

# PIXE Monitored Laboratory Feeding Test for Bioaccumulation of Copper

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**Abstract:** In the last decades several natural disasters have resulted from heavy metal pollution all over the world, and also in Hungary. Increasing quantities of flashy runoff from urban, industrial, and road surfaces that are being caused by climate changes have amplified this problem worldwide by now, also heavy metals are considered to be the most dangerous inorganic micro-pollutants. Recently, several studies have dealt with the environmental monitoring of these elements, and some of them pointed out that living organs like plants, shell, fish can be suitable candidates for this purpose as they accumulate heavy-metal pollutants in themselves. In the recent study silver crucian (*Carassius auratus*) was used as model specie. The fishes were fed with Cu enriched nutrient. The amount of copper in the fish food was 500 mg/kg. Feeding times of 7, 14, 21 and 28 days were applied. At the end of the feeding period fish was sacrificed, then dried, mashed and pill samples were prepared. Copper concentration in the pills was measured by PIXE (particle induced x-ray emission) method in the ATOMKI (Institute of Nuclear Research of the Hungarian Academy of Sciences, Debrecen, Hungary). On the basis of the results it can be concluded that the concentration of copper in the samples can be measured by PIXE. It was also proven that the Cu concentration in the pills is proportional with the amount of copper that the fish consumed during the experiment. Consequently, these fish are suitable indicator organisms for heavy metal pollution.

**Key words:** Heavy metal, PIXE, copper exposition, climate change, nonpoint source pollution.

## 1. Introduction

Heavy metals are natural ingredients of our environment. However, their secretion in the metabolic pathways is very limited hence we must face a continuous increase of heavy metal load of anthropogenic origin, also amplified by the extra quantities and intensities of flood and runoff waters as presently being caused by the changing climate [1]. This represents a serious ganger for the flora and the fauna [2-5]. Heavy metal contamination of watercourses can be established by investigating living organisms. Using appropriate model species one can determine the heavy metal accumulation proportional

to the degree of loading and time of exposition. According to the Water Framework Directive of the European Union (DIRECTIVE 2000/60/EC) ecological quality of surface and underground water sources should be judged on the basis of status of organisms living in water.

Hence search for model species capable to signal heavy metal contamination is imminent.

Fish species are good candidates for this role as heavy metals are accumulated in their different and well separable organs. Chemical analysis of the metal content of these organs can show the ways of heavy metal exposition [6].

Fishes are at the highest level of trophic food chain so they have a high bio-concentration factor. Their

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relatively long life cycle makes them ideal candidates to monitor pollution factors of their living environment for long period of time [7]. Their pollutant content can be determined provided heavy metal loading propagating in the food chain reaches the trophic level. However, metal concentration in fish organs depends on several other factors [4, 8]. The processes of uptake, storage and secretion may have significant role in addition to the half life of the metal content in living organisms. The size and age of the fish may also have a substantial effect [9-11]. The most important effect of heavy metals on the flora and fauna is that living organisms can not get rid of them via metabolic pathways so they will be accumulated in the bodies. Moreover, it is also important fact that heavy metal pollutants can not degrade through natural processes in the soil or in the water either [12].

## 2. Methods and Data

Feeding experiments were performed in four periods of 7, 14, 21 and 28 days, respectively in the Fish' Laboratory at the University of Kaposvár, Hungary between January 30 and February 27, 2006. The main purpose of our experiments was to monitor the copper content in fishes under different experimental conditions and exposition time. Copper concentrations in the fish pills were measured by PIXE (particle induced x-ray emission) method in the Institute of Nuclear Research of the Hungarian Academy of Sciences, Debrecen, Hungary.

Copper is an essential microelement generally found in living organisms as part of essential enzymes. However, it is also an important pollutant as high amount of copper can get into the biosphere by anthropogenic effects spread by water and air, the basic transport routes of non-point source pollution [13]. Penetrating into the living organisms and by accumulating over a threshold limit it is a toxic element. Physical and chemical characteristics of the water environment highly influence the toxicity of copper on fishes. In general, the narrower the optimal

effect-range of any chemical and higher its enrichment the more pronounced its deterioration effect is. So for essential heavy metals there are deficiency, optimal, tolerable and lethal concentration levels.

A common characteristic of all toxicology tests is that lethality ratio of the living organism is investigated. However, the toxicology test has a negative result when no death of the organism is observed. Nevertheless, all living organism has a tolerance spectrum in which region they do not die because of the pollutant.

For a long time well tolerable copper content range for fishes is somewhere in the range of 0.001-0.01 mg/L, depending on physical and chemical characteristics of water and sensitivity of the fish species. The more the carbohydrate in the feed, the more copper is accumulated in the liver. Independently, the highest exposition time of the copper content is in the liver and the lowest are in the brain and the kidney. The average copper content of fishes is around 0.5 mg/kg.

A careful search of the literature revealed only a very few studies on copper poison through feed for both cold and warm water fishes [14].

Our experimental model was silver crucian (*Carassius auratus L.*) purchased from fish farm of Dinnyés.

This species was chosen as model because of its wide tolerance range. So it has rather high immunity for heavy metal loading which was supposed. This assumption was right as no animal was lost during the experiment.

The feed was special crucian nutrient (ALLER SAFIR) with energy content of 17.3 ME MJ/kg (4,145 kcal/kg) while the composition of the dry material was as follows:

- Raw protein: 45%;
- Raw fat: 20%;
- Carbohydrate: 16%;
- Ash: 8%;
- Cellulose: 2%;

Phosphorous: 1%.

The copper was administered and mixed into the feed as  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  and 1 kg of feed contained 500 mg copper (500 ppm Cu). During the experiment fishes were kept in separated 16 fish tanks. Feeding frequency was once per day. And 1 hour after feeding when fishes did not eat any more, all remaining feed was removed from the container to prevent the long time dissolution to influence the results. Several parameters for each specimen were monitored including starting weight and length, weight increase, copper consumption and feed consumption. The experiments lasted for 28 days and four specimens were removed every week, put down and stored in refrigerator till processing.

During the processing of the fishes they were dried, powdered and tablets were pressed. Copper content was determined by PIXE method. In this technique, the sample is bombarded with high energy protons. They interact with the electrons of the sample. X-ray photons are emitted when vacancies of a lower shell are filled from the valence shell and their energy is characteristic for the emitting element. The intensity of the X-ray radiation is proportional to its concentration. PIXE station built at the Van de Graaff accelerator of the Institute of Nuclear Research of the Hungarian

Academy of Sciences, Debrecen, Hungary has wide range applications including elemental analysis of biological samples [15, 16].

### 3. Results

Table 1 contains starting and end mass and length, daily weight gain, copper consumption and nutrient consumption for all specimen. Fig. 1 shows the relationship between the Cu content and Cu feed while Fig. 2 contains the dependence of weight gain on the feed consumption. Detailed PIXE data of dried, powdered and pressed samples were collected in Table 2.

### 4. Discussion

According to our results the average copper content of specimen shows a trend for all experimental cycle.

Remaining food was removed from the fish tank after each feeding to prevent dissolving copper influencing the results.

Because of the small size of these fish specimen PIXE method was utilized to determine copper content of tablets pressed from the dried bodies.

**Table 1** Weight, length increase, feed and copper consumption and accumulated copper content of silver crucian specimen.

No. of fish tank	Experimental cycle (days)	Mass (g)	Length (mm)	Weight gain (g)	Daily weight gain (g/day)	Nutrient consumption (g)	Cu consumption (mg)	Cu (ppm)
1	21	36.65	109	5.14	0.245	11.08	5.54	9
2	21	32.73	101	1.6	0.076	11.29	5.625	9
3	28	29.4	98	5.06	0.181	14.1	7.05	9
4	7	25.92	99	1.68	0.24	3.7	1.85	7
5	14	24.03	92	3.78	0.27	7.05	3.525	6
6	28	28.3	95	6.64	0.237	11.72	5.86	11
7	28	24.62	92	5.39	0.193	15.53	7.765	9
8	7	32.39	104	1.4	0.2	4.36	2.18	7
9	14	21.13	88	1.33	0.095	5.43	2.715	10
10	21	27.83	98	3.65	0.174	8.91	4.455	7
11	7	22.32	93	-2.99	-0.427	2.73	1.365	7
12	21	17.16	85	0.6	0.029	5.69	2.845	7
13	7	21.71	90	1.5	0.214	3.52	1.76	6
14	14	25.36	92	2.51	0.179	6.78	3.39	5
15	28	34.97	105	3.36	0.12	9.06	4.53	8
16	14	30.5	102	2.91	0.208	6.68	3.34	9

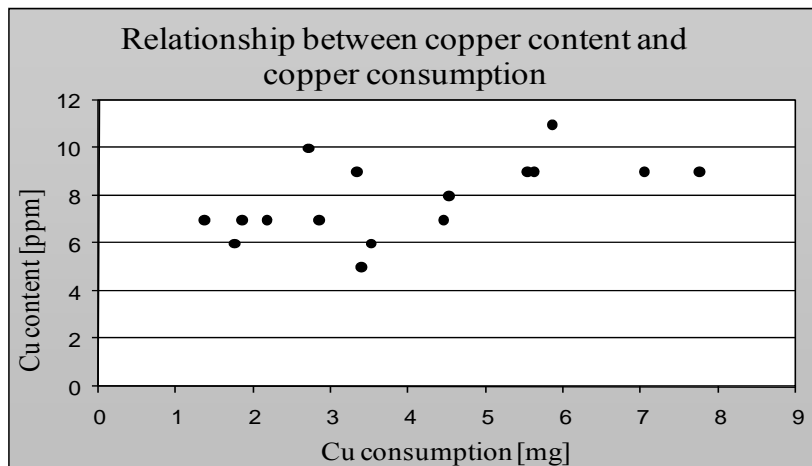
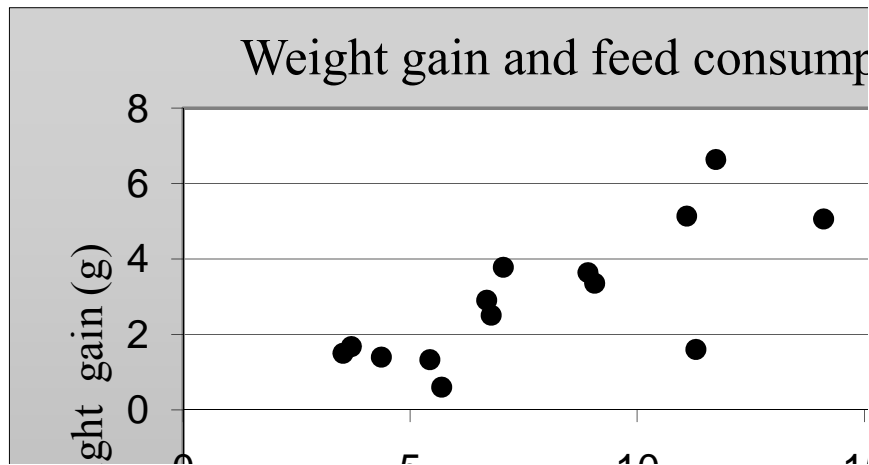


Fig. 1 Relationship between copper content and copper consumption.



**Fig. 2 Relationship between weight gain and feed consumption.****Table 2 PIXE analysis data of samples.**

No. of fish tank	Cycle (day)	Weight gain (g)	Copper consumption (g)	Cu content (ppm)	Average Cu (ppm)
4	7	1.68	1.85	7	
8	7	1.4	2.18	7	6.75
11	7	-2.99	1.365	7	
13	7	1.5	1.76	6	
5	14	3.78	3.525	6	
9	14	1.33	2.715	10	7.50
14	14	2.51	3.39	5	
16	14	2.91	3.34	9	
1	21	5.14	5.54	9	
2	21	1.6	5.625	9	8.00
10	21	3.65	3.65	7	
12	21	0.6	2.845	7	
3	28	5.06	7.05	9	
6	28	6.64	5.86	11	9.25
7	28	5.39	7.765	9	
15	28	3.36	4.53	8	

The highest copper content was measured for specimen 6 which was fed with copper containing nutrient for 28 days. This observation also supports that the longer the exposition the higher the accumulated copper. The main purpose of the study was to detect the effect of copper exposition. The results successfully proved that copper concentration of fish specimen can be detected even at short exposition time and can be measured by PIXE method.

## 5. Conclusions

PIXE is a good analytical method to determine heavy metal content of low concentrations.

The present study is a good example for successful multidisciplinary co-operation.

This type of monitoring work becomes ever more important with the climate change induced drastic increase of diffuse-source water pollution (see downloadable reports and articles of [www.climatewater.org](http://www.climatewater.org)) [17].

The set of counter actions and measures to control diffuse and non point source loads may also be called ecohydrological management [18].

Nevertheless in the case of heavy metals the hydrological part of this planning procedure becomes dominating e.g. to keep pollutants (and water) in the place they occur (for example by the so-called Dutch drains).

Another solution for surface runoff waters in an urban environment may be a series of ponds where (sadly and sorrowfully) the sediments and thus the living organisms, plants and animals, of the first ponds are sacrificed to uptake the metals for the sake of saving the final recipients rivers, lakes and seas [18].

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