THE BIOCHEMISTRY OF HEMOLYMPH AND THE CONCENTRATION OF SOME HEAVY METALS IN THE EXOSKELETON OF THE HELIX ASPERSA SNAIL IN TWO LOCALITITES IN KOSOVO

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Abstract
The concentration of Pb, Cd, Zn and Fe in the soil, their bioaccumulation in the exoskeleton of the Helix aspersa snail, as well as the concentration of total proteins and hemocyanin in its hemolymph was researched.
Snails of the sample group (N=24) were collected in the close vicinity of the "Sharrcem" Hani i Elezit cement factory, whereas those of the control group (N=24) were taken in the village of Zhur near Prizren. The weight of the snails was approximately the same.
The analysis of heavy metals was done with the Perkin Elmer – ICP OES Optima 2100 DV equipment whereas, the concentration of hemocyanin has been indirectly measured through copper with the sodium diethyldithiocarbamate. Total proteins were measured by Biuret's method.
The research results show that compared to the location of Zhur, the concentration of lead, cadmium, iron and zinc (ppm) in the soil samples and the exoskeleton of snails from the contaminated location ("Sharrcem"), are 2–6.5 times higher whereas, the quantity of total proteins ($P<0.01$) and the hemocyanin, ($P<0.05$)) in their hemolymph, is significantly lower.
The preliminary analysis of the results show that the biochemistry of the hemolymph depends on the accumulation level of heavy metals in the organism, while exoskeleton of the snail Helix aspersa has a higher capacity for the accumulation of Zn (about 5–20 times more than its concentration on the soil), but not for the Pb, Cd and Fe.

Keywords: *Helix aspersa, hemolymph, heavy metals, exoskeleton*

Introduction

Environmental pollution by heavy metals is an environmental problem due to rapid development in industry, technology, urbanization and agriculture. Heavy metals are dangerous to living beings, especially for human. The main sources are the various industries as well as organic and inorganic untreated waste (Zhou et al., 1998)

Some metals, in small quantities are essential but others as Pb, Cr, Cd, Ni are toxic as interference in enzymatic and metabolic systems of the body. Because of their extensive use in many segments they can penetrate into the body, stored and express their toxic effect (Hey et.al 2006; Irwin 1997).

Metals cannot be degraded further, and their toxic effects may be evident (Clark, 1992). They also can bioaccumulate and may have toxic effects even in very small concentrations (Davey et., 1973). The concentration of lead in different environments is highly variable. Its content in soil varies by country (in rural area 5-40 mg/kg, while in urban area to 50-300 mg/kg and in the vicinity of smelters 20-20000 mg/kg) (Ewers and Schlipkoter, 1991).

According to the WHO, in 2002, Kosovo is ranked as one of the ten most contaminated regions of Europe. Currently the main sources of pollution are power plants of Kosovo. Monitoring shows that only one of the five Power Plant Units – "Kosova A", within an hour releases about 25 tons of dust and ash. The emission level is about 74 times higher than European standards (Kosovo Strategic Environmental Assessment, 2001). Currently about 700,000 citizen's inhalation mixtures of toxic gases that are emitted from power plants A and B (Plani i Kosovës për Veprim në Mjedis 2006 – 2010). Also transport nowadays considered to be one of the greatest contributors to air pollution. From industrial and thermo units installed in this area, released into the air without any control hundreds - thousands of tons of sulfur dioxide (SO2), nitrogen oxides (NOx), ammonia (NH3), ozone (O3), dust, smoke, lead (Pb), cadmium (Cd), radionuclide, dioxin (Plani i Kosovës për Veprim në Mjedis 2006 – 2010).

As a major source of pollution in the territory of Kosovo are: "Ferronikel" in Drenas ,Cement Factory "Sharrcem" Hani Elezit, central heating system (Prishtina, Gjakova, Mitrovica) (Plani i Kosovës për Veprim në Mjedis 2006 – 2010).

Terrestrial invertebrates and gastropods of Helix sp. accumulate various pollutants such heavy metals, agrochemicals, urban pollutants as well as radio nuclides (Beeby and Richmond, 2002, 2003, Regoli et al., 2005). Snail is a good bio-indicator in the term of environmental pollution evaluation, because of its sensitivity in environmental changes (Adeyeye, 1996). On the other hand, the polluting substances released from the chimneys of Kosovo's Power Plants in Kastriot have carcinogenic effects which affect the snails also. Given that toxic gases, particles of coal burning dust released into the air and the radionuclides mixed in dust, deposited on land, water, plants surface such as leaves, where they can close the stomas, prevent the light to reach the leaves, but they may absorbed through the root system also and deposited in various tissues and organs of the plant (Berger and Dallinger, 1993). The snail's intoxication is a result of feeding with contaminated plants in polluted regions. Accumulated pollutants could be transported through different routes by

hemolymph and blood cells in the other organs including the shell (Beeby and Richmond, 2002, Regoli et al., 2005).
The aim of this research was to evaluate the effect of environmental pollution by heavy metals in the biochemistry of hemolymph and their concentration in the exoskeleton of the snail Helix aspersa.

Material and Methods

Material

The investigation was conducted with natural populations of snail Helix aspersa. For this research were used a total of 48 snails, 24 test and 24 as control. The test groups of snails were taken in close vicinity of the factory "Sharcem" in Han Elezit, while control group in a region far away from the factory and unaffected by industrial pollution, respectively in village Zhur of Prizren.
The reason why exactly the snail is used as biomonitor, is because the mollusks generally have a wide distribution, high capacity for heavy metal accumulation, easy to be determined, and are sufficient in the study area and have adequate size to provide an appropriate sample for analysis (Rayment and Barry, 2000).
For analysis of the concentration of heavy metals were used: soil samples and animal material - shell of snail Helix Aspersa. Samples of soil and animal material were taken in two localities of Kosovo, in close vicinity of the factory "Sharcem" in Han Elezit and Zhur village of Prizren.
METHOD (ICP-OES)
The concentration of heavy metals (Pb, Cd, Zn and Fe) in the soil and shell was done by flame Atomic Absorption Spectroscopy (AAS), in the spectrometer type Perkin Elmer –ICP OES Optima 2100 DV. Preparation of soil samples was done according to method of EPA method 3052 (1996), while the preparations of snail samples were done according to method Blanusa and Breski (1981). Hemocyanin concentration is measured indirectly through the sodium-copper dietilditiokarbamat and reading is done with modern spectrophotometer GENESYS 10 UV / VIS at wavelength 480 nm (Hc). Extinction coefficient for total protein is read with the same spectrophotometer at 580 nm wavelength (Berth and Delanghe, 2004)

Statistics

Results of this research are expressed in arithmetic average ±, standard deviation (SD). Statistical analysis was done using statistical software, Microsoft Excel and student t-test

Results

The total proteins and hemocyanin in hemolymph of Helix aspersa snails is presented in (Table 1). The data indicate for a significant decrease ($P<0.001$) in total protein and hemocyanin concentration to the snails from vicinity of “Sharrcem” factory at Hani i Elezit compared with the control group of snails taken in village Zhur of Prizren.
Regarding heavy metals concentration (Pb, Cd, Zn and Fe) in the soil and shell of snails Helix aspersa, also there is a significant difference in two localities. Their concentration is higly significant ($P<0.001$) in the soil and snails shell form the vicinity of “Sharrcem” factory

compared to the locality of Zhur in Prizren (Table 2). These variations values also may see in the figures 1, 2 and 3.

Tab. 1 Summary presentation of total protein and hemocianines concentration in hemolymph of snails taken from two locations in Kosovo (Hani i Elezit and Zhur)

Location	Parameters	
	Total proteins (mmol/L)	**Hemocyanin (g%)**
Zhur	26.6±3.2 (24)	14.4±2.6 (24)
"Sharrcem"	17.9±3.8 (24)	9.45±5 (24)
Significance	P<0.001	P<0.05

Fig. 1 Graph of concentrations values of total proteins and hemocyanin in hemolymph of snails Helix aspersa from polluted locality Hani i Elezit near "Sharrcem" locality and unpolluted locality Zhur – Prizren.

Tab. 2 Comparisons values of heavy metals concentration in soil and shell samples of snails Helix aspersa taken near the factory "Sharcem"-Hani i Elezit and locality of the village Zhur-Prizren

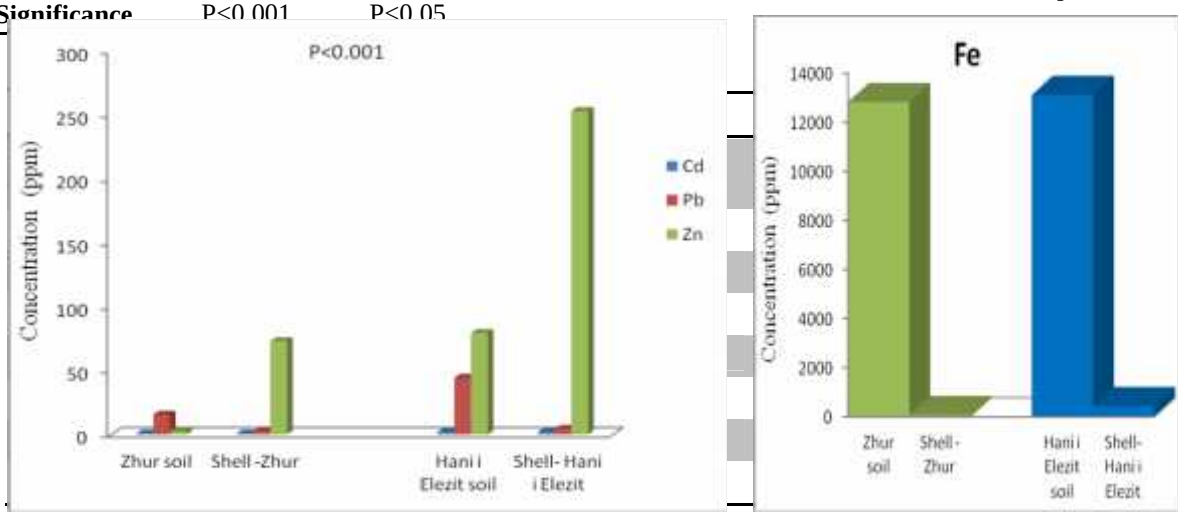
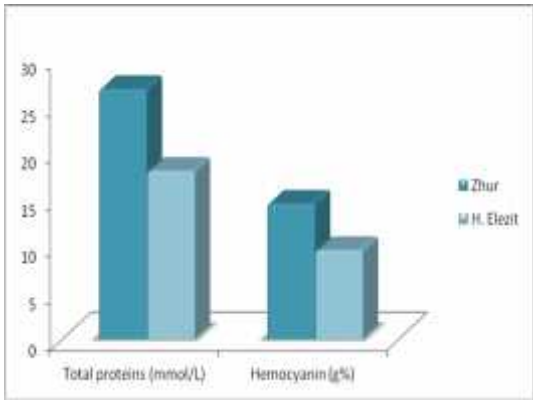


Fig. 2 Graph of concentration of heavy metals (excluding Fe) in soil and shell of snails from two localities, Hani I Elezit and Zhur – Prizren

Fig. 3 A high rate of hezavy metal concentration in soil and shell also in the snails Helix aspersa taken near the "Sharrcem"- Hani I Elezit and Zhur - Prizren



Discussion

Data from the literature show that terrestrial mollusks, snails are a good species for monitoring of traces metals, agricultural chemicals, urban pollution and electromagnetic exposure, because the effects of pollutants can be seen for a very short time (Abdullah and Mustafa, 2002; Tay et. al, 2003; Beeby and Richmond, 2002, 2003; Viard et. al. 2004; Regolli et. al., 2005; Lowe et. al, 2006). Mollusks advantages regarding heavy metal accumulation are numerous, because they have wide distribution, high capacity for accumulation of heavy metals, easy to recognize, abundant in the study area and also have sufficient size to provide adequate sample for analysis (Rayment and

Barry, 2000). Terrestrial gastropods (Helix pomatia, Helix aspersa), were used as an experimental model to determine differences in the concentrations of heavy metals content, Pb, Cu, Fe, Cd, Zn, Mg (Menta and Parsi, 2001).

The high concentration of zinc in the skeleton of snails in contaminated region is consistent with the results obtained in laying hens exposed to industrial pollution at the base of lead metallurgy in Mitrovica (Bakalli et. Al, 1991). Otherwise authors concerned with high concentrations of zinc in the liver, kidney, heart and accumulation of Cd and Pb in these bodies relate to the protective effect of zinc, which is partly associated with increased induction of the synthesis of metallothioneins level, as well as antagonism of cadmium and zinc, which operates in anti oxidative level of protection system of the body.

It is worth mentioning that the opposite trend in the concentration of heavy metals in the skeleton has total protein and hemocianines in hemolymph of snail Helix aspersa.

Therefore, the results of research concerning with lower significant scale of total protein and hemocyanin in hemolymph of snail Helix aspersa close surroundings "Sharcem" are consistent with the literature data (Rozhaja et. al. 1980) who found lower levels of the protein concentration in serum of turtles and mice of this region, compared with control animals. Regarding the comment on the change of the concentration of hemocyanin in snails hemolymph in the contaminated region, it should be noted that based on the literature data, it can be linked with respiratory pigment hemocyanin, since this pigment to mollusks represents 90-98% of the total protein of hem lymph (Korzuev Aljakrinsja, 1972). Also, when it comes to hemocyanin as respiratory pigment, the results of this research are consistent with the research results of Serafinski and Szullakowska (1977), who notice decrease concentration of hemocyanin in hemolymph of snails Planorbius corneus exposed to different concentrations of lead nitrate (0.001-10 mg / l) for 91 days.

Agnieszka Zawisza-Raszka (2010) explained the mechanisms of decrease of the total protein concentration and hemocyanin in snail hemolymph in the contaminated region as reduction of liver ability (in this case hepatopancreas, as the analogue of vertebrate liver by its function) in protein synthesis, while another explanation according Dorosenko (1951) is that the decrease in protein concentration might be related to the degradation of abnormal proteins in the body (gluconeogenesis). According Boselli (1954) loss of 1gr protein in plasma is equivalent of losing of 30 gr of tissue protein.

This research shows that Helix aspersa snails are good species for monitoring environmental pollution from industrial pollution, urban, traffic, heavy metals, and agrochemicals.

Conclusions

Based on the results of this research can be concluded as follows:

- The concentration of Pb, Cd, Zn and Fe is significantly higher in soil samples and in the shell of snail Helix aspersa at the close surroundings of “Sharcem” – Hani i Elezit compared with their concentration in the soil and shell samples of Zhur locality in Prizren;
- Significantly lower concentration of total protein ($P < 0.001$) and hemocyanin ($p < 0.05$ level) in hemolymph of snails from polluted region – “sharrcem”, in comparison with their concentration in snails of control group, Zhur - Prizren.

Snail research in this regard has practical importance, since they are used as food and can serve as a source of human intoxication with heavy metals.

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