METAL ACCUMULATION IN INSECTS (ORTHOPTERA, ACRIDIDAE) NEAR A COPPER SMELTER AND COPPER-FLOTATION FACTORY (PIRDOP, BULGARIA)

I. Karadjova[1], E. Markova[2]
[1] Faculty of Chemistry, University of Sofia
[2] Faculty of Biology, University of Sofia "St. Kliment Ohridski", Department of Ecology and Environmental Protection, blvd. Dragan Tzankov 8, Sofia–1164, Bulgaria
Correspondence to: Emilia Markova
E-mail: markovae@abv.bg

ABSTRACT
This study reports on accumulation of Cd, Co, Cu, Fe, Mn, Ni and Pb in insect species (grasshoppers) from different sites in the vicinity of a copper smelter and copper-flotation factory in mining region Pirdop, Bulgaria. Higher concentrations of Pb and Cd were found in the summer and autumn captured insects from the sample sites closer to the flotation factory than those from the control site, indicating, that elevated levels of these metals in this mining region is an important factor influencing body burdens. Observed differences were associated with higher metal concentrations in the plants from these sites because positive correlation between Cd and Pb concentrations in grasshoppers and plants for summer and autumn samples was found. It should be pointed that, for spring captured insects differences in Pb and Cd content for all sample saites under investigation were less significant. The concentrations of Cu, Fe, and Mn are more dependent on the feeding properties of the insects than on the distance from the emitter. In general metal body burdens for Cd, Cu, Mn, Pb and Ni were higher in summer captured insects than in spring irrespective of site or metal.

Keywords: Acrididae, grasshoppers, Pirdop, pollution, trace metals

Introduction
The area around copper smelter and copper-flotation factory near copper mine provide an opportunity to study the effects of geological and antropogenic factors on plants and animal communities. Several studies have been undertaken on the accumulations of metals in soils, plants and vegetables from this region and, as one would expect, concentrations of metals are usually highest at the sites closest to the smelter (2). However there is a lack of information for the metal accumulation in herbivorous insects. Data presented in the literature for the ecophysiological response of invertebrates to chronic and acute metal stress and their potential use as bioindicators are ambiguous (6; 3; 7). Generally higher metal concentrations have been reported in invertebrates collected from more contaminated sites and considerable differences have been observed between the metal concentrations of the various group of invertebrates (1; 4; 5). There is a growing evidence of a connection between air pollution and the degree of damage inflicted by herbivorous insects; however, the ecological significance of these relationships is still unclear (8). This paper aims to describe the patterns of metal (Cd, Cu, Co, Fe, Mn, Ni and Pb) accumulation in herbivorous insects - grasshoppers, (Orthoptera, Acrididae) at selected sites in the vicinity of a copper flotation factory and copper smelter in mining region Pirdop, Bulgaria. The samples are collected during spring, summer and autumn.

Materials and methods
Grasshoppers (Orthoptera, Acrididae) have one generation per year and their life cycle is characterized by four distinct stages. Single egg masses are laid in the soil at the end of October, where they remain throughout the winter. Larvae hatch in early spring, feed from may to June and pass through 4-5 instars. Adults insects were collected seasonally in the middle of June (spring), at the end of august (summer) and in the middle of October (autumn). Five sample sites were chosen with respect to prevailing winds and based on

previous investigations for soil and plant pollution. Site 1 is located far from the cooper flotation factory and copper smelter and is control, unpolluted point for this region. Sites 4 and 2 are located at distances of 0.5 and 3 km north respectively from copper flotation factory, site 5 is between copper flotation factory and copper smelter and site 3 is 2 km west from copper flotation factory. Five parallel samples were analyzed from each sample site.

Ultrasonic bath was used to clean invertebrates of surface deposits. After drying to constant weight (60-70 °C for 22-24 h) samples were digested using nitric acid.

Metal concentrations of all samples were analyzed by flame atomic absorption spectrometry, Perkin Elmer 1100B (Cu, Fe and Mn) and electrothermal atomic absorption spectrometry, Perkin Ellmer Zeeman 3030 (Cd, Ni and Pb). Calibration standards were prepared from Titrisol concentrates (Merck) and standard addition method was used for calibration. Analytical blanks and standard reference material (TORT -1 Lobster Hepatopankreas, National Research Council Canada) were treated simultaneously in the same manner to confirm accuracy of processes. All samples consist of individual animals, i.e. no samples were pooled.

Statistical analysis of the data was carried out using Kruskal-Wallis H-test and Mann-Whitney U-test, differences were considered significant at $P<0.05$ (9). These nonparametric comparison tests were considered after all values obtained were examined for normality. The Kolmogorov-Smirnov test was used as an estimate of goodness-of-fit to normality and only a very limited number of data sets revealed a good fit to normal distribution.

Results and Discussion

Analytical results obtained are presented at **Fig. 1** for all studied elements, seasons and sites. As can be expected in spring insignificant differences were observed in metal concentration for all investigated sample sites. Only for Fe relatively higher contents were found for site 4 and site 5, which are closer to the emission source.

However grasshoppers clearly showed sites-dependent metal accumulation patterns for Cd and Pb in summer samples. Taking into account, that the main pollutants emitted in this region are Cu, Cd, Fe and Pb, significantly elevated concentrations found for Cd and Pb in order site 5>site 4>site 3 confirm the expectation that the animals' body burden reflects site pollution ($p<0.05$, U-test). Less contrasting differences were found for Cu, while Fe, Mn and Ni ranges were not significantly different between all sites ($p<0.05$, H-test). It might be assumed that because Cu, Fe and Mn are essential ions in insect metabolism there are physiological mechanism, which aids in its regulations and prevent toxic levels. One explanation for site independent nickel distribution is that the volatility of this element is much lower in comparison with Cd and Pb. Taking into account that grasshoppers are herbivorous insects, pooled plant samples were analyzed from the same sample sites. An analysis of the correlation coefficient (linear, r^2) between metal concentrations in pooled plant samples and insects revealed significant ($P<0.05$) relationship for Cd ($r^2=0.95$) and Pb ($r^2=0.93$).

It should be pointed, that Cu, Cd Mn, Ni and Pb concentrations differ significantly with seasons. In general for all sample sites summer captured individuals accumulated more Cd, Cu, Mn, Ni and Pb then spring captured animals. This accumulation is more pronounced for Cd and Pb and less expressed for Cu, Mn and Ni. Season accumulation pattern (spring-summer) is well established for insects captured from more polluted sites e.g. site 4 and site 5. Season accumulation (summer-autumn) was observed only for Pb.

In autumn site-specific metal accumulation patterns were found only for Pb. For all other investigated elements same levels of content were analyzed for five sample sites.

Site-independent and season independent accumulation may explain the rather tangled picture of Fe levels in grasshoppers. Probably some specific feeding and life habits of these insects reflect on Fe accumulation.

Conclusion

- Cadmium and lead the main pollutants of the region, became heavily accumulated in grasshoppers at the most contaminated sites.
- Possitive correlation was found between Cd and Pb concentrations in pooled plant samples and in insects from the same sample site.
- Iron and manganese appeared to be under homeostatic control.
- The expression of site-specificity of copper another main pollutant for this region and Ni is not clear.

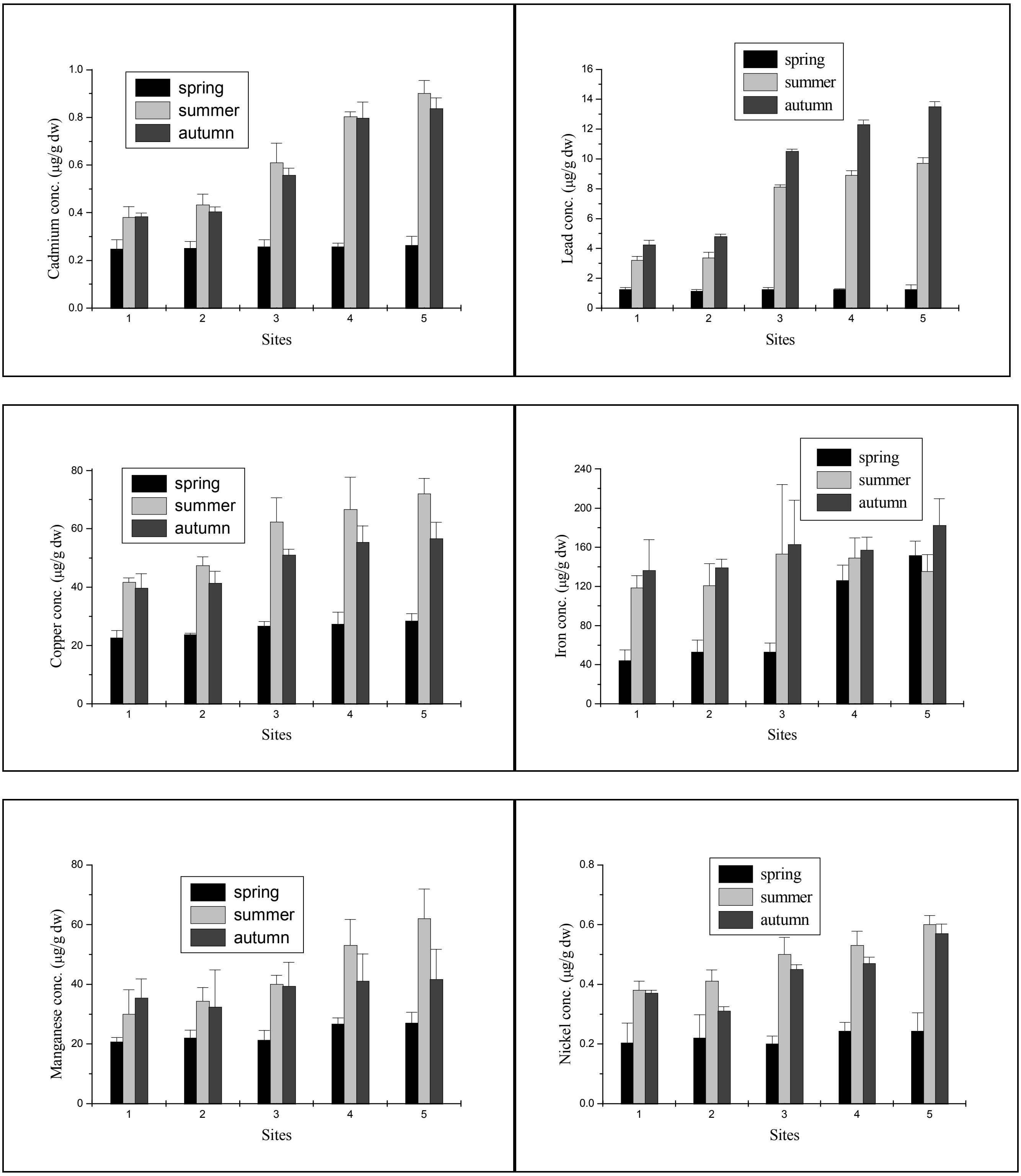

Fig. 1. Median and quartiles of metal concentrations in grasshoppers at different sites for spring, summer and autumn

REFERENCES

1. **Beeby A**. (1991). In: Metal Ecotoxicology. Concepts and Applications, (M.C. Newman & A. W. McIntosh, ed.) Lewis Publ., London, UK, 65-89.
2. **Djingova R., Kuleff I., Andreev N.** (1993). Chemosphere, **27:** 1385-1396.
3. **Hopkin S. P.** (1989). Ecophysiology of Metals in Terrestrial Invertebrates. Elsevier, London, UK. (ISBN 1-85166-312-6).
4. **Hunter B. A., Hunter L. M., Jonson M. S., Thomson D. J.** (1987). Arch. Environ. Contam. Toxicol., **16**: 711-16.
5. **Janssen M. P. M., Hogervorst R. F.** (1993). Environ Pollut., **79** : 181-9.
6. **Martin M. H., Coughtrey P. J.** (1982). Biological monitoring of heavy metal pollution. Land and Air. Applied science publishers, London and New York.
7. **Posthuma L., Van Straalen N. M.** (1993). Comp. Biochem. Physiol., **106 C**: 11-38.
8. **Riemer J., Whittaker J. B.** (1989). In: Insect-plant Interaction, Vol. 1 (E.A. Bernays, ed.). CRC Press, Boca raton, FL, USA, 73-106.
9. **Zar J. H.** (1974). Biostatistical Analysis. Prentice-Hall, Inc., Englewood Cliffs, NJ, USA, 620 p.