

COMPARATIVE ANALYSIS OF SOME BIOCHEMICAL PARAMETERS IN HEMOLYMPH OF GARDEN SNAIL (*HELIX POMATIA L.*) OF THE KASTRIOT AND FERIZAJ REGIONS, KOSOVO

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ABSTRACT

In current work the concentration of glucose, total proteins, cholesterol in hemolymph of two local population's of Garden snail Helix pomatia L. (N=27) were analyzed. The test group of animals (N=17) were taken in the region contaminated by the power plants of Kosovo in Obiliq, while the snails of control group were from another region of Kosovo – Ferizaj, which is unaffected by industrial pollution and is far away from power plants. The investigation results have shown that the concentration of the cholesterol and total protein in the hemolymph of snails of control group were significantly higher $P < 0.001$. Whereas, concentration of glucose was also significantly higher $P < 0.01$ but with a lower rate than two abovementioned parameters compared with the snails of the polluted region. Statistical results were calculated using software MINITAB 16.

The investigation shows that the Garden snail can serve as bio-indicator, whereas the changing of the hemolymph chemistry as marker for the biochemical indication of the environmental pollution effects.

Keywords: *Helix pomatia L., hemolymph, glucose, cholesterol, total proteins*

Introduction

Environmental pollution in Kosovo has arisen at high level. The causes of this pollution are different, as: uncontrolled coal exploitation for Power Plants, mineral resources of Pb, Zn, Cr, obsolete technology, inadequate and unprofessional use of agrochemicals, food additives, dense traffic of old cars with incomplete combustion of fuel, without catalyzators, which release toxic gases, aerosols, cancerogenous benzopyren, large and uncontrolled urbanization, lack of water treatment plants, lack of sanitary landfills, etc. (Adrović F., 1997). Due to its proximity to the Power Plants and other above-mentioned problems, Pristina region is considered among the most polluted areas in Kosovo. Therefore, compared to other

European countries, Kosovo ranks in the tenth's position of the countries with the higher degree of pollution (WHO, 2002).

In economic and mining aspect, coal, which represents one of the main natural fuel for generating electricity in Kosovo dominates compared to other minerals (98%). According a pre-calculation, lignite reserves in Kosovo brought about 14.3 billion tons, enough to produce electricity for the next 100 years, (Zeqiri, 1984). When

the lignite of medium quality burned in power plants with 200MW capacity and 6300 tons of coal daily consumption, if the electro-filters work normally, every day in the atmosphere released 382 tons of SO₂, 60 tons of NO_x, 1.4 tons of CO as well as large quantities of ash particle (250 tons within 24 h, especially at night and weekends

when electro-filters are inactive), and the surface of degraded by ash and mining is about 10,000 ha in all Kosovo (Despotović, 1989). The Power Plant "Kosovo-A", has five blocks (units) with a capacity 790 MW/h and Power Plant "Kosova - B" has two units with a capacity of 678 MW/h. According to the "Strategic Environmental Assessment for Kosovo 2001", the amount of dust release from "Kosovo - A" units (power plants) is 25 tons, while for "Kosovo - B", 1.03 tones of dust per hour (this level exceeds about 74 times European standards). Human and ecotoxicological risk exists due to the respiratory and cardiovascular diseases as well as carcinogenic and mutagenic effects of arising as a consequence of complex chemical mixture (Maynard, 2004).

Terrestrial invertebrates and gastropods of *Helix* sp. accumulate various pollutants such heavy metals, agrochemicals, urban pollutants as well as radionuclide (Beeby and Richmond, 2002, 2003; Regoli et al., 2005). Snails play an important role in nature with diverse functions and may act as sensitive indicator of environmental change (Adeyeye, 1996). On the other hand, the polluting substances released from the chimneys of Kosovo's Power Plants in Kastriot have carcinogenic effects which manifest the snails

MATERIAL AND METHODS

Material

The investigation was conducted with natural populations of garden snail *Helix pomatia* L. For this research were used a total of 27 garden snails, 17 test and 10 control. The test groups of snails were taken in the region surrounding the Kosovo's Power Plant (Obiliq), while control in a region far away from Power Plants and free of pollution (Ferizaj).

The reason why exactly garden 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

also. Given that toxic gases, particles of coal burning dust released into the air and the radionuclide 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 B. and Dallinger R., 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 organs e.g. hepatopancreas, which is a gland that support the food digestion and is the main organ for detoxication and metabolism (Beeby and Richmond, 2002; Regolli, 2002; Regolli et. al., 2005).

To evaluate the effects of this complex environmental pollution in biological systems, respectively local populations of animals of polluted region was defined to apply passive biomonitoring version and garden snail *Helix pomatia* L. as biomonitor, in two locations in Kosovo with high degree of pollution and various etiologies (in Obiliq as polluted region and Ferizaj as control region).

size to provide an appropriate sample for analysis (Rayment and Barry, 2000).

Another advantage is that, because of their special relation with the ecosystem (soil and plants), of the terrestrial gastropods, effects of pollutants in the body can be identified for a very short time (Scheifler, 2002).

To avoid changes in the content of hemolymph and tissues depending on the age or weight (Burton 1971, 1971b; Coughrey and Martin, 1970) or by the season or implications of circadian rhythms in results (Abdussamad et al., 2010), were selected the snails of the approximately the same body weight and analyzes were performed at the same time also.

Methods

For laboratory analyses were used standard methods and reagents prepared by HUMAN firm. Hemolymph centrifugation was performed on centrifuge (10 min/3000rpm), while biochemical parameters were determined using apparatus for automatic analysis Humalyzer 2000 Human.

Snail hemolymph glucose concentration was determined by enzymatic method, GOD-PAP method, enzymatic colorimetric test (Barham D and Trinder P. 1972).

Total proteins were measured by Biuret's method. For analysis were used 20 μ l of hemolymph, mixed with 1ml reagent and then is centrifuged (10min/3000rpm). The centrifuged content is running in the cuvette, to read absorbance in spectrophotometer at 545 nm wavelength. Read

values are expressed in the unit g/L. During this process, peptide linkages of proteins react with Cu⁺⁺ ions in an alkaline medium and form colored complex: peptide-Cu⁺⁺. Created complex then read in spectrophotometers according to the colorimetric-photometric method with standard 80 at the wavelength 545 nm and is proportional to the total protein concentration in the sample provided (Alma Hiller, A. et al., 1976):

For the quantitative determination of cholesterol in snail's hemolymph was use the CHOD-PAP method (LCF), with standard 5.17 mmol/l. In this method, 20 μ l of hemolymph were mixed with 1ml reagent, and then centrifuged for 10min in 3000rpm. Then the content is put on spectrophotometer cuvette to read absorbance at 520 nm wavelength. The obtained values are expressed in the unit mmol / L (Richmond, 1973).

Results

The research results are presented in Table 1. The table shows that there is a significant decrease of glucose, total protein and cholesterol concentration in snail's hemolymph of polluted region around the Kosovo's Power Plants in Obiliq compared to control snails taken in the

region unaffected by industrial pollution, in Ferizaj respectively (Table 1 and Fig. A and B).

Results of this investigation were expressed as the arithmetical average values \pm (plus-minus) standard deviation (SD). For statistical analysis were also used statistical manuals and software (MINITAB 16).

Table 1: The concentration of glucose, total proteins and cholesterol in the hemolymph of the garden snail *Helix pomatia* L. taken from the regions of Obiliq and Ferizaj

| Locality | Glucose (mmol/l) | Total proteins (g/l) | Cholesterol (mmol/l) |
|------------------------------|---|---|---|
| Ferizaj Control | 0.8\pm0.12 (10) | 44.18\pm13.25 (10) | 1.04\pm0.15 (10) |
| Obiliq (Power Plants) | 0.58\pm0.14 (17) | 24.9\pm9.03 (17) | 0.36\pm0.06 (17) |
| Significance | ** | *** | *** |

** Significant, ***High significances

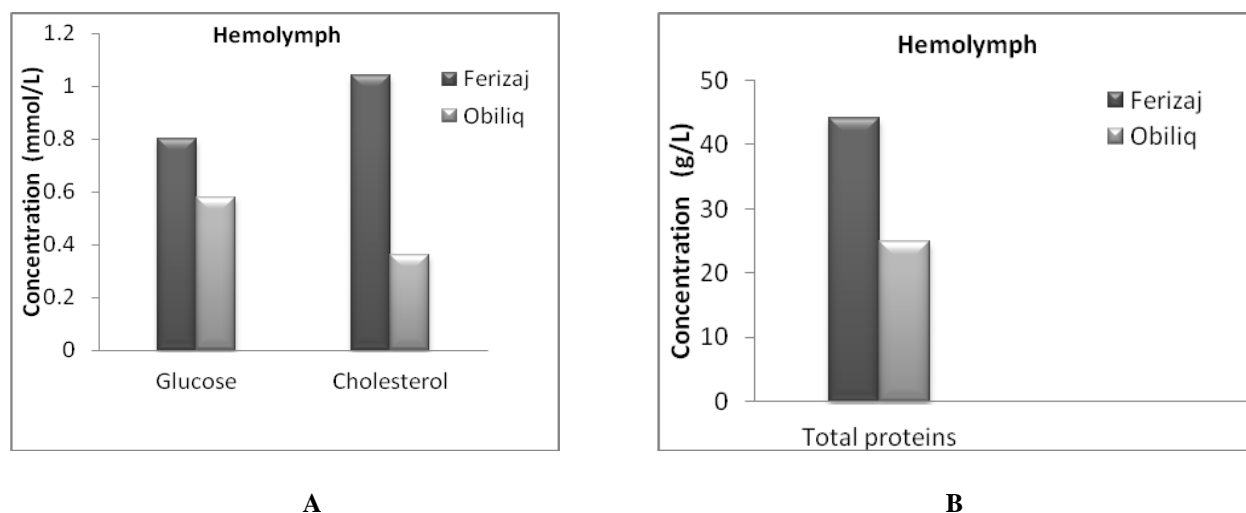


Figure 1. Comparative assessment of glucose and cholesterol (A) and total proteins (B) concentration (mmol/L, g/L respectively) in the hemolymph of garden snail *Helix pomatia L.* of Ferizaj and Obiliq regions

Discussion

Obtained results through passive monitoring on some biochemical parameters (glucose, total protein and cholesterol) in hemolymph of garden snail from polluted region of Power Plants in Obiliq are approximately consistent with the results of some earlier researches obtained by passive or active monitoring of the vineyard snails *Helix pomatia L.* (Halili et al., 1980; Cakaj et al., 2004), earthworms *Lumbricus terrestris L.* (Gashi et al., 2005, 2006), etc. Authors concerned with the analysis of hemolymph of the natural local population of vineyard snail in body homogenate of local natural populations of earthworm taken in ash dump of Kosovo's Power Plants, found decrease (reduction) of the total protein concentration, change in glucose concentration, total lipids and cholesterol compared with the corresponding values of these parameters obtained with the analysis of hemolymph or body homogenate of the control group of animals.

Regarding the effect of the ash from Kosova's Power Plant in Obiliq in glicemy, we didn't found any particular publication, but hypoglycemia of

the snails from contaminated region in Obiliq may be associated with the data of Tarun Balain et al (2011) who found that high doses of toxicants result in hypoglycemia to the birds poisoned with insecticide Imidacloprid. In fact the authors conclude that the effects of toxicants in glicemy depend on the dose, so e.g. high doses of agrochemicals reduce blood glucose concentration, whereas low doses result in hyperglycemia. The authors concerned, this fluctuation of glicemy of the poisoned birds clarify as follows: as a consequence of the high doses of toxicant, the glycogen "depot" (muscle and liver) damaged and reduced and it leads in a decrease of the level of blood glycemia, while hyperglycemia associate with the process of glyconeogenesis.

Our results are also consistent to the findings of Bislim et al. (2002, 2003), who found similar changes in some biochemical parameters (glucose, AST, ALT, ALP, CK) in the blood serum and histopathological changes in the kidney and liver of poultry birds Hybro treated for 30 days with standard food mixed with the ash of Kosovo's Power Plants compared with control birds.

In this context, we must emphasize the researches results of Halili et al. (1985), whom found glycemic homeostasis disorder in the snails of the heavy metal contaminated region in Zvecan or snails intoxicated with the lead acetate, compared to control snails. On the other hand Cheng Wan Hee (2008) by analyzing of the heavy metals concentration in snail (*Nerita lineate*) soft tissues, shell and operculum from a region contaminated with heavy metals, found high level of Cd, Cu, Fe, Ni, Pb and Zn concentration, derived from different paths.

Heavy metals are involved in the metabolism of hydrocarbons by attacking the endocrine system (hepatopancreas) and the enzymes involved in these processes (Anderson et al., 1997).

Liver is the target organ for heavy metals activity. The effect of toxic heavy metals in the liver and kidneys interferes with the level of energetic metabolism by reducing the amount of glycogen in the liver as well as its detoxification function and disturbing the normal function of endocrine glands (Squibb and Fowler, 1981)

Regarding the total proteins level, our results show a decrease – hypoproteinemia in the snails of the test group compared with control and this difference is in significant degree ($P < 0.001$). According to the literature data, the causes of hypoproteinemia is the increasing of protein degradation process or disruption of protein metabolism, because the heavy metals and radionuclides present in ash bind to RNA and block the protein biosynthesis process on ribosome's level (CIESM 2002).

Reducing of ability of the liver (hepatopancreas) to produce proteins, might be as a consequence of blocking of the aminoacids reactive groups by pollutants, such as carboxyl and alpha lysine groups, phenoxy group of tyrosine, SH (sulfhydryl group) group of cysteine (Kohler H., 1996), or as a result of damage of the ribosomal complex and RNA due to the heavy metal connection for RNA (Vallee BL and Ulmer DD, 1972).

Hypoproteinemia was found by El-Demerdash et al. (2006) also in the rabbits treated with hexavalent chromium (dose 5mg/kg b.w.) for a period of 10 weeks.

A decrease in total protein was observed by Vinodhini et al. (2008), in the liver of fish (*Cyprinus Carpio L.*) exposed to heavy metal concentration medium. According to these authors the decrease of total protein was due to pathological processes caused by heavy metals, including kidney damage and elimination of proteins from the urine, then in the blood flow or as a result of hemorrhages in the peritoneal cavity, associated with the reduction of protein and RNA in the liver (Jain et al., 1996).

Related to cholesterol concentration in hemolymph, the results of this research are consistent with findings of Nedjoud Gara (2012), who found a significant reduction of lipids in the snails, treated with heavy metals, and this proportion depended from the concentrations of heavy metals. Aourousseau (2002) suggest that free oxygenated radicals express their toxic effect through the lipid degradation during the β -oxidation process.

A significant decrease ($P < 0.001$) of total lipids was shown by Torreblanca et al. (2004), in the crab *Procambarus clarkii* exposed to lethal and sub-lethal doses of mercury (Hg) for 96 hours. Ortel (1991) also finds a significant decrease of total lipid values in the butterfly *Pimpla turionellae* after exposure to cadmium.

In a research conducted by Shin et al. (2001), found a significant decrease of triglycerides values in the butterflies *Galleria mellonella* exposed to cadmium chloride (CdCl_2) at different concentrations compared with non-exposed control groups.

Triglycerides and cholesterol are known as compounds that take a part in the structure of total lipids. Lipids homeostasis is essential for the main functions of the liver, where any change in serum triglycerides concentration shows a liver dysfunction (Kaplan et al., 1988). In addition, abnormal accumulation of fat in experimental animals may be due to the lack of a balance

between production and its use (Moore et al., 1988).

Conclusions

In this research the effects of pollution from Kosovo's Power Plants in biochemical changes of hemolymph of natural populations of the vineyard snail *Helix pomatia* L. were evaluated.

Based on the obtained results, it is clear that there is a significant decrease of the abovementioned biochemical parameters (glucose, cholesterol and

total protein) of snails from the region polluted by Kosovo's Power Plants (Obiliq), compared with the control group of snails taken from region of Ferizaj, free of industrial pollution.

Therefore, based on the results of this research can be concluded that the vineyard snail *Helix pomatia* L. may serve as a good experimental model and as a biomonitor to evaluate the effects of industrial pollution.

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