ABSTRACT

Ecotoxicological studies primarily focus on the cellular effects of heavy metal contaminations in insects. However, in spiders - significant predators of many insect species - mechanisms determining the resistance to high metal concentrations are still poorly understood. Until now, only a few studies have focused on the effects of heavy metal contaminations on the immune system of spiders. Quantitative and qualitative assessments of the status of hemocytes and changes in the proteome profile of hemolymph may help understand and recognize the reactions of the spider immune system in terms of compensatory/adaptive mechanisms determining the tolerance of high metal concentrations in the body.

This study assessed the immune potential of the hemolymph of female *Steatoda grossa* (Theridiidae) spiders after long-term exposure to cadmium and copper, which are administered orally via *Drosophila hydei* victims cultured on media supplemented with cadmium and copper salts in sublethal doses, for both the predators and their victims (0.25 $CdCl_2$; 0.23 $CuSO_4$), under laboratory conditions. Qualitative and quantitative assessments of the status of hemolymph were carried out after using natural (bacterial suspensions: *Staphylococcus aureus*; G+ and *Pseudomonas fluorescens*; G−) and artificial (PMA; phorbol myristate acetate) immunostimulants, and the status of hemocytes and the characteristics of the hemolymph proteome were compared between the control and the exposed individuals in terms of food contamination with metals. The parameters analyzed and the methods used are presented in the following table:

Parameter	Method/technique
Cadmium and copper concentration	Atomic absorption spectrometry (AAS)
Quantitative assessment of hemocytes	Bürker hemocytometer, light microscopy
Quantitative assessment of hemocyte subpopulations in hemolymph	Flow cytometry
Qualitative assessment of the status of hemocytes	Transmission electron microscopy (TEM)
Quantitative evaluation of hemolymph proteome	Two-dimensional difference gel electrophoresis (2D-DIGE)
Qualitative evaluation of hemolymph proteome	Mass spectrometry (MALDI– TOF MS)
Quantitative assessment of cecropin-like and defensin-like peptides	Enzyme-linked immunosorbent assay (ELISA)
Quantitative assessment of metallothionein-like peptides (MTs)	Enzyme-linked immunosorbent assay (ELISA)

Long-term exposure to copper resulted in lower bioaccumulation of this metal in the body than cadmium, confirming more efficient mechanisms that regulate the concentration of biogenic metals, including its faster excretion from the body. Following metal intoxication, a reduction in the total number of hemocytes was observed in the spider hemolymph. Bacterial suspensions and PMA effectively increased the total number of hemocytes, but the effectiveness of immunostimulants depended on exposure to metals. G+ and G− bacterial suspensions increased the number of hemocytes in the individuals intoxicated with metals, whereas PMA had a similar effect in the control group. Long-term exposure to metal-contaminated food also changed the quantitative proportions of the individual hemocyte subpopulations, reducing the proportion of small nongranular cells and increasing the proportion of granular cells. Cadmium exposure increased apoptotic and necrotic changes in hemocytes. Copper, at the used concentration level and the set exposure time, did not significantly affect the changes in the ultrastructure of hemocytes. Metal supplementation of the spider diet changed the protein profile of the hemolymph. To a higher extent than copper, cadmium favored the appearance of proteins characterized by altered activity. Among the identified proteins, the hemocyanin content was the highest, containing various chains and actin. Immunostimulation increased the level of cecropin-like and defensin-like peptides, but only in control individuals. Long-term exposure to the metal-contaminated diet disturbed the synthesis of the abovementioned antibacterial proteins in the hemolymph of the immunostimulated individuals. Metals in the diet also increased the synthesis of MTs in the hemolymph. However, the level of these proteins after immunostimulation depended on the type of metal. The highest level of MTs was found in the individuals intoxicated with copper after immunostimulation with G− bacterial suspension.

In female *S. grossa* spiders, dietary intoxication with metals evoked diverse responses in terms of both the ability to absorb metals and the influence of these contaminants on the quantitative and qualitative changes in hemocytes and proteins in the hemolymph.