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Aldona CIĄGŁO[1], Anna KUCZKOWSKA-KUŹNIAR[1],
Władysław ZAMACHOWSKI[1], Robert STAWARZ[1]
and Grzegorz FORMICKI[1]

ACCUMULATION OF HEAVY METALS AT EARLY STAGES OF *Phrynohyas resinifictrix* (GOELDI) ONTOGENESIS

KUMULACJA METALI CIĘŻKICH WE WCZESNYCH STADIACH ONTOGENEZY *Phrynohyas resinifictrix* (GOELDI)

Abstract: The research focused on an assessment of interrelations between copper, zinc, magnesium and cadmium regarding the rate of their accumulation in *Phrynohyas resinifictrix* organisms. Group A consisted of organisms subjected to the effect of solution containing 2 mg/dm^3 Mg^{2+}, Zn^{2+} and Cd^{2+}, whereas group B to 4 mg/dm^3 Mg^{2+}, Zn^{2+} and 2 mg/dm^3 Cd^{2+}. Significant changes in copper and zinc concentrations were registered during the experiment. Copper content in group A was 5.97 μg/g d.m. after 2 hrs and 367.521 μg/g d.m. after a week ($p = 0.002$). In the group B copper concentration was 12.914 μg/g d.m. after 2 hrs and 234.372 μg/g d.m. ($p = 0.013$) after 2 weeks. Zinc level in group A was 282.779 μg/g d.m. after 2 hrs and 750.051 μg/g d.m. after a week ($p = 0.003$).

Keywords: mineral composition, Cd, Cu, Zn, Mg, amphibian, *Phrynohyas resinifictrix* larvae

Heavy metal concentrations in surface waters may differ greatly and their bioavailability is connected with water chemical composition [1]. Inorganic metal compounds show the strongest toxic properties, since they are readily soluble and strongly dissociating, therefore they easily penetrate through cell membranes. Interrelations between heavy metals, eg Zn-Cu-Cd play a special metabolic role. Mutual biochemical relations between these metals occur both at their natural and toxic contents. Cadmium present in the blood serum and corpuscules dislodges zinc, which is subsequently bound by metallothioneins. This protein serves to maintain zinc homeo-

[1] Department of Vertebrate Zoology and Human Biology, Institute of Biology, Pedagogical University in Kraków, ul. Podbrzezie 3, 31–054 Kraków, Poland, phone: +48 12 662 67 20, fax: +48 12 662 66 82, email: aldonaputala@wp.pl, akucz156@wp.pl

stasis, it is also a factor protecting organism against toxic effect of cadmium. Biochemical mechanisms of cadmium and copper antagonism involve, similar as in the relationship between cadmium and zinc, replacement of copper by cadmium in proteins. The metabolic disturbances connected with low level or lack of copper may be an indirect result of cadmium toxicity.

Material and methods

The experiment focused on the measurement of degree and rate of copper, zinc, magnesium and cadmium cumulation at early stages of *Phrynohyas resinifictrix* ontogenesis. The research was conducted on 105 larvae from the culture initiated in the zoological garden in Plock and continued at the Department of Vertebrate Zoology of the Pedagogical University in Krakow. Analyzed were 105 larvae divided into three groups. The control group consisted of larvae living in a spring water. In group A the tadpoles were placed in a solution containing 2 mg/dm^3 Mg, Zn and Cd and in group B in a solution with 4 mg/dm^3 Mg and Zn and 2 mg/dm^3 Cd. 5 tadpoles were captured from each group after 2 hrs, 4 hrs, 6 hrs, 8 hrs and 16 hrs, after a week and two weeks later. The organisms were killed and dried in a thermostat (at 105 ^{o}C) until their dry mass was established. Subsequently the material was incinerated in a muffle furnace (at 450 ^{o}C), the ash was dissolved in 2 cm^3 of nitric acid(V) in 65 % concentration. When the prepared mixture was poured into volumetric flasks, the samples were filled up with (spectrally pure) distilled water to the volume of 5 cm^3 and thoroughly mixed. The solutions prepared in this way served for an assessment of heavy metal concentrations. The content of cadmium and copper were determined using EA9C electrochemical analyzer, while magnesium and zinc concentrations using BUCK200A atomic absorption spectrophotometer. The data were given in milligrams per a gram of dry mass, and subjected to statistical analysis using Friedman ANOVA and Kruskal-Wallis ANOVA tests.

Results

A comparison of the control groups revealed statistically significant differences only in copper content ($p = 0.022$). For group A statistically significant changes in the contents of analysed metals were registered for copper ($p = 0.002$) and zinc ($p = 0.003$). For group B the only statistically significant differences were noted for copper ($p = 0.013$). Copper content in group A after 2 hrs was 5.97 μg/g d.m. and after 1 week 367.521 μg/g d.m., whereas in group B copper concentrations were 12.914 μg/g d.m. after 2 hrs and 234.372 μg/g d.m. after 2 weeks. Zinc contents in group A were 282.779 μg/g d.m. after 2 hrs and 750.051 μg/g d.m. after 1 week ($p = 0.003$).

Statistical analysis based on Kruskal-Wallis ANOVA test shows statistically significant differences between groups for copper after 4 hrs ($p = 0.031$), 16 hrs ($p = 0.039$), after 1 week ($p = 0.009$) and after 2 weeks ($p = 0.007$). After 4 hrs

the highest copper concentrations were noted in group B and the lowest in the control group. On the other hand, after 16 hrs the highest copper content was registered in group A. The results obtained after 1 and 2 weeks point to the greatest differences in this element content between groups, where the highest increase was found in groups A and B. Changes in copper content were presented in Figs. 1–4.

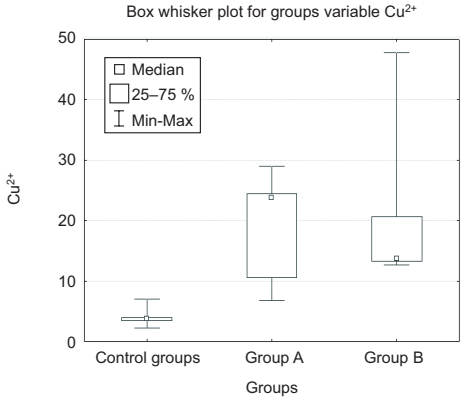

Fig. 1. Comparison of copper content in *Phrynohyas resinifictrix* larvae organisms between groups after hrs of experiment

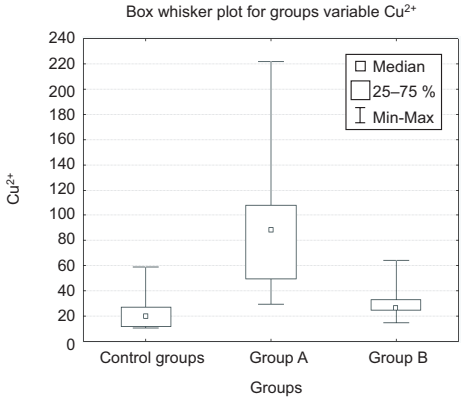

Fig. 2. Comparison of copper content in *Phrynohyas resinifictrix* larvae organisms between groups after 16hrs of experiment

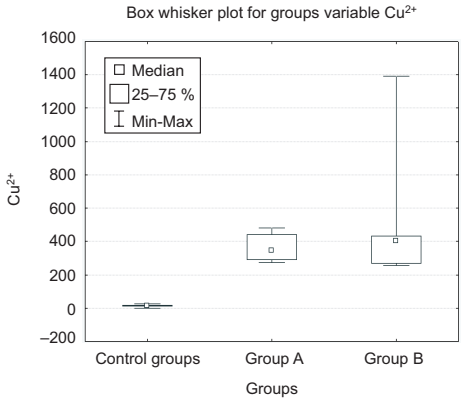

Fig. 3. Comparison of copper content in *Phrynohyas resinifictrix* larvae organisms between groups after 1 week of experiment

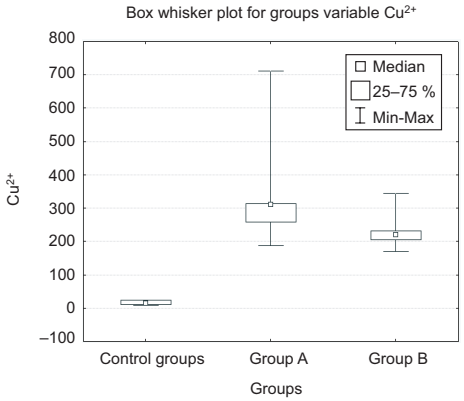

Fig. 4. Comparison of copper content in *Phrynohyas resinifictrix* larvae organisms between groups after 2 weeks of experiment

For zinc, statistically significant differences were registered after 1 week ($p = 0.003$) and after 2 weeks ($p = 0.026$). These measurements noted the highest concentrations in groups A and B. Changes in zinc contents were shown in Figs. 5 and 6.

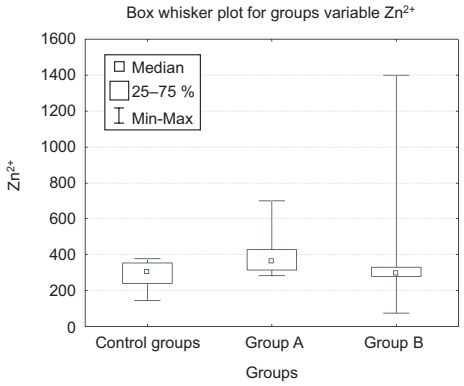

Fig. 5. Comparison of zinc content in *Phrynohyas resinifictrix* larvae organisms between groups after 16 hrs of experiment

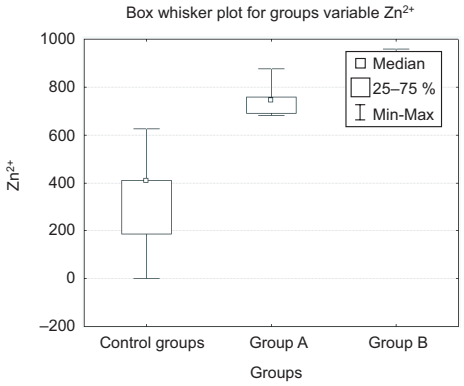

Fig. 6. Comparison of zinc content in *Phrynohyas resinifictrix* larvae organisms between groups after 1 week of experiment

Discussion

Our research revealed that copper concentrations increased during the experiment. Copper is an element crucial for organism functioning. However, copper excess is strongly toxic. The degree of this metal assimilation depends on its form in feed but also on the organism species, its development stage and also on the content of other elements in the diet, among others zinc and cadmium. Increasing copper concentrations may be connected with the organism protection against toxic effect of cadmium [2]. The most frequent is Cu-Zn antagonism, which explains many symptoms associated with copper deficiency. It involves the competition between both cations in the process of absorption from the alimentary system. Generally, a balance between these metals is maintained in the organism for the benefit of zinc, which is often associated with increased copper excretion from the organism, and also with increased zinc to copper ratio in the adipose tissue. Greater zinc content in food leads to a decrease in copper concentration in the liver, heart and blood serum. The result of copper and zinc linking is less toxic than their separate effects. This result may be ascribed to a replacement and competition of these elements for available bonds in protein synthesis, but also to the species properties [3]. On the basis of the above-mentioned information and the data obtained from the discussed experiment, it may be assumed that after 1 week and 2 weeks antagonistic effect of Zn and Cu becomes apparent in the organisms of tadpoles. Application of 4 mg/dm^3 Zn in group B points mainly to the effect of zinc on copper content, but interactions between copper and cadmium are also known.

Zinc is an element crucial for the proper development of larvae [4]. In animal organisms zinc cooperates in metabolic processes with other elements, mainly with copper, whose metabolism is disturbed by cadmium. Both zinc and cadmium reveal similar properties in forming metal-protein complexes and therefore their physiological effect is antagonistic. Zinc may have a limiting effect on cadmium cumulation [5]. The analysis of zinc ions influence on cadmium level in group A may suggest that after one

and two weeks significant changes in zinc content in tadpole organisms led to a slight decrease in cadmium level after two weeks. However, it may be supposed that this process is not only the effect of zinc activity. Changes in zinc and cadmium concentrations in group B are not significant, but the duration of zinc effect on cadmium caused similar changes in its content as in group A.

The analysis of magnesium concentrations did not reveal any marked changes in the test groups. However, it is difficult to determine the direct influence of magnesium on the other elements [6]. Most probably solutions with 2 mg/dm^3 and 4 mg/dm^3 of magnesium applied for the experiment and considering high level of this element in the control did not have any marked effect on the level of other metals cumulation in the organisms.

The antagonism between cadmium and copper occurs especially in young organisms, for which even low concentrations of cadmium in diet affect copper content in the liver and kidneys. Elevated copper concentrations in organism lowers the rate of cadmium uptake and even contributes to its elimination [7]. In the presented experiment such interactions might have occurred in group A, where copper reveals significant changes of its contents measured after 4 hrs, 16 hrs, after 1 week and 2 weeks, whereas cadmium in larvae organisms does not reveal any differences in cumulation. It means that changes in cadmium concentrations in the analyzed test group are not significant despite almost constant cumulation rate of this metal in all registered measurements, whereas copper reveals high concentrations after 16 hrs and after one week. The analysis of group B concerning the antagonism between copper and cadmium shows changes similar to those which occur in group A.

Biochemical function of cadmium in animal organisms has not been fully explained so far. Selvi [8] and Flament [9] stated that increasing level of this metal accumulation in amphibian organisms affects the metamorphosis, the course of gonadogenesis and behavioural changes. In our experiment we have registered increased cadmium cumulation in larvae organisms already after 16 hrs and after 1 week, and high levels persisting after 2 weeks in groups A and B. On the other hand, we cannot state a direct effect of cadmium because of tadpoles exposure to cadmium in the presence of zinc which we applied in our experiment. We may only assume that if cadmium concentration were higher, such phenomena would probably occur.

The interactions found between the individual elements are more pronounced when early stages of *Phrynohyas resinifictrix* ontogenesis were treated with 1:1:1 (Mg : Zn : Cd) ratio than for 2:2:1 (Mg : Zn : Cd) ratio.

Conclusions

1. A significant increase in copper content after 4 hrs, 16 hrs, after 1 week and 2 weeks later, and elevated zinc content after 1 and 2 weeks were assessed in the organisms of *Phrynohyas resinifictrix* larvae subjected to the effect of 2 mg/dm^3 Mg^{2+}, Zn^{2+} and Cd^{2+} and 4 mg/dm^3 Mg^{2+}, Zn^{2+} and 2 mg/dm^3 Cd^{2+}.

2. No significant changes of cadmium or magnesium contents were assessed in *Phrynohyas resinifictrix* larvae organisms.

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KUMULACJA METALI CIĘŻKICH WE WCZESNYCH STADIACH ONTOGENEZY *Phrynohyas resinifictrix* (GOELDI)

Zakład Zoologii Kręgowców i Biologii Człowieka, Instytut Biologii
Uniwersytet Pedagogiczny w Krakowie

Abstrakt: Badania dotyczyły oceny współzależności między miedzią, cynkiem, magnezem i kadmem w odniesieniu do tempa ich kumulacji w organizmach *Phrynohyas resinifictrix*. Grupę A stanowiły organizmy poddane działaniu roztworu zawierającego 2 mg/dm^3 Mg^{2+}, Zn^{2+} i Cd^{2+}, a grupę B 4 mg/dm^3 Mg^{2+} i Zn^{2+}, 2 mg/dm^3 Cd^{2+}. W czasie trwania eksperymentu stwierdzono istotne zmiany zawartości miedzi i cynku. Zawartość miedzi w grupie A wynosiła 5,97 μg/g s.m. po 2 h, a po 1 tygodniu 367,521 μg/g s.m. ($p = 0.002$). W grupie B zawartość miedzi wynosiła 12,914 μg/g s.m. po 2 h, a po 2 tygodniach 234,372 μg/g s.m. ($p = 0{,}013$). Poziom cynku w grupie A wynosił 282,779 μg/g s.m. po 2 h i 750,051 μg/g s.m. po 1 tygodniu ($p = 0.003$).

Słowa kluczowe: mineralny skład, Cd, Cu, Zn, Mg, płaz, larwy *Phrynohyas resinifictrix*