Mercury distribution in the main compartments of the eutrophic Lake Candia (Northern Italy)

Maria Chiara FONTANELLA, Oscar RAVERA*, Gian Maria BEONE[1], Nicoletta RICCARDI and Ilenia CATTANI[1]

CNR - Institute of Ecosystem Study, Largo Tonolli 50, I – 28922 Verbania Pallanza, Italy
[1]Università Cattolica del Sacro Cuore, Istituto di Chimica Agraria e Ambientale, Via E. Parmense 84, 29100 Piacenza, Italy
*e-mail corresponding author: oscar.ravera@ise.cnr.it

ABSTRACT

Total mercury (T-Hg) and organic mercury (mainly methylmercury, MeHg) concentrations in the most important compartments (water, sediment, macrophytes, zooplankton, mussels and fish) of the shallow and eutrophic Lake Candia (Turin, Northern Italy) were measured. The decreasing sequence of the T-Hg concentrations is as follows: cat-fish (143 µg kg^{-1} d.w.), zooplankton (77 µg kg^{-1} d.w.), Unio pictorum mancus (37.9 µg kg^{-1} d.w.), macrophytes (28.9 µg kg^{-1} d.w.). The content of mercury in mussel tissues increased with the size of the animal, but the relationship between Hg concentration and tissue weight was negative, indicating that the rate of mercury accumulation was lower than the tissue growth rate. The amount of mercury accumulated in the mussels living in the lake sediments was estimated to be 0.54 µg m^{-2}. The importance of mercury biomagnification is also discussed.

Key words: fish, mollusc, macrophytes, zooplankton, mercury, biomagnification

1. INTRODUCTION

Interest in mercury contamination focuses primarily on its organic compounds, particularly methylmercury (MeHg), due to its high toxicity. A diet based on fish highly contaminated by mercury discharged with industrial effluents caused the poisoning of fishermen and cats in Minamata Bay (Japan) and attracted attention to a serious mercury-induced neurological and cardiovascular diseases (Sorensen *et al*. 1990). Since then mercury and its compounds have been regarded by national and international norms (e.g., WHO-IPCS 1990) as the most toxic of heavy metals. The organic forms (e.g., dimethylmercury) are more volatile, more toxic, and more readily available to organisms than the inorganic compounds, and have a different distribution in the environment.

When mercury and its inorganic species are transferred from point sources (ores, rocks, effluents) or diffused sources (atmosphere) to a water body, they may be transformed into organo-metals (e.g., monomethylmercury, dimethylmercury) by bacterial and algal activity (Jensen & Jernelov 1969; Jernelov & Lann 1971; Mason *et al*. 1996). In fresh and brackish waters the sulphate-reducing bacteria are the most efficient microrganisms in the methylation process, which mainly occurs at the sediment-water interface and, to a lesser extent, in the water column. The opposite process, demethylation, is also mainly due to bacterial activity at the sediment surface. Therefore, the MeHg concentration in the aquatic environment is the result of the balance between the methylation and the demethylation processes (Campeau & Bartha 1984).

Experimental studies have focused on the relationships between the two processes (e.g., Rambal *et al*. 1986; Hintelman & Evans 1997) and on the influence of biotic and abiotic factors. For instance, Gardfeldt *et al*. (2003) demonstrated that methylation is substantially driven by biotic factors, while it is poorly affected by abiotic conditions. A relatively important role has been attributed to solar radiation, which seems to be involved in both the formation and decomposition of MeHg. The influence of light is obviously limited to the photic layer, where the highest photosynthetic activity is sustained by phytoplankton. The release of soluble extracellular organic substances (e.g., glycine) by planktonic algae stimulates the bacterial activity, thus indirectly contributing to the production and decomposition of MeHg.

Aquatic animals take up MeHg from both water and food, but generally food seems to be the most important source of MeHg for zooplankton (Tsui & Wang 2004) and fish (Hall *et al*. 1997). The ability of organisms to survive in environments heavily polluted by toxic metals (e.g., mercury) varies with the species. The capacity of some species for accumulating in their bodies metal concentrations exceeding their physiological needs by 2 or 3 orders of magnitude without evident damage justifies the use of some of these species as bioindicators of accumulation for one or more pollutants. One of the commonly used bioindicators for mercury is fish, with muscle the tissue analysed, even though the highest mercury concentration occurs in liver and kidney (Jernelov & Lann 1971). However, muscle has some important advantages; for example, most of the fish's weight derives from muscle, which may be easily isolated from other tissues, the organic mercury percentage

in the muscle is fairly high, and it is an important food for several species, including man.

The study of mercury distribution, and particularly that of its organic forms, is not only justified by its high toxicity and its persistence in the environment, but also to the fact that it is easily and readily available to animals and has a tendency to increase its concentration along the trophic chain (biomagnification).

Continuous progress in analytical chemistry means that researchers are now able to tackle the problems of the biogeochemical cycle of mercury and its physical and chemical forms.

The main objective of this research was to assess the concentration and distribution of total mercury (T-Hg) and its organic forms (MeHg) in the major compartments (water, sediment, macrophytes, zooplankton, bivalve and fish) of a shallow and eutrophic lake (Lake Candia, Turin, Northern Italy). This lake is characterized by low toxic metal concentrations and receives mercury only from atmospheric depositions.

2. MATERIALS AND METHODS

2.1. Study site

The research was carried out in Lake Candia (Northern Italy, latitude N 45°19'32"; longitude E 7°54'38"). The most important morphometric characteristics are reported in table 1. Further details on this shallow and eutrophic lake may be found in Ravera *et al*. (2007a).

2.2. Sampling and sample preparation

Macrophytes (*Trapa natans*, *Myriophyllum spicatum*, *Nymphoides peltata*) and mussels (*Unio pictorum mancus*) were collected at random by hand. The mean value of the mussel shell length was 78 mm (49÷95 mm). The young of the year (YOY) fish (*Ictalurus melas*) of 4-5 cm length were captured with a nylon net and the plankton with a conical net (mesh size = 85 μm).

Tab. 1. Morphometric characteristics of Lake Candia (Giussani & Galanti 1992).

Altitude (m a.s.l.)	226
Watershed area (km^2)	9.9
Lake area (km^2)	1.5
Mean depth (m)	3.8
Maximum depth (m)	7.7

The plankton sample was divided into two sub-samples: a small one was preserved in a 5% neutralized formaldehyde solution and subsequently used for microscopical analyses; a larger sample was used for chemical analyses. The plankton sample was almost exclusively made up of Crustaceans (Cladocera and Copepoda); *Chaoborus flavicans* larvae and Rotifers (*Asplanchna priodonta*) were present in a very low density. Phytoplankton consisted of only a few small colonies of *Microcystis* sp. (Tab. 2). Due to the small size of the young cat-fish (*Ictalurus melas*), the entire body was analyzed.

Surface water samples were filtered (Millipore 0.45 μm) soon after collection and preserved in plastic bottles. Surface sediment was collected using a plexiglass core sampler (inner diameter = 7 cm).

All the samples were preserved in plastic bags placed in an ice-box and brought to the laboratory, where they were kept at -20°C until the chemical analyses were performed, within 20-25 days after sample collection.

Tab. 2. The most abundant species of zooplankton in Lake Candia sample.

Cladocerans
Ceriodaphnia quadrangula
Ceriodaphnia pulchella
Daphnia hyalina-galeata-cucullata complex
Diaphanosoma brachyurum
Eubosmina coregoni
Copepods
Eudiaptomus gracilis
Mesocyclops leuckarti
Thermocyclops crassus

2.3. Chemical analyses

Total mercury (T-Hg) was measured by using solid/liquid atomic absorption spectrometer Hg analyser (Advanced Mercury Analyzer, AMA 254 model, FKV) based on combustion of the sample in oxygen atmosphere and amalgamation preconcentration on a single gold trap. Precision and accuracy were checked using standard reference materials (mussel tissue BCR 278 and estuarine sediment ERM 580) and replicate analysis. The quality control gave good precision (SD <5%) for almost all samples.

The organic species of mercury (ethylmercury and methylmercury) were determined by HPLC-ICP-MS (HPLC 1100 Agilent model coupled with an ICP-MS 7500 ce Agilent model) following the procedure described by Cattani *et al*. (2008). The only organic form found in the samples was methylmercury (MeHg). The water samples were injected directly into HPLC-ICP-MS. The extraction from 1 g of lyophilized solid samples was obtained with the addition of 9 mL HCl (7.6% w/v) and 1 mL mercaptoethanol (10% w/v). The mixture was placed for 45 minutes in ultrasonic bath with ice in the water to prevent excessive warming. An aliquot of clear solution was brought to pH 6.8 with ammonia (10% w/v). After being diluted ten times with deionized water (18 MΩ-cm resistance, Millipore® system), the sample solutions were filtered through a 0.45 μm membrane before HPLC-ICP-MS analysis. The m/z signal adopted was 202. More details of working parameters of the instruments are reported in Cattani *et al*. (2008). The extractions and HPLC-ICP-MS measurements were made on the same day. Spike addition, replicate determinations and analysis of certified standard
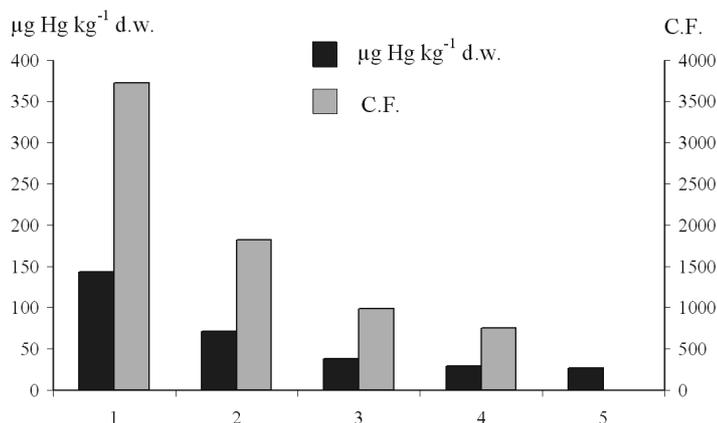
Fig. 1. Concentrations of mercury (µg kg⁻¹ d.w.) and concentration factors (C.F.) in the most important compartments of Lake Candia. 1) fish, 2) zooplankton, 3) mussel, 4) macrophytes, 5) sediments
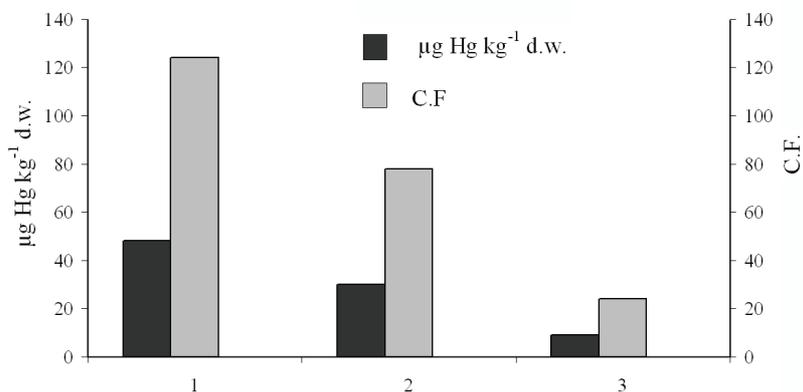

Fig. 2. Concentrations of mercury (µg kg⁻¹ d.w.) and concentration factors (C.F.) in macrophytes 1) *Trapa natans*, 2) *Myriophyllum spicatum*, 3) *Nymphoides peltata.*

ERM 580, estuarine sediment certified for the MeHg and T-Hg concentration, were carried out to verify the precision and accuracy of the measurements.

2.4. Calculations

The concentration factor (C.F.) was calculated as the ratio between the concentration of the T-Hg in the organism (related to wet weight) and its concentration in the filtered water.

Spearman's coefficient of rank correlation was calculated to measure the intensity of association observed between the concentration of mercury in the mussel tissues, and the mussel size. Non-parametric correlation analysis was used, since the data were not normally distributed as verified by a normality test according to the Kolmogoroff-Smirnoff method.

3. RESULTS

The T-Hg and MeHg concentrations in the most important compartments of Lake Candia and the concentration factor (C.F.) values are reported in figure 1.

Since the T-Hg concentration in the filtered water (0.005 µg L⁻¹) is the same, the C.F. values for all the compartments analyzed are proportional to the T-Hg concentrations in each of them.

The decreasing sequence of the T-Hg concentrations is the following: *Ictalurus melas* > zooplankton > *Unio pictorum mancus* (bivalve) > macrophytes > sediments > filtered water. The MeHg percentage of the T-Hg is 13% in the zooplankton but decreases to 2.8% in the macrophytes, which is a value of the same order of magnitude as that measured in the sediments: 3.3% in the first cm and 3.7% in the second centimetre.

The mean concentration of T-Hg in the macrophytes was fairly low (Fig. 2), but the differences between species were quite wide and decreased from Trapa natans (47.6 µg kg⁻¹ dw) to Myriophyllum spicatum (30.0 µg kg⁻¹ dw) and *Nymphoides peltata* (9.12 µg kg⁻¹ dw). The MeHg concentrations were 1.67 µg kg⁻¹ dw in *Trapa natans* and 0.40 µg kg⁻¹ dw in the other two species.

The content of mercury in mussel tissues increased with animal dry weight, but a tendency towards a decrease of mercury concentration in the tissues with
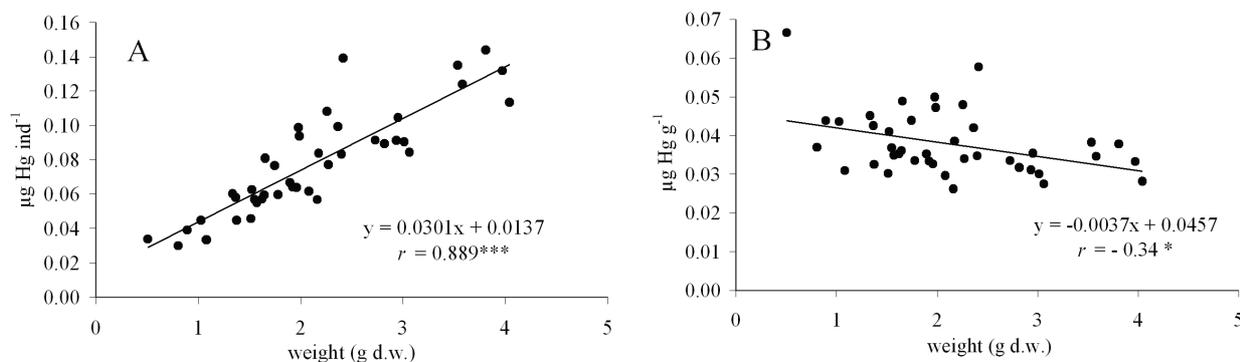
Fig. 3. Relationship between *Unio* soft tissue weight and mercury content in the mean individual mussel (**A**) and between soft tissue weight and mercury concentration in   mussel tissues (**B**). * = *p* <0.05; *** = *p* <0.001.

the increase in tissue weight was quite evident, even though the relationship was not statistically significant (Fig. 3).

4. DISCUSSION

Mercury concentration in aquatic organisms is the combined result of several variables, such as the concentration of the available forms in the water and food, the age and size of the organism and its place in the food-web.

4.1. Fish

Because of the high mercury concentration in fish and the large percentage of its organic forms, which are also more toxic, fish are excellent indicators for estimating the mercury contamination level of the environment. In fact, the T-Hg concentration in *Ictalurus melas* from Lake Candia (143 µg kg^{-1} d.w.) was about twice that measured in net plankton (77 µg kg^{-1}) and about five times higher than the mean concentration in the macrophytes (28.9 µg kg^{-1}). Ulbrich *et al.* (2007) analyzed the T-Hg in fish species from various water bodies from Kazakhstan, finding the highest values and the widest variability in *Perca fluviatilis* (190÷1680 µg kg^{-1}) and *Tinca tinca* (160÷1120 µg kg^{-1}); Grieb *et al.* (1990) measured concentrations from 40 to 360 µg kg^{-1} in *Perca fluviatilis* from various water bodies in Michigan.

The major drawback of using fish as indicators regards their mobility and the migratory behaviour of some species; this can make it difficult to establish whether all the mercury measured in the fish was accumulated in the habitat from which it was captured. In our case, a very low mobility can be reasonably assumed, since YOY cat fish live in swarms which explore a limited littoral area.

4.2. Zooplankton

Due to their short life-span and rapid rates of metal uptake and loss, zooplankters are very useful indicators of the present mercury pollution level (Tsui & Wang 2004). The specific composition and the size structure of zooplankton varies widely not only with the environment but also with the season; as a consequence, wide variations over time of T-Hg and MeHg concentrations may be expected, even if the mercury concentration in the water is constant. The samples from lake Candia were essentially composed of crustaceans (Tab. 2), with T-Hg and MeHg concentrations (77 µg kg^{-1} and 9.32 µg kg^{-1}) falling in the very wide range reported in the literature. For example, Sorensen et al. (1990) reported for net-plankton concentrations of T-Hg from 9.50 µg kg^{-1} d.w. to 209 µg kg^{-1} d.w., while Nguyen *et al*. (2005) calculated for Lake Balaton (Hungary) copepods the mean concentration of T-Hg of 0.31÷6.8 µg kg^{-1}. The variability of the MeHg concentrations is not lower than that of T-Hg; for example, Wescott & Kalff (1996) reported concentrations of organic mercury from 19 to 448 µg MeHg kg^{-1} d.w. and Back & Watras (1995) concentrations from 1 to 479 µg kg^{-1}.

To obtain homogenous zooplankton samples, Watras & Bloom (1992) were the first to measure the T-Hg and MeHg concentrations in samples composed of 5 to 20 zooplankters. A very interesting research was performed by Back & Watras (1995) on the MeHg concentrations in single species of zooplankton from 12 lakes (Wisconsin, USA). The concentrations varied with the species and the environment; for example, the MeHg concentration in *Holopedium gibberum* varied from 40 to 479 µg kg^{-1} d.w. and in *Diaptomus minutum* and *D. oregonensis* from 22 to 66 µg kg^{-1}. In addition, the MeHg concentrations in four species of *Daphnia (D. pulex, D. galeata, D. mendotae* and *D. ambigua*) varied from 1 to 211 µg kg^{-1}.

4.3. Macrophytes

Wide interspecific variation in T-Hg and MeHg concentrations was measured for macrophytes (*Trapa natans*, *Myriophyllum spicatum*, *Nymphoides peltata*), all of which presented a low MeHg concentration in comparison to fish and zooplankton. This might suggest that at least a part of the mercury measured in the macrophytes was not metabolized, but adsorbed on the plant surface.

In spite of their low mercury concentrations, macrophytes may play an important role in environments in which their biomass is abundant. In this case the total content of mercury in the macrophytes assumes great importance from a biogeochemical point of view.

4.4. Molluscs

Since the freshwater mussel *Dreissena polymorpha*, commonly used as a bioindicator of the environment pollution level (Busch & Schuchardt 1991; De Kock & Bowmer 1993; Camusso *et al.* 2001) has not yet invaded Lake Candia, it seemed interesting to use as a bioindicator for mercury another common freshwater mussel (*Unio pictorum mancus*), which has been successfully tested for other metals on previous occasions (Beone & Ravera 2003; Ravera *et al.* 2003a, 2003b, 2005, 2007a, 2007b). The T-Hg mean concentration in the soft tissues of *Unio* from Lake Candia, 38 µg kg^{-1} d.w., is of the same order of magnitude as that measured in *Dreissena* from the largest Northern Italian lakes (Camusso *et al.* 2001). As an example, the mean concentration of T-Hg in *Dreissena* ranges from 49 µg kg^{-1} d.w. in Lake Lugano to 53 µg kg^{-1} d.w. in Lake Como and 65 µg kg^{-1} d.w. in Lake Garda and Lake Iseo. The highest concentration (158 µg kg^{-1} d.w.) measured by the same authors, in animals from Lake Maggiore, could be explained by the relatively high pollution level of the sampling site (Pallanza Gulf), which was affected by various industrial effluents during the 1960s and 70s.

The concentration of metal in the mussel tissues is the result of a balance between the tissue growth-rate and the metal accumulation rate. If the tissue growth rate exceeds the accumulation rate, the concentration of mercury decreases with the mussel's size, while the opposite occurs when the mussel grows more slowly than the rate of metal accumulation. Therefore the negative relationship between mercury concentration and mussel size that we observed in the eutrophic Lake Candia could be the result of a dilution effect due to rapid mussel growth enhanced by high food availability (Fig. 3).

To evaluate the potential impact of a population on its environment, the element (in this case mercury) content can be estimated when population density is known. In Lake Candia, for instance, given a mean value of 6.93 individuals m^{-2} and a mean individual T-Hg content of 0.078 µg, the total content of Hg in the mussel tissues is 0.54 µg Hg m^{-2} (Ravera 2008). This value, which represents the amount of mercury sequestered by the live mussels, and which will be returned to the environment after their death, is commonly neglected in spite of its importance from an ecological point of view.

4.5 Biomagnification

Some authors attribute the high mercury concentration measured in large-sized fish to their high longevity, which gives them a sufficiently long time to accumulate a larger amount of mercury than fish species with a shorter life span (e.g., Knauer & Martin 1972; Williams & Weiss 1973). Presently there is general agreement about attributing the mercury concentration in different species to their position in the food chain (Cabana & Rasmussen 1994; Kidd *et al.* 2003; Hammerschmidt & Fitzgerald 2006; Orihel *et al.* 2007; Campbell *et al.* 2008). The abundant information on this concept, called "biomagnification", demonstrates that mercury concentration increases from the lower (primary producers) to the middle (herbivores) and upper (carnivores) trophic levels. An example is provided by Hammerschmidt & Fitzgerald (2006), whose study of the MeHg concentration in an aquatic food chain found the following sequence of increase: filtered water (0.00003 ng g^{-1} ww) < seston (0.5 ng g^{-1} ww) < zooplankton (1.1 ng g^{-1} ww) < fish (27 ng g^{-1} ww). Studies on biomagnification contribute to extending knowledge of the trophic structure of a community through the assessment of the distribution of pollutants (such as mercury) in the different taxa. Nevertheless, while it is relatively easy to study linear food chains (e.g., grass-cow-man), more difficulties arise when we are faced with the more complicated food webs which commonly occur in the majority of environments. In fact, most preys are generally preyed upon by different predators, most species are omnivorous and feed on different trophic levels, and seasonal and age-dependent shifts in diet are quite common. A significant contribution to solving these problems was made by the use of stable isotopes (^{13}C and ^{15}N), which made it possible to investigate the food web in greater detail and to identify the trophic relationships between species (e.g., Fry 1991). For instance, some very detailed studies used this technique to demonstrate mercury biomagnification across the food web of tropical lakes (Campbell *et al.* 2003a; Kidd *et al.*, 2003; Campbell *et al.* 2008; Poste *et al.* 2008).

Since fish is the most important source of MeHg for man (Fitzgerald & Clarkson 1991), the studies on food chains ending with fish are also useful from a practical point of view. In fact, if we know the human diet and the mercury concentration in the successive levels of the food chain, the amount of mercury taken up daily by man can be predicted. Even though our data are not sufficient to build up a trophic food chain, we found clear evidence of biomagnification from the lower to the upper trophic levels. There is a clear increase from the 1st level, represented by macrophytes (mean concentration = 28.9 µg T-Hg kg^{-1} d.w.) to the 2nd level, represented by Unio (37.9 µg T-Hg kg^{-1} d.w.) and net-zooplankton (77 µg kg^{-1}), and to the 3rd level, represented by young cat-fish (143 µg kg^{-1} d.w.). The different concentrations found in *Unio* and zooplankton are probably due to differences in their diet: while the diet of zooplankton essentially consists of phytoplankton and seston, mussels mainly feed on phytoplankton and

sediment organic particles. Unfortunately, only T-Hg was measured in the cat-fish, but since organic mercury in fish is generally higher than 95% (e.g., Bloom 1992), we can reasonably assume that the percentage of MeHg in the cat-fish would be higher than that calculated in other compartments.

5. CONCLUSIONS

The level of mercury contamination in Lake Candia is fairly low. The highest value, which was measured in young cat-fish (143 µg kg^{-1} d.w.), is 1.4 times lower than the threshold recommended by W.H.O. (1990; 200 µg kg^{-1}) for this fish, which represents the sole source of protein for some populations; for example, fishermen living in some Pacific islands.

Data on environments with a low mercury concentration, such as Lake Candia, can be used to establish a background value which is particularly useful for evaluating the level of pollution in other environments.

Useful indicators of mercury contamination are fish, mussel (*Unio pictorum mancus*) and net-zooplankton; fish, for their capacity to accumulate mercury in their tissues without any apparent damage, the bivalve for its sedentary behaviour, while the zooplankton's short life span makes it a useful indicator of the daily pollution level. Conversely, macrophytes cannot be recommended as a useful indicator because of their low capacity to accumulate mercury and its organic forms.

To assess the actual importance of mercury accumulation in the various compartments of the ecosystem, the metal content in each compartment, in addition to the metal concentration, should be estimated. This would provide useful information on the dynamics of mercury in the environment and on the relative impact of each community compartment on the metal biogeochemical cycle.

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