Assessment of Stress Situations in the Grey Garden Slug, *Deroceras reticulatum*, Caused by Heavy Metal Intoxication: Semi-Quantification of the 70 kD Stress Protein (Hsp70)

Beurteilung schwermetallinduzierter Streßsituationen bei der Genetzten Ackerschnecke *Deroceras reticulatum*: Semi-Quantifikation des 70 kD-Streßproteins (Hsp70)

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Mature specimens of the grey garden slug, *Deroceras reticulatum* (Müller) were kept in microcosms containing a ground of raw humus and food (leaves of lettuce, carrot slices, powdered with $CaCO_3$) *ad libitum*. To characterize stress conditions inducing hsp70 the animals were kept under different temperature conditions: (1) constantly at 10°C, (2) constantly at 15°C, (3) at 10°C with a subsequent heat shock for 2 h at 20°C, or (4) at 10°C with a subsequent heat shock for 2 h at 25°C. Total protein was extracted, separated *via* SDS-PAGE, and hsp70 detected by a monoclonal antibody in the immunoblot. A single, well stained band of hsp70 could be observed in the slugs subjected to heat shock (20°C as well as 25°C) and also in the animals kept constantly at 15°C. For 10°C, only a weak band could be detected in the immunoblot (Fig.1). These observations revealed stress conditions for temperature ≥15°C and made subsequent toxicity experiments necessary to be carried out at 10°C.

To analyse stress response to environmentally relevant heavy metals, slugs were exposed to either 10, 50, or 100 mg/kg Cd^{2+} (as $CdCl_2$), to 100, 500, or 1,000 mg/kg Pb^{2+} (as $PbCl_2$), or to 500, 1,000, or 5,000 mg/kg Zn^{2+} (as $ZnCl_2$) at 10°C. Substrate and food were soaked with the respective solution (controls: tap water) and the experiment was carried out for 3 weeks. Constant amount of total protein (quantification by BCA-assay) was processed as mentioned above. Compared to the controls, concentration of hsp70 in the homogenates of the whole animals increased after contamination with any of the tested metal ions in nearly all examined samples. Darkest staining of the protein band occurred in specimens contaminated with 500 mg/kg Pb^{2+}, 1,000 mg/kg Pb^{2+}, or 5,000 mg/kg Zn^{2+} (Fig.2). Image analysis quantification of the mean relative grey value of all examined specimens revealed -in relation to the metal ion concentrations slugs were exposed to- different patterns of stress response of *D. reticulatum* to the three tested metals. For cadmium and lead, a significant increase in hsp70 content could be proven in animals exposed to 10 mg/kg Cd^{2+} or 100 mg/kg Pb^{2+}, for higher concentrations of the respective ion hsp70 protein content, however, remained nearly constant. In contrast, increased zinc contamination resulted in elevated hsp70 levels even up to the highest appied concentration. These results show induction of hsp70 to be a useful biomarker to assess stress conditions caused by different heavy metals especially in comparatively low concentrations.

Fig. 1

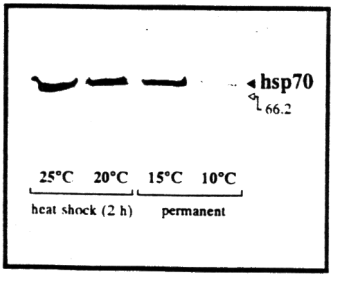
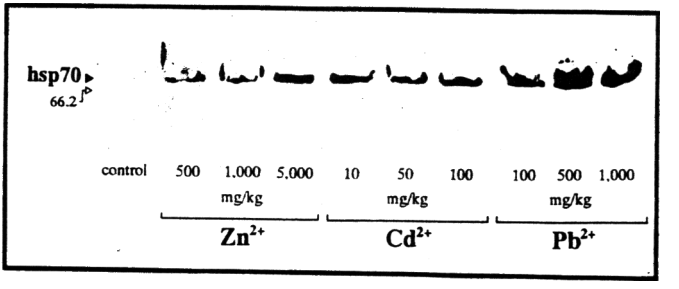


Fig. 2



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