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Effects of the Metals Lead and Zinc on the Growth, Development, and Reproduction of *Pardosa Astrigera* (Araneae: Lycosidae)

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Abstract Metal pollution is a serious environmental problem worldwide, and severely threatens biological diversity and human health. In order to investigate the effects of metals on a potential indicator species of wolf spider, *Pardosa astrigera* (Araneae: Lycosidae), 3rd-instar spiderlings were exposed to metals by using sublethal concentrations of $PbCl_2$ and $ZnCl_2$ solutions as their drinking water. The total durations of development of *P. astrigera* from the 3rd to 6th instars were significantly longer than that of the control group, and females showed a sharp reduction in total egg number. Body weights were significantly decreased in the mature spiders exposed to $PbCl_2$ solutions and $ZnCl_2$ solutions of high concentration (100 mM). These results suggest that detoxifying strategies deployed by *P. astrigera* against metal intoxication incur the costs of delayed development, and reduced growth and reproduction.

Keywords *Pardosa astrigera* · Metal · Body weight · Developmental duration · Total egg number

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One consequence of rapid development, industrialization, and urbanization is that the number and amounts of metals entering the soil, air, and water continue to grow (Sorvari et al. 2007). Metal pollution is now a serious issue in many countries, affecting a multitude of environments (Srivastava and Goyal 2010). Metals can accumulate in plants, and via food webs can cause harmful effects to herbivorous and pollinating insects and their predators, among other members of the biota. Biological indicator species are species that can be measured as surrogates for environmental health and levels of pollution before adverse environmental impacts become obvious. This field of ecotoxicological research, as it relates to the accumulation of metals, focuses on the responses of indicator species to determine, for example, the rate and extent of metal accumulation in the body. A great deal of attention has been paid to the effects of metal pollution on insects (van Ooik et al. 2007). Metals can be absorbed from the soil through the roots of plants, and can also be absorbed through the leaves in gases or dust collected from the atmosphere, and then enter herbivorous animals such as cicadas, locust and sawflies (Thornton 1991). However, the impacts of metal pollution on spiders, key predators of insects, remain unclear (Hendrickx et al. 2003a).

Recent research into spiders as indicator species has concentrated on the contamination and accumulation of metals (Larsen et al. 1994; Wilczek et al. 2004). When spiders are exposed to environmental pollutants, growth and reproduction can be sharply reduced due to an increased detoxification effort (Hendrickx et al. 2003b). Spiders, at the individual level, have great potential as biological indicators of metal contamination in soil, because of their position in the food web and consequent biological magnification (Maelfait 1996; Marc et al. 1999; Jung et al. 2007). The spider webs can also serve as indicators of metal pollution in the atmosphere (Shao et al. 2006).

The toxic effects of metals can cause harm to insects in several ways, including changing the duration of development; reducing the pupation rate, body weight, number of eggs laid, and hatching success; increasing mortality; and leading to declines in population size (Mousavi et al. 2003; Hayford and Ferrington 2005); Jung et al. (2005) reported that the metal cadmium can significantly reduce the rate of increase of body weight in spiderlings of *Pardosa astrigera*.

In China, there were diseases resulted from long-term exposure to the sub-lethal concentration of lead (Pb) such as neurasthenic syndrome and hypothyroidism. Pb is an important raw material in industrial production, and it usually enters the human body through inhalation of vapors or dust. Toxic effects on human systems include, mainly, lesions in the nervous system, and cause a lifelong injury of the blood system, cardiovascular system, and skeletal system. Use of pesticides and chemical fertilizers containing zinc (Zn) in agriculture leads to accumulate this metal in the environment. Zn comes into the soil in the form of liquid, and excess Zn can cause zinc fever (Jiang et al. 2009).

Spiders occupy an important part of the predatory arthropod fauna in terrestrial ecosystems (Riechert and Lockley 1984). They also play a large role in the regulation of pest species in agriculture (Barrion and Litsinger 1995). Wolf spiders are generalist predators of other terrestrial invertebrates, and because they live on the surface of the ground, which may be contaminated with metals, their role in the bioaccumulation and transfer of Pb and Zn in the food chain should be understood thoroughly. *Pardosa astrigera* has the independent way of life, and is an active predator in the terrestrial environment, common, and widely distributed in various ecosystems. It has been recently suggested as an important bioindicator species to evaluate the terrestrial environment (Jung et al. 2005). In this study, we exposed *P. astrigera* to sublethal concentrations of Pb and Zn, by adding salts of these metals in the drinking water of spiders, to test the effects on growth, development, and reproduction of the spider.

Materials and Methods

Subadult individuals of *P. astrigera* were collected from cotton fields on the campus of Huazhong Agricultural University, Wuhan, China, in November 2007. Spiders were kept individually in cylindrical glass tubes (diameter 2 cm, height 12 cm) with a layer of sponge (1.5 cm thick) moistened with distilled water on the bottom. The tubes were plugged with cotton. The spiders were kept in a chamber at 24°C and relative humidity of 60%–80% under a light: dark cycle of 14:10 h (lights turned on at 08:00 hs). We fed the spiders with adults of *Drosophila* sp. and *Tendipes* sp. every 2 days. Two days post-maturation (determined by completion of their final molt), females and males were placed together for mating. Males were removed after the female deposited the first egg sac. Following the emergence of 2nd-instar spiderlings, and dispersal from the female's abdomen, siblings from different egg sacs were separated and reared individually with flies. Once the 2nd-instar spiderlings molted, they were used for the following experiments.

Third-instar spiderlings were reared individually in cylindrical glass tubes (diameter 2 cm, height 12 cm). We added metal salt ($PbCl_2$ and $ZnCl_2$) solutions, using a dipping tube to moisten the sponge lining in the bottom of each tube. We established seven experimental groups, and used three concentrations of $PbCl_2$ solution (1, 5, and 10 mM), three concentrations of $ZnCl_2$ solution (10, 50, and 100 mM), and water for a control group. We chose these concentrations according to Jung et al. (2005) and then carried out a preliminary experiment. Each experimental treatment comprised 50 spiders, and was repeated three times. We fed the spiderlings with adults of *Drosophila* sp. and *Tendipes* sp., and replaced the sponges every second day. Molts were recorded when exuviae were observed in the tube, and the time between molts was used as our measure of developmental duration. Sexually mature males and females were then weighed and housed together for mating, after which the males were removed. We collected all egg sacs laid by the females, and counted the total number of eggs.

Data are expressed as mean ± S.D. For our six experimental manipulations and one control group, we measured the developmental duration, body mass, and total number of eggs produced. Data were compared for statistical significance using Duncan's multiple range tests (SAS Institute, 1997). The data were tested for homogeneity of variance using Levene's test of equality of error variances. Normality was checked by the Kolmogorov–Smirnov test. The differences in the developmental durations, body weights, and total number of eggs between the two metals (Pb and Zn) were compared by the *t*-test (SPSS version 13.0).

Results and Discussion

Differences in the developmental duration of *P. astrigera* between the experimental groups were apparent (Table 1). The total developmental duration from the 3rd to the 6th instar was significantly longer in spiders exposed to Pb and Zn compared to the control (Table 1). This suggests that

Table 1 The developmental duration (d) of *Pardosa astrigera* exposed to different concentrations of $PbCl_2$ and $ZnCl_2$

Stage	Water (Control)	Concentration of $PbCl_2$ and $ZnCl_2$ (mM)					
		$PbCl_2$			$ZnCl_2$		
		1	5	10	10	50	100
3rd instar	12.25 ± 0.45a	12.34 ± 0.56a	11.67 ± 0.67a	12.04 ± 0.43a	13.78 ± 0.69b	13.46 ± 0.28b	13.23 ± 0.12b
4th instar	11.07 ± 0.82a	11.44 ± 0.34a	12.89 ± 0.59b	13.02 ± 0.42b	11.92 ± 0.23a	12.90 ± 0.65b	12.98 ± 0.78b
5th instar	12.72 ± 0.41a	14.24 ± 0.66b	16.19 ± 0.48b	17.41 ± 0.38b	13.30 ± 0.64a	13.42 ± 0.49b	13.78 ± 1.20b
6th instar	14.18 ± 0.98a	18.36 ± 1.34b	19.10 ± 0.72b	20.23 ± 0.79b	16.33 ± 0.78b	16.06 ± 0.54b	17.37 ± 0.55b
Total	49.22 ± 3.23a	56.38 ± 6.23b	59.85 ± 6.74b	62.70 ± 5.98b	55.33 ± 5.03b	55.84 ± 6.42b	57.36 ± 5.86b

Duncan's multiple range test, LSD. Data ended with different letter denote a significant difference($p < 0.05$)

Table 2 The body weight (mg) of mature *Pardosa astrigera* exposed to metal salt solutions

Sex	Water (Control)	Concentration of $PbCl_2$ and $ZnCl_2$ (mM)					
		$PbCl_2$			$ZnCl_2$		
		1	5	10	10	50	100
Female	40.8 ± 9.0a	35.3 ± 7.3b	36.2 ± 12.1b	33.8 ± 9.2b	37.8 ± 8.7a	32.9 ± 3.2b	34.3 ± 10.3b
Male	32.7 ± 4.5a	26.7 ± 9.8b	28.5 ± 10.9b	25.9 ± 10.5b	30.9 ± 3.4a	31.0 ± 5.2a	28.7 ± 8.2b

Duncan's multiple range test, LSD. Data ended with different letter denote a significant difference ($p < 0.05$)

when *P. astrigera* is exposed to Pb and Zn, its development time lengthens. However, there was no difference between Pb and Zn in their effects on the developmental duration of *P. astrigera* ($t = 1.8$, n = 9, $p = 0.11$).

We found that exposure to $PbCl_2$ solution in any concentration resulted in adult spiders with lower body mass compared to the control ($p < 0.05$) (Table 2). Exposure to $ZnCl_2$ only affected the body mass of adults at higher concentrations: an effect was only found for females at and above 50 mM, and for males at 100 mM. There was no difference between Pb and Zn in the effects on female body mass ($t = 0.062$, n = 9, $p = 0.48$), but the body mass of males exposed to Pb was lower than those exposed to Zn ($t = 2.95$, n = 9, $p = 0.02$).

Females exposed to Pb (n = 9, $p = 0.012$) and Zn (n = 9, $p = 0.017$) both produced fewer eggs than control animals (Fig. 1). This effect was stronger in Pb-treated than in Zn-treated females ($t = 3.25$, n = 9, $p = 0.009$).

In order to protect themselves from the harmful effects of metals, animals deploy certain detoxification mechanisms, which allow their organs to conduct normal physiological functions (Hendrickx et al. 2003a). Spiders acquire metals from their prey and detoxify them by deactivation, sequestering them in the intercellular pellet of midget diverticulae. This kind of detoxifying process can effectively enhance the viability of the spider in a metal-polluted environment, but it is certain that this kind of defense mechanism incurs the cost of slowing the growth, development, and reproduction of the spider

In the present study, as *P. astrigera* was exposed to different concentrations of $PbCl_2$ and $ZnCl_2$, its developmental duration lengthened, the rate of weight gain slowed, and the total number of eggs laid by the females was greatly reduced. The concentration of $PbCl_2$ was only one-tenth that of $ZnCl_2$, but the influence of $PbCl_2$ on body weight and total number of egg produced by females of *P. astrigera* was greater than that of $ZnCl_2$, suggesting that Pb is more toxic to the spiders.

Metal pollution at sublethal doses will most likely change reproductive strategies of insects and spiders (Hendrickx et al. 2003b; Sibly and Calow 1989; Parry et al. 2001). According to the optimal life-history model, when environmental resources decrease, animals can employ two kinds of reproductive strategies, one of which is to reduce the number of offspring but produce larger eggs (Stearns 1992). The larger descendants have a higher probability of survival, earlier maturation, and more rapid development. An alternative strategy is to produce smaller eggs, but in larger numbers, to improve reproductive capacity (Tamate and Maekawa 2000); Hendrickx et al. (2003a) reported that a population of *P. astrigera* with a high metal content produced markedly fewer, but larger eggs. Our results were similar to those of Hendrickx et al. (2003b). When *P. astrigera* was subjected to the stress of Pb or Zn, female

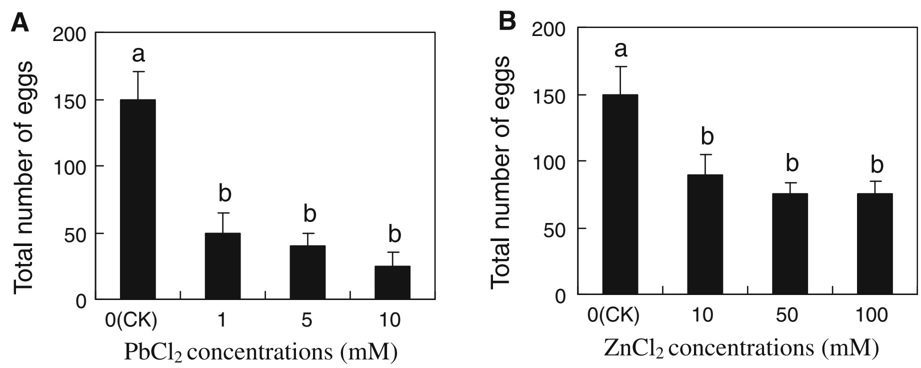

Fig. 1 The total number of eggs produced by females of *Pardosa astrigera* exposed to different metal solutions ($PbCl_2$ and $ZnCl_2$). Duncan's multiple range test, LSD. *Different letters* above the columns denote a significant difference ($p < 0.05$)

spiders laid fewer eggs than the control group. We did not measure the size of the eggs, and have no information about whether *P. astrigera* populations can lay fewer but larger eggs to adapt metal stress, or not. Further work is needed to resolve this question.

In studying the effects of metals on insects, these substances are commonly added to the food (Eeva et al. 2004; Nursita et al. 2005). Since spiders are primarily carnivorous, they require behavioral cues from the prey to initiate attack and feeding (Riechert and Luczak 1982) and most species of spiders must be reared by using live prey (Foelix 1996). There are no artificial diets appropriate for rearing spiders (Amalin et al. 1999). Therefore, Jung et al. (2005) dipped fruit flies in a metal salt solution and used them to feed the spiders. Their method allows strict control of the amount of metal absorbed by the spider. Our present study followed from an investigation of the effects of sublethal doses of pesticides on spiders (Tietjen and Cady 2007) and allowed the spider to take in metals by adding a metal salt to its drinking water. Although we cannot accurately measure the dose of metals received by the spider, our method is easy to carry out, and we obtained the same effectiveness as exposure to metals by dietary uptake (Jung et al. 2005). In this latter study, the contents of Pb and Zn did not show a clear correlation with the metal contents in the sediments. This report is somewhat different from our findings, but demonstrates that dietary uptake of metals through the food chain may function as a more important route of exposure to metals than direct contact with the environment such as with contaminated soils and sediments.

In general, lead and zinc had chronic effects on *P. astrigera,* while the spiders exhibited no apparent symptoms of acute intoxication. *Pardosa astrigera* can be employed as a specific indicator for metal exposure, and also for the evaluation of chronic effects of metals in food chains after long-term exposure.

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