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BIOMONITORING OF ATMOSPHERIC POLLUTION: POSSIBILITIES AND FUTURE CHALLENGES

Heavy metal accumulation in lizards living near a phosphate treatment plant: possible transfer of contaminants from aquatic to terrestrial food webs

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Abstract We investigated the accumulation of heavy metals in Bosk's fringe-toed lizards (*Acanthodactylus boskianus*) living in Gabès region (southeastern Tunisia), in relation to habitat, diet, and distance from the Gabès-Ghannouche factory complex of phosphate treatment. More specifically, we compared the concentrations of cadmium, lead, and zinc in the stomach contents and samples of the liver, kidney, and tail from lizards living in four sites corresponding to different combinations of habitat (coastal dunes vs backshore) and distance from the factory complex (<500 vs 20 km). Examination of stomach contents showed that lizards living on the coastal dunes mainly feed on littoral amphipods, while those living in the backshore feed exclusively on terrestrial invertebrates. The concentrations of heavy metals in lizard tissues were overall positively correlated with those in the preys they ingested. Moreover, there was a general tendency towards increased concentrations of cadmium, lead, and zinc in the samples from lizards living on coastal dunes compared to those from the other sites, although some differences still lacked statistical significance. These results suggest that the highest contamination of lizards living on coastal dunes was probably related to the ingestion of contaminated amphipods. Thus, amphipods and Bosk's fringe-toed lizards seem to provide an important link between the marine and terrestrial food webs, with higher concentrations appearing to accumulate from materials released into the sea rather than the terrestrial environment. With regard to metal distribution among tissues, our results were overall in agreement with previous findings in other reptiles. In particular, cadmium was most concentrated in the liver samples, stressing once more the role of the liver as a storage organ of Cd. Moreover, high concentrations of the three assessed metals were found in the kidney samples, showing the role of the kidney as an active site of heavy metal accumulation.

Keywords *Acanthodactylus boskianus* · Environmental pollution · Heavy metals · Trophic transfer · Tunisia

Introduction

Pollution of the natural environment by heavy metals is a worldwide problem that influences the functional and structural integrity of aquatic and terrestrial ecosystems (Harte et al. 1991; Farkas et al. 2001). Because of their high toxicity, long persistence, bioaccumulation, and sometimes biomagnification in the food chains, heavy metals constitute serious threats against wild animals, particularly those occupying high trophic levels (Zhuang et al. 2009). Previous studies have focused on the transfer of heavy metals along the food chains in various aquatic (Van Straalen and Ernst 1991; Amiard-Triquet et al. 1980; Quinn et al. 2003) and terrestrial ecosystems (Inouye et al. 2007; Ping et al. 2009). However, the possible movements of these chemicals between the aquatic and terrestrial food webs through littoral organisms have rarely been investigated (e.g., Walters et al. 2008; Mogren et al. 2013; Soucek et al. 2013). This issue is nonetheless of great interest for understanding the extent of the effects of heavy metals released in the natural environments.

Gabès region, in southeastern Tunisia, represents one polluted area where such a transfer is expected to contribute to the exportation of heavy metals from the marine to the bordering

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terrestrial ecosystems. This area is nowadays considered as a pollution hotspot mainly due to the operation of the Gabès-Ghannouche factory complex of phosphate treatment for acid and fertilizer production since the early 1970s (Béjaoui et al. 2004). With the absence of scavenging processes and treatment plans for industrial wastes, persistent toxic elements and heavy metals find their way into the sea. Alarmingly, 10,000 to 12,000 tonnes of phosphogypsum, containing heavy metals mainly, cadmium (Cd), lead (Pb), zinc (Zn), copper (Cu), and chromium (Cr), are released in the sea per day (Béjaoui et al. 2004, Ayadi et al. 2015). Previous studies have shown high levels of heavy metals in the coastal sediments close to the factory complex (Illou 1999, Ayadi et al. 2015). Particularly high concentrations were recorded in the site where the effluents are released: Cd, >100 ppm; Pb, 10–12 ppm; Zn, >1000 ppm; Cu, 50–60 ppm; and Cr, 100–120 ppm (Ayadi et al. 2015). The accumulation of heavy metals in the tissues of some marine animals, mainly molluscs (Rabaoui et al. 2013) and fishes (Messaoudi et al. 2008), has also been demonstrated. However, information about the possible contamination of terrestrial animals inhabiting littoral areas through the consumption of contaminated marine and littoral preys is lacking.

One of these terrestrial predators that we expected to be particularly exposed to heavy metals in this littoral area is the Bosk's fringe-toed lizard, *Acanthodactylus boskianus*. This lizard is one of the most abundant lacertids in Gabès region, where it occurs in different habitats including coastal dunes and sandy inland areas (Schleich et al. 1996; Nouira and Blanc 2003; Geniez et al. 2004). It is an opportunistic predator that feeds on various invertebrates and changes its diet in relation to prey availability (Carretero 1997; Kalbousi and Nouira 2004). Previously, we have noticed that the diet of Bosk's fringe-toed lizards inhabiting the coastal dunes close to Gabès City is mainly composed of *Talitrus saltator*, an abundant and widespread amphipod in the intertidal area. Although no accurate data are available, amphipods are likely to be contaminated by heavy metals discharged into the sea. The ingestion of these possibly contaminated preys, together with the dermal contact and the accidental intake of sediment and sea water, is likely to favor the contamination of lizards. We thus expected lizards living in the sandy beaches close to the factory complex to suffer higher exposure to heavy metals than those living in the nearby backshore and preying exclusively on terrestrial invertebrates. We also expected the proximity to the factory complex to be associated with increased exposure to heavy metals in both habitats.

The aim of this work was to test these expectations using data on Cd, Pb, and Zn concentrations in the stomach contents and samples of the liver, kidney, and tail from Bosk's fringe-toed lizards. The liver and kidney were sampled because they are known to play the role of detoxifying organs and to accumulate heavy metals (Liu et al. 2000; Sabolic et al. 2001; Trinchella et al. 2006). We also sampled the tail because it represents a combination of fat, blood, muscle, bone, and skin tissues, thus providing a general assessment of metal accumulation in the whole organism (Fletcher et al. 2006). We compared the accumulation of heavy metals in lizards living in four sites corresponding to different combinations of habitat (coastal dunes vs backshore) and distance from the factory complex (nearby vs faraway). By doing so, we also aimed to add to the ecotoxicological literature new data from one particularly polluted but largely unexplored area.

Materials and methods

Study sites and data collection

Fieldwork was carried out in two localities in the coastal area of Gabès: Ghannouche (less than 500 m from the factory complex) and Limaoua (20 km from the factory complex) (Fig. 1). At both localities, we sampled lizards in two sampling sites, corresponding to two habitat types: sandy beach vs sandy backshore (Fig. 1). Our sampling scheme was thus designed to collect data from four sites corresponding to different habitat-locality combinations. As stressed above, Ghannouche beach is known to be contaminated by heavy metal released into the sea by the phosphate treatment plant. Ghannouche backshore is supposed to be less contaminated, although detailed information on heavy metal concentrations in this site is lacking. Nonetheless, this terrestrial area could also be affected by the dispersion of heavy metals, presumably by the wind, from the solid waste directly deposited into the plant, as it has been shown in similar industrialized areas (e.g., Aoun et al. 2010). Limaoua locality is situated in an industry-free area and is less affected by pollution.

Lizards were caught by hand and carried alive in separate cloth bags to the laboratory for sampling stomach contents and tissues. Only sexually mature lizards, identified later by gonad examination after dissection, were considered in the study sample. Upon arrival at the laboratory, each lizard was weighed (to nearest 1 mg), and snout-vent length measured (to nearest 0.01 mm). Then, the lizard was anesthetized by ether and dissected, and its liver and kidneys removed, drained with filter paper, and placed in a separate Eppendorf tube. The stomach was also removed and opened, and its contents emptied into a separate Eppendorf tube. A small portion (2 cm) of the tail was also taken using a plastic blade and placed in a separate Eppendorf tube.

For heavy metal determination, subsamples of the removed tissues and stomach contents were dried to constant weight at 80 °C for 48 h, ground to a powder using a mortar and pestle, and then charred in a furnace for 2 h at 250 C. The charring procedure was used to accelerate the decomposition of the samples, putting the different elements in their most oxidized

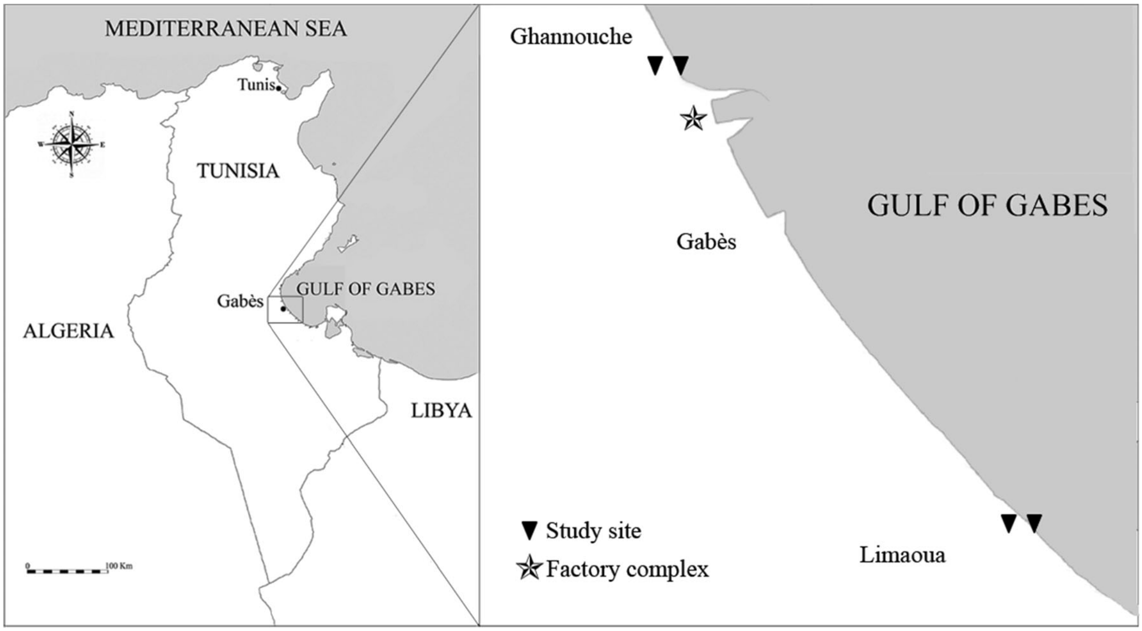

Fig. 1 Map of the study area showing the location of sampling sites

ionic states (Perez et al. 2000). The dry residues were then digested with 10 mL of analytical grade nitric acid (HNO_3) for 6 h at 70 °C. The digested solutions were then diluted to volume in 25 mL using double-distilled water. Finally, Cd, Zn, and Pb concentrations were determined by means of an atomic absorption spectrophotometer (Avanta GBC spectrometer, Australia), using an air–acetylene flame. Equipment was calibrated using working standards (Cd 0, 3, 5, 15 μg/mL; Pb 0, 10, 15, 20 μg/mL; Zn 0, 3, 5, 15, 50 μg/mL), with satisfactory recoveries (more than 80 %). All concentrations are expressed in micrograms per gram on a dry-weight basis using weights obtained from oven-dried samples.

Data analyses

The collected data were first used to check whether the concentrations of heavy metals in stomach contents were correlated with those in the sampled tissues, by means of Pearson's correlation coefficient. We also conducted MANOVA followed by ANOVAs and Duncan post hoc test to investigate whether metal concentrations varied significantly among sites. In these analyses, lizard size (snout-vent length) was considered as a covariate to account for its possible effects. Finally, we checked whether metal concentrations varied among the three sampled tissues by means of linear mixed models, taking into account lizard identity as a random factor.

In all analyses, data normality and variance homogeneity were verified prior to statistical analysis. All statistical tests and analyses were conducted using SAS software (SAS Institute 1999). Means are reported ±SE throughout the manuscript.

Results and discussion

We sampled a total of 47 lizards (Ghannouche beach 13, Ghannouche backshore 11, Limaoua beach 12, Limaoua backshore 11). Mean snout-vent length (±SE) was 53.11 (±3.21) in Ghannouche beach, 66.44 (±2.90) in Ghannouche backshore, 58.83 (±2.92) in Limaoua beach, and 50.04 (±3.11) in Limaoua backshore. Examination of their stomach contents showed a diet composed of various invertebrates (Table 1). Amphipods were exclusively present in the diets of lizards from Ghannouche and Limaoua beaches, with high and non-significantly different frequencies (Table 2; χ^2_1=2.138; p=0.1437). Knowing that ingestion and, to a lesser extent, dermal contact are the two primary routes of contaminant exposure in lizards (Hopkins et al. 2002; Smith et al. 2007), we expected lizards living in Ghannouche beach to accumulate higher concentration of heavy metals than those living in the other sites. Overall, our results gave support to this hypothesis.

Metal concentrations could not be determined in some samples, so that sample size was not always equal to the number of studied lizards. The mean Cd, Pb, and Zn concentrations in tissue samples and stomach contents are shown in Fig. 2. Metal concentrations in the liver and kidney samples were positively correlated with metal concentrations in stomach contents (Table 2). However, different results were found for metal concentration in the tail (Table 2).

After controlling for size effect, there was overall significant differences in metal concentrations in stomach contents and tissues among sites (MANOVA: Wilks' λ=0.021, $F_{36,60}$= 4.506, p=0.0001). Individual ANOVAs and Duncan post hoc tests showed a general tendency towards increased metal

Table 1 Degree of presence (%) of identified prey classes in the stomachs of sampled lizard and results of chi^2 test for the comparisons between sites

Prey class	Ghannouche		Limaoua		Chi^2 test	
	Beach	Backshore	Beach	Backshore	χ^2_3	p
Orthoptera	38	55	42	45	0.86	0.8700
Coleoptera	92	82	67	91	2.53	0.4600
Tenebrionid larvae	46	82	58	73	3.83	0.2700
Ants	77	82	67	100	4.33	0.2200
Spiders	77	9	8	9	0.021	0.9900
Flies	54	73	33	45	3.75	0.2800
Butterflies	38	73	42	82	6.86	0.0700
Amphipods	85	0	58	0	27.50	<0.0001

concentrations in lizards from Ghannouche beach compared to those from the other sites, although some differences between Ghannouche beach and Ghannouche backshore were not statistically significant (Fig. 2). Overall, these results are consistent with the general finding that the contamination of lizards by heavy metals increased close to the source of contamination (e.g., Campbell and Campbell 2000; Burger et al. 2004; Fletcher et al. 2006).

As mentioned above, examination of stomach contents showed that lizards living in the backshore exclusively preyed on terrestrial invertebrates, while those living on the coastal dunes mainly preyed on amphipods. Investigation of heavy metals in the stomach contents showed significantly higher levels of Cd in samples from Ghannouche beach compared to those from the backshore. Pb and Zn levels also tended to be higher in Ghannouche beach compared to Ghannouche backshore, but the trends were still non-significant. We believe that larger data are needed to increase the statistical power and to obtain more accurate results. As the concentrations of heavy metals in lizard tissues were frequently positively correlated with those in their stomach contents, we infer that ingestion of contaminated amphipods contributes to the higher contamination of lizards living on the coastal dunes. Crustaceans are often used as indicators of metal pollution in littoral environments (Rainbow 2002). In our study area, amphipods and Bosk's fringe-toed lizards seem to provide an important link between the marine and terrestrial food webs, favoring the transfer of heavy metals released in the sea by the factory complex to the terrestrial environment.

Concerning metal distribution among tissues, the results of linear mixed models, accounting for lizard identity as a random effect, showed that metal concentrations varied significantly among the three sampled tissues (Cd: $F_{2,85}$=43.95, p<0.0001; Pb: $F_{2,85}$=83.56, p<0.0001; Zn $F_{2,80}$=40.20, p<0.0001). However, different patterns were observed for the three assessed metals (Fig. 2). Liver samples showed the highest concentrations of Cd compared to the kidney and tail samples (Fig. 2). The levels of Cd in the liver samples were almost twice as large as those in the ingested preys (Fig. 2). These results are consistent with previous findings in other reptiles. For example, in a controlled laboratory study on the lizard *Podarcis siculus*, Trinchella et al. (2006) have demonstrated that cadmium concentration was twofold higher in the liver than in the kidney. Moreover, Cd concentrations were the highest in the livers of water snakes compared with the other tissues examined (Hopkins et al. 2002; Campbell et al. 2005). All these findings, together with our results, show that the liver has a high potential for Cd accumulation, providing a storage organ for this metal (Loumbourdis 1997; Campbell et al. 2005; Trinchella et al. 2006; Mann et al. 2007).

Our results also showed high levels of the three assessed metals in the kidney samples (Fig. 2). In particular, Pb and Zn were the most concentrated in the kidney samples (Fig. 2). The concentrations of these two metals in the kidney samples were respectively eight times and twice larger than those in the ingested preys (Fig. 2). These results give support to the

Table 2 Pearson correlation coefficients between metal concentrations in the sampled organs and stomach contents

	Cd			Pb			Zn		
	Liver	Kidney	Tail	Liver	Kidney	Tail	Liver	Kidney	Tail
Stomach contents	0.714**	0.451**	0.239	0.423**	0.491**	0.326*	0.410**	0.238	0.256
Liver	–	0.540**	0.287*	–	0.435**	0.147	–	0.300*	0.498**
Kidney	–	–	0.301*	–	–	0.122	–	–	0.010

*p<0.05; **p<0.0, level of significance

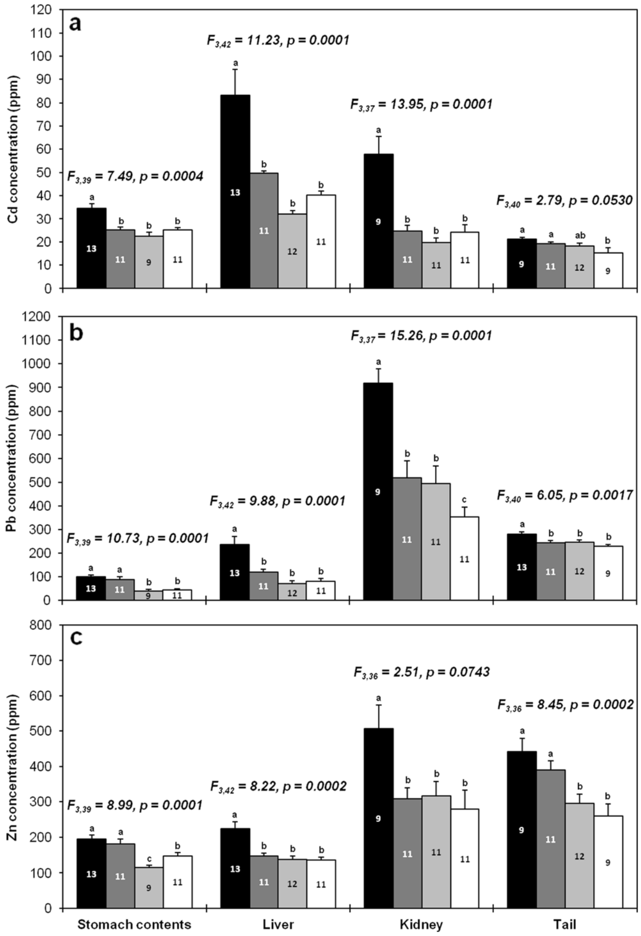

Fig. 2 Mean (±SE) concentrations of Cd (**a**), Pb (**b**), and Zn (**c**) in the tissues and stomach contents of the lizards sampled form Ghannouche beach (*black*), Ghannouche backshore (*dark gray*), Limaoua beach (*light gray*), and Limaoua backshore (*white*). The *numbers* in the bars indicate sample sizes. *F*-ratios and associated *p* values are derived from the ANOVAs of metal concentration as a function of site, accounting for lizard size as a covariate. The *letters* on the bars show the results of Duncan post hoc test for mean grouping

conclusions of previous works highlighting the role of kidney as an active site of heavy metal accumulation (e.g., Liu et al. 2000; Sabolic et al. 2001).

With regard to the tail samples, it has previously been suggested that lizard tail provides a powerful and non-destructive indicator of heavy metals (Fletcher et al. 2006; Kinney et al. 2008). This opinion was supported by our results concerning Zn and Pb, but not by those concerning Cd. Indeed, the levels of Zn in the tail samples were as high as those in the kidney samples and exceeded twice those in the ingested preys (Fig. 2). Moreover, the levels of Pb in the tail samples were twice as large as in the liver samples and four times larger than those in the ingested preys (Fig. 2). However, the tail samples showed relatively low levels of Cd even compared to those of stomach contents (Fig. 2). These results suggest that the usefulness of the tail as an indicator of bioaccumulation depends on the involved metal.

Conclusion

In conclusion, the results of this study showed remarkably high levels of Cd, Pb, and Zn in Bosk's fringe-toed lizards living on the coastal dunes close to the Gabès-Ghannouche factory complex of phosphate treatment, suggesting severe contamination of this area. Our results also showed that due to its opportunistic predatory behavior and to the inclusion of littoral preys in its diet, this lizard species provides a suitable indicator of heavy metal contamination. It can thus be used as a biomonitor of the polluted littoral area close to the Gabès-Ghannouche factory complex. However, the lack of detailed knowledge on the effects of heavy metals makes it difficult to speculate about the biologic significance of each metal or the existence of synergistic effects. Future researches are thus needed to investigate this issue.

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