

## Heavy metals and detoxifying enzymes in spiders from coal and metallurgic dumps near Ostrava (Czech Republic)

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### ABSTRACT

Detoxifying enzymes: carboxylesterases (CarE) [EC 3. 1. 1. 1] and glutathione S-transferases (GST) [EC 2. 5. 1. 18] were analysed and metal concentration (Cd, Pb, Cu, Zn) were monitored in tissues of web-building spiders: *Linyphia triangularis*, *Metellina segmentata*, *Araneus diadematus*. Spiders were collected in coal- and metallurgic dumps (HG and HH respectively) located on the Ostrava borders, and in Brenna-Bukowa (Beskidy Mts., Poland) being the control site.

Accumulation of heavy metals in spiders was both species (within the site) and site dependent. Only *M. segmentata* from HH being in the early stages of reclamation, had significantly higher burdens of Pb and Zn than individuals of the the same species from HG. All examined species of spiders, independent of the dump origin and stage of their reclamation, showed better abilities to detoxify xenobiotics by conjugation reactions (against the DCNB) than the spiders from the control site. The spiders inhabiting HH had also significantly higher GST activity, measured as CDNB conjugation with glutathione. On the other hand, *L. triangularis* and *A. diadematus* from the HG accumulated greater amounts of metals paralell to enhanced activity of GST, while the activity of CarE was generally lower in spiders from polluted sites in comparison with those from the control site, and the hydrolysis rate has not been correlated with their metal burdens. Only in *M. segmentata* from HH a high load of Cd and Pb was accompanied with high activity level of CarE.

### INTRODUCTION

Post-industrial dumps, due to their different stages of reclamation, are sites interesting enough not only for the study of plant succession or animal communities but also the development of adaptive mechanisms allowing them to live in these severely polluted environments. Earlier studies on spiders from areas near Ostrava (Ostrava-Karvina Industrial District)

revealed a presence of quite numerous populations. The species composition differed depending on the dump origin and stage of its reclamation (Majkus 1988). Chemical composition of the dumps and their specific microclimatic conditions stimulate various protective mechanisms in inhabiting organisms necessary for neutralisation of numerous industrial toxins, especially those easily transferred in food chain. Spiders, as the secondary consumers, are particularly vulnerable to various xenobiotics, including heavy metals (Marczyk *et al.* 1993). The existence in polluted habitats depends strongly on their tolerance of various xenobiotics entering the body mainly through the digestive tract. Higher tolerance involves, among some other mechanisms, appropriate changes in activity patterns of numerous detoxifying enzymes (Wilkinson 1980). Carboxylesterases (EC 3. 1. 1. 1) and glutathione S-transferases (EC 2. 5. 1. 18) that neutralise many endogenous and exogenous substrates are important as an alternative for oxidative degradation of organic xenobiotics (Yu 1982; Lindroth 1989). Analysis of activity patterns of these enzymes may provide useful indices of sublethal stress from industrial pollutants (Depledge & Fossi 1994). Differences in feeding activity, behaviour and body size among various species of spiders, explain well the alterations in their detoxifying abilities (Wilczek & Migula 1996). In this study we evaluated whether body burdens with heavy metals may affect detoxifying abilities in spiders from industrial dumps varying in chemical composition and advancement of reclamation.

#### **MATERIAL AND METHODS**

Adult females of *Linyphia triangularis* (Linyphiidae), *Metellina segmentata* (Metidae) and *Araneus diadematus* (Araneidae) were examined. These species differ in their strategy of prey searching, hunting intensity and the type of web-spinning (Foelix 1982; Heimer & Nentwig 1991).

The material was collected in 1994 and 1995 from metallurgic (Hrabovka) and coal (Dolu Lidice) dumps at the outskirts of Ostrava (Ostrava-Karvina Industrial District, Czech Republic). Control samples were from the mountainous area at Brenna-Bukowa (Beskid Śląski Mts., Poland). The metallurgic dump covering 108.6 ha, consists mainly of cinders from the iron and steel industry. The dump was established nearly fifty years ago and the rough materials are still stowed away there. The dump's material has a strong alkaline pH and contains high levels of phosphorus, calcium, magnesium, iron, manganese, copper, chromium, lead, and lower contents of cadmium (Hrabcova 1988; Sobotkova 1992). The coal dump was created in the process of piling the residues from coal mining, mainly rocks and carbonaceous shale. The dump was closed in 1966 and recently has been completely reclaimed (Majkus 1988). Characteristics of the main aerial pollutants of these areas are given in Tab. 1.

Captured spiders were anaesthetised on ice, weighed and homogenised at 0-4 °C in 1:50 vv, using Tris-HCl pH 7.85. Homogenised samples were centrifuged at 15,000 g for 10 min. The surface fat coats and pellets were discarded and the supernatants served as a crude enzyme extracts. Activity of carboxylesterases was measured spectrophotometrically at 600 nm, according to Van Asperen (1962) using  $\alpha$ -naphthyl acetate as a substrate. Glutathione S-transferase was assayed by measurements of conjugation rate of 3,4-dichloronitrobenzene (DCNB) and 1-chloro-2,4-dinitrobenzene (CDNB) with glutathione (Yu 1982) at 344 nm and 340 nm, respectively. Assays of each enzyme was repeated 6 times for each species from a given sampling site. The protein concentration was determined by the method of Bradford (1976). Contents of Cd, Pb, Cu, Zn were measured in dried, then acid digested samples by a flame and flameless atomic absorption spectrophotometry. Mean values for each metal were calculated from 8 determinations for each species from a site. Differences between means were tested by analysis of variance, using the LSD test for homogeneity testing at  $p < 0.05$ .

Tab. 1. Concentrations of selected chemical compounds in the suspended dust - seasonal means (July - October) for Ostrava and the annual means for Brenna (Hygienicke Stanice v Ostrave, 1994; Woj. Urząd Stat., Katowice, 1994).

SITES	SO <sub>2</sub> (mg/m <sup>3</sup> )	NO <sub>2</sub> (mg/m <sup>3</sup> )	pyren (ng/m <sup>3</sup> )	benzo a-pyren (ng/m <sup>3</sup> )	benzo a-antracen (ng/m <sup>3</sup> )	dibenzo -(a,h) antracen (ng/m <sup>3</sup> )	Cd (mg/m <sup>3</sup> )	Pb (mg/m <sup>3</sup> )	Zn (mg/m <sup>3</sup> )
Ostrava	47	55	20.6	6.7	12.3	20.7	0.0051	0.061	0.0067
Brenna	40	8	Nd	Nd	Nd	Nd	Nd	Nd	Nd

## RESULTS

### Carboxylesterases (CarE)

Spiders of *M. segmentata* from both dumps, had higher CarE activity than those from the control group (Tab. 3, 4). The highest activity, almost double that measured in control material, was stated in samples of this species from metallurgic dump (Tab. 3). In other species CarE activity was significantly lower than in appropriate controls. The activity level in *L. triangularis* was almost 50 % lower than that of the control site, while in *A. diadematus* these differences were 10 % and 50 % for coal and metallurgic dump, respectively. The tissue CarE activity level in material from 1995 was significantly lower than that of 1994, both in spiders from metallurgic and coal dumps (Fig. 1).

### Glutathione S-transferases (GST)

GST activity, measured against DCNB, was always higher in all the spiders from polluted areas. The highest GST activity was measured in *A. diadematus* from the metallurgic and coal dumps (Tab. 3, 4). Site-related differences were confirmed only in the case of *L. triangularis*. The spiders inhabiting the metallurgic dump showed also significantly higher GST activity, measured as the CDNB conjugation rate with glutathione. Time-related differences were indicated for two substrates: DCNB and CDNB in *M. segmentata* and *A. diadematus*, with higher GST activity in 1995 (Fig. 2, 3).

Tab. 2. Mean metal concentrations ( $\mu\text{g Me/g dry weight} \pm \text{SE}$ ) in spiders from various localities.

Species	Metal	Localities		
		coal dump	metallurgic dump	control site
<i>Linyphia triangularis</i>	Cd	4.98a	4.06a	4.97a
		$\pm 0.235$	$\pm 0.203$	$\pm 0.235$
	Pb	9.31a	8.93a	5.19b
		$\pm 0.810$	$\pm 0.700$	$\pm 0.520$
	Cu	75.33a	55.33b	41.37c
		$\pm 3.520$	$\pm 6.810$	$\pm 1.930$
Zn	752.44a	683.16a	365.74b	
	$\pm 41.262$	$\pm 89.201$	$\pm 23.177$	
<i>Metellina segmentata</i>	Cd	5.65a	6.24a	5.43a
		$\pm 0.262$	$\pm 0.405$	$\pm 0.272$
	Pb	6.36a	11.52b	7.12a
		$\pm 0.241$	$\pm 1.543$	$\pm 0.610$
	Cu	77.75a	84.57a	41.74b
		$\pm 2.910$	$\pm 8.060$	$\pm 1.363$
Zn	689.52a	810.62b	462.80c	
	$\pm 32.205$	$\pm 53.740$	$\pm 42.901$	
<i>Araneus diadematus</i>	Cd	3.42a	2.62ab	1.73b
		$\pm 0.451$	$\pm 0.130$	$\pm 0.380$
	Pb	9.33a	5.60b	3.30c
		$\pm 0.185$	$\pm 0.423$	$\pm 0.400$
	Cu	58.02a	50.92a	46.74a
		$\pm 5.510$	$\pm 8.375$	13.360
Zn	644.85a	657.04a	379.54b	
	$\pm 51.53$	$\pm 76.272$	$\pm 40.050$	

abc - different letters denote statistically differences of a given metal concentration in spiders from various sites (at  $p < 0,05$ ).

### Contents of heavy metals (Cd, Cu, Pb, Zn) in spiders

In *M. segmentata* and *L. triangularis* there were no significant interspecific differences in body burdens of cadmium from different locations (Tab. 2). *M. segmentata* from the metallurgic dump accumulated the highest levels of cadmium. The lowest cadmium levels occurred in specimens of *A. diadematus* from different locations, reaching half of these measured in other species (Tab. 2).

Significantly higher levels of copper, as compared to the control, were stated in *L. triangularis* and *M. segmentata* from both polluted areas. The highest copper concentration, almost double that found in the control group, was found in *M. segmentata* from the metallurgic dump. Also, *M. segmentata* and *L. triangularis* from the coal dump had similar levels of this element. Irrespectively of location, the lowest concentrations of copper occurred in *A. diadematus* (Tab. 2).

Elevated lead contents in the tissues were characteristic of all species collected from industrial dumps as compared to the control group. In comparison to other locations the lead concentration in *M. segmentata* from the metallurgic dump was 50 % higher at times. The lowest lead concentrations were in *A. diadematus* from the coal dump (Tab. 2).

In all specimens from polluted areas the zinc concentrations were significantly higher than in the control spiders. The highest level was in *M. segmentata* from the metallurgic dump. Accumulation of zinc in *L. triangularis* and *A. diadematus* from both analysed dumps was similar (Tab. 2).

### DISCUSSION

Environmental pollutants may cause strong negative effects on predators and detritivores as they are on upper positions in trophic chains (Clausen 1984; Nuorteva 1990). All spiders from environmentally degraded areas, irrespective of their systematic position and behavioural differences, had higher body burdens of metals than individuals from the control area in Beskid Śląski Mts. This would be the result of their polyphagic selection of food with high assimilation rates but low metabolism rate of hepatopancreas, where heavy metals are accumulated at high concentrations (Moulder & Reichle 1972; Hopkin 1989; Salo *et al.* 1991). The elements' migration into the body may be also facilitated by local abiotic conditions, such as climate, rainfall, wind direction, and acidification, caused by elevated concentrations of SO<sub>2</sub> and NO<sub>2</sub>. Actually, the high NO<sub>2</sub> concentrations observed near Ostrava might have facilitated heavy metal transport into the spiders' tissues despite highly alkaline dump material.

Our results showed significant differences in metal loads of spiders, probably influenced by prevailing conditions of different sites, species, and types of heavy metals. This supports the needs for further biomonitoring

studies, as heavy metal concentration in a given environment does not directly reflect actual exposure, increased accumulation or hazard for the organism. Most of the analysed elements reached the highest degree of accumulation in tissues of *M. segmentata*, especially in specimens from the metallurgic dump. Despite lower accumulation of metals in *A. diadematus* and *L. triangularis*, it is noteworthy that Linyphiids from the coal dump accumulated copper, while Araneids accumulated lead. Such differences in heavy metal contents of spiders could result from individual body burdens of heavy metal in their prey and their hunting activity (Salo *et al.* 1991; Nuorteva 1990). In the diet of Araneidae and Linyphiidae there is a considerable amount of herbivorous insects (Nentwig 1983), accumulating much lower levels of toxic substances than the detritivorous and carnivorous insects (Hunter *et al.* 1987). This might have contributed to lower heavy metal accumulation in *A. diadematus* and *L. triangularis* but was not confirmed for *M. segmentata*. A high intra- and interspecific variance in heavy metal accumulation may also be due to the fact that the polyphagous spiders catch all kinds of insects in their webs: herbivorous and detritivorous, carnivorous and parasitic ones. The kind of prey and their number depend on such factors as the environmental and climatic conditions or prey population dynamics in a certain area. The dumps where spiders were collected, due to varying progress of reclamation, offer different conditions for preying, with the coal dump, mostly reclaimed, presenting the best variety.

The accumulation of heavy metals in an organism depends on its physiological properties and characteristics of a particular element. Numerous invertebrate species, including spiders, avoid toxic substances by their elimination with faeces. Low levels of heavy metals in Araneids may result from the strategy used, also in severally polluted environments. On the other hand, elevated accumulation of heavy metals in *M. segmentata* exemplifies a different strategy involving the storage of toxic substances as intracellular mineral granules in their tissues (Hopkin 1989). Histochemical studies confirmed the presence of such granules in the midgut gland of spiders (Ludwig & Alberti 1988)

As the spiders accumulated high levels of metals we hypothesised about their negative effects on functioning of detoxifying enzymes. CarE and GST, selected in our studies, consist of several isoenzymes, which activity can be modified by various toxins of natural as well as industrial origin (Lindroth 1989; Grant *et al.* 1991; Iio *et al.* 1993). The interspecific differences in the activity patterns of analysed enzymes, which we observed within and between selected locations, may have resulted from an increased rate of hydrolysis or conjugation as the effects of increased concentrations of inductors of a specific isoenzyme. Glutathione transferases are a group of cytosolic enzymes, which catalyse the conjugation of reduced glutathione with a wide range of lipophilic

toxicants bearing electrophilic sites. The conjugates are further transformed to more water-soluble metabolites, which are easily extractable. This is regarded as an important detoxification mechanism, known in various groups of animals. Our results showed that a conjugation due to GST activity plays an important role in the neutralisation of several environmental toxins in spiders. All studied species from polluted areas, despite their hunting intensity, spatial distribution and systematic position, showed a significant increase in the transferase activity measured against DCNB. Concomitantly all spiders collected from the metallurgic dump had higher GST activity measured against CDNB. This may be explained as the direct effects of high concentrations of metals and organic compounds (Fair 1986; Freundt & Ibrahim 1991; Yamaguchi *et al.* 1991), such as aromatic hydrocarbons prevailing near Ostrava. Possibly increased detoxification processes and elevated concentrations of existing isoenzymes involve expression of the genes responsible for synthesis of transferases (Yu 1982). Seasonal variations in transferase activity suggest also a stimulating effect of local pollutants. However, this also indicates interspecific differences in sensitivity to particular industrial toxins. The increased rate of cytoplasmic biotransformation processes involving either CarE or GST activity was stated only in *M. segmentata*. These reactions were well exposed in specimens collected from the metallurgic dump, with the highest concentrations of heavy metals in their tissues. Although specimens of *L. triangularis* and *A. diadematus* collected from the coal dump had a high GST activity, in case of CarE the activity was significantly lower than in control animals. These species accumulated also high levels of lead and zinc. Low CarE activity probably resulted from the inhibitory action of these metals on enzymes, interacting directly with the active centre of the enzyme or by the inhibition of the gene expression for these proteins (Polek & Fric 1980). Such xenobiotic-enzyme relations were species-specific, because in *M. segmentata* from the metallurgic dump high metal loads correlated well with the activity of both analysed detoxifying enzymes. Quantitative differences in esterase activity between species and even between strains (as a result of esterase gene amplification) are known as one of adaptive mechanisms against environmental xenobiotics (Callaghan *et al.* 1991). CarE may be used as a biomarker of exposure to various pollutants when a species is available for enzymatic assays. Transferases are good indicators of metals toxicity in a given species.

Our studies suggest that functional abilities of a given spider species inhabiting various dumps depend on mobilisation of adaptive mechanisms increasing their tolerance to high metal loads as well as effective detoxification of many organic xenobiotics, also by the conjugation reactions. Metabolic differences among the species are the overall effects of various interacting pollutants and local changes in their microhabitats including specificity of adaptive mechanisms of particular species.

Tab. 3. Means specific enzymes activity ( CarE - nmol/min/mg  $\pm$ SE; GST -  $\mu$ mol/min/mg protein  $\pm$  SE) in spiders from various localities - data from 1994.

Localities	Enzymes	Species		
		<i>Linyphia triangularis</i>	<i>Metellina segmentata</i>	<i>Araneus diadematus</i>
coal dump	CarE	65.07	163.3	84.33
		$\pm 2.66$	$\pm 13.67$	$\pm 2.51$
	GST 1	2.62	2.23	3.89
		$\pm 0.42$	$\pm 0.12$	$\pm 0.26$
	GST 2	68.35	59.12	55.72
		$\pm 2.24$	$\pm 6.63$	$\pm 4.58$
metallurgic dump	CarE	131.23	194.67	42.22
		$\pm 11.29$	$\pm 13.4$	$\pm 7.57$
	GST 1	1.69	2.5	3.98
		$\pm 0.16$	$\pm 0.13$	$\pm 0.81$
	GST 2	115.50	94.5	91.47
		$\pm 4.19$	$\pm 8.37$	$\pm 12.16$
control site	CarE	130.25	109.85	91.76
		$\pm 10.14$	$\pm 6.17$	$\pm 3.66$
	GST 1	1.01	1.57	1.75
		$\pm 0.12$	$\pm 0.15$	$\pm 0.32$
	GST 2	-	-	-
		-	-	-

GST 1-glutathione S-transferase (DCNB), GST 2-glutathione S-transferase (CDNB).

Tab. 4. Homogeneity analysis of enzyme activity in three species of spiders from the various localities.

ENZYMES	Homogeneity		
	<i>L. trian.</i>	<i>M. segm.</i>	<i>A. diad.</i>
GST 1	<u>B HH HG</u>	<u>B HG HH</u>	<u>B HG HH</u>
GST 2	HG HH	HG HH	HG HH
CarE	HG <u>B HH</u>	B HG HH	HH <u>HG B</u>

Localities are placed in an increased order of enzyme activity. Homogenous groups are underlined (LSD- test). B - Brenna, HG - coal dump, HH - metallurgic dump; GST1 - glutathione S-transferase (DCNB), GST2 - glutathione S-transferase (CDNB), CarE - carboksyesterases.

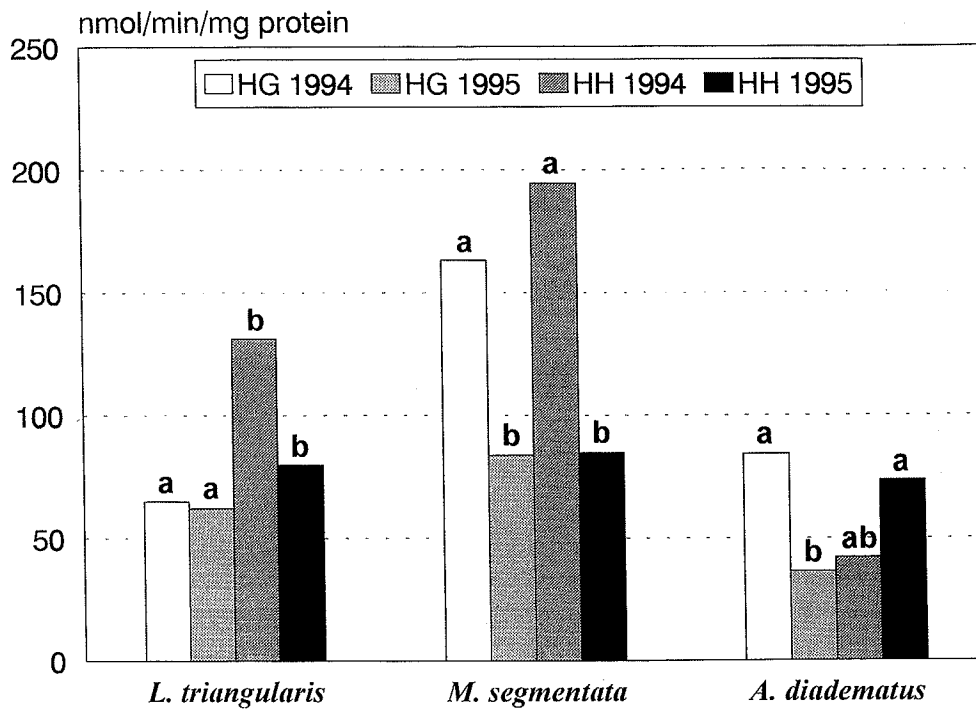


Fig.1. Time related (1994-1995) activity of carboxylesterases in spiders from coal (HG) and metallurgic dumps (HH). Homogenous groups are labelled by the same letters a, b, c.

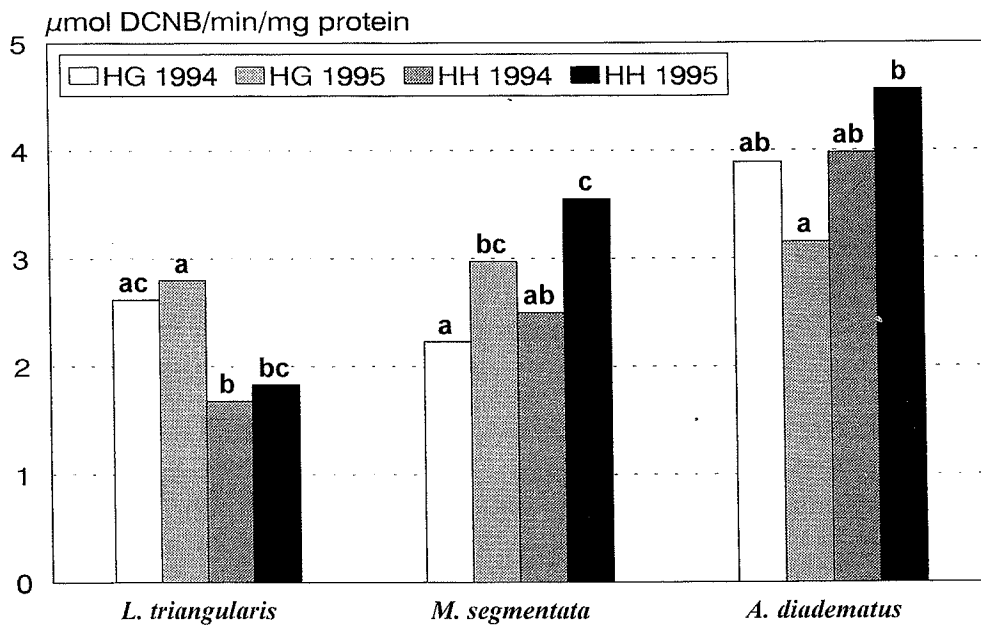


Fig. 2. Time related (1994-1995) activity of glutathione S-transferase (DCNB) in spiders from coal (HG) and metallurgic dumps (HH). Homogenous groups are labelled by the same letters a, b, c.

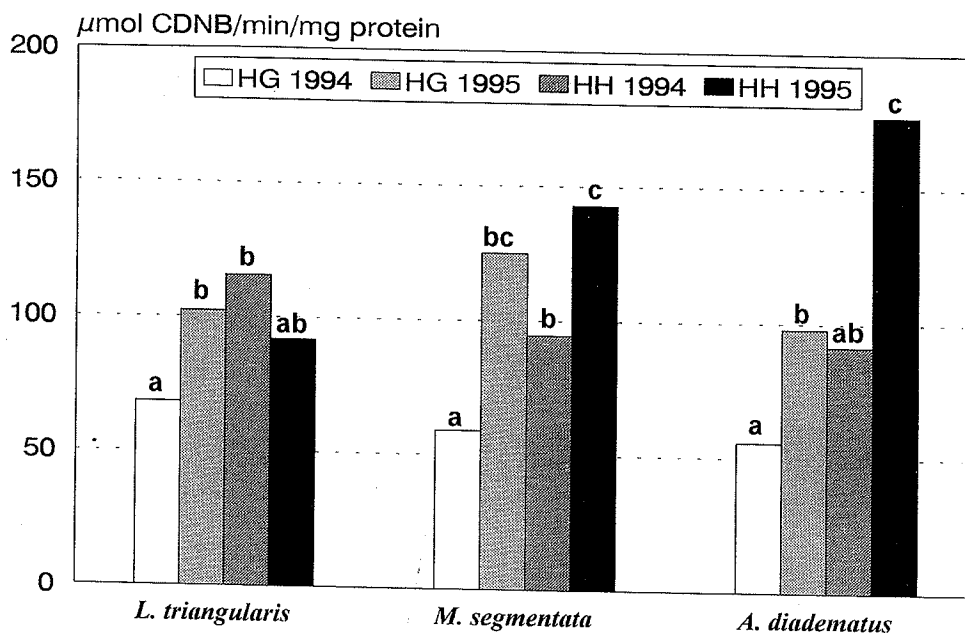


Fig. 3. Time related (1994-1995) activity of glutathione S-transferase (CDNB) in spiders from coal (HG) and metallurgic dumps (HH). Homogenous groups are labelled by the same letters a, b, c.

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