TRANSFER FACTORS OF HEAVY METALS IN AQUATIC ORGANISMS OF DIFFERENT TROPHIC LEVELS

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Introduction

Lake ecosystems are in particular vulnerable to heavy metal pollution, and their bio-accumulation as well as bio-magnification are always a threat. Transferfactors (tf), as the quotient of:

$$\frac{\text{Concentration of pollutant in an organism of trophic level (X)}}{\text{Concentration of pollutant in an organism of trophic level (X-1)}}$$

This indicates whether heavy metal bio-magnification takes place through the trophic chain. A (tf) greater than 1 underlines bio-accumulation when (X) represents and organism and (X-1) represents the water. A study on the transfer of Cd, Pb, Cu and Zn through the trophic chain of the river Loire estuary ecosystem proved the existence of bio-accumulation, but not bio-magnification ([1]). Copper as an essential trace metal for many systems such as haemocyanin, the respiratory protein of many invertebrates etc., was found in high concentrations but with a very low tf. On the contrary zinc had a tf 1 and possibly was bio-magnified through trophic levels ([1]). Ioannina lake (Pamvotis), a 22.8 square kilometre shallow water basin, is an ecosystem organised in 4 trophic levels. ([2]) Thiobacteria are the main decomposers. Trophic level:

I. consists of producers, represented by emerged and floating macrophytes and planktonic chlorophytes and diatoms ([3]).
II. consists of a-order consumers, represented by vegetarian zooplankton and fish.
III. consists of b-order consumers, such as small fish and insect larvae which feed on zooplankton and fish eggs. In the same level omnivorous organisms, like carp, eel, astacus and molluscs, are included.
IV. consists of humans who are c-order consumers and form the top of the trophic pyramid.

The present work is an investigation of the extent of the environmental pollution from heavy metals on the trophic chain of the lake.

The concentrations of Fe, Zn, Cu, Pb as well as Ca and Mg were measured in water, aquatic plants, fish and lake organisms. Also the transfer factors in the successive trophic levels were estimated and discussed.

Materials And Methods

Materials

A. Lake water
Sixty (60) lake water samples taken from fifteen (15) selected positions in the lake were analysed. These positions in the lake were identified by means of numbered floaters.
From these 15 positions, samples were taken from the surface of the lake during August 1981.
Furthermore 15 surface samples were also collected in Winter 1982 and were analysed only for Pb, with and without extraction. In addition, during Spring 1982, 30 further samples were examined. Fifteen of them were surface samples and fifteen were collected from 2 m depth.

The water sampler was a Lamotte, Model 1060, van Dorn type.

B. Water plants Twenty water plant samples were collected.
P1 sampling during March 1982 included old plant-societies of the genders Ceratophyllum, Myriophyllum and Potamogeton.
Only in one lake position (no 14) were these plant species found. In the rest of the sampling positions no plant societies were present. P2, P3 and P4 samplings were examined in May 1982, in a period where young plant societies (lake positions, Nrs 7, 14, 15), of the above mentioned genders, as well as some Nuphar ones were abundantly found. P5 sampling, during July 1982, included new plant societies of the same type as previously found.

C. Fish and water organisms Fifty six (56) samples were analysed from fish caught during February-March 1982 and November 1982. The species analysed were:
a) Paraphoxinus epiroticus b) Rutilus rutilus
c) Tinca tinca
d) Cyprinus carpio
e) Barbus albanicus
f) Astacus fluviatilis
g) Eggs of Afluviatilis

These are all omnivorous fish. The analysed specimens from the species P. Ipirotikus, R. rutilus, and B. albanicus included muscles and bones, just as they are consumed.

Methods

The samples were measured by atomic absorption spectrometry (AAS) using Perkin Elmer atomic absorption spectrometers, Models 560 and 3030 for flame measurements and for graphite furnace measurements, a system consisting of a Perkin-Elmer 272 spectrometer, HGA 400 furnace, As 1 autosampler and a model 56 recorder.

A. Water analysis
The water samples were either
a) filtered through Gelman (GN-6, 0.45 m) filters and acidified to pH 2 with NHO3, or
b) after extraction in methyl-isobutyl-ketone (MIKB) (4).
Standard solutions for Ca, Mg, Fe, Zn, Cu, and Pb were prepared according to analyctical methods for AAS.
Standard solutions for Ca and Mg were diluted in 5% LaCl3 solution and HCl to 0.25% (w/v) La concentration.

B. B. Plant and animal tissues analysis
Tissues were first dried then ashed at high temperature (plant tissues between 475-500 degree Celsius for 2-4 hours and animal tissues at 525 degree Celsius for 2 hours). The ash was then dissolved in 5 ml 20% NHO3 plus 0.6 ml HCL and diluted to 25 ml final volume.
Ca and Mg were determined by flame AAS after dilution with lanthanum chloride. Zn, Cu and Fe were determined directly by flame AAS.
Lead was determined by first extracting with ammonium pyrrolider dithiocarbamate into methyl-isobytul-ketone and then analysing the extracts by graphite furnace AAS.

Results And Discussion

Tables 1, 2 and 3 give the concentrations of the six metals Ca, Mg, Fe, Zn, Cu, Pb in surface water samples collected from 15 sites on Ioannina lake. The following observations can be made:

Table 1
Concentration of metals in lake water. Surface samples. Autumn 1981. Spring 1982.

Samp. Posit.	Ca μg/ml		Mg μg/ml		Fe μg/ml		Zn μg/ml		Cu μg/ml		Pb μg/ml	
	A	S	A	S	A	S	A	S	A	S	A	S
$\bar{x}$		20.94	7.68	3.97	0.0	0.013	0.09	0.0	0.0	0.02	0.2	0.12

Table 2
Concentration of metals in lake water. Surface and 2 m deep samples. Spring 1982.

Sampl. posit.	Ca μg/ml		Mg μg/ml		Fe μg/ml		Zn μg/ml		Cu μg/ml		Pb μg/ml	
	0m	2m	0m	2m	0m	2m	0m	2m	0m	2m	0m	2m
$\bar{x}$	20.86		3.96		0.019		0.00		0.02		0.13	

Table 3
Lead concentration in lake water. Surface samples. January 1982.

Filtration conc-μg/ml	Extraction conc-μg/ml
15 0.2	15 0.2
S.Conc-20μg/ml Abc.0.179	S.MIBK:conc 20μg/ml Abc: 0.635

Table 4

Concentration in μg/g of metals, in aquatic plants.

Sampling dates: P1= 5/3/83 P2, P3, P4= 5/5/82 P5= 3/7/82

Samples: Plant societies consisting of Ceratophyllum, Myriophyllum, Nuphar, Potamogeton.

Metal / Sample	Ca(mg/g)	Mg(mg/g)	Fe(mg/g)	Zn(mg/g)	Ca(μg/g)	Pb(μg/g)
$\bar{X}$	301.8	16.38	5.08	0.06	19.2	7.4

Table 6

Concentration in μg/g of metals in fish: Cyprinus carpio, Barbus albanicus, Astacus fluviatilis. Eggs of A. fluviatilis.

Metal / Sample	Ca(mg/g)	Mg(mg/g)	Fe(mg/g)	Zn(mg/g)	Cu(μg/g)	Pb(μg/g)
	1. Cyprinus carpio					
15.2.82	200	15.3	0.21	0.80	27	2
5.3.82	52	14.8	1.50	0.90	116	3
$\bar{X}$	126	15.05	0.85	0.85	71.5	2.5
	2. Barbus albanicus					
5.3.82	$\bar{X}$:246	13.9	0.26	0.80	31	1
	3. Astacus fluviatilis					
5.3.82	$\bar{X}$:161	15.0	0.95	2.25	207	22
	Eggs Astacus fluviatilis					
5.3.82	$\bar{X}$: 23	20.6	1.91	1.16	1.26	37

Table 5
Transfer factors

ftCa = 14,467
ftMg = 4,136
ftFe = 267,368
ftZn = 20,000
ftCu = 1,200
ftPb = 56.92

Table 7
Transfer factors

ftCa = 0.41
ftMg = 0.91
ftFe = 0.15
ftZn = 14.16
ftCu = 3.72
ftPb = 0.33

Table 8
Transfer factors

ftCa = 0.81
ftMg = 0.84
ftFe = 0.05
ftZn = 13.3
ftCu = 1.6
ftPb = 0.13

Table 9
Transfer factors

ftCa = 0.53
ftMg = 0.91
ftFe = 0.18
ftZn = 37.16
ftCu = 10.78
ftPb = 2.97

1. The concentrations of the materials Cu, Fe and Zn in the lake water are very low, almost negligible.
2. There are slight chances of the concentration of Ca of various samples ranging from 19.2 to 24.7 ìg/ml for magnesium respectively.
3. There is a decrease in the concentration of Mg and Pb between autumn and spring, because the lake water is also used for irrigation purposes during the dry period of the year (April-September) and also because of the excessive evaporation from the surface of the lake during the summer period. Conversely during the winter a dilution occurs through rainfall.
4. There is no statistically significant difference in trace element concentration between water samples from 0m and 2m depths.

The calcium and magnesium concentrations in the lake water are probably derived from the geological basement of the basin which consists of lime and dolomite as well as from marble industries cited in this areas. These two elements regulate the hardness of the lake water which varies between 140-150 ìg/ml CaCo3. It is also known that natural water possessing a hardness of 20-125 ìg/ml acts protectively towards heavy metal accumulation ([6,7,8,9]).

Lead shows a steady concentration of 0.2 ìg/ml during autumn in all analysed sampling positions of the lake. However during spring it varies considerably ranging from 0.0 to 0.3 ìg/ml Pb. The Pb concentrations are 10 times higher than the permitted limits for natural waters. It must be pointed out that there is no statistical difference between the two techniques, filtration and extraction, used for Pb-analysis (Table 3). Filtration, as the less time-consuming method, was followed in this work. The main sources for metal pollution of Ioannina lake are the following:

1. Garages, motor workshops and car washing stations, are activities releasing mainly Pb but also Cu, Fe, Zn ([11, 12, 13, 14]). It is characteristic that people occupational involved in such activities present elevated Pb blood concentration ([15]).
2. Traffic statistics revealed that 10.000 cars and 3.000 motorbikes constantly circulate in the city while 600 vehicles/day pass through, as Ioannina is a major crossing place in western Greece. The deposition of Pb is a common problem in roads with heavy traffic and particles floating in the air over 250 m of diameter are carrying almost 50% of metal pollutants ([14, 16]).
   In Ioannina the rainwash drainage net system of the roads terminates through 14 ducts to the lake. It carries away pollutants like Pb, Hg, Zn and other toxic metals coming from fuels, coal, old house paint and roofs. Corrosive acid rains may dissolve heavy metals through contact with metallic surfaces. These toxic solutions end in the lake thus contributing to its pollution.
3. A municipal sewage system is under construction and so far septic tanks were the only solution.
   Illegal connection of septic tanks to the rainwash net system leads metals into the lake. Organisms experimentally brought up in sewage whit metal amounts smaller than 0.1 ppm produced remarkable metal accumulation ([17]).
   Another source of polluting metals is the piping system used for water supply and drainage purposes. This is mainly constructed of lead tubes containing also traces of Cu and Zn. Soft acid water and lead piping is a dangerous combination for human health ([16, 18]).
   It is estimated that nearly 175 Kg of metals reach the lake in a year, through all the above mentioned sources.
4. Effluents from animal farms also reach the lake. Pig and poultry farms are well developed in the area and it is known that their discharges contain trace metals from the food and the use of disinfectants ([9, 19, 20]).
5. Furthermore fertilisers and biocides in use in over 66,000,000 square kilometres of arable land increase the metal burden of the basin. The surface land formations are mainly argilic with an increased retaining ability for metal ions. Also, the solubility of the metals is decreased and thus their absorption from plants is minimised ([17]).

The heavy rains and the high rainfall levels (1262 mm/year) of the area contribute to the carrying away of the surface particles to the lake. Plants are the producers of the tropic chain and metals found in them are either absorbed from bottom sediments or are deposited on their shoots and leaves. Samples were withdrawn from plant masses of all species mentioned above (Table 4).

Higher amounts of Ca were detected in plants collected during May 1982 (P 2-4) compared to those samples of March and July 1982 (P5). On the contrary the old plant colonies (P1) exposed for longer time to bio-accumulation revealed higher concentration of heavy metals than was expected. The question is why calcium in P1 sampling does not follow the tendency for increased concentrations found for the other metals (Mg, Fe, Zn, Cu, Pb).

Samples of P5 were exposed to bio-accumulation for 2-3 months (May-July). In comparison to P2, P3, P4, plant samples which were exposed for 1 month (April) revealed a paradoxical behaviour of calcium. This alkaline earth metal shows its highest concentration in younger colonies (P2, P3, P4) varying from 377-392 mg/g Ca which is decreased after 3 months to 137 mg/g and stabilised after 1 year at 223 mg/g Ca.

The other metals Mg, Fe, Cu, Zn, Pb show a gradual increase in their concentration in plants in relation to the time of exposure. Transfer factor (Table 5) estimated for spring season water samples and plant specimens are all greater than 1. This is proof of metal bio-accumulation from level I to level II. Also the high transfer factors confirm that lake sediments must be seriously polluted. Sediment samples seem to be abiotic in many sites of the lake but this observation must be confirmed by future investigation.

Six species of class b consumers (see materials) were analysed. These species are the domination ones among the animal populations of the lake. The lake at present is considered as an ecologically undergrated system, a fact proved by

a. the reduced productivity
b. the limitation of species diversion and
c. the elimination of species like trout and eel.

Table 10
Transfer factors

Table 12
Transfer factors

Table 14
Transfer factors

Table 16
Transfer factors

ftCa = 0.07	ftCa = 0.64	ftCa = 0.78	ftCa = 0.86
ftMg = 1.25	ftMg = 0.96	ftMg = 0.69	ftMg = 0.71
ftFe = 0.37	ftFe = 0.15	ftFe = 0.13	ftFe = 0.07
ftZn = 19.3	ftZn = 10.5	ftZn = 21.3	ftZn = 9.66
ftCu = 0.06	ftCu = 2.13	ftCu = 2.55	ftCu = 1.33
ftPb = 5	ftPb = 0.40	ftPb = 0.31	ftPb = 0.31

Table 11

Concentration in ìg/g of metals, in Tinca tinga

4. Tinca tinca

Metal / Sample	Ca(mg/g)	Mg(mg/g)	Fe(mg/g)	Zn(mg/g)	Cu(μg/g)	Pb(μg/g)
15.2.82	255	12.5	0.48	0.68	17	1
5.3.82	167	17.0	1.14	0.59	52	2
10.11.82	163	18.0	0.81	0.63	54	6
$\bar{x}$	195	15.83	0.81	0.63	41	3

Table 13

Concentration in ìg/g of metals, in Paraphoxinus epiroticus.

5. Paraphoxinus epiroticus

Metal / Sample	Ca(mg/g)	Mg(mg/g)	Fe(mg/g)	Zn(mg/g)	Cu(μg/g)	Pb(μg/g)
15.2.82	272	10.2	0.81	1.02	48	2
5.3.82	168	12.0	0.75	1.46	34	1
10.11.82	273	11.9	0.56	1.38	65	4
$\bar{x}$	237.6	11.36	0.70	1.28	49	2.33

Table 15

Concentration in ìg/g of metals, in Rutilus rutilus.

6. Rutilus rutilus

Metal / sample	Ca(mg/g)	Mg(mg/g)	Fe(mg/g)	Zn(mg/g)	Cu(μg/g)	Pb(μg/g)
15.2.82	222	12.9	0.23	0.76	20	1
5.3.82	287	10.3	0.55	0.56	26	2
10.11.82	274	11.7	0.42	0.42	31	4
$\bar{x}$	261	11.63	0.4	0.58	25.66	2.33

Additionally C. carpio has been replaced, by R. rutilus, despite its resistance and adaptation to unfavourable lake conditions. Tables 6, 11, 13, 15, demonstrates that metal concentration in fish is higher than in the water, which indicates the bio-accumulation. Transfer factors from level II to level III (Tables 7, 8, 9, 10, 12, 14, 16) show that Zn and Cu are bio-magnified in all species investigated. The amounts of lead found in A. fluviatillis and especially in its eggs have exceeded the permitted limits, and have become dangerous for consumption. The high-concentration of lead in eggs of crayfish (A. fluviatilis) may be due to

a. their attachment at the back edge of cephalothorax, very close to the respiratory apparatus (gills) where a large volume of water is filtered and
b. the fact that crayfish is mainly a benthic organism i.e. the adobement side of the body crawls on the lake bottom where most metals have settled.

T. tinca, a vegetarian fish, reveals the highest concentration Mg and Pb (Table 11). A fluviatillis is followed by C. carpio and P. epiroticus in magnitude of Cu and Zn concentrations.

It is remarkable that the Pb concentrations in T. tinca, P. epiroticus and R. rutilus in autumn sampling exceeded the permitted limits while in spring season sampling metal concentrations were within limits (Tables 11, 13, 15).

In conclusion, the metals Ca, Mg, Fe, Cu, Zn in lake water are within permitted levels, except Pb which is 10 times higher.

All metals exhibit bio-accumulation, because their concentration in all trophic levels is higher than those of the water, but within permitted limits, except Pb which is 1-18,5 x higher.

Transfer factors, for Zn and Cu, between tropic levels I-III, are greater than 1 thus the bio-magnification of the 2 metals has been established in the ecosystem of the lake.

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