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Copper and zinc in four freshwater fish species from Lake Pamvotis (Greece)

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Abstract

Lake ecosystems are vulnerable to heavy-metal pollution. Fish samples are considered as one of the most indicative factors, in freshwater systems, for the estimation of trace metals pollution potential. Lake Pamvotis (NW Greece) is a typical Mediterranean ecosystem of great importance in regard to biodiversity and to aesthetic value. The fish species found most commonly in the lake are *Cyprinus carpio*, *Silurus aristotelis*, *Rutilus ylikiensis*, and *Carassius gibelio*. The aim of the present study is to evaluate the level of contamination of two essential heavy metals (copper and zinc) appearing at high concentrations in lake water in the above four fish species. Metal concentrations were measured by atomic absorption spectroscopy in three different tissues (muscle, liver, gonads) in order to assess the fish contamination. A two-factor analysis of variance, based on the procedure of general linear models, was employed in which fish species (four levels) and fish tissue (three levels) were examined for potential influence on Cu and Zn concentrations. Differences between level means per factor were treated using Tukey's multiple comparisons of means.

The study showed that *C. carpio* and *R. ylikiensis* presented the highest metal content. Tissues analysis revealed that liver and gonads accumulated the highest levels of Cu and Zn. Metal concentration in the edible part of the examined fish (muscle) were in the safety-permissible levels for human consumption.

Keywords: Heavy metals; Fish; Lake Pamvotis; Contamination

1. Introduction

The concern on the effects of anthropogenic pollution of freshwater ecosystems is growing. Heavy metals from natural and anthropogenic sources are continually released into aquatic ecosystems, and they are a serious threat because of their toxicity, long persistence, bioaccumulation, and biomagnification in the food chain (Eisler, 1988). Fish samples are considered as one of the most indicative factors, in freshwater systems, for the estimation of trace metals pollution potential (Barak and Mason, 1990; Evans et al., 1993; Rashed, 2001). Lake Pamvotis (NW Greece) (Fig. 1) is a typical Mediterranean ecosystem of great importance in regard to biodiversity and to aesthetic value. Due to its morphological and climatological characteristics, Lake Pamvotis is a suitable habitat for a variety of species, especially fish of both conservation and commercial importance (Economidis, 1991). The fish species found most commonly in the lake are *Cyprinus carpio*, *Silurus aristotelis*, *Rutilus ylikiensis*, and *Carassius gibelio*. *C. carpio* (Linnaeus, 1758) locally named 'cyprinos,' is considered as native in South-East Greece (Thessaly, Macedonia, and Thrace) (Economidis, 1991). *C. carpio* was introduced in Pamvotis lake in the 1920s.

C. gibelio (Bloch, 1782) locally named 'petalouda' was introduced in Pamvotis lake during the 1980s. *S. aristotelis* (Agassiz, 1856) locally named 'glanidi' (Aristotle's catfish) is an endemic Greek species found in the Acheloos River system (Trichonis, Lyssimachia, Ozeros Amvrakia lakes, and Acheloos River) (Economidis, 1991). There is a historical interest about *S. aristotelis* because Aristotelis (384–322 B.C.) described its spawning behavior.

R. ylikiensis (Stephaniids, 1939) locally named 'dromitsa' (Ylikiensis roach) is an endemic Greek species

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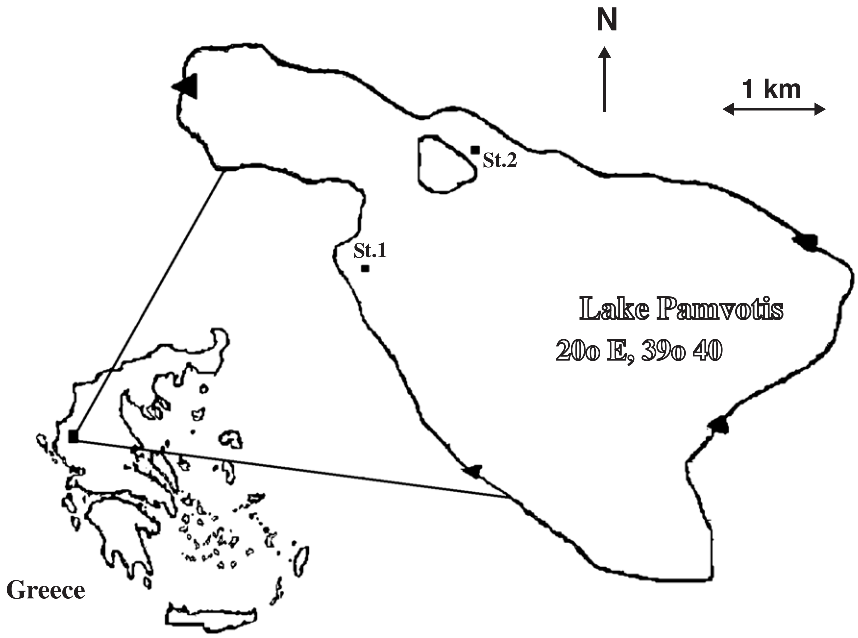

Fig. 1. Lake Pamvotis (Greece) map, sampling stations, and main point-pollution sources (triangles).

distributed in Central and West Greece (Yliki, Paralimni lakes in Boeotis, Trichonis, Lyssimachia, Ozeros, Amvrakia lakes, and Acheloos River) (Economidis, 1991). The above two species were introduced from Trichonis lake to Pamvotis lake in the 1950s. These species are considered as "data deficient" in the IUCN Red List of the World Conservation Monitoring Centre.

Although there have been monitoring studies of the water quality in Lake Pamvotis (Albanis et al., 1986; Stalikas et al., 1994; Kagalou et al., 2001; Papagiannis et al., 2002), only limited efforts to monitor contaminant burdens in fish fauna have been undertaken (Kalfakakou and Kallistratos 1987). According to Kalfakakou and Kallistratos (1987), the main sources of metal pollution in Lake Pamvotis are the following: garages, motor workshops, car washing stations, and silver crafting. All these sources release various metals such as Pb, Cu, Zn, and Fe and, through the rainwash drainage net system, move to the lake. Moreover, effluents from animal farms containing fertilizers and pesticides reach the lake, enhancing the lake's metal burden (Albanis et al., 1986; Kalfakakou and Kallistratos, 1987).

The aim of the present study is to evaluate the level of contamination of two essential heavy metals (copper and zinc) appearing at high concentrations in the lake water (Kalfakakou and Kallistratos, 1987; Papagiannis et al., 2002) in these four fish species which appears to have great economic and ecological importance in W Greece. Populations of *S. aristotelis*, *R. ylikiensis*, and *C. carpio* are currently in rapid decline because of environmental degradation due to human activities, which is intensified by the characteristic irregularity of the Mediterranean climate. Information on contaminant levels in Greek freshwater fish is rather scarce. Especially for Lake Pamvotis, previous data are extremely limited. Our preliminary results provide information for the background levels of metals in common fish species of the lake, contributing to the effective monitoring of both environmental quality and the health of the organisms inhabiting the lake ecosystem.

2. Materials and methods

2.1. Sample collection

Ten specimens of each of the following fish species (*C. carpio*, *C. gibelio*, *S. aristotelis*, and *R. ylikiensis*) were caught during two surveys (March–April 2001), from two sampling locations (Fig. 1).The sampling stations were chosen according our previous study concerning water-quality monitoring (Papagiannis et al., 2002). The fish were then transferred to the laboratory where we recorded their sex, age, total body length, and total wet weight (Table 1). The sex of all specimens was recorded by microscopic examination of the gonads. The ages of *R. ylikiensis*, *C. carpio*, and *C. gibelio* were determined from scales, which were removed from the left side between the posterior end of the pectoral fin and the anterior end of the dorsal fin. The age of *S. aristotelis* was determined from the otoliths which were removed from the inner ear of the fish.

Accurately weighed (0.8–1.3 g) subsamples of muscle (edible part), gonads, and liver were freeze-dried and homogenized before analysis (Miao et al., 2001).Then, they were digested with 3 ml HNO_3 (65% Suprapur) in Teflon bombs and were put into a microwave digestion system (Kingston and Jassie, 1988). After cooling and transfer into a 20-ml

Table 1
Some morphometric and biological characteristics of four freshwater fish species from Lake Pamvotis

Species	Weight range (mean) (g)	Length range (mean) (cm)	Age (years)	Number of individuals	Male/female
Cyprinus carpio	350–450 (400)	15–30 (25)	2–3+	10	5♂/5♀
Silurus aristotelis	100–150 (125)	10–22 (18)	4–5+	10	6♂/4♀
Carassius gibelio	250–350 (300)	14–25 (20)	1–3+	10	3♂/7♀
Rutilus ylikiensis	5–10 (7.5)	5–9 (7)	1+	10	5♂/5♀

polypropylene volumetric flask and rinsed with double-distilled water, samples underwent analysis of copper and zinc.

2.2. Analytical procedure

Metal concentrations (μg/g dry weight) were measured by atomic absorption spectroscopy using a Perkin Elmer spectrometer, model 560, equipped with graphite furnace HGA-400 and background correction. The quality assurance of the results was controlled by using reference material CRM-278 Mussel tissues. Ten replicates of one sample (muscle) were run and the following coefficients of variation were found: Cu, 1.9%, and Zn, 2.1%.

2.3. Statistical analysis

A two-factor analysis of variance, based on the procedure of general linear models, was employed in which fish species (four levels) and fish tissue (three levels) were examined for potential influence on Cu and Zn concentrations. Differences between level means per factor were treated using Tukey's multiple comparisons of means. Prior to statistical analysis on main effects and their interaction, normality and homoscedasticity of residuals were tested, and they were found to be statistically valid only after logarithmic transformation of the initial data (Ter Braak, 1987).

3. Results and discussion

Concentrations of heavy metals detected in the muscle, gonad, and liver samples are shown in Figs. 2 and 3. Both Zn and Cu concentrations between fish species were statistically significant ($p < 0.0001$, $df = 3$) (Figs. 4a and 5a), and also, the statistic comparison among fish tissues showed great significant ($p < 0.0001$, $df = 2$) (Figs. 4b and 5b). Tukey's multiple comparisons between mean metal concentrations per factor (specie or tissue) showed the following order (values in brackets are in antilog form). Concerning Cu concentrations for fish species: *C. carpio* = *R. ylikiensis* > *C. gibelio* = *S. aristotelis* (3.08 = 2.63 > 1.51 = 1.19) and for tissues: liver > gonads > muscles (4.42 > 2.74 > 0.61). The corresponding pattern for Zn mean concentrations was *R. ylikiensis* > *C. carpio* = *C. gibelio* > *S. aristotelis* (95.39 > 52.81 = 46.21 > 18.09) and for tissues: liver > gonads > muscles (107.92 > 51.43 > 16.75). It appears that concentrations of both metals behave in a similar way in the examined tissues. Concentrations were high in liver than in any other tissues, and lowest in the muscle (Figs. 4b and 5b). Liver tissue displayed 1.6 to 2 times higher metal concentrations than those presented in gonads and more than 6–7 times from those in muscles. *C. carpio* and *R. ylikiensis* concentrate equally higher mean copper quantities compared to those of *C. gibelio* and *S. aristotelis* (Fig. 4a). Higher mean zinc concentrations were found in *R. ylikiensis* than in *C. carpio* and *C. gibelio* and the lowest concentration detected in *S. aristotelis* (Fig. 5a).

Cu and Zn are essential elements and are carefully regulated by physiological mechanisms in most organisms (Eisler, 1988). However, they are regarded as potential hazards that can endanger both animal and human health. Knowledge of their concentrations in fish is therefore important both with respect to nature management and human consumption of fish (Amundsen et al., 1997).

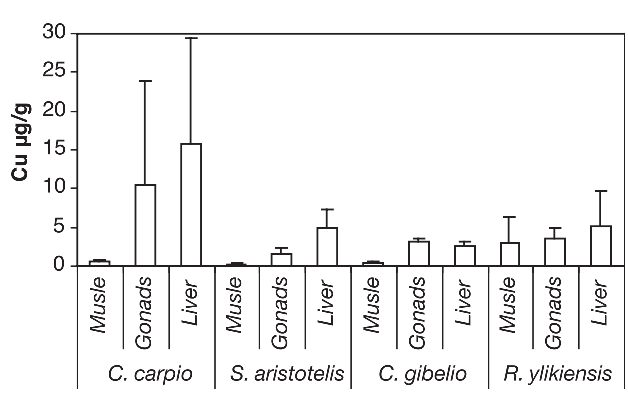

Fig. 2. Mean Cu concentrations (μg/g dry weight) in different tissues of four freshwater fish species from Lake Pamvotis.

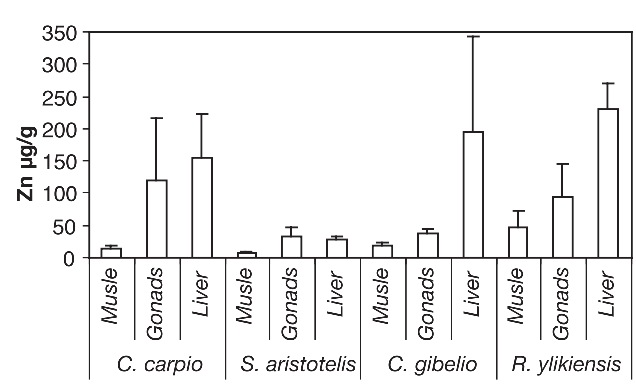

Fig. 3. Mean Zn concentrations (μg/g dry weight) in different tissues of four freshwater fish species from Lake Pamvotis.

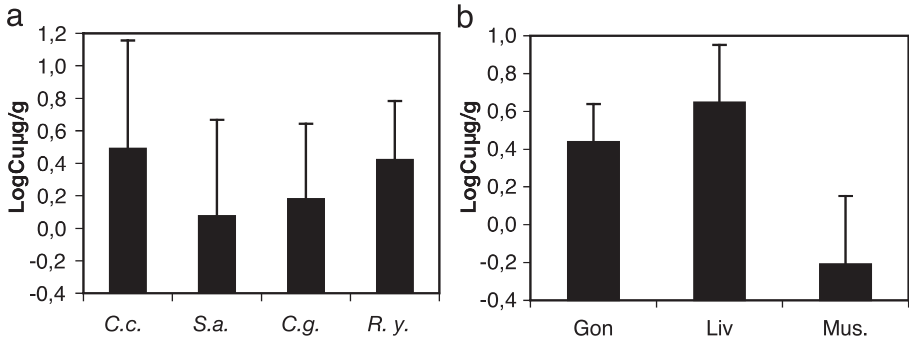

Fig. 4. (a) Log_{10} mean Cu concentrations (μg/g dry weight) in four freshwater fish species from Lake Pamvotis. (b) Log_{10} mean Cu concentrations (μg/g dry weight) in different tissues of four freshwater fish species from Lake Pamvotis.

It has been demonstrated that bioavailability and toxicity of Zn and Cu in aquatic organisms depend on the total concentration of each metal in the water (Chen et al., 2000; Rashed, 2001). Mean water concentrations of dissolved Cu and Zn in Lake Pamvotis (0.12–0.14 μg/l for Cu and 1.21–1.24 μg/l for Zn; Papagiannis and Kagalou, 2001) are close to the upper recommended limits suggested by EEC guidelines (EEC, 46399/86) for surface freshwater quality for cyprinids.

According to previous studies concerning water metal concentrations in Lake Pamvotis and its fish species, assessing the bioaccumulation factor (BAF) of Zn, Cu, and Pb, it indicates that the bioaccumulation occurred through water to muscle and liver, since the BAF factors for Zn and Cu were greater than 1 (Kalfakakou and Kallistratos, 1987; Papagiannis et al., 2002). Higher Zn bioaccumulation was revealed for *R. ylikiensis* and the lowest for *S. aristotelis*. Regarding Cu bioaccumulation (Papagiannis et al., 2002), higher BAF was found for *C. carpio* and the lowest for *S. aristotelis*, reflecting the differences in metal content between the species. One possible explanation for the higher metal concentrations displayed in *R. ylikiensis* is that these individuals were the youngest among the species (Table 1) appearing with higher growth and metabolic rates (Leonardos et al., 2003), while *S. aristotelis* specimens were the oldest. Negative correlation between fish age and metal concentrations in fish tissues was reported in the literature (Allen-Gil and Martynov, 1995). The lack of similar data does not permit us to attribute the observed differences of metal accumulation among these fish species. More comparative data are required in order to explain age-dependent metal accumulation in fish species.

Dietary habits may also have an impact on metal concentrations in different species. Previous studies have suggested that Zn may be biomagnified in a variety of aquatic food webs and lake types (Chen et al., 2000). Strong relationships were also found between Zn concentrations in zooplankton and fish (Spry et al., 1988). In our study, fish feeding mostly on invertebrates, such as *R. ylikiensis*, display higher concentrations of Zn than omnivores (*C. carpio*, *C. gibelio*) and piscivores (*S. aristotelis*). It seems that Zn concentrations is inversely related to the trophic status of the fish. These results are in agreement with those of Amundsen et al. (1997) regarding trophic status and heavy-metal contamination in different freshwater fish species. Although there is no clear evidence about Cu dietary transfer (Eisler, 1988), many studies have demonstrated that diet is the most important route of copper accumulation in aquatic animals, and food choice influences body burden of copper (Sindayigaya et al., 1994; Fisher and Reinfelder, 1995). According to Eisler (1988), little or no biomagnification of copper is evident in freshwater food chains;

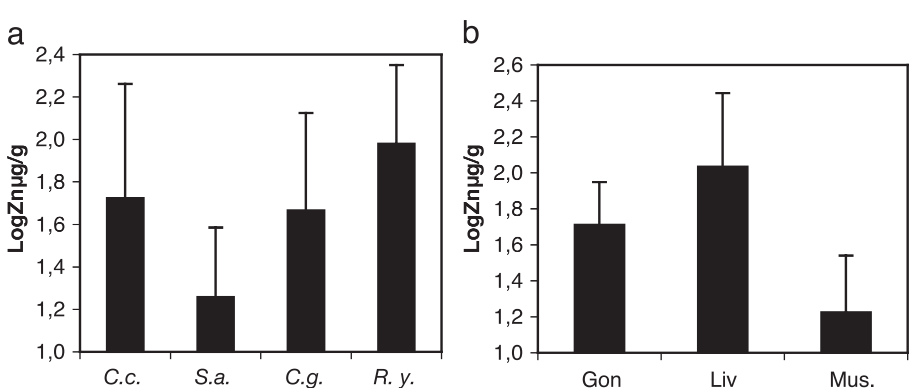

Fig. 5. (a) Log_{10} mean Zn concentrations (μg/g dry weight) in four freshwater fish species from Lake Pamvotis. (b) Log_{10} mean Zn concentrations (μg/g dry weight) in different tissues of four freshwater fish species from Lake Pamvotis.

therefore, species such as *S. aristotelis* which are mostly predators appear the lowest in Cu content. However, the observed variability of metal concentrations in these fish must be seen in the wide perspective of other variables such as habitat, seasonal variations, and individual affinity for metal uptake. Differences in life history patterns that influence exposure (including trophic levels and geographic distribution of life stages) are additional possible explanations for the variation observed in metal concentration between different fish species (Allen-Gil and Martynov, 1995).

Higher metal concentrations were found in liver tissue, while the lowest were detected in muscle tissues. This finding is in agreement with those of other studies regarding the differences between heavy-metal accumulation in fish tissues (Carpene and Vasak, 1989; Allen-Gil and Martynov, 1995). Bioaccumulation in liver was observed for most metals (Sims and Presley, 1976; Sindayigaya et al., 1994), since the liver is the major organ involved in xenobiotic metabolism in fish (Romeo et al., 1994). Organisms retain both metals, Cu and Zn, through specific binding proteins known as metallothioneins in their liver (Allen-Gil and Martynov, 1995). Metallothioneins play an important role in metal homeostasis and in protection against heavy-metal toxicity (Olsson et al., 1989). Metallothioneins for lead binding has been discovered in kidney, liver, and brain (Carpene and Vasak, 1989; Beck, 1992). The low concentrations of Cu and Zn in the muscles of the examined fish species may reflect the low levels of these binding proteins in the muscle (Allen-Gil and Martynov, 1995). The gonads showed higher concentrations of Cu and Zn than those in muscles. Sindayigaya et al. (1994) also found higher levels of Cu and Zn in the gonads than in muscles of two fish species in Lake Tanganyika, Burundi. It remains very difficult to compare the metal concentrations even between the same tissues of different species because of the different feeding habits and the differences in the aquatic environments concerning the type and the level of water pollution. Unfortunately, there are no data concerning metal concentration in the fish species found in Lake Pamvotis. The only comparative data are those referred by Bobori (1996) concerning Cu and Zn concentration in perch (*Perca fluviatilis*) in a similar Mediterranean lake in Greece (Lake Koronia). Values for metal concentration in the present study are lower than those referred by Bobori (1996), and this may be partially attributed to the fact that Koronia is a more heavily polluted lake than Lake Pamvotis. (Papagiannis and Kagalou, 2001)

There are no guidelines on acceptable levels of Cu and Zn in the edible parts of fish suggested by EEC or FAO/WHO. Comparisons with the Canadian food standards (Cu: 100 μg/g; Zn: 100 μg/g), Hungarian standards (Cu: 60 μg/g; Zn: 80 μg/g), and Australian accepted limits (Cu: 10 μg/g; Zn: 150 μg/g) demonstrate that there is metal contamination, but it is lower than the guidelines, in the edible part of the examined fish. On the basis of the recommended daily dietary allowances (RDA) for safe consumption of fish muscle, the allowed intake is regulated at 50–350 mg of metal per 100 g serving of the muscle (NRC, 1980). According to our results, the examined fish were not associated with enhanced metal content in their muscle and were safe within the limits for human consumption.

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