ACTIVITY OF GLUTATHIONE S-TRANSFERASE IN *Cepaea vindobonensis* (GASTROPODA: HELICIDAE) FROM POLLUTED AREA

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Abstract
This study investigates the activity of snail glutathione-S-transferase (GST) and metal bioaccumulation for assessing ecotoxicological effects of urban metal pollution in Belgrade city, Serbia. This investigation was performed in the viscera of roadside Cepaea vindobonensis snails collected from two different sites in the city. The results showed that mean concentrations of the measured metals, cadmium (Cd) and lead (Pb) were higher in site next to the major federal highway (HS) when compared to the background levels of the reference site (RS) 1.5 km away from the highway. The pattern of metal accumulation at both sites was Pb > Cd. At the HS site, snails displayed higher mean of GST activity compared to snails from the RS. The activity of GST enzyme was positively related to metal concentrations. The tested antioxidant enzyme is sensitive parameter that could be used as biomarker in snails exposed to the actual metals in the environment. The overall results of this study showed the importance of C. vindobonensis as a sentinel organism for biomonitoring the biologic impact of atmospheric pollution in urban areas.

Keywords: *Cepaea vindobonensis*, cadmium, lead, glutathione-S-transferase

INTRODUCTION

The release of inorganic contaminants such metals in natural ecosystems can consequently pose environmental and human health risks. In the case of urban areas affected by high traffic density, most of the metal pollution primarily comes from atmospheric depositions. Heavy metals like cadmium (Cd) and lead (Pb) can contaminate soils from atmospheric deposits, continuous application of large amounts of fertilizer, disposal of industrial waste sludge and vehicle emissions [1]. The presence of heavy metals in terrestrial ecosystems is of special concern for their high toxicity and ability to be biomagnified through the food web [2,3].

Metal pollution causes oxidative stress which can induce the activity of antioxidants in exposed organisms. Chronic exposures to metals lead to excessive production of reactive oxygen species (ROS) such as superoxide anion radical ($O_2^{\cdot -}$), hydroxyl radical (•OH) and hydrogen peroxide (H_2O_2). This can result in a shift in the redox status of the cell, thereby causing damage to biomolecules such as lipids, nucleic acids and proteins and consequently to oxidative stress in the organisms [4].

Aerobic organisms have a variety of enzymatic and nonenzymatic antioxidant defenses that maintain endogenous reactive oxygen species (ROS) at relatively low levels and attenuate the damage related to their high reactivity. Glutathione-S-transferase (GST) is a detoxifying and antioxidant enzyme that protects cells from oxidative stress. This enzyme catalyzes the conjugation of glutathione (GSH) with a variety of electrophilic metabolites and heavy metal ions resulting from detoxification of both reactive intermediates and oxygen radicals [5].

Soil organisms, like terrestrial snails as an important part of the invertebrate biomass, are continuously exposed to soil inorganic contaminants [1]. Snails accumulate substantial quantities of pollutants in their bodies, often without lethal consequences [6,7]. Due to their capacity to accumulate heavy metals in their tissues and limited ability to excrete them, terrestrial or land snails have been extensively studied as bioindicator organisms of environmental contamination by metals [7].

Previous studies have shown that land snail can be good biomonitoring organisms and hyper-accumulators of heavy metals pollution [7,8]. The land snails are involved in transfer of trace elements in food webs, because of their position in trophic webs - detrivorous and herbivorous species, being dietary item of numerous invertebrate and vertebrate predators [9].

The objective of this study was to investigate the impact of vehicle traffic on soil contamination using the urban land snail *Cepaea vindobonensis* (C. Pfeiffer, 1828) which is very abundant and widespread gastropod species in Balkan Peninsula [10]. Concentrations of cadmium (Cd) and lead (Pb) were determined in soil and snail samples. In order to measure the effects produced by high environmental heavy metal concentrations, the oxidative biomarker GST in snail was assessed.

MATERIALS AND METHODS

Sampling sites

Soil samples and snails were collected from two sites in Belgrade city, Serbia, during the April of 2017. The first site (HS, Lat. 44°48'43.65"N, Long. 20°25'17.12"E) was chosen due to its close proximity to the major federal highway - a permanent source of soil pollution via heavy metal atmospheric deposition. The second site, designated as referent site (RS, Lat. 44°49'08.81"N, Long. 20°26'27.06"E) was 1.5 km away from the highway and placed in the city park “Ušće”. Three subsamples of soil (~100 g) were randomly taken at a depth of 10 cm from each site using a plastic grab. After sampling, soils were packed in polyethylene bags, transported to the laboratory and stored at 4 °C until further treatment. The adult snails (around 60 individuals per site) were collected from sampling sites by hand, washed with distilled water and transferred into clean plastic bags. Upon arrival at the laboratory, the samples were stored at –80 °C until further analysis.

Heavy metal extraction and analysis

Adult snails belonging to the *C. vindobonensis* were classified according to the presence of a clear white or brown-black lip at the mouth of their shell [11]. Before performing the metal analyses, the soft bodies of the snails were separated from the shell and dried in an oven (60°C) to a constant dry mass. The dried samples were ground into a fine powder by mortar and pestle. Snails were generally analyzed individually, but when their dry mass was lower than 0.1g, three to five individuals were pooled to obtain a sufficient biomass for metal analysis.

Soil and tissue digestion was conducted according to the procedure of Thevenon and Poté [12]. Analysis in the digests of soils and soft tissues was performed by inductively-coupled plasma optical spectrometry (ICP/OES) and comprised assessment of the concentrations of Cd and Pb. The values are expressed by the mean ± standard deviation (SD) in the analysis of three subsamples for each soil and tissue sample. Metals were analyzed in triplicate and concentration was expressed in mg kg^{-1} of dry mass.

Determination of enzyme activity

The whole soft tissue (n = 10) was minced and homogenized in 5 volumes of 25 mmol l^{-1} sucrose containing 10 mmol l^{-1} Tris–HCl, pH 7.5 using a Janke & Kunkel (Staufen, Germany) IKA-Werk Ultra-Turrax homogenizer [13] and sonicated for 15 s at 10 kHz on ice [14]. Sonicates were centrifuged at 100,000 × *g* for 90 min at 4°C. Protein content and enzyme assay were performed with the supernatant fraction.

Protein content was determined by the Folin–Phenol reaction as described by Lowry *et al.* [15] using bovine serum albumin as a standard. The activity of GST toward 1-chloro-2,4-dinitrobenzene as a substrate was determined according to Habig *et al.* [16]. The reaction rate was recorded at 340 nm and expressed as nmol of glutathione (GSH) min^{-1} mg $protein^{-1}$.

Statistical analyses

With the aim of detecting normality of data and homogeneity of variances the Kolmogorov–Smirnov test and Levene's test were used, respectively. Significant differences were analyzed using one-way ANOVA and post-hoc Tukey honest significant difference (HSD) multiple-comparison test. $P < 0.05$ was considered significant. All analyses were performed using SAS 9.1.3 software (SAS Institute, Cary, NC, USA).

RESULTS AND DISCUSSION

In order to determine the level of *C. vindobonensis* biomarker response and heavy metal contamination of soil, the concentrations of the most toxic heavy metals Cd and Pb were measured in soil and snails' samples at two sites with different pollution pressure (Table 1). Notably, HS site which showed increased Cd and Pb levels during sampling is located near major road network that is abundant source of Cd and Pb pollution exposure from highway traffic. At both sites, higher values of Pb were measured in soil and snails' samples compared to Cd concentration. Lower heavy metal concentrations were registered in the RS site, which validates its selection as a reference site.

It can be seen from Table 1 that accumulation in the organism is higher than that of the medium from which the metal was taken and the organism can be considered as concentrator as proposed by Dallinger [17]. Only soil concentrations of Cd exceeded maximum allowed concentration (MAC) values at site HS according to Serbian legal regulations, Official Gazette of FRY, No.23/94 (Table 1), thereby placing it in the range of chemical concentration that is occasionally associated with adverse biological effects. In addition, the adverse biological effects of Pb cannot be ruled out, even though its concentration did not exceed MAC value (Table 1).

The detrimental effect of Cd takes several forms, such as blocking of signalling receptors, interactions with some enzymes, induction of oxidative stress, genotoxic and necrotic effects

[18]. Cadmium presents a higher ecological risk than Pb and any other metal, which is due to the high toxicity coefficient of Cd despite its lower concentration (Table 1). Therefore, Cd can be regarded as one of the most probable stressors affecting GST activity. Consequently, GST activity showed positive relation with concentrations of Cd and Pb.

Table 1 *Cadmium (Cd) and lead (Pb) content (mg kg^{-1} dry weight) in the soil and snail Cepaea vindobonensis collected from referent site (RS) and polluted site (HS). Metal concentrations are presented as average concentration levels measured in these specimens (for soil n = 3 per site; for snails n = 10 per site). Significant differences in metal concentrations between referent and polluted samples were calculated by one-way ANOVA. The level of statistical significance of differences between the sites was defined as $p < 0.05$. Statistical significance is marked with an asterisk: ** $p < 0.01$ and *** $p < 0.001$.*

Metal concentration (mg kg^{-1} dry weight), mean ± SD	**Cd**		**Pb**	
Locality	RS	HS	RS	HS
Soil	1.75 ± 0.09	3.46 ± 0.1**	15.88 ± 1.77	46.49 ± 2.63**
Snail	2.65 ± 0.11	6.19 ± 0.56***	11.25 ± 0.84	36.73 ± 2.08**
MAC[a]	3		100	

[a]MAC(s) - maximum allowed concentrations of potentially toxic elements in the soil as prescribed by Serbian legal regulations, Official Gazette of FRY, No.23/94.

Data on GST activity in snails collected from two studied sites are presented in Figure 1. At the site located in the close proximity to pollution source (HS), snails showed higher GST activity compared to snails from the site RS with significant statistical difference ($p < 0.01$).

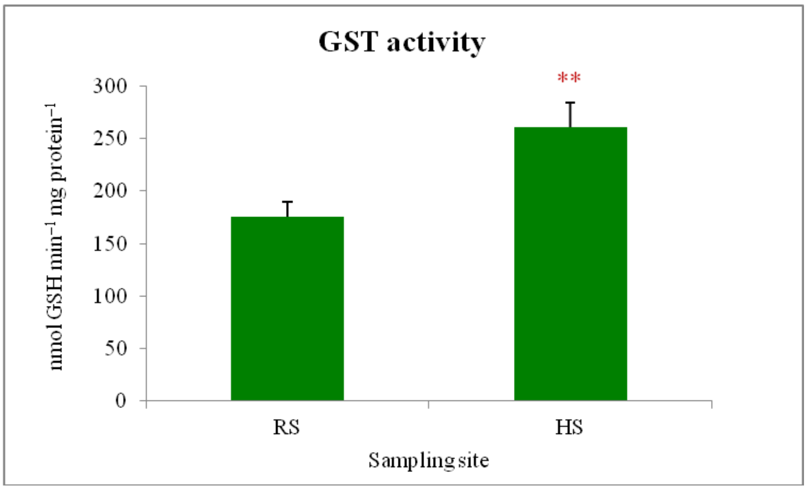

Figure 1 *Activity of glutathione S-transferase (GST) in viscera of Cepaea vindobonenesis sampled from referent (RS) and polluted (HS) site during April 2017. Data are presented as mean + SD (n = 10 for each site). Significant difference between referent and polluted samples were calculated by one-way ANOVA and marked with an asterisks, ** $p < 0.01$*

GST activity was significantly elevated in polluted snails, indicating that Cd and Pb can induce oxidative stress by producing ROS, and GST may be involved in cellular protection against metal-induced toxicity. Pollution of the soil with heavy metals is often less visible and direct than other types of land pollution, but its effects on terrestrial ecosystems and biota are long-lasting and severe. Different types of interactions, like that synergic and antagonic relationships found between heavy metal ions may increase their toxic potential. These findings are very important, because in the environment heavy metals usually co-occur in complex mixtures [19]. It is already shown that GST enzyme in land snail *Cantareus apertus* is responsive to Cd and Pb and their combination [20]. The induction of GST activity indicates an adaptation of the organism to enhanced pollution stress like is previously demonstrated [21].

CONCLUSION

The results in this study confirm that snail *C. vindobonensis* is able to accumulate Cd and Pb in concentrations higher than that in surrounding soil accompanied by significant difference in the GST values in the field. Increased GST activity suggests that the detoxification process against pro-oxidation forces, which are mediated by this enzyme, is induced. Therefore, this study suggests *C. vindobonensis* is a suitable sentinel organism and GST as suitable biomarker for terrestrial heavy metal biomonitoring.

ACKNOWLEDGEMENT

This work was supported by the Serbian Ministry of Education, Science and Technological Development (TR 37009).

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