Co-occurrence of polychlorinated biphenyls, cyanotoxins and trace elements in commercial fish species from a freshwater protected area (Pertusillo Lake, Southern Italy).

Abstract

A total of 79 fish samples covering nine species were collected in a preliminary investigation on a SCI (Site of Community Importance) water reservoir (Pertusillo Lake, Southern Italy) created for drinking purpose and located in a territory used for drilling activities. Analyses for microcystins (MYCs) and cylindrospermopsins (CYLs) presence were performed using Elisa assays, while 10 fish samples were analyzed also for trace elements by atomic adsorption spectrophotometry and for polychlorinated biphenyls (PCBs) by GC-MS operated in EI mode. The results showed the compresence of important cyanotoxins and industrial contaminants in fish. More extended studies are needed to evaluate the combined effects of these contaminants on the lake ecosystem and ichthyic fauna, in order to establish an available risk assessment for human population in the lake region.

Keywords: microcystins, cylindrospermopsins, trace elements, polychlorinated biphenyls, fish, bioaccumulation, Pertusillo Lake.

Introduction

In the past century, the development of industry and agriculture often caused the release or production of organic and inorganic pollutants in the environment, posing threats to wildlife and human health. Several studies have shown the presence of anthropic contaminants in inland waters of various continents, as found in lakes polluted by industries (Yu et al., 2012; Hao et al., 2013; Jia et al., 2014). A particular class of these contaminants, microcystins (MYCs), the commonest biotoxins of Cyanobacteria, are a family of more than 90 potent eptapeptide hepatotoxins (Teixeira et al 1993, Codd 1995, Jochimsen et al 1998, Gacsi et al 2009) acting as specific inhibitors of protein phosphatases (PPs) of type 1, 2A, 3 (for MC-LA; Prinsep et al 1992), 4 and 5 (Hastie et al 2005), and to a lesser extent of type 2B (Mackintosh et al 1990). The inhibition of PP1 and PP2A results in an increased phosphorylation of proteins in liver cells, affecting several cellular processes (Dawson, 1998). MYCs are responsible for liver failure and death in humans (Falconer et al., 1983; Azevedo et al., 2002; Crux et al., 1993), wild animals, livestock and aquatic life (Sivonen and Jones, 1999; Mwaura et al., 2004). Indirect evidence supporting tumour promotion of human cancer from MYCs exposure comes from the studies of Yu (1989), Ueno et al. (1996) and Zhou et al. (2002) in China, Fleming et al. (2002) in Florida, and Svircev et al. (2009) in Serbia. They can induce oxidative DNA damage (Zegura et al., 2003), genotoxicity (Bouaicha et al., 2005) and cause the activation of proto-oncogenes c-jun, c-fos and c-myc (Li et al., 2009). In addition, MYCs from contaminated lakes can percolate and contaminate groundwater proportionally to the duration of toxic bloom events (Eynard et al 2000, Messineo et al 2006). Their association with primary carcinogens in the aquatic environment is a problematic event. Several large scale fish death outbreaks have been associated to massive occurrence of Cyanobacteria in waterbodies (Jewel et al., 2003; Zimba et al., 2006). MYCs concentrations between 0.34 µg/kg (Magalhaes et al., 2001) and 36.42 µg/kg (Bruno et al., 2009) were measured in the muscle tissue of wild or farmed fish, indicating that even the consumption of contaminated fish muscle might constitute a threat for human health. Cylindrospermopsin (CYN), another common cyanotoxin, is a sulfated-guanidinium alkaloid with hepatotoxic, nephrotoxic and thymotoxic effects (Terao et al., 1994; Banker et al., 1997). CYN has *in vitro* and *in vivo* mutagenic, endocrine-disrupting and carcinogenic activity (Shaw et al., 2000; Shen et al., 2002; Bain et al., 2007; Young et al., 2008; Zegura et al., 2011), showing neurotoxic activity in fish (Guzman-Guillen et al., 2015). Aside from microcystins, other toxic substances of major concern contaminating the environment are toxic metals, namely mercury (Hg), cadmium (Cd) and lead (Pb), and organic contaminants, including polychlorinated biphenyls

(PCBs). As a consequence of their environmental persistence and potential for bioaccumulation, these chemicals are widespread throughout the ecosystem, causing toxic problems to all life forms. Fish, in particular, have the ability to accumulate these contaminants and, often, have been employed to assess environmental contamination (Tekin-Ozan and Kir, 2008). More attention should be devoted to contaminant levels in fish especially when significant alterations in industrial development can result in large pollutant releases into the environment. Common carp is a good species for bioaccumulation monitoring, being bottom feeder fish that do not migrate extensively, reproduce rapidly and have long life spans (up to 38 yrs.) (Pérez-Fuentetaja et al., 2010).

Being fish an important food source and a major part of many natural food chains, the objective of the present study was to investigate the presence of these specific contaminants in fish from lake Pertusillo, an extended Italian reservoir part of a national park, which neighbourhood is interested by intense drilling activities often accused of causing serious water and sediment pollution in the lake.

Materials and methods

Site description

Lake Pertusillo is an artificial reservoir of the Italian region Basilicata, located at the conjunction of the three municipal lands of Grumento Nova, Montemurro and Spinoso towns (fig.1). Created between 1957 and 1962 by damming the River Agri, its surface area is 7.5 km^2 and its depth reaches 90 m. The mean renewal time is six months (Calderoni and Mosello, 1978). Thick and beautiful woods surround it, covering its shores; the lake is a Site of Community Importance (SCI) for the preservation of natural habitats (European Commission Habitats Directive 92/43/EEC) and a Special Protection Zone (SPZ) (European Union Directive on the Conservation of Wild Bird

Directive 79/409/EEC). As part of the National Park of Val d'Agri the lake is used for angling and rowing, and its waters are used for drinking and irrigation purposes by the Basilicata and Apulia Regions. Lake Pertusillo is about eight kilometers distant from a center of petroleum refining and in 2016, during an incident, 400 oil tons were spilled from this center in the site groundwater. In 2010, 2011, 2012, 2013, 2014 and 2015 fish deaths occurred in the lake, which cause was not found. In 2010, 2011, 2012 and 2017 huge dinoflagellate blooms, covering the lake surface, occurred in spring and winter.

Sample collection

Samplings from June, 2010 to March, 2013 and in May, 2016 and April, 2017 were carried out in six stations (Rifreddo, **R**; Madonna Grumentina, **MG**; Spinoso, **S**; Montemurro Bridge, **MB**; Lake Damming, **LD**; Masseria Crisci; **MC**) of the lake. Seventy nine adult fish covering 10 species and thirty water samples were analyzed. The analyzed fish species were the zoobenthivorous species *Cyprinus carpio* (carp, 30 individuals), *Carassius carassius* (crucian carp, 10 individuals) and *Cyprinus carpio specularis* (mirror carp, 2 individual), the carnivorous species *Lepomis gibbosus* (pumpkinseed, 2 individuals), *Perca fluviatilis* (perch, 9 individuals), *Scardinius erythrophtalmus* (rudd, 1 individual), *Ictalurus melas* (catfish, 1 individual), *Alburnus alborella* (bleak, 9 individuals), *Squalius cephalus* (chub, 5 individuals) and *Micropterus salmoides* (black bass, 10 individuals). Fish captured by angling were ice–stored and transported to the laboratory. Thirty surface water samples were collected in 20 samplings by filling 1 L Pyrex glass bottles 10-20 cm below the water surface from two stations (**S** shore and **R** shore).

Fish tissue cylindrospermopsin (CYN) extraction

Cylindrospermopsin extraction from tissue samples was performed according to Saker et al. (1999, 2004) mod.: tissue (5 g, muscle) was homogenized in 10 mL 100% MeOH for 15 min. using a Potter Homogenizer (Polytron), then sonicated 5 min. at 30–40 °C in an ultrasonic bath (Elgasonic Swiss made, 25 kHz) at room temperature, to disrupt cells. The sample was then centrifuged for 5 min. at 5000 g and the supernatant decanted and filtered . The extraction was repeated on the pellet, the sample was centrifuged and the supernatant filtered on the same filter previously used. The filter and the funnel were washed three times with little volumes of MeOH; the two extracts and washings were collected together, then dried by rotavapor at 40 °C; the residue re-suspended in 2 mL distilled water was then stored at -30 °C until analysis.

Fish tissue microcystin (MYC) extraction

Five grams (wet weight) of muscle tissue from each fish was extracted. The sample was homogenized in 10mL MeOH for 15min. using an Ultra-Turrax T8 (IKA Werke, Staufen, Germany) grinder and then sonicated for 5 min. at 30–40∘C in an ultrasonic bath (Elgasonic Swiss made, 25 kHz) to disrupt cell membranes. The sample was centrifuged for 5min. at 5000 g and the supernatant decanted and filtered on a paper filter. The extraction was repeated on the pellet, the sample was centrifuged, and the supernatant filtered on the same filter previously used. The filter and the funnel were washed three times with little volumes ofMeOH; the two supernatants and the washings were gathered, then reduced to a small volume (1-2 mL) by rotary evaporator (Bùchi, Switzerland) at 40∘C, and diluted to 5mL with MeOH. One mL (for fish) of the extract (corresponding to 1 g of tissue) were then added with 1mL of distilled water and loaded onto a HLB SPE Waters OASIS cartridge, preconditioned with 1mL MeOH followed by 1mL of distilled water. The column was washed with 1mL of 5% MeOH in distilled water. Microcystins were eluted by 1 mL of MeOH. The MeOH eluate was dried by rotary evaporator at 40°C; the residue, dissolved in 2 mL distilled water, was stored at −30 °C for subsequent microcystin analysis with the EnviroGard Elisa kit.

CYN and MYC analysis by ELISA assays

Muscle tissue extracts from 17 fish caught in 2012 in MG and S stations were analyzed using the Abraxis Cylindrospermopsin ELISA Microtiter Plate immunoassay (Abraxis Bioscience CA). ELISA assays were performed in accordance with the manufacturer’s instructions using the calibration concentrations suggested. The Abraxis immunoassay declares the detection limit is 40 ppb, with percentage coefficients of variation below 10% for standard and below 15% for samples. The final reaction solution absorbances of the kit were measured at 450 nm with an Anthos 2010 microplate spectrophotometer (Anthos – Labtech, Salzburg, Austria).
Muscle tissue extracts from 79 fish samples were analyzed using the EnviroGard Microcystins Plate Kit (Strategic Diagnostics Inc., Newark, DE, USA), a direct competitive ELISA for quantitative detection of microcystins and nodularins (limit of quantification, LOQ = 0.1 ppb). This immunoassay does not differentiate between microcystin-LR and two other microcystin variants (MC-RR and MC-YR) but detects their presence to differing degrees. The concentrations at 50% inhibition (50% B/Bo absorbance signal) for these compounds (ppb) are: microcystin-LR 0.31, microcystin-RR 0.32, microcystin-YR 0.38. The final reaction solution absorbances of the kit were measured at 450 nm with an Anthos 2010 microplate spectrophotometer (Anthos – Labtech, Salzburg, Austria). The analytical method to determine microcystins in water and fish samples was previously validated according to the decision 2002/657/CEE (De Pace et al., 2014).

Sample handling and trace elements and PCB analysis

Ten specimens of *Cyprinus carpio* (common carp) caught from two stations (MC, LD) of Pertusillo Lake in April, 2017 (figure 2,3) were analysed also for trace elements and PCBs. After sampling, the specimens were stored in ice boxes with dry ice, transferred to the laboratory and immediately kept in a deep freezer. Subsequently, the frozen fish samples were thawed and biometric measurement were made (weight range: 868–3195 g, mean: 1296±697 g; length range: 37.0–60.0 cm, mean: 43.1±6.9 cm). From each specimen the muscle tissue was dissected, homogenized and analyzed. The extractive analytical procedure and the instrumental conditions to determine trace element concentrations have been described in detail elsewhere (Barone et al., 2013). Briefly, about 0.5 g of the samples were digested to a transparent solution with a mixture of HNO_3-$HClO_4$ (8:3) for cadmium (Cd), lead (Pb), chromium (Cr), copper (Cu) and zinc (Zn) determination and with a mixture of H_2SO_4-HNO_3 (1:1) for mercury (Hg). The completely digested samples were allowed to cool temperature and diluted with deionized water according to the method recommended by Official Italian Agencies (G.U.R.I., 1994). The content of elements was determined by atomic absorption spectrophotometry (Shimadzu AA 7000). Zn was analysed by flame, Cd, Pb, Cr, and Cu by using a graphite furnace (high density tube) (GFA-7000), Hg was measured by using a hydride vapour generator (HVG-1) after reduction by $NaBH_4$. Concerning PCBs, the concentrations of indicator PCBs (28, 52, 101, 138, 153 and 180) were determined using analytical procedures previously described and validated (Storelli, 2014). Briefly, about 40 g of powder were mixed with Na_2SO_4 and spiked with PCB 143 used as internal standard. The mixture was extracted with hexane:acetone (9:1) and the extracts were concentrated in order to determine the fat content by gravimetry. Next the extract was dissolved in hexane and cleaned by passing through 8 g of acid silica (H_2SO_4, 44% w. w.), using 50 mL of a mixture of hexane/dichloromethane (1/1, v/v) for elution of the analytes. The eluate was evaporated to dryness and redissolved in 100 mL of iso-octane. For the analysis of PCBs, a Thermo Trace GC connected with a Thermo PolarisQ MS operated in electron impact ionization (EI) mode was equipped with a 30 m, i.d. 0.25 mm and 0.25

µm Rtx 200 capillary column (Thermo, Austin, Texas, USA). The MS was used in the SIM mode with two ions monitored for each PCBs homologue group in specific windows. One ml of the cleaned extract was injected in splitless mode (injector temperature 90 °C then to 300 °C with 70 °C/min), splitless time 1.50 min, pulse pressure time 1.50 min, pressure pulse 25 psi. Helium was used as carrier gas at constant flow (1.0 ml/min). The temperature of the Rtx 200 column was held at 90 °C for 1.50 min, then increased to 180 °C at a rate of 15 °C/min, further increased to 280 °C at a rate of 5 °C/min, further increased to 300 °C at a rate of 40 °C/min, held for 7 min.

Quality assurance

Reference tissue (Tort-2 Lobster Hepatopancreas, National Research Council of Canada, Ottawa, Ontario, Canada) was treated and analysed in the same way as the samples. Results (Hg: 0.28 ± 0.03; Cd: 26.2 ± 2.4; Pb: 0.32 ± 0.18; Cr: 0.73 ± 0.16; Cu: 101 ± 13; Zn: 188 ± 12 µg g^{-1} dry weight) were in good agreement with the certified values (Hg: 0.27 ± 0.06; Cd: 26.7 ± 0.60; Pb: 0.35 ± 0.13; Cr: 0.77 ± 0.15; Cu: 106 ± 10; Zn: 180 ± 6 µg g^{-1} dry weight) and the standard deviations were low, proving good repeatability of the methods. The results for standard reference material displayed recoveries of the elements ranging from 91 to 104% (n = 3).The limit of detection (LOD) (Hg: 5; Cd: 0.12; Pb: 10; Cr: 5; Cu: 26; Zn: 24 ng g^{-1} wet weight) was defined as the concentration corresponding to three times the standard deviation of blanks, and the standards of quantification (LOQs) were the following: Hg: 13; Cd: 0.30; Pb: 38; Cr: 16; Cu: 81; Zn: 87 ng g^{-1} wet weight. Two blank samples were analysed together with each sample batch. Metal concentrations in blanks were below the detection limits in all the analyses. Blanks and calibration standard solutions were similarly analysed as the digested sample solution, and calibration curves were constructed. Analyses were duplicated to check the reproducibility of the results. Relative standard deviations among replicates were always less than 10%. Recovery tests were performed for the investigated metals in selected samples by spiking analysed samples with aliquots of the metal standards and then carrying out digestion. The recovery percentages ranged from 96 to 99%. Metal concentrations are presented as μg g^{-1} wet weight basis. For PCBs quality control was performed through the analysis of procedural blanks, a duplicate sample and a standard reference material [CRM349 for PCBs (cod liver oils) (BCR, Brussels)] within each batch of samples. The recovery percentage of the standard reference material was within the range of 86 and 105%. For the samples and standard reference materials, the relative standard deviations (RSD) were <10% for all the detected compounds. The limit of detection (LOD) for PCBs ranged from 0.02 to 0.50 ng g^{-1} on a lipid weight basis, while the limit of quantification (LOQ) varied from 0.20 to 1.30 ng g^{-1} on a lipid weight basis. Appropriate standard solution was added to the samples and recovery values were between 82 and 104%. The trace element and PCB concentrations in the samples were expressed as μg g^{-1} and ng g^{-1} wet weight, respectively.

Statistical analysis

Kruskal-Wallis test was conducted to verify the difference in the levels of trace metal and PCB accumulation, while simple linear regression coefficient was used to examine the correlations between PCBs and specimen length. To investigate size influence on PCB accumulation, the length of fish was chosen, because less subject to fluctuation than body weight (Diaz et al., 1994). The level of significance was set at $p < 0.05$.

Microscopic observations

The water samples were stored in ice chests and transported to the laboratory. For microscopic observations water subsamples were analyzed by an inverted microscope (Leitz Labovert FS) according to Utermőhl (1931) and Lund et al. (1958), using 25 ml sedimentation chambers for phytoplankton identification and cell density estimation.

Results and discussion

Trace element and PCB concentrations

The trace element concentrations detected in the study showed Zn values ranging from 1.15 to 4.32 μg g^{-1} wet weight (2.31 μg g^{-1} wet weight), while Cu showed much lower concentrations, ranging from 0.15 to 0.61 μg g^{-1} wet weight (0.36 μg g^{-1} wet weight) ($p < 0.001$) (fig.2). The considerable difference in levels between these two metals is not unique to the species here studied, being part of a general picture suggesting muscle tissue not to be considered a specific physiological site for Cu (Zia and Khan, 1989). Cr levels were very low too, ranging from 0.02 to 0.05 μg g^{-1} wet weight (0.03 μg g^{-1} wet weight) ($p < 0.001$) . Among non-essential metals the highest concentrations were recorded for Hg with values between 0.27 and 0.53 μg g^{-1} wet weight (0.40 μg g^{-1} wet weight), followed by Pb showing levels from 0.05 to 0.28 μg g^{-1} wet weight (0.14 μg g^{-1} wet weight), while Cd registered the lowest values between 0.03 and 0.05 μg g^{-1} wet weight (0.04 μg g^{-1} wet weight) ($p < 0.001$). A comparison with data in the literature shows a wide concentration heterogeneity for all metals studied. However, our Hg levels are very similar to those found by Stong et al. (2013) in common carp from Lake Chapala in Mexico, but very higher than those reported by Vicarova et al. (2016) in the same species from three reservoirs in the Czech Republic. For Cd and Pb, the levels in this study are in line with values reported by Yancheva et al. (2014) in muscle tissue of common carp from Topolnitsa reservoir in Bulgaria. For essential metals, our Cr values are in good agreement with results found in the muscle tissues of common carp from the uncontaminated fishponds in the Czech Republic (Čelechovská et al., 2007) and Kabul River in Pakistan (Yousafzai et al., 2017). In contrast, our Zn values are lower than those reported by Yousafzai et al. (2017) and by Čelechovská et al. (2007) in muscle tissue of common carp from the Keban Dam Lake in Turkey and the fishponds in the Czech Republic, respectively. Regarding Cu concentrations, samples analysed in this study showed levels of the same order of magnitude of those reported for common carp from the Czech Republic (Čelechovská et al., 2007). To safeguard public health, concentration standards in fish for some heavy metals have been established by the European Commission). In particular, Hg, Pb and Cd limit values at 0.50, 0.30 and 0.05 $\mu g\ g^{-1}$ wet weight respectively, have been fixed (Official Journal of the European Union, 2006, 2014, 2015). In this context, no analysed fish sample showed concentrations exceeding the European Directive proposed limits for Pb and Cd while for Hg, slightly exceeding levels were registered in two samples (0.51 and 0.53 $\mu g\ g^{-1}$ wet weight).There are no European guidelines for fish consumption established as regards Cu, Zn and Cr, but the UK Food Standards Committee's Report fixed Zn and Cu limits at 50 and 20 $\mu g\ g^{-1}$ wet weight respectively, while the Western Australian Food and Drug Regulation List (Usero et al., 2003) fixed Cr limits at 5.5 $\mu g\ g^{-1}$ wet weight. Our detected results were always lower than these human consumption limits.

The subset of six PCB congeners here tested were selected by the International Council for the Exploration of the Sea (ICES) as contamination indicators, due to their easy quantification compared to the other non-dioxin-like PCBs, however representing all relevant degrees of chlorination. The data analysis showed that PCBs 153 and 138 were the most frequently detected congeners (detection in 100% of samples), while PCBs 101 and 180 were detected with 50% and 70% frequency, respectively, and PCBs 28 and 52 were below the detection limits in all samples examined. The total concentrations of indicator PCBs were 95.8-202.5 ng g^{-1} lipid weight, with a mean value of 148.6 ng g^{-1} lipid weight. PCBs 153 and 138 with mean values of 62.6 ng g^{-1} lipid weight and 55.4 ng g^{-1} lipid weight were the highest in concentration, followed by PCB 180 showing a mean concentration of 18.7 ng g^{-1} lipid weight and PCB 101 exibithing the lower mean value equal to 11.9 ng g^{-1} lipid weight. The PCB bioconcentration in aquatic organisms correlates with the degree of chlorination, the stereochemistry and lipophilicity (Fox et al., 1994). Generally, congeners with a high chlorination grade are more difficult to metabolise and eliminate than less chlorinated congeners. Our data well fit this general picture, being low chlorinated congeners PCBs 28 and 52 below the detection limit, PCB 101 contributing for 8%, while hexa- and heptachlorinated biphenyls 138, 153 and 180 together constituted a consistent percentage of the total PCB burden representing 92%. Generally, the largest and potentially oldest fish exhibit higher PCB levels than younger organisms. Despite of this, no correlation between fish length and total PCB concentrations was observed ($R = 0.42$; $P > 0.05$) in the present study, probably as consequence of scarce PCB contamination in the Pertusillo basin. These PCBs have been recommended by the EU as indicators of PCB contamination because generally they represent approximately half of the total ndl-like PCBs existing in food. In fact, the European Food Safety Authority (EFSA) Scientific Panel regarding Contaminants in the Food Chain (CONTAM Panel) recommends the sum of these six PCBs as an appropriate marker for risk assessment of ndl-PCBs. Regulation No. 1259/2011 of the European Union (EU) (Official Journal of the European Union, 2011) has set *de novo* maximum tolerable levels for the sum of the six indicators non-dioxin-like PCBs in muscle meat of freshwater fish that, apart from some exceptions, is of 125 ng g^{-1} wet weight. Our results presented on a lipid weight basis have, hence, been converted to wet weight basis to conform to legal standard. According to this, the sum of six "indicator" congener concentrations was below the conventional permissible consumption limit in all samples examined (1.27 ng g^{-1} wet weight).

Microcystin and cylindrospermopsin concentration

Superficial fortnightly water samples taken from March to April 2012 and from October 2012 to March 2013 were analyzed for phytoplankton presence. In these winter samples only 16 species were detected; the lack of summer samples, due to difficulties in carrying out regular water samplings, did not allow a complete evaluation of phytoplanktonic composition. In a few summer samples analyzed by the Basilicata Agency for Environmental Protection (ARPAB) in 2014, 9 other species were detected (ARPAB, 2015). The poor presence of phytoplanktonic species detected in this study may also be due to the need for column samplings and more systematic monitoring. However, even in the past the lake showed the presence of a limited number of species (29 species detected, Ruggiu and Saraceni, 1978). No cyanotoxins were detected in the analyzed water samples.
In fish 86% of total tissue samples were positive for MYC presence, at concentration values ranging from a minimum of 0.19 ng/g to a maximum of 2.01 ng/g b.w. (fig. 4-6). *Micropterus salmoides*, *Carassius carassius* and *Cyprinus carpio* were the species with highest concentration capacity and averages. ELISA analyses showed the presence of CYN in 64% of samples, with maximum concentrations at 0.78 ng/g in muscle (fig. 7). *Cyprinus carpio* and *Perca fluviatilis* were the species with highest concentration capacity and averages. ARPAB phytoplankton analyses in summer 2014 showed the presence of *Aphanizomenon* sp., which could take account for CYN presence (Messineo et al., 2010).

In May, 2016 fifteen fish samples from four stations (2 carps from MG , 2 carps from MB, 5 chubs and 6 perchs from MC) were analyzed for MYC presence (fig. 5), showing the highest mean content (0.72 ng/g), in perchs. In the following year (April, 2017) nine samples (5 carps from MC and 4 carps from LD) showed a mean content (0.91 and 0.93 ng/g, respectively, fig.6) higher than that of 2016 carps (0.29 and 0.28 ng/g, respectively). The toxicity of microcystins in fish depends on the balance between accumulation and metabolism (Ito et al., 2002); the observed species-specific sensitivities have been interpreted as the result of anatomical, physiological and behavioral differences among the various fish orders (Tencalla and Dietrich, 1997; Fischer and Dietrich, 2000):

the detoxification capacities *via* the glutathione-S-transferase pathway are species-specific dependent, too (Cazenave et al., 2006).

CYN accumulation in ichthyic fauna was previously investigated in crayfish (*Cherax quadricarinatus*), rainbow fish (*Melanotaenia eachamensis*) (Saker and Eaglesham, 1999), freshwater mussels (*Anodonta cygnea*) (Saker et al., 2004), salmonids (*Salmo trutta*) (Messineo et al., 2010) and finfish (Berry et al. 2012).

The acute Tolerable Daily Intake (TDI) guideline for MC-LR, proposed by WHO in 1998 for an adult of 60 kg b.w. (0.04 μg/kg body weight/day, Chorus and Bartram, 1999) was revised by USEPA in 2006, with new proposed guidelines developed for acute and chronic risk (0.006 and 0.003 microcystin μg/kg b.w./day, respectively; US Environmental Protection Agency, 2006), but no guidelines for cancerogenicity were proposed, due to the insufficient adequacy of the available studies. In the same 2006 the International Agency for Research on Cancer classified microcystin-LR as possibly carcinogenic to humans (group 2B: IARC, 2010). Case-control studies in southwest China recently confirmed the link between MYC serum levels and hepatocellular carcinoma occurrence in humans (Zheng et al., 2017).

For an adult human weighing 60 kg and ingesting 300 g serving of fish muscle, the microcystin level in 14.5 % of muscle samples analyzed from 2010 to 2012 was 1.6 -fold the recommended TDI acute value of EPA, and 36.3% of muscle samples were even 3.3 -fold the recommended chronic value.

Contaminant classes like polycyclic aromatic hydrocarbons, trace elements, PCBs and microcystins are known to produce synergistic effects on organisms: in fish heavy metals may cause enhanced toxic effects if combined (Espina et al., 1997), *in vitro* and *in vivo* studies on cyanobacterial extracts, PCB 153 and fluoranthene (Bartova et al., 2011) provide evidence on synergistic effects of tumor promotion.

In Italy microcystin contaminations in ichthyic fauna were detected in several lakes (Bruno et al., 2009; Bruno et al., 2012). MYCs demonstrated to be a recurrent component among the lake Pertusillo main contaminants, being detected in fish tissue all along the duration of the study. No MYC producing cyanobacteria were found in our phytoplankton analyses but several benthic species are MYC producers, too, and an extended monitoring for phytobenthic toxic species in the sediments of the lake would be needed, to investigate the reason why a higher presence of these toxins was detected in the cyprinid species.

Zn levels detected by ARPAB lake water monitoring in 2014 (between 5 and 83 µg/l; ARPAB, 2015) are known to increase the growth and intracellular MYC production in *Microcystis aeruginosa* cultures (Polyak et al., 2013). A recent meta-analysis has also shown that persistent organic pollutants, among which PCBs, are able to stimulate cyanobacterial growth (Harris and Smith, 2016).

A more extended monitoring is needed to define the presence of these different contaminants in ichthyic fauna, their role in the recurrent fish deaths in the lake, and the exposure risk of people of the lake region by consuming contaminated lake fish. As Pertusillo Lake is part of a SCI zone, the PCBs content in lake fish could endanger also the fish-eating birds, through biomagnification. Lake Pertusillo is mesotrophic-eutrophic (ARPAB, 2015), and several episodes of algal blooms occurred in the lake during the last seven years Organisms are usually exposed not only to isolated environmental pollutants, but to chemical mixtures which individual components may be present at concentrations lower than their safety threshold levels.

Although the concentrations of metals and PCBs detected in the analysed fish samples are not high, the presence of these different compounds in association with microcystins suggests the possibility of future cyanobacterial toxic blooms in this environment through their growth stimulation, pointing out the need for restoration programs to improve the trophic conditions of the reservoir. Moreover, given the presence of the industrial activities of oil drilling in the area, further studies are needed to investigate the potential contamination of oil compounds in Pertusillo ichthyic fauna.

Conclusions

The ichthyic fauna of Pertusillo appears to be interested by multiple contaminant concentrations. The MYC production may be synergistically influenced and enhanced in the aquatic environment by some trace element concentrations.

Waiting for new tools to assess the overall impact of these pollutants on aquatic life and human health, the managing policy remains the exploration and implementation of cost-effective and appropriate remediation, coupled with the search for environmentally more benign products and processes, which should aim to minimize introduction of critical pollutants into the aquatic environment.

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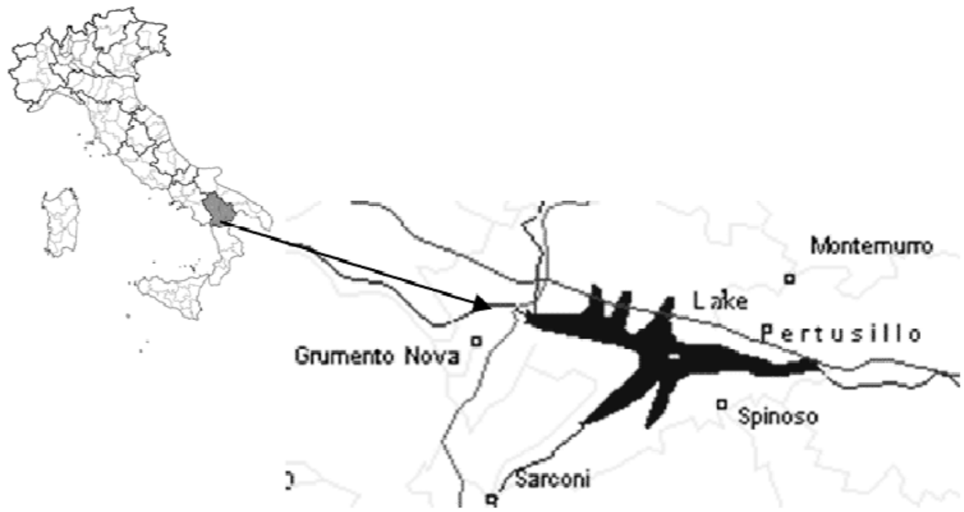
Legend

Figure 1. Study site and station coordinates.



Sampling station	N	E
Masseria Crisci **MC**	40.28977	15.95180
Rifreddo **R**	40.28710	15.9527
Spinoso **S**	40.28044	15.96638
Madonna Grumentina **MG**	40.29172	15.92957
Montemurro Bridge **MB**	40.28238	15.9825
Lake Damming **LD**	40.27522	15.99157

Figure 2. Trace element concentrations in common carp.

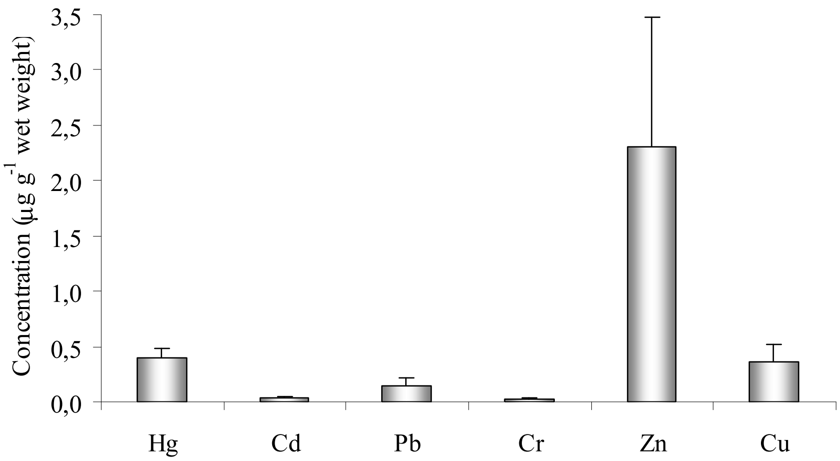


Figure 3. Concentrations of six PCB indicator congeners in common carp.

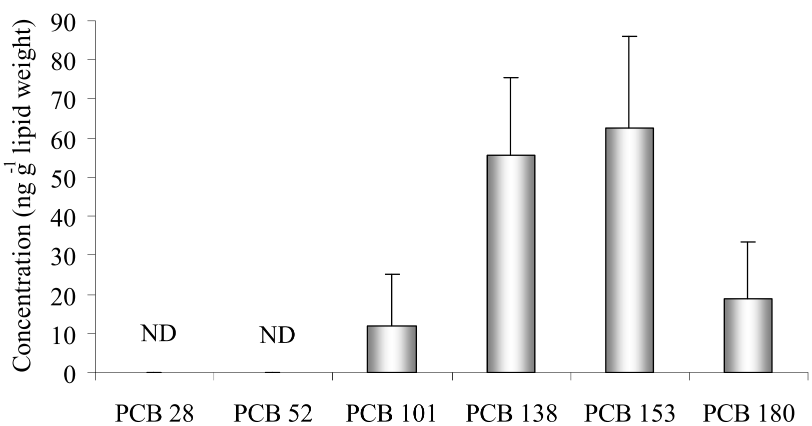


Table 1. Phytoplanktonic species identified in the superficial samplings of 2012.

Phytoplanktonic species	
Cyanobacteria	*Coelosphaerium kutzingianum* Nageli
Diatomeae	*Asterionella formosa* Hassall
	Cyclotella kutzingiana Thwaites
	Cymbella sp. C. Agardh
	Fragilaria crotonensis Kitton
	Gyrosigma attenuatum (Kutzing) Rabenhorst
	Melosira italica (Ehrenberg) Kutzing
	Melosira varians C. Agardh
	Navicula ssp. Bory de Saint-Vincent
	Nitzschia acicularis (Kutzing) W. Smith
	Rhizosolenia sp. Ehrenberg
	Stephanodiscus astraea (Ehrenberg) Grunow
Chlorophyceae	*Oocystis lacustris* Chodat
Conjugatophyceae	*Closterium kǜtzingii* Brébisson
	Closterium pronum Brébisson
Dinophyceae	*Ceratium hirundinella* (O.F.Müller) Dujardin

Figure 4. Microcystin concentration in fish muscle tissue (all the stations) during three years (2010-2012).

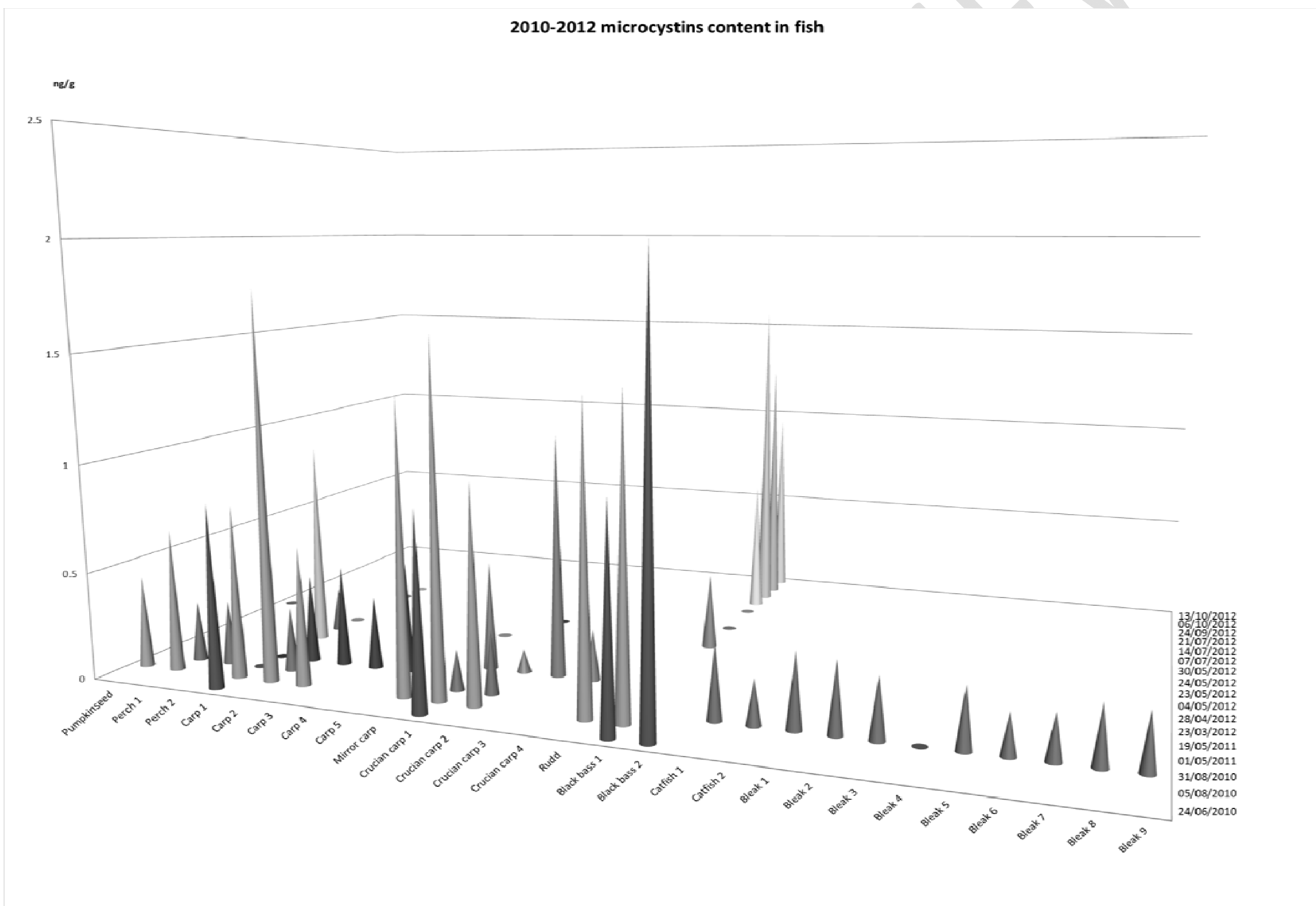


Figure 5. Microcystin concentration in fish samples from three lake stations (MG, MB, MC) in May, 2016.

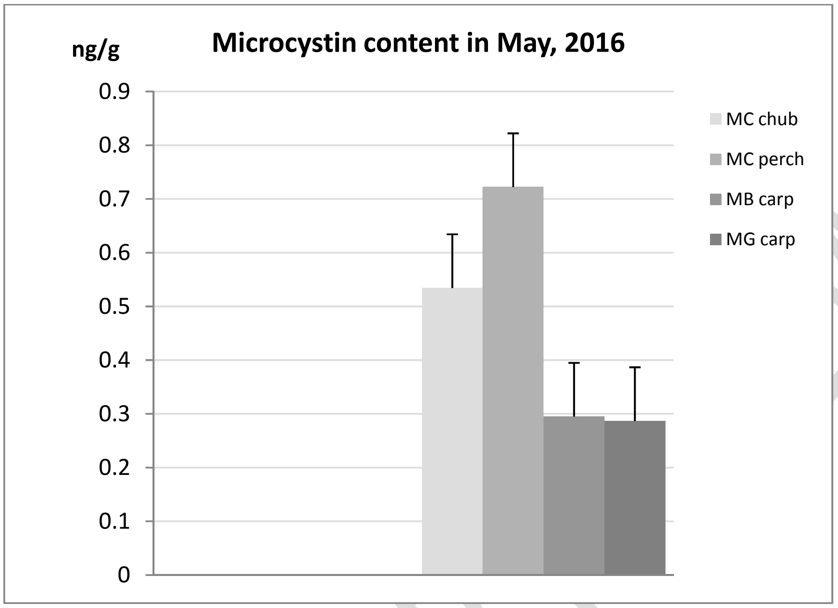


Figure 6. Microcystin concentration in fish samples from two lake stations (MC, LD) in April, 2017.

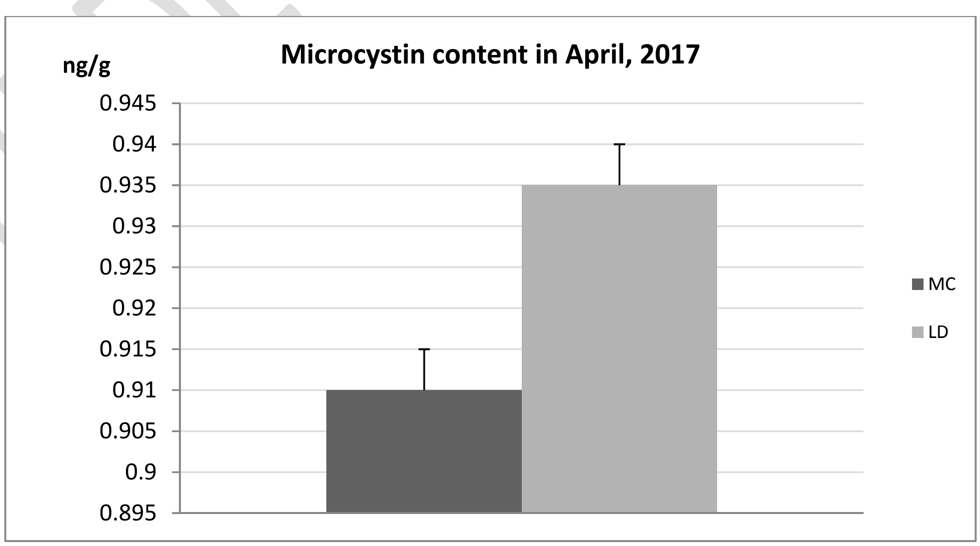


Figure 7. Cylindrospermopsin concentration in fish muscle tissue during 2012.

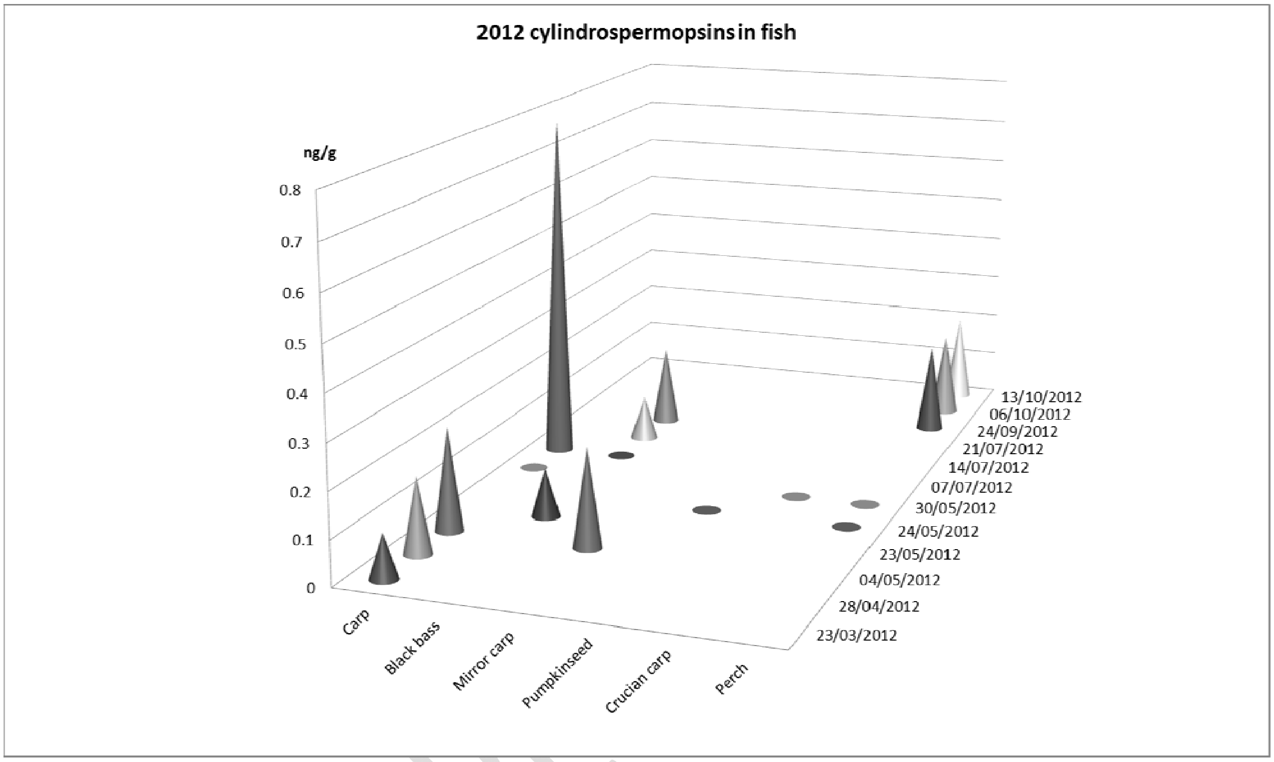


Figure legend

Figure 1. Study site and sampling stations

Figure 2. Trace element concentrations in common carp.

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Table 1. Phytoplanktonic species identified in the superficial samplings of 2012.