFR contamination of fish tissues is reported in Table IV-3.
Average values of the zooplankton sampling sites were considered because fish can move for long distances and feed on zooplankton growing in different areas of the lake. Specific concentrations of zooplankton sampled in different sites are listed in Table IV-4, while different PBDE congener composition in fish tissues are reported in Table IV-5. Analytical results on novel BFR analysis showed that PBEB was never detected in any of the zooplankton sample analyzed in this study (<LOD), while HBB and BTBPE concentrations ranged from 1.0 to 3.9 ng/g l.w. and from 7.4 to 13.0 ng/g l.w. respectively.

Table IV-2 Zooplankton lipid content (%) and BRF contamination (ng/g l.w.) from spring to winter 2011

	Spring	Summer	Autumn	Winter
Lipids (%)	19.2	16.2	10.6	20.5
PBEB	<LOD	<LOD	<LOD	<LOD
HBB	0.7	1.0	3.9	1.5
BTBPE	7.1	7.4	13.3	7.6
DBDPE	<LOD	<LOD	<LOD	<LOD
HBCD	28.6	64.5	100.6	166.7
BDE TOT	377.1	569.0	2087.9	766.9

LOD: limit of detection

Table IV-3 BRF concentrations (ng/g l.w.) in muscle and liver of fish sampled in Lake Maggiore from spring to winter 2011

	Spring				Summer				Autumn				Winter			
	S (I)	W (I)	S (II)	W(II)	S (I)	W (I)	S (II)	W(II)	S (I)	W (I)	S (II)	W(II)	S (I)	W (I)	S (II)	W(II)
Muscle																
PBEB	<LOD	<LOD	na	na	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
HBB	<LOD	<LOD	na	na	<LOD	<LOD	<LOD	0.4	<LOD	<LOD	0.5	0.7	<LOD	<LOD	0.2	2.4
BTBPE	0.3	0.4	na	na	1.3	4.6	1.0	0.8	0.2	0.9	0.5	0.2	15.6	2.3	0.2	25.3
DBDPE	<LOD	<LOD	na	na	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
HBCD	146.1	64.0	na	na	313.3	110.4	162.5	151.7	13.3	279.6	453.8	291.3	574.3	791.6	360.5	433.4
BDE TOT	326.0	149.1	na	na	1763.2	908.7	365.6	432.6	375.4	440.3	589.2	170.6	921.1	397.1	255.5	516.3
Liver																
PBEB	0.7	<LOD	na	na	0.9	0.2	0.9	<LOD	<LOD	<LOD	<LOD	<LOD	0.6	<LOD	na	na
HBB	0.2	0.3	na	na	0.3	<LOD	0.3	<LOD	<LOD	0.5	<LOD	<LOD	<LOD	2.3	na	na
BTBPE	0.5	1.3	na	na	22.7	5.4	8.4	0.4	0.1	3.7	1.2	<LOD	4.1	4.8	na	na
DBDPE	<LOD	<LOD	na	na	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	na	na
HBCD	331.6	110.3	na	na	169.1	75.8	70.5	27.0	75.5	259.8	284.4	275.9	136.8	1232.1	na	na
BDE TOT	411.4	74.5	na	na	235.3	81.0	132.6	54.7	41.7	109.7	156.6	420.1	356.5	351.3	na	na

S (I) and S (II): young and old Shad; W (I) and W (II): young and old Whitefish; LOD: limit of detection; na: sample not available

Table IV-4 Sample specific concentration of BFRs (ng/g l.w.) determined in zooplankton from Lake Maggiore

	Spring 2011			Summer 2011			Autumn 2011			Winter 2011		
	Baveno	Ghiffa	Lesa	Baveno	Ghiffa	Lesa	Baveno	Ghiffa	Lesa	Baveno	Ghiffa	Lesa
PBEB	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
HBB	0.62	0.67	0.76	1.66	0.09	1.30	10.02	0.98	0.59	3.02	0.26	1.18
HBCD	16.57	40.53	28.57	35.99	16.86	140.58	191.37	37.25	73.14	190.87	87.02	222.26
BTBPE	3.83	10.68	6.67	12.68	1.36	8.03	9.80	10.20	20.00	7.02	6.73	9.04
DBDPE	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
BDE-28	1.66	1.23	1.46	4.44	3.56	2.25	16.69	1.33	2.12	1.94	1.08	3.66
BDE-47	40.46	31.05	48.57	141.61	65.45	73.02	525.49	49.59	16.20	89.42	104.46	168.04
BDE-99	6.51	10.89	6.68	11.02	6.91	16.54	25.69	15.77	8.56	12.50	17.61	12.48
BDE-100	5.38	5.46	10.52	2.67	<LOD	7.56	24.31	3.98	1.45	6.25	6.47	6.27
BDE-154	<LOD	2.02	<LOD	8.25	<LOD	4.44	33.12	4.45	<LOD	7.56	<LOD	<LOD
BDE-153	3.72	2.29	2.26	8.18	6.28	4.83	32.10	3.69	3.27	6.72	11.58	10.41
BDE-183	4.25	0.61	2.70	9.23	5.24	4.06	11.76	2.85	0.83	3.41	2.96	3.51
BDE-188	0.03	0.03	0.05	0.37	<LOD	0.09	0.82	0.41	<LOD	0.21	0.49	0.58
BDE-179	0.07	0.21	0.32	1.26	0.17	0.69	2.47	0.47	0.20	0.29	0.26	<LOD
BDE-202	0.03	0.08	<LOD	0.07	0.03	<LOD	1.00	0.67	0.43	0.19	2.83	0.50
BDE-201	0.05	0.07	0.08	0.50	0.18	0.36	1.69	1.63	1.45	0.19	4.94	0.31
BDE-208	0.46	0.49	0.47	1.27	0.14	0.77	10.35	8.24	6.27	1.50	3.48	0.43
BDE-207	0.62	1.37	0.72	3.58	0.10	1.24	18.24	17.25	10.59	3.46	4.42	0.53
BDE-206	0.51	0.97	1.11	2.85	0.07	1.13	19.75	15.12	10.59	3.50	2.02	1.08
BDE-209	245.71	247.37	442.86	394.16	416.06	496.35	1921.57	1980.39	1450.98	740.38	798.08	254.81
ΣBDE	309.5	304.1	517.8	589.4	504.2	613.3	2645.0	2105.8	1512.9	877.5	960.7	462.6

Table IV-5 Concentrations of different PBDE congeners (ng/g l.w.) in fish muscle and liver from spring to winter 2011

	Spring				Summer				Autumn				Winter			
	S (I)	W (I)	S (II)	W(II)	S (I)	W (I)	S (II)	W(II)	S (I)	W (I)	S (II)	W(II)	S (I)	W (I)	S (II)	W(II)
Muscle																
BDE-28	0.7	1.0	na	na	2.7	0.5	1.1	0.5	0.2	0.8	3.7	0.4	1.8	2.2	1.8	2.9
BDE-47	169.8	60.2	na	na	510.2	196.3	256.3	296.7	279.5	91.7	474.6	29.1	663.0	82.2	188.0	109.9
BDE-99	30.8	7.0	na	na	61.8	37.8	8.2	24.6	26.3	87.5	23.9	18.3	56.5	67.9	17.8	101.3
BDE-100	13.4	3.4	na	na	42.1	17.1	4.3	8.1	15.4	11.6	14.3	4.6	30.4	5.2	8.7	31.3
BDE-154	1.4	0.5	na	na	8.1	3.4	2.6	3.4	7.0	11.1	6.1	2.8	7.2	9.1	4.4	13.2
BDE-153	0.7	0.3	na	na	2.7	1.8	1.9	2.0	3.5	2.8	4.1	2.1	0.7	0.7	2.7	7.4
BDE-183	0.3	0.1	na	na	0.8	0.5	0.3	0.4	0.7	0.6	0.4	<LOD	2.2	2.2	0.7	2.8
BDE-188	0.9	<LOD	na	na	1.8	0.2	0.4	<LOD	0.2	<LOD	1.4	<LOD	1.4	2.5	0.2	0.4
BDE-179	0.6	0.2	na	na	0.9	4.6	0.6	<LOD	0.3	<LOD	1.1	1.2	0.8	2.8	3.1	0.8
BDE-202	0.3	<LOD	na	na	0.9	1.0	0.6	<LOD	0.2	<LOD	1.1	1.4	0.5	1.0	0.2	0.6
BDE-201	0.4	<LOD	na	na	<LOD	1.0	0.2	<LOD	<LOD	<LOD	0.3	0.3	0.4	1.9	<LOD	<LOD
BDE-208	1.1	<LOD	na	na	1.3	0.7	0.3	<LOD	<LOD	0.4	<LOD	<LOD	<LOD	1.0	<LOD	0.8
BDE-207	2.0	0.6	na	na	4.0	1.9	0.4	<LOD	0.1	0.6	<LOD	<LOD	<LOD	1.9	<LOD	1.6
BDE-206	2.0	0.2	na	na	2.7	1.6	0.4	<LOD	0.1	0.6	<LOD	<LOD	<LOD	2.1	<LOD	1.4
BDE-209	101.5	75.8	na	na	1123.3	640.2	88.0	97.0	41.9	232.6	58.2	110.4	156.2	214.4	28.0	242.0

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Liver																
BDE-28	1.0	0.3	na	na	0.6	<LOD	0.9	1.8	0.4	1.2	0.5	0.4	<LOD	<LOD	na	na
BDE-47	60.6	11.9	na	na	82.5	5.8	75.1	23.6	12.3	44.6	92.3	150.0	137.4	76.5	na	na
BDE-99	19.7	9.3	na	na	27.7	<LOD	4.0	0.6	2.0	17.7	4.6	72.8	34.7	62.0	na	na
BDE-100	11.7	2.5	na	na	20.0	<LOD	1.8	0.5	2.4	7.5	3.7	12.0	13.8	23.6	na	na
BDE-154	4.6	1.4	na	na	15.8	3.9	7.6	4.8	1.0	3.7	8.5	5.3	30.8	<LOD	na	na
BDE-153	<LOD	<LOD	na	na	<LOD	<LOD	<LOD	2.1	<LOD	<LOD	5.5	<LOD	18.5	<LOD	na	na
BDE-183	<LOD	<LOD	na	na	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	na	na
BDE-188	0.1	<LOD	na	na	0.9	0.2	0.4	0.3	<LOD	0.4	0.4	<LOD	1.3	0.5	na	na
BDE-179	0.2	<LOD	na	na	0.5	<LOD	0.3	0.2	<LOD	0.6	0.4	<LOD	1.5	0.7	na	na
BDE-202	<LOD	<LOD	na	na	0.5	0.1	0.2	0.1	<LOD	0.3	0.3	<LOD	0.6	0.9	na	na
BDE-201	0.1	<LOD	na	na	<LOD	0.3	0.1	<LOD	<LOD	0.1	0.2	<LOD	0.9	1.1	na	na
BDE-208	0.5	0.1	na	na	<LOD	0.2	0.2	<LOD	0.1	<LOD	0.1	0.4	<LOD	0.9	na	na
BDE-207	0.9	0.1	na	na	0.7	0.4	0.2	<LOD	0.1	0.4	0.4	0.9	0.7	<LOD	na	na
BDE-206	0.7	0.1	na	na	0.7	0.3	0.2	0.1	0.2	0.2	0.3	0.7	0.5	13.2	na	na
BDE-209	311.4	48.8	na	na	85.5	69.9	41.7	20.7	23.1	32.9	39.4	177.7	115.8	171.6	na	na

Regarding DBDPE contamination, despite the concentration levels (up to 30 ng/g d.w.) measured in the sediments of Lake Maggiore (Poma et al., submitted), DBDPE was below the detection limit in all the considered zooplankton samples, probably because of its high log K_{ow} value (log K_{ow} = 11), which reduced the potential bioaccumulation in organisms as mentioned by other studies (Law et al. 2006). Similar to zooplankton samples, DBDPE and PBEB were never detected in fish muscle, while PBEB concentrations in fish livers ranged from <LOD to 0.9 ng/g l.w. Also previous literature studies did not report detectable levels of DBDPE in freshwater biological samples (Klosterhaus et al., 2012; Covaci et al., 2011; Law et al., 2006), while only a few researchers have reported on the occurrence of PBEB in wildlife living in aquatic environments (Klosterhaus et al., 2012; Arp et al., 2011). HBB concentrations in fish muscle ranged from <LOD to 2.4 ng/g l.w, and in fish livers it was detected with concentrations ranging from <LOD to 2.3 ng/g l.w. A few studies in the literature have investigated the presence of HBB in the aquatic environment; for example, it was not detected in fish from San Francisco Bay (<LOD), while higher concentrations of HBB (on wet weight basis) were measured in mud carp from an e-waste recycling site in South China (up to 2450 ng/g l.w.) (Klosterhaus et al., 2012; Wu et al., 2010). BTBPE was detected ranging from 0.2 to 25 ng/g l.w in fish muscle, and from 0.1 to 23 ng/g l.w. in livers. Our findings are consistent with other studies conducted worldwide, which reported BTBPE accumulation in wildlife at concentrations generally less than 5 ng/g l.w. (on wet weight basis) (Covaci et al., 2011).

The concentrations of HBCD and PBDEs in the zooplankton samples were much higher than those measured for novel BFRs, from one to two orders of magnitude, ranging from 29 to 167 ng/g l.w. and from 379 to 2094 ng/g l.w. respectively. In

particular, mean results showed that zooplankton samples collected in the autumn and winter of 2011 were significantly more contaminated than those sampled in the spring and summer of 2011 ($p<0.01$). As reported elsewhere (Poma et al., submitted), it was hypothesized that a high contamination of the lake due to HBCD and PBDEs in the second half of 2011 could be determined by the Northern Italian meteorological conditions in the summer of 2011. The occurrence of heavy rains, in fact, could have caused an additional input of contaminated suspended particle matter transported by water flows. The fact that zooplankton is expected to respond quickly to fluctuation of pollutants occurring in the water column (Bettinetti et al., 2010) could explain this behaviour. Moreover, considering each season (Table IV-4), it was observed that Baveno, Ghiffa and Lesa were generally similarly contaminated by PBDEs and this might be related to the very similar taxa composition of the zooplankton samples in the three sampling sites (Fig. IV-1).

In fish, the variability of concentrations, considering different seasons, species and age, was very high. For example, HBCD was detected with concentrations ranging from 13 to 792 ng/g l.w., and from 27 to 1232 ng/g l.w in fish muscle and liver respectively. Concentrations reported for HBCD in aquatic wildlife vary widely by species, tissue, geographic region, and proximity to sources (Klosterhaus et al., 2012; Covaci et al., 2006; de Wit et al., 2010). The concentrations of HBCD in shad and whitefish muscles were generally one order of magnitude higher than concentrations reported on wet weight basis in fish from San Francisco Bay and from Lake Winnipeg (Canada) (Klosterhaus et al., 2012; Law et al., 2006) (considered as low contaminated areas), and consistent with those measured in Swiss fish by Gerecke et al. (2003). Conversely, in this study the HBCD levels were one order of magnitude lower than those

observed on wet weight basis by Wu et al. (2010) in fish from an e-waste recycling area in South China.
Considering the total PBDE concentrations in fish, values ranged from 149 to 1763 ng/g l.w. in muscles and from 42 to 420 ng/g l.w. in livers. The high variability observed in the fish tissue contamination could lead us to hypothesize that Lake Maggiore is still subjected to local inputs of PBDEs, particularly explained by the use of Deca-BDE technical formulation and by the past use of Penta- and Octa- mixtures in the lake basin. Similar conclusions were also suggested from the analysis of sediments and mussels collected in 2011 in different sites from Lake Maggiore (Poma et al., submitted).

4.3.3 PBDE congener patterns

The mean relative distribution pattern of PBDE congeners in zooplankton and fish tissues is shown in Fig. IV-3. Zooplankton PBDE pattern showed a clear predominance of BDE-209 (>80%), similar to the percentage composition of Deca-BDE technical formulation, followed by BDE-47 (12%) > BDE-99 > BDE-100. Because zooplankton is expected to respond quickly to pollutant fluctuation occurring in the water column (Bettinetti et al., 2010), it is likely that the high percentage of BDE-209 in zooplankton could be due to the presence of recent inputs of deca-BDE arriving to the lake through its tributaries.
In fish, differences in the PBDE congener distribution for the two species could be observed. In whitefish (I) and (II) the congener pattern was very similar between liver and muscle sample, with % of BDE-209 > -47 > -99 > -100; on the contrary, in shad the relative contribution of BDE-209 was generally lower than BDE-47, and its relative proportion in S (I) and S (II) decreased from liver to muscle of 15 and 50% respectively. Moreover, the presence of hepta- (BDE-188 and -179) and octa- (BDE-201 and -202) BDE congeners was observed both in fish

muscle and in liver tissues (Table IV-5), while it is known that they are not present in any BDE technical formulations (Viganò et al., 2011).

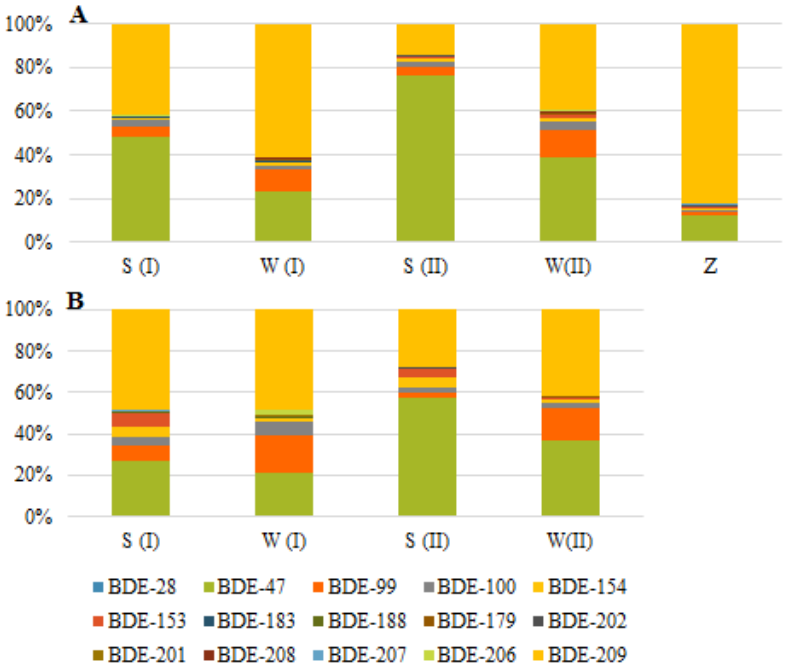

Fig. IV-3 Mean relative distribution pattern of PBDE congeners in zooplankton and fish (A: muscle, B: liver)

These data are consistent with the hypothesis that fish have a metabolic capacity to biotransform PBDEs via debromination pathways, as suggested by several studies (Stapleton et al., 2004; Stapleton et al., 2006; La Guardia et al., 2007; Kierkegaard et al., 1999). In particular, Stapleton et al (2006) proposed that the liver acted as a sink for BDE-209, and that it is reasonable that BDE-209 debromination takes place in it, being a primary tissue

involved in the biotransformation of organic compounds. This could be confirmed also by the higher presence of lower brominated congeners (BDE-99, -100, -154, -153) in the liver than in muscle considered in this work. Moreover, it has been suggested that there are species-specific differences in the biotransformation/debromination capacity of BDE-209 in fish, and that this capacity could differ in the extent, and probably the rate, of debromination (Stapleton et al., 2006; Stapleton 2006). This theory could explain why differences in bioaccumulation of BDE-209 were found between whitefish and shad, regardless of their age.

4.3.4 Biomagnification of BFRs

Previous studies demonstrated that different BDE congeners can biomagnify in aquatic organisms through the food web (Hu et al., 2010; Law et al., 2006). We evaluated the potential BFR bioaccumulation in Lake Maggiore by calculating both the BMF_{TL}, in order to examine the direct predator/prey relationships, and the TMF. In the factor calculation, only the mean BFR muscle concentrations of pelagic fish were considered (spring and summer), being directly related to zooplankton samples. Subsequently, we compared the two calculated factors in order to determine if certain BMF_{TL} predator/prey combinations result in greater or less accumulation than that indicated by TMFs. The calculated values of BMF_{TL} and TMF are reported in Table IV-6. The slope (m), the R^2 of the regression lines between TL and the concentration of the considered chemicals, used to calculate the TMF, and the significance of correlations (*p*) are also reported.

Table IV-6 BMF_{TL} calculated considering the predator/prey relationship of the different fish species with respect to zooplankton; TMF calculated values; slope (m), R^2, and *p*-values of the regression between BFR Log concentration and the TL of the Lake Maggiore organisms

	BMF_{TL}				**mean BMF_{TL}**	**TMF**	slope (m) ± SD	R^2	*p*
	S(I)	W(I)	S(II)	W(II)					
HBCD	2.4	1.3	2.1	1.9	**1.9**	**1.8**	0.25±0.12	0.58	0.13
BTBPE	0.3	0.6	0.3	0.3	**0.4**	**0.3**	-0.46±0.11	0.84	0.03
BDE-28	0.8	0.6	0.6	0.4	**0.6**	**0.6**	-0.23±0.10	0.61	0.05
BDE-47	2.4	1.4	2.2	2.3	**2.1**	**1.8**	0.26±0.15	0.50	0.18
BDE-99	2.3	1.5	0.9	1.7	**1.6**	**1.5**	0.17±0.18	0.23	0.37
BDE-100	2.3	1.3	0.8	1.2	**1.4**	**1.3**	0.10±0.18	0.09	0.62
BDE-154	1.1	0.7	0.8	0.9	**0.8**	**0.8**	-0.11±0.11	0.27	0.51
BDE-153	0.6	0.5	0.6	0.6	**0.6**	**0.5**	-0.28±0.09	0.76	0.05
BDE-183	0.3	0.3	0.2	0.3	**0.3**	**0.3**	-0.57±0.06	0.97	0.02
BDE-188	3.5	1.2	1.9	-	**2.2**	**2.3**	0.36±0.25	0.41	0.37
BDE-179	1.3	2.2	1.2	-	**1.6**	**1.5**	0.17±0.05	0.80	0.04
BDE-202	3.7	4.0	4.1	-	**3.9**	**4.1**	0.61±0.07	0.96	0.00
BDE-201	1.5	2.1	0.9	-	**1.5**	**2.1**	0.32±0.20	0.46	0.31
BDE-208	1.5	1.1	0.7	-	**1.1**	**1.2**	0.06±0.16	0.05	0.83
BDE-207	1.6	1.0	0.5	-	**1.0**	**1.0**	-0.01±0.19	0.00	0.98
BDE-206	1.5	0.9	0.6	-	**1.0**	**0.9**	-0.05±0.19	0.03	0.82
BDE-209	1.3	1.0	0.4	0.5	**0.8**	**0.7**	-0.16±0.18	0.21	0.33

The results showed that BMF_{TL}s>1 were determined for HBCD (1.9), and BDE-47, -99, -100 (2.1, 1.6 and 1.4 respectively), confirming the hypothesis that the biomagnification of these chemicals occurred in the fish species of the lake. Moreover, BMF_{TL}s>1 were observed also for BDE-188 (2.2), -179 (1.6), -202 (3.9), -201 (1.5), -208 (1.1), and BMF_{TL}s=1 were calculated for BDE-207 and -206. Concerning these hepta- to nona-congeners, we suggest that it is likely that the metabolically mediated debromination in fish of higher to lower brominated congeners may increase the BMF_{TL} for these compounds, and that their bioformation may consequently lead to an apparent

increase in BMF_{TL} values. This hypothesis is supported by literature studies where BDE-209 debromination in fish tissues was investigated. Stapleton et al. (2006) identified several hepta-, octa-, and nona-BDE congeners as BDE-209 debromination products in *in vivo* laboratory study on fish tissues, while La Guardia et al. (2007) detected BDE-179, -188, -201, and -202 in fish from a wastewater receiving stream, reinforcing the theory that metabolic debromination of BDE-209 does occur in the aquatic environment also in real conditions. Moreover, the octa-brominated congener BDE-202 was found to be the dominant debromination product by Stapleton et al. (2006), confirming the highest BMF_{TL} of BDE-202 calculated in this study.

Comparing the calculated values of BMF_{TL} and TMF (Table IV-6 and Fig. IV-4), a significant correlation could be observed (R^2 = 0.9603, *p*-value<0.01) between the two factors, probably because the considered food web has direct fish-zooplankton relationships.

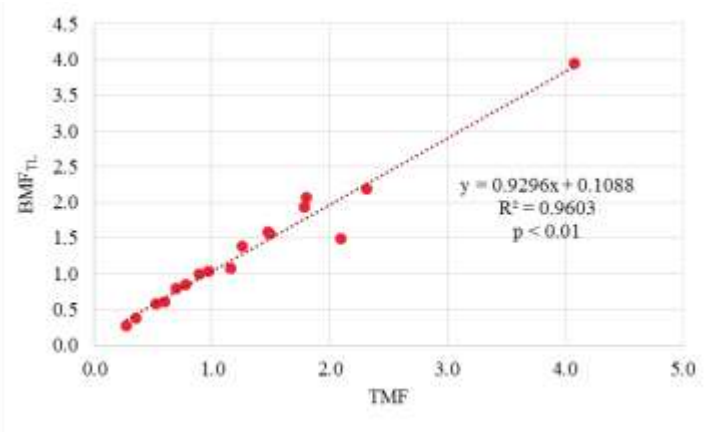

Fig. IV-4 Correlation between BMF_{TL} and TMF values calculated in the present study

It has been suggested that potential discrepancies between BMF_{TL} and TMF could derive from the difficulty of single trophic interactions to represent the overall degree of biomagnification that may occur in a complex food web (Conder et al., 2012). On the basis of our results, we suggest that the use of BMF_{TL} to investigate the biomagnification potential of organic chemical compounds may be an appropriate approach when a simple trophic food web is considered. In addition, a comparison between our results and literature data was also undertaken, pointing out that the TMFs calculated in the present work were very similar to those measured in several other literature studies and confirming that HBCD and some PBDE congeners are able to biomagnify within food webs. For example, Wu et al. (2010) determined a TMF of 1.82 ($p = 0.12$) for HBCD in aquatic species from an e-waste recycling site in South China,Van Ael et al. (2013) determined a TMF of 1.17 for BDE-100 in biological samples from the Scheldt Estuary, while Hu et al. (2010) and Yu et al. (2012) calculated the TMF of BDE-47, -99, and -100 obtaining values of 1.31, 1.39, 1.82 and 1.97, 1.59, 2.95 respectively in food webs of Chinese lakes.

4.4 CONCLUSIONS

The concentrations of novel BFRs detected in the biological samples were greatly lower than those measured for PBDEs and HBCD, and this might be related to their more limited usage in electrical and electronic equipment compared to PBDEs and HBCD. Moreover, this work pointed out that PBDEs are still the most present and abundant BFRs in the lake wildlife, despite the fact that the Penta- and Octa-BDE technical formulations were phased out several years ago, confirming the heavy contamination of the Lake due to these compounds. The concentration levels of HBCD in the wildlife of Lake Maggiore were measured in the same order of magnitude of PBDEs, suggesting the wide use of this compound in the basin. Anyway, we suggest the hypothesis that the presence of HBCD in the Lake Maggiore could probably originate from diffused sources rather than local inputs of contamination. Considering the trophic interactions of nBFRs, HBCD and PBDEs in the biological samples, a significant positive correlation was observed between BMF_{TL} and TMF, probably because the considered food web has direct fish-zooplankton relationships. On the basis of our results, we suggest that the use of BMF_{TL} to investigate the biomagnification potential of organic chemical compounds may be an appropriate approach when a simple trophic food web is considered. In addition, our results have confirmed that HBCD and some PBDE congeners can biomagnify within food webs.

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4.6 REFERENCES

Alaee, M., Arias, P., Sjödin, A., Bergman, Å., 2003. An overview of commercially used brominated flame retardants, their application, their use pattern in different countries/regions and possible modes of release. Environment International 29(6), 683-689.

Arnot, J. A., Gobas, F.A.P.C., 2006. A review of bioconcentration factor (BCF) and bioaccumulation factor (BAF) assessments for organic chemicals in aquatic organisms. Environmental Reviews 14(4), 257-297.

Arp, H.P.H., Moskeland, T., Andersson, P.L., et al., 2011. Presence and partitioning properties of the flame retardants pentabromotoluene, pentabromoethylbenzene and hexabromobenzene near suspected source zones in Norway. Journal of Environmental Monitoring 13(3), 505-513. DOI: 10.1039/c0em00258e.

Berg, A., Grimaldi, E., 1965. Biologia delle due forme di Coregone (*Coregonus* sp.) del Lago Maggiore. Memorie dell'Istituto Italiano di Idrobiologia 18, 25-196.

Bettinetti, R., Galassi, S., Guzzella, L., Quadroni, S., Volta, P., 2010. The role of zooplankton in DDT biomagnification in a pelagic food web of Lake Maggiore (Northern Italy). Environmental Science and Pollution Research 17(9), 1508-1518.

Betts, K., 2008. New flame retardants detected in indoor and outdoor environments. Environmental Science & Technology 42(18), 6778-6778.

Borgå, K., Kidd, K.A., Muir, D.C.G., et al., 2012 (Epub 2011 Aug 26). Trophic magnification factors: considerations of ecology, ecosystems, and study design. Integrated Environmental Assessment and Management 8(1), 64-84. DOI: 10.1002/ieam.244.

BSEF, Bromine Science Environmental Forum, 2013. http://www.bsef.com/regulation/international-regulations/what-are-the-united-nations-conventions

CIPAIS (Commissione Internazionale per la Protezione delle Acque Italo-Svizzere), 2012. Indagini su DDT e sostanze pericolose nell'ecosistema del Lago Maggiore. Rapporto annuale 2012 (in Italian).

Coat, S., Monti, D., Bouchon, C., et al., (2009). Trophic relationships in a tropical stream food web assessed by stable isotope analysis. Freshwater Biology 54(5), 1028-1041. DOI: 10.1111/j.1365-2427.2008.02149.x.

Conder, J.M., Gobas, F.A.P.C., Borgå, K., et al., 2012. Use of trophic magnification factors and related measures to characterize bioaccumulation potential of chemicals. Integrated Environmental Assessment and Management 8(1), 85-97.

Covaci, A., Harrad, S., Abdallah, M.A-E, Ali, N., Law, R.J., Herzke, D., et al, 2011. Novel brominated flame retardants: a review of their analysis, environmental fate and behaviour. Environment International 37(2), 532–556.

Covaci, A., Gerecke, A.C., Law, R.J., et al., 2006. Hexabromocyclododecanes (HBCDs) in the environment and humans: A review. Environmental Science & Technology 40(12), 3679-3688. DOI: 10.1021/es0602492.

Cox, P., Drys, G., 2003. Directive 2002/95/EC of the European Parliament and of the Council of 27 January 2003 on the restriction of the use of certain hazardous substances in electrical and electronic equipment. Official Journal of the European Union 37, 19-23.

Cox, P., Efthymiou, P., 2003. Directive 2003/11/EC of the European Parliament and of the Council of February 6 2003 amending for the 24th time Council Directive 76/669/EEC relating to restrictions on the marketing and use of certain dangerous substances and preparations (pentabromodiphenyl ether, octabromodiphenyl ether). Official Journal of the European Union 42, 45-46.

Cullon, D.L., Yunker, M.B., Christensen, J.R., et al., 2012. Biomagnification of polychlorinated biphenyls in a harbor seal (*Phoca vitulina*) food web from the strait of Georgia, British Columbia, Canada. Environmental Toxicology and Chemistry 31(11), 2445-2455.

De Jourdan, B., Hanson, M., Muir, D.C.G., Solomon, K.R., 2013. Environmental Fate of Three Novel Brominated Flame Retardants in Aquatic Mesocosms. Environmental Toxicology and Chemistry 32(5), 1060-1068.

De Niro, M.J., Epstein, S., 1978. Influence of diet on the distribution of carbon isotopes in animals. Geochimica et Cosmochimica 42(5), 495-506.

de Wit, C.A., Herzke, D., Vorkamp, K., 2010. Brominated flame retardants in the Arctic environment - trends and new candidates. Science of the Total Environment 408(15), 2885-2918. DOI: 10.1016/j.scitotenv.2009.08.037.

de Wit, C.A., 2002. An overview of brominated flame retardants in the environment. Chemosphere 46(5), 583–624.

Fisk, A.T., Hobson, K.A., Norstrom, R.J., 2001. Influence of chemical and biological factors on trophic transfer of persistent organic pollutants in the northwater polynya marine food web. Environmental Science & Technology 35(8), 1700-1700. DOI: 10.1021/es010719m.

Gauthier, L.T., Hebert, C.E., Weseloh D.V.C., Letcher, R.J., 2007. Current-use flame retardants in the eggs of herring gulls (*Larus argentatus*) from the Laurentian Great Lakes. Environmental Science & Technology 41(13), 4561-4567.

Gerecke, A.C., Kohler, M., Zennegg, M., Schmid, P., Heeb, N.V., 2003. Detection of α-isomer dominated HBCD (hexabromocyclododecane) in Swiss fish at levels comparable to PBDEs (polybrominated diphenyl ethers). Organohalogen Compounds 61, 155-158.

Great Lakes Chemical Corporation, 2004. Report on Firemaster 550. http://www.prnewswire.com/news-releases/great-lakes-chemical-

corporation-completes-phase-out-of-two-flame-retardants-54043892.html

Guerra, P., Eljarrat, E., Barcelò, D., 2010. Analysis and occurrence of emerging brominated flame retardants in the Llobregat River basin. Journal of Hydrology 383(1-2), 39-43.

Hoh, E., Zhu, L., Hites, R.A., 2005. Novel flame retardants, 1, 2-Bis(2, 4, 6-ribromophenoxy)ethane and 2, 3, 4, 5, 6-pentabromoethylbenzene, in United States' environmental samples. Environmental Science & Technology 39(8), 2472-2477.

Hu, G-c., Dai, J-y., Xu, Z-c., et al., 2010. Bioaccumulation behavior of polybrominated diphenyl ethers (PBDEs) in the freshwater food chain of Baiyangdian Lake, North China. Environment International 36(4), 309-315.

Kierkegaard, A., Balk, L., Tjarnlund, U., et al., 1999. Dietary uptake and biological effects of decabromodiphenyl ether in rainbow trout (*Oncorhynchus mykiss*). Environmental Science & Technology 33(10), 1612-1617. DOI: 10.1021/es9807082.

Klosterhaus, S.L., Stapleton, H.M., La Guardia, M.J., Greig, D.J., 2012. Brominated and chlorinated flame retardants in San Francisco Bay sediments and wildlife. Environment International 47, 56-65.

Infantino, A., Morselli, M., Di Guardo, A., 2013. Integration of a dynamic organism model into the DynA Model: Development and application to the case of DDT in Lake Maggiore, Italy. Science of the Total Environment 454-455, 358-365.

La Guardia, M.J., Hale, R.C., Harvey, E., 2007. Evidence of debromination of decabromodiphenyl ether (BDE-209) in biota from a wastewater receiving stream. Environmental Science & Technology 41(19), 6663-6670.

Law, K., Halldorson, T., Danell, R., Stern, G., Gewurtz, S., Alaee, M., et al., 2006. Bioaccumulation and trophic transfer of some brominated flame retardants in a lake Winnipeg (Canada) food web. Environmental Toxicology and Chemistry 25(8), 2177-2186.

Lopez, P., Brandsma, S.A., Leonards, P.E.G., de Boer, J., 2011. Optimization and development of analytical methods for the determination of new brominated flame retardants and polybrominated diphenyl ethers in sediments and suspended particulate matter. Analytical and Bioanalytical Chemistry 400(3), 871-883.

Matthews, B., Mazumder, A., 2003. Compositional and interlake variability of zooplankton affect baseline stable isotope signatures. Limnology and Oceanography 48(5), 1977-1987.

Perga, M.E., Gerdeaux, D., 2005. 'Are fish what they eat' all year round? Oecologia 144(4), 598-606. DOI: 10.1007/s00442-005-0069-5.

Post, D.M., 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology 83(3), 703–718.

Salamova, A., Hites, R.A., 2011. Discontinued and alternative brominated flame retardants in the atmosphere and precipitation from the Great Lakes Basin. Environmental Science & Technology 45(20), 8698–8706.

Song, S.B., Xu, Y., Zhou, B.S., 2006. Effects of hexachlorobenzene on antioxidant status of liver and brain of common carp (Cyprinus carpio). Chemosphere 65(4), 699-706.

Stapleton, H.M., Alaee, M., Letcher, R.J., et al., 2004. Debromination of the flame retardant decabromodiphenyl ether by juvenile carp (*Cyprinus carpio*) following dietary exposure. Source: Environmental Science & Technology 38(1), 112-119. DOI: 10.1021/es034746j.

Stapleton, H.M., Brazil, B., Holbrook, R.D., et al., 2006. In vivo and in vitro debromination of decabromodiphenyl ether (BDE 209) by juvenile rainbow trout and common carp. Environmental Science & Technology 40(15), 4653-4658. DOI: 10.1021/es060573x.

Stapleton, H.M., 2006. Brominated Flame Retardants: Assessing DecaBDE Debromination in the Environment. EEN epha environment network.

UNEP, Stockholm Convention on persistent organic pollutants (POPs), 2010. http://chm.pops.int/.

U.S. EPA Environmental Protection Agency, 2009. Polybrominated diphenyl ethers (PBDEs) Action Plan. http://www.epa.gov/oppt/existingchemicals/pubs/actionplans/pbdes_ap_2009_1230_final.pdf

Van Ael, E., Covaci, A., Das, K., et al., 2013. Factors influencing the bioaccumulation of persistent organic pollutants in food webs of the scheldt estuary. Environmental Science & Technology 47(19), 11221-11231. DOI: 10.1021/es400307s.

Verreault, J., Gebbink, W., Gauthier, L.T., Gabrielsen, G.W., Letcher, R.J., 2007. Brominated flame retardants in Glaucous Gulls from the Norwegian arctic: more than just an issue of polybrominated diphenyl ethers. Environmental Science & Technology 41(14), 4925-4931.

Viganò, L., Roscioli C., Guzzella L., 2011. Decabromodiphenyl ether (BDE-209) enters the food web of the River Po and is metabolically debrominated in resident cyprinid fishes. Science of the Total Environment 409(23), 4966–4972.

Visconti, A., Manca, M., 2011. Seasonal changes in the $\delta^{13}C$ and $\delta^{15}N$ signatures of the Lago Maggiore pelagic food web. Journal of Limnology 70(2), 263-271.

Visconti, A., Volta, P., Fadda, A., Di Guardo, A., Manca, M., 2013. Seasonality, littoral vs. pelagic carbon sources and stepwise 15N-enrichment of pelagic food web in a deep subalpine lake: the role of planktivorous fish. Canadian Journal of Fisheries and Aquatic Sciences. Published on the web 27 November 2013, 10.1139/cjfas-2013-0178.

Volta, P., Tremolada, P., Neri, M.C., Giussani, G., Galassi, S., 2009. Age-dependent bioaccumulation of organochlorine compounds in fish and their selective biotransformation in top predators from Lake Maggiore (Italy). Water Air & Soil Pollution 197(1-4), 193-209.

Volta, P., Oggioni, A., Bettinetti, R., Jeppesen, E., 2011. Assessing lake typologies and indicator fish species for Italian natural lakes using past fish richness and assemblages. Hydrobiologia 671, 227-240.

Wu, J.P., Guan, Y.T., Zhang, Y., Luo, X.J., Zhi, H., Chen, S.J., Mai, B.X., 2010. Trophodynamics of hexabromocyclododecanes and several other non-PBDE brominated flame retardants in a freshwater food web. Environmental Science & Technology 44(14), 5490-5495.

Wu, J.P., Guan, Y.T., Zhang, Y., Luo, X.J., Zhi, H., Chen, S.J., Mai, B.X., 2011. Several current-use, non-PBDE brominated flame retardants are highly bioaccumulative: evidence from field determined bioaccumulation factors. Environment International 37(1), 210-215.

Wu, J.P., Zhang, Y., Luo, X.J., et al., 2012. A review of polybrominated diphenyl ethers and alternative brominated flame retardants in wildlife from China: Levels, trends, and bioaccumulation characteristics. Journal of Environmental Sciences (China) 24(2), 183-194.

Yu, Y.X., Zhang, S.H., Huang, N.B., Li, J.L., Pang, Y.P., Zhang, X.Y., Yu, Z.Q., Xu, Z.G., 2012. Polybrominated diphenyl ethers and polychlorinated biphenyls in freshwater fish from Taihu Lake, China: their levels and the factors that influence biomagnification. Environmental Toxicology and Chemistry 31(3), 542-549.

CHAPTER V

GENERAL CONCLUSIONS

In this thesis, Lake Maggiore contamination due to novel BFRs, PBDEs and HBCD was investigated in abiotic and biologic matrices, providing information on their levels, distribution patterns, temporal trends, potential bioaccumulation processes, and possible correlations among them.

It was pointed out that the lake and river sediments had weak concentrations of PBEB, HBB, and BTBPE, but a not negligible contamination by HBCD, probably deriving from a variety of industrial sources in the lake basin rather than a single industrial point emission. On the contrary, DBDPE was always detected in the Lake Maggiore sediments, showing a moderately high contamination. BDE-209 was the predominant congener in all the considered samples, still highlighting the current use of Deca-BDE formulation in the Lake Maggiore basin. Moreover, a limited but still detectable presence of congeners BDE-47, 99 and 100 in the sediments might confirm the hypothesis that also technical Penta-BDE formulation had an important use in the lake basin. In addition, a positive correlation between DBDPE and BDE-209 was observed, confirming a wide and important use of DBDPE in the lake basin and the hypothesis that this compound will soon become one of the most important nBFRs used in Northern Italy.

The concentrations of novel BFRs detected in all the biological samples were considerably lower than those measured for PBDEs and HBCD, and this might be related to their more limited usage in electrical and electronical equipment. Interestingly, despite the high concentrations measured in the sediments, DBDPE was never detected in all the considered biological samples, most likely because of its high log K_{ow} value

(log K_{ow} = 11), which can reduce the potential bioaccumulation in organisms. Considering these results, we suggest that currently no environmental risk related to the presence of novel BFRs has been evidenced. It was also pointed out that PBDEs and HBCD are the most abundant and common BFRs in the lake aquatic wildlife, suggesting their wide use in the lake basin and confirming their role in the contamination of Lake Maggiore. Moreover, the results confirmed the theory that there are species-specific differences in the biotransformation/-debromination capacity of the different BDE congeners in fish, and that this capacity could differ in the extent, and probably the rate, of debromination.

The potential BFR bioaccumulation in the lake aquatic food web was investigated by considering different biomagnification factors (BMF, $BMF_{TL,}$ and TMF), highlighting factors more than 1 for HBCD, and some tetra- and penta-BDE congeners, confirming the hypothesis that the biomagnification of these chemicals occurred in the lake fish species. Moreover, factors > 1 were also obtained for several higher brominated BDEs (especially hepta- and octa-BDEs), but we suggest that it is probable that the metabolically mediated debromination in fish of higher to lower brominated congeners may increase the biomagnification factors for these compounds, and that their bioformation may consequently lead to an apparent increase of factor values. On the basis of the calculated factors and on the trophic interactions of BFRs in the biological samples, we suggest that the use of the BMF normalized on trophic levels (BMF_{TL}) might be an appropriate approach to investigate the biomagnification potential of organic chemical compounds when a simple trophic food web is considered.