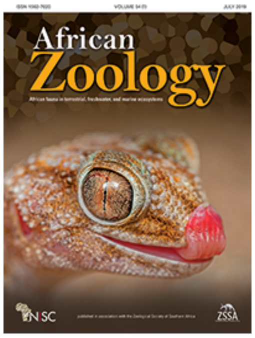

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Variations in heavy metal concentrations among trophic levels of the food webs in two agroecosystems

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Excessive accumulation of trace metal in soil represents a growing environmental problem posing severe risks to biota, humans and ecosystems. Concentrations of Cd, Pb, Cu and Zn were determined in soil, as well as in representatives of some trophic levels in the terrestrial food webs in two Egyptian agroecosystems; El-Manzala (a rural area located in the northeast of the River Nile Delta) and El-Tebbin (an industrial area located in South Cairo). Representatives of trophic levels included arthropods, amphibians, reptiles, birds and small mammals. Metal concentrations were determined in the leaves of wild plants, bodies of arthropods and livers of vertebrates. Levels of metals in the soil, plants and most animal species were higher in El-Tebbin than El-Manzala. Minimal concentrations of metals were detected more often in plants and in the cattle egret, whereas maximum values were common in the soil, amphibians and a mantid. Accumulation of metals was more frequent in arthropods and other taxa at lower trophic levels of food webs, suggesting that the transfer of metals along the vertebrate food web may be of relatively minor importance. However, of all the metals examined, only Pb was bioaccumulated to any appreciable extent in some of the higher trophic levels. A significant increase in liver mass and relative liver mass of the Norway rat from the polluted site was observed.

Keywords: environmental problems, liver, plant, soil, trace elements, wild animals

Introduction

Excessive accumulation of trace metals in soil, as a result of increasing anthropogenic activities, represents a growing environmental problem posing severe risks to biota, humans and ecosystems (Shaapera et al. 2013; Burger et al. 2017; Yan et al. 2018). Trace metals from both natural and contaminated sources enter food webs either directly through inhalation and direct contact with organisms or indirectly through soils; where some of them can be transferred up through food chains (Gall et al. 2015; Ali and Khan 2018). Metal accumulation has been found in a wide range of terrestrial organisms, including arthropods (Zhang et al. 2010; Zhang et al. 2014; Butt and Aziz 2016), amphibians (Shaapera et al. 2013; Zocche et al. 2013; Taiwo et al. 2014; Intamat et al. 2016; Singh et al. 2016), reptiles (Campbell and Campbell 2001; Márquez-Ferrando et al. 2009; Malik et al. 2013; Burger et al. 2017), birds (Lebedeva 1997; Ferreira 2011; Abduljalee et al. 2012; van Riper and Lester 2016) and small mammals (Milton et al. 2003; Bonilla-Valverde et al. 2004; Sánchez-Chardi et al. 2009; Ljungvall et al. 2017; Mukhacheva 2017). Estimation of total heavy metals in soil provides a convenient measure of trace element pollution, but it does not predict ecosystem quality, as does the evaluation of pollutant concentrations in the biota (Vizzini et al. 2013).

In addition, determination of trace metal concentrations in tissues of wild animals can provide early warning of environmental disturbances and identify bioindicator species (Sánchez-Chardi et al. 2009). By their trophic position, arthropods, amphibians and lizards are expected to have higher potential for trace element accumulation than the other groups of animals (Torres and Johnson 2001; Sparling et al. 2010; Ali and Khan 2018). We consider these suitable species to assess environmental quality, especially arthropods that are very abundant in terrestrial ecosystems, with high biomass and are important primary consumers (Mackay et al. 1997). Invertebrates, as a group, have been found to provide important links in transferring heavy metals from producers to higher trophic levels (Milton et al. 2003; Gasparik et al. 2004; Vandecasteele et al. 2004). Amphibians could also be utilised as bioindicators to follow changes in their natural surroundings and in ecotoxicological investigations (Henry 2000) because they are responsive to the surroundings both in aquatic and terrestrial environments. Probable reasons for their apparent sensitivity include semi-permeable skins and multiple life cycle phases (Alford and Richards 1999; Ali and Khan 2018). Adult toads and frogs that prey on arthropods are a constituent of food items for some animals, so they are potentially a significant metal-transferring link between invertebrates and other vertebrates (Sparling et al. 2010). Except for a few turtle and lizard taxa, reptiles are exclusively carnivorous and many occupy high trophic levels within food webs. What is more, many reptiles are long-lived and have small home ranges compared with similar-sized endotherms. Consequently, reptiles are prone to long-term toxicant exposure and resultant biomagnification (Hopkins 2000; Shelby and Mendonça

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2001; Bergeron et al. 2007). Nevertheless, they remain among the least investigated vertebrate groups in terms of ecotoxicology (Bonnet et al. 2002; Ali and Khan 2018) and the ecotoxicological studies have been confined to crocodiles and turtles (Campbell and Campbell 2001; Todd et al. 2010).

Wild mammals have been documented to be exposed to trace elements in polluted areas and to bioaccumulate them in different organs (Pereira et al. 2006).

Besides the bioaccumulation levels, it is important to investigate the physiological changes of long-term exposure to heavy metals in order to form a real picture of the deleterious effects of pollution on the biota in their natural environment. Information on these changes can be obtained to a certain extent by morphological parameters of vertebrates. Actually, the toxic levels of some metals may cause an expansion in relative organ weight, which may be diagnostic for histopathological alterations (e.g. Ma and Talmage 2001; Sánchez-Chardi et al. 2007). For example, histopathological and mass changes have been recorded in the liver and other organs of rodents exposed to a mixture of trace metals near an abandoned pyrite mine (Pereira et al. 2006). Similarly, several previous studies have compared body mass and relative hepatic mass in small mammals inhabiting heavy metal polluted and reference sites (e.g. Bartels et al. 1979; Ma and Talmage 2001; Milton et al. 2003; Sánchez-Chardi et al. 2007). These studies have showed a significant increase in body mass and relative hepatic mass in mammals from the contaminated sites.

Although some studies of ecotoxicology in Egypt focus on aquatic ecosystems (e.g. Barakat et al. 2012; El-Moselhy et al. 2014; El-Shazly et al. 2016; Bream et al. 2017), no studies have examined the prevalence and distribution of heavy metals in the trophic levels of terrestrial ecosystems. During the past century, certain parts of south Cairo have been heavily impacted by industrial activities (e.g. petroleum coke factory, ferrous and non-ferrous metallurgical work, chemical industry, power station and cement industry), including El-Tebbin region, which accounts for approximately 16.5% of Egypt's total industry (Soliman et al. 2017). The current accumulation of contaminants or heavy metals in El-Tebbin region has led to critical environmental issues and agricultural soils around industrial zones are expected to be heavily polluted with trace elements. On the other hand, El-Manzala is a rural area in the north-eastern part of the Nile River Delta, where pollution could be attributed largely to agricultural activities. El-Manzala extends through three Egyptian governorates (Damietta, Dakahlia and Al-Sharqia) and covers a distance of ~45 km along the Lake Manzala's southern shore (Figure 1). The specific objectives of the current study were to monitor and compare the contamination (cadmium, lead, copper and zinc) of soil and selected taxa in the terrestrial food webs within and between the two areas and to investigate the relative liver masses of some components of these food webs.

Materials and methods

Sample collection and preparation

Samples were collected during the summers of 2014 (soil and plant) and 2015 (animal species) from different trophic levels representing the food webs of the agroecosystems in El-Tebbin and El-Manzala regions. Samples were taken from seven sites at different distances at El-Tebbin, up to 10 km downwind from the main sources of the industrial pollution (Figure 1). In El-Manzala, samples were taken from six sites at different distances located ~2–8 km inland from the southern side of Lake Manzala; sites M1 and M2 in Damietta, sites M3 to M5 in Dakahlia and site M6 in Al-Sharqia (Figure 1). The collected animal species, with their localities and numbers, are given in Table 1. All samples (except birds) were collected from the grass strips along the edges of farmland in the two regions, where plants are naturally abundant. Arthropods were sampled by hand picking and sweep nets and euthanised with alcohol. Amphibians and reptiles (lizards and snakes) were collected by hand and killed by chloroform. Small mammals (rodents) were collected in Sherman live traps. Birds, including owls, hawks and cattle egrets, were collected by bownet traps, baited inside with mice. Small mammals and birds were sacrificed by carbon dioxide asphyxiation according to AVMA guidelines for the euthanasia of animals (AVMA 2013). Following euthenisation, vertebrate individuals were weighed, dissected and their livers were weighed and put in labelled plastic containers.

All samples were kept in ice coolers for their safe arrival in the laboratory, where these were preserved at −20 °C until the tissues were processed. The most abundant wild plants (*Paspalum distichum* L. in El-Tebbin and *Cynodon dactylon* (L.) in El Manzala) were sampled at the same sites and stored in polythene bags. The leaves and stems were washed with ultrapure water to remove metals attached to the surface. Five topsoil samples (0–15 cm) were taken at random from each of the sites and thoroughly mixed, with ~1 kg of soil used for lab analysis. Soil, plants, arthropods and liver samples were oven-dried (70 °C) until a constant mass was attained, ground to a homogenous powder and preserved in polythene bags. Some analysed animal samples consisted of several pooled individuals to obtain 0.5 g of dry mass. The sampling positions were recorded using GPS technology and ArcGIS 9.3 was used to develop the location map.

Heavy metal analysis

Soil digestion was based on the protocol described by Soliman et al. (2017). Soil samples (0.5 g) were placed in a digesting flask and pre-digested with 12 ml of 37% HCl: 65% HNO_3 (3:1) mixture for 24 h at room temperature. The suspension was then digested to near dryness on a thermostatically controlled hotplate at 90 °C in a fume cupboard, following which 2.5 ml of 37% HCl and 2.5 ml of 30% H_2O_2 were added to complete the digestion. The resultant mixture was heated again and then cooled to ambient temperature. The flask walls were washed with 10 ml of ultrapure water and the suspension was then filtered through Whatman filter paper (No. 41) in a volumetric flask, diluted to 50 ml and stored in polyethylene bottles at 4 °C for later analyses. Digestion of plant and animal samples was carried out in a similar way, but samples were allowed to stand for 24 h with 10.0 ml of 65% HNO_3 and only 2.0 ml of 30% H_2O_2 was added to aid the digestion of the organic matter. The filtered solutions

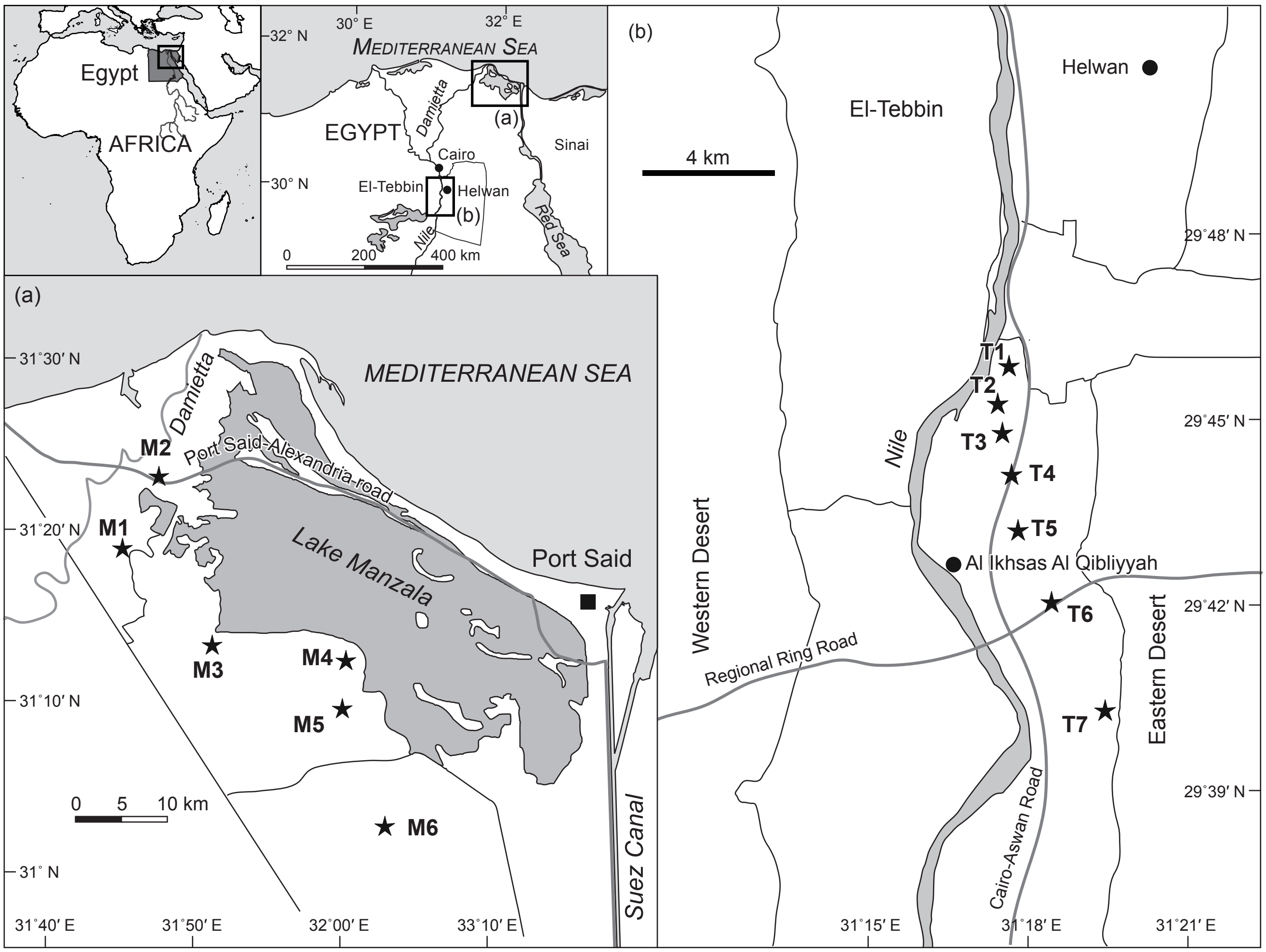

Figure 1: Map of El-Manzala and El-Tebbin showing the sites where soil, plants and animals were collected

were diluted up to 25 ml. Concentrations of Cd, Pb, Cu and Zn were determined by inductively coupled plasma (ICP-AES-Jobin Yovin ultima2, France).

Wavelengths and detection limits of the ICP for the analysed metals were: 226.502 nm and 0.0023 mg l^{-1} for Cd; 220.353 nm and 0.028 mg l^{-1} for Pb; 324.754 nm and 0.0036 mg l^{-1} for Cu; 213.856 nm and 0.0012 mg l^{-1} for Zn, respectively. A certified reference material and a reagent process blank were performed for each analytical batch to assess accuracy and mean values of three replicates were calculated for each determination. Metal concentrations were expressed as mg kg^{-1} dry mass. Chemicals, stock solutions and reagents were all of analytical grade (Merck). All glassware and plastic materials were washed with distilled water before use, soaked in 2N nitric acid overnight and then rinsed thoroughly with ultrapure water.

Data analyses

Most datasets did not reveal a good fit to normal distribution and there were large differences in variance between treatment groups, and the sample sizes were small. Therefore, Kruskal–Wallis *H*-tests were used to test for significant differences in metal concentrations between the taxa for each food web, whereas Mann–Whitney *U*-tests were used to test for significant differences in metal concentrations between the two regions for each taxon (SPSS Statistics 2008). The differences in body masses, liver masses and percentage liver mass of vertebrates, common to both sites, were analysed for sites with a Student's *t*-test (SPSS Statistics 2008). In all tests, $p \leq 0.05$ was considered significant. All treatments were replicated at least three times in the experiment.

Results and discussion

Metal concentration in the soil, plants and animals

Concentrations of Cd, Pb, Cu and Zn in the soil, plant and arthropods and in vertebrates from El-Manzala and El-Tebbin are shown in Table 2, Figure 2 and Figure 3, respectively. When compared with the background levels of trace elements in non-polluted agricultural soils in Egypt (El-Sharabasy and Ibrahim 2010), the El-Tebbin region was considered potentially contaminated (Table 2). The highest concentrations of all the tested metals were detected in the industrially polluted area, El-Tebbin;

Table 1: Localities, feeding habits and numbers of animal species collected from El-Manzala and El-Tebbin areas (refer to Figure 1 for site codes). Food habits: Herbivore (Herb); Primary carnivore (1° Carn); Secondary carnivore (2° Carn); Top carnivore (Top Carn); Omnivore (Omni).

Taxon (Common and Latin names)	Food habits	Number of animal species collected at each site												
		El-Manzala						El-Tebbin						
		M1	M2	M3	M4	M5	M6	T1	T2	T3	T4	T5	T6	T7
Arthropods														
Green-stripe-winged grasshopper (*Aiolopus thalassinus* (Fabr.))	Herb	28	24	25	21	35	22	30	43	39	26	51	42	32
Egyptian mantis (*Miomantis paykullii* Stål)	1° Carn				34	22			17	13			38	
Amphibians														
Egyptian toad (*Amietophrynus regularis* (Reuss))	2° Carn		3	2		2		1	3			1		
Mascarene grass frog (*Ptychadena mascareniensis* (Dumeril and Bibron))	2° Carn		4		3	5					4			6
Reptiles														
Lizards														
Bridled mabuya (*Trachylepis vittata* (Olivier))	2° Carn		4		2		2	3			5			2
Ocellated skink (*Chalcides ocellatus* (Forskål))	2° Carn			2		4				3	2		2	1
Wedge-snouted skink (*Chalcides sepsoides* (Audouin))	2° Carn	2	1											
Common wall gecko (*Tarentola mauritanica* (Linnaeus))	2° Carn								2			2		
Snakes														
Forskal sand snake (*Psammophis schokari* (Forskål))	2° Carn		2		3				2	1		3		
Flowered racer (*Coluber florulentus* (Geoffroy Saint-Hilaire))	2° Carn	1	1				2							
Birds														
Cattle egret (*Bubulcus ibis* (Linnaeus))	2° Carn	3		2			4			3		3		
Little owl (*Athene noctua* (Scopoli))	Top Carn	1	3	1										
Common kestrel (*Falco tinnunculus* (Linnaeus))	Top Carn				2		2			1				2
Black-winged kite (*Elanus caeruleus* (Desfontaines))	Top Carn				1	1								
Small mammals														
Norway rat (*Rattus norvegicus* (Berkenhout))	Omni			3			3		2		2		1	
House mouse (*Mus musculus* (Linnaeus))	Omni	3					5							

Table 2: Mean metal concentrations (mg kg^{-1} dry mass) in soil samples from El-Manzala and El-Tebbin regions

Region	Heavy metal							
	Cd		Pb		Cu		Zn	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
El-Manzala	2.4a	0.7	4.6a	1.2	55.5a	8.4	87.3a	8.8
El-Tebbin	19.4a	9.46	51.2b	22.4	115.1a	36.1	282.3b	82.8
Reference soil*	1.0		50.0		30.0		100.0	

Means within each column followed by the same letters are not significantly different (Mann–Whitney *U*-test, $p > 0.05$).
*background levels of trace elements in non-polluted agricultural soils in Egypt (El-Sharabasy and Ibrahim 2010).

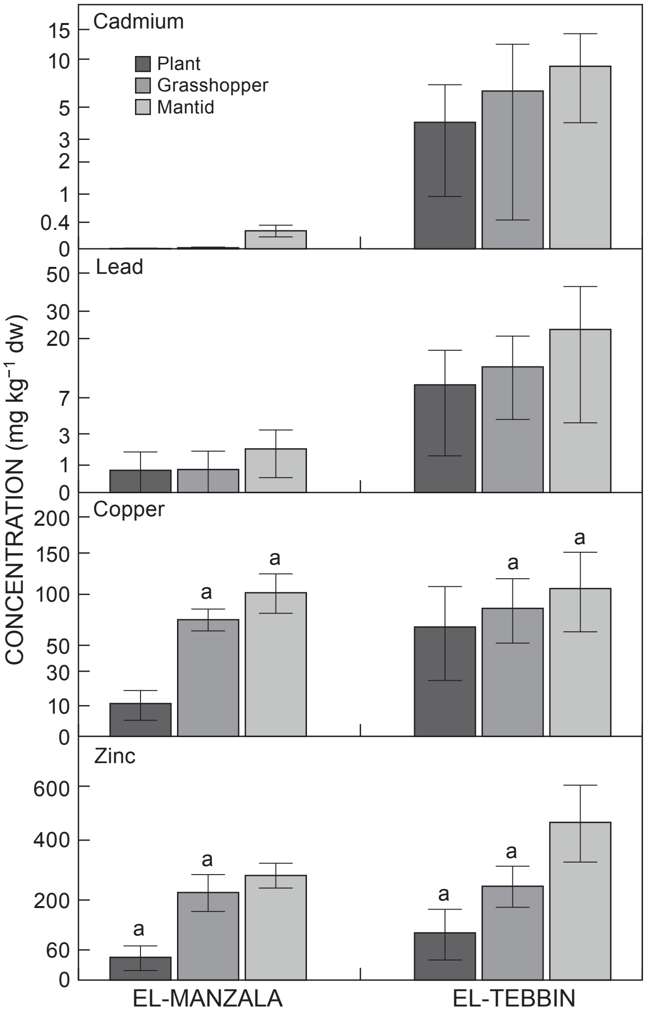

Figure 2: Mean metal concentrations in plants and arthropods from El-Manzala and El-Tebbin regions. Data are presented as mean ± SE in mg kg^{-1} dry mass. The letter 'a' indicates that metal concentrations in the same taxon are not significantly different between the two regions (Mann–Whitney *U*-test, $p > 0.05$)

where the maximum concentrations of the non-essential heavy metals (Cd and Pb) were found in the soil and the maximum concentration of the essential ones (Cu and Zn) were found in the Egyptian mantis, *Miomantis paykullii* Stål and the Mascarene grass frog, *Ptychadena mascareniensis* (Dumeril and Bibron), respectively. Moreover, levels of trace metals in the soil, plants and animal species in El-Tebbin region were higher than in El-Manzala region and most of these differences were significant (Mann–Whitney *U*-test, $p \leq 0.05$), particularly for Cd (except for soil, *Psammophis schokari* (Forskål) and *Falco tinnunculus* (Linnaeus)) and Pb (except for *Bubulcus ibis* (Linnaeus) and *P. schokari*). However, the concentration of Zn in most species of the two regions did not differ significantly. Furthermore, Cu concentrations in the ocellated skink, *Chalcides ocellatus* (Forsskål) and the cattle egret *B. ibis*, Zn content in the Forsskål sand snake, *P. schokari*, Bridled mabuya, *Trachylepis vittata* (Olivier) and the Norway rat, *Rattus norvegicus* (Berkenhout) collected from El-Manzala were slightly higher than those collected from El-Tebbin region (Figure 3). Karadjova and Markova (2009) reported higher Cd and Pb concentrations in grasshoppers captured from sites in Bulgaria that are closer to the copper smelter and copper-flotation factory than those from the control site.

Likewise, in a study on different aquatic and terrestrial predatory insects (waterstriders, antlions, ants and dragonfly larvae) at Koverhar, Hanko Peninsula, Nummelin et al. (2007) observed that in most cases, metal (Fe, Mn, Zn, Cu, Ni and Cd) concentration in species collected from sites close to an iron and steel factory were higher than those collected from the control sites. It can therefore be assumed that biota living in zones of greater metal concentrations in the soil would contain greater body burdens of trace element than biota from zones of low metal concentrations in the soil.

The similar metal concentrations in birds and rodents from the polluted site with those from the reference site in the current study were previously reported in the Algerian mouse *Mus spretus* (Bonilla-Valverde et al. 2004) and in the shrew *Crocidura russula* (Sánchez-Chardi et al. 2009). However, the differences in the concentration of Cu in rodent livers observed among the two locations suggest that internal Cu regulation may prove inefficient for small mammals inhabiting highly contaminated areas. This result is in agreement with earlier studies performed on small mammals inhabiting contaminated sites (Hunter and Johnson 1982; Laurinolli and Bendell-Young 1996; Torres and Johnson 2001).

The levels of trace metals varied significantly among soil, plants and animal species in the two study areas. Concentrations of cadmium ($H = 53.31$, $p < 0.001$) and Cu ($H = 51.56$, $p < 0.001$) at El-Manzala had the highest statistical mean ranks among the taxa followed by Zn ($H = 50.88$, $p < 0.001$) and Pb ($H = 44.26$, $p < 0.001$), while Cu ($H = 34.29$, $p = 0.001$) and Cd ($H = 32.24$, $p = 0.001$) concentrations at El-Tebbin had the highest statistical mean ranks among the taxa followed by Pb ($H = 31.58$, $p = 0.002$) and Zn ($H = 25.58$, $p = 0.012$). On an average, arthropods, amphibians and lizards concentrated more trace element than the other food web components (Figures 2 and 3). The lowest concentrations of trace elements were detected more often in plants and in the cattle egret, whereas the maximum values were common in the soil, amphibians and mantid. Relatively high concentrations of metals were also registered in lizards. Certain taxa of the food web may be more likely to accumulate one metal over another, especially if they are in close proximity to metal-rich soils or consume contaminated food. Several factors may be involved; however, the primary factors can be attributed to their different ecological niche, food type and physiological responses (Gall et al. 2015).

In the industrially polluted site, birds and small mammals generally exhibited lower levels of trace elements (except Zn), despite the contamination found in the soil and the other lower trophic categories. A number of factors may explain this result, in particular: (1) metabolic regulation that maintains a constant internal metal concentration, independent of environmental concentrations and prevents high bioaccumulation in small mammals and birds (e.g. Talmage and Walton 1991; Goyer 1997; Ma

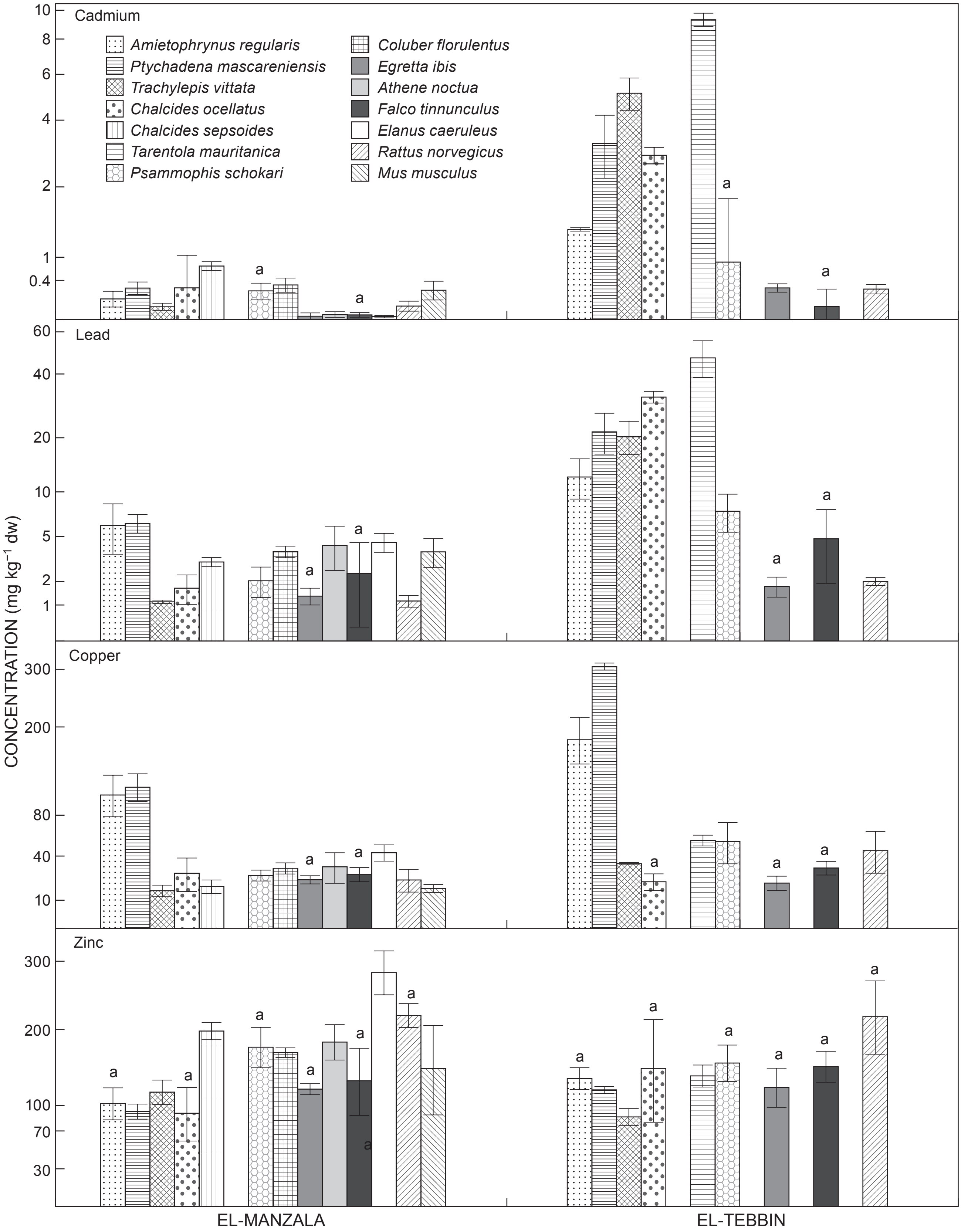

Figure 3: Mean metal concentrations in selected vertebrate species from El-Manzala and El-Tebbin regions. Data are presented as mean ± SE in mg kg⁻¹ dry mass. The letter ‘a’ indicates that metal concentrations in the same taxon are not significantly different between the two regions (Mann–Whitney *U*-test, $p > 0.05$). (*Ch. sepsoides, Co. florulentus, Mu. musculus, At. noctua,* and *E. caeruleus* were collected only from El-Manzala and *Ta. mauritanica* was collected only from El-Tebbin)

and Talmage 2001); (2) organisms at higher trophic levels may exhibit more efficient excretion of certain elements (Vizzini et al. 2013); and (3) as active flyers, raptors reflect environmental pollution at a broad spatial scale.

Non-essential heavy metals exhibited lower concentrations in living organisms than essential ones. The concentrations of Cd varied from 0.11 (hawk) to 19.4 mg kg^{-1} (soil) in El-Tebbin and from 0.004 (plants) to 2.4 mg kg^{-1} (soil) in El-Manzala.; Pb concentration ranged between 1.7 (cattle egret) and 51.2 mg kg^{-1} (soil) in El-Tebbin and between 0.76 (plants) and 6.1 mg kg^{-1} (amphibians) in El-Manzala. The concentration of Cu varied from 11.0 (plants) to 108.5 mg kg^{-1} (amphibians) in El-Manzala and from 19.8 (cattle egret) to 242.6 mg kg^{-1} (amphibians) in El-Tebbin and Zn concentration ranged from 42.4 (plants) to 278.6 mg kg^{-1} (mantid) in El-Manzala and from 104.0 (plants) to 463.7 mg kg^{-1} (mantid) in El-Tebbin. Concentrations of essential metals (e.g. Zn and Cu) are partially controlled by metallothionein and metallothionein-like proteins found in invertebrates and vertebrates (Phipps et al. 2002; Newman and Unger 2003). These polypeptides coordinate the uptake, accumulation and excretion rates of these metals in organisms making food chain bioaccumulation improbable. Moreover, Cu is the metal in haemocyanin of some invertebrates (Anderson et al. 1978). Therefore, some physiological regulations of these elements are possible. In contrast, Cd and Pb have not been known to be vital to biological systems (Anderson et al. 1978) and their significant higher concentrations in El-Tebbin for species common to both sites mirror the higher potential inputs at El-Tebbin and the absence of physiological mechanisms for controlling Cd and Pb body burdens.

Cadmium and Pb concentrations in livers of raptors reported in this study (Figure 3) were much lower than those found in the common buzzards (*Buteo buteo*) from different areas of Sicily, Italy, whereas the Cu and Zn concentrations of both studies were comparable (Licata et al. 2010). On the other hand, our results showed that Pb and Zn concentrations in liver were higher than those noted in eight raptor species from Calabria region in Italy (Annalisa et al. 2008). Nevertheless, the investigated metals were lower than levels that can give rise to toxic effects in the raptors studied (Eisler 1986). Concentrations for Cu and Zn were much higher in livers of the Norway rat and house mouse in our study than in the liver of the Algerian mouse (*Mus spretus*) in a study conducted by Marques et al. (2008) around an abandoned mining area in South Portugal. In another study carried out along a metal pollution gradient in Belgium, Cd concentrations were much higher in the liver of the wood mouse (*Apodemus sylvaticus*) than in rodents in our study (Rogival et al. 2007). In contrast, Cu and Zn concentrations were higher in livers of our rodents, whereas Pb concentrations were similar between the two studies. The Zn concentration was much higher and the Cd concentration was much lower in the liver of small mammals in our study than in the shrew *C. russula* inhabiting the protected wetland of Doñana, Spain (Sánchez-Chardi et al. 2009). The results also showed comparable accumulation of Pb and Cu in the livers of small mammals of the two studies.

The variations of metal concentrations in species of the same and different trophic levels (Figures 2 and 3) imply that species, more than the trophic category, was the factor which best explained the variability observed in the concentration of metals in terrestrial organisms. This may be owing to their species-specific ability to actively excrete heavy metal ions and/or their ecological characteristics (Van Straalen and Van Wensem 1986. This species factor assumption is conforming to Mackay et al. (1997), who stated that “it may be the physiology of an organism and not the trophic level which determines the internal concentration of heavy metals”. However, Hernández et al. (1999) reported that both species and trophic level were key factors determining heavy metal concentration. It is not right to deduce that all of the contamination of biota at investigated sites can be attributed to the movement of trace elements through food chains. For example, Hirao and Patterson (1974) observed that aerial deposition of Pb is a very serious source of contamination. Furthermore, heavy metal contaminants may be ingested by accident with soil on diet items, inhaled, or absorbed through the skin of some animals, such as amphibians (Alford and Richards 1999).

Relative liver mass

The liver is the most important detoxifying tissue. This organ plays an essential role in food conversion, detoxification of chemicals and vitellogenesis for reproduction purposes (Sánchez-Chardi et al. 2009). Among all the vertebrate species common to both investigated areas, liver mass and relative liver mass were significantly higher only in the Norway rat (*R. norvegicus*) specimens from the polluted area ($t = -7.007$, $p < 0.001$; $t = -5.751$, $p = 0.005$, respectively) (Table 3). This increase in the liver mass and relative liver mass may be characteristic of significant histological and physiological changes and could be illuminated by exposure to toxic levels of xenobiotics (e.g. Cd and Pb) (Sánchez-Chardi et al. 2009). The liver concentrations of Cd, Pb and Cu were significantly higher in Norway rats from the polluted area (El-Tebbin), whereas no significant difference was observed for Zn (Figure 3). Likewise, Sánchez-Chardi et al. (2009) noted a significant increase in liver mass and percentage liver mass of the shrew *C. russula* from the polluted site. Moreover, in their study, the analysis of shrews from the polluted site showed both histological alterations in the livers and genotoxic effects in the blood, which were consistent with the morphological parameters. Similar results were previously obtained for rodents exposed to trace metal pollution (Ma and Talmage 2001; Pereira et al. 2006; Sánchez-Chardi et al. 2007).

In the current study, two issues suggest the results must be interpreted with caution. First, the small sample size of vertebrate species may have decreased the ability to detect real differences in metal concentrations between these species (type 2 error). Second, two different tissues were used to determine metal concentrations in animal species (bodies of invertebrates and livers of vertebrates). Levels of metals in the liver are correlated to levels in other body tissues, but may be accumulated in the liver at concentrations higher than average body concentrations (Linde et al. 2004). However, in this study, levels of metals

Table 3: Body mass (BW), liver mass (LW) and relative liver mass for vertebrates compared by site

Taxa	El-Manzala				El-Tebbin			
	n	BW	LW	% liver mass	*n*	BW	LW	% liver mass
Am. regularis	7	31.5 ± 1.4	1.4 ± 0.05	4.4 ± 0.36	5	32.0 ± 2.4	1.3 ± 0.10	3.9 ± 0.4
Pt. mascareniensis	12	5.0 ± 0.7	0.2 ± 0.02	3.5 ± 0.61	10	5.7 ± 1.0	0.2 ± 0.01	3.8 ± 1.2
Tr. vittata	8	15.3 ± 1.3	0.3 ± 0.03	2.2 ± 0.45	10	15.6 ± 1.8	0.3 ± 0.04	1.6 ± 0.3
Ch. ocellatus	6	22.4 ± 3.1	0.5 ± 0.05	1.9 ± 0.11	8	27.9 ± 3.2	0.7 ± 0.17	2.9 ± 1.3
Ps. schokari	5	62.6 ± 1.0	3.3 ± 0.21	5.1 ± 0.42	6	61.4 ± 1.8	2.8 ± 0.12	4.6 ± 0.3
R. norvegicus	6	270.8 ± 13.1	10.8 ± 0.54	4.2 ± 0.12	5	293.9 ± 3.6	17.5 ± 0.78**	6.1 ± 0.3*
B. ibis	9	345.4 ± 23.9	13.7 ± 0.92	4.0 ± 0.64	6	316.6 ± 13.8	12.4 ± 1.40	4.1 ± 0.4
F. tinnunculus	4	198.7 ± 5.6	5.7 ± 0.55	2.8 ± 0.32	3	186.8 ± 5.7	4.6 ± 0.71	2.4 ± 0.5

Data are presented as mean ± SE in g wet mass. *n* is the number of individuals.
* < 0.01, ** < 0.005.

in the bodies of invertebrates were higher than their levels in the livers of birds and small mammals.

In conclusion, our findings indicated significant differences in concentrations of the individual heavy metals between species of the same and different trophic levels. This implies that physiological and biochemical processes of the species, more than food items, were the factors that best explained the variability observed in the concentration of metals in terrestrial taxa. Accumulation of heavy metals was more frequent in arthropods and other taxa at lower trophic levels of the food web, suggesting the possibility of increasingly efficient excretion of metals for the animals at higher trophic levels and that the transfer of trace metals along the vertebrate food web may be of relatively minor importance. Accordingly, trace element bioaccumulation is not a rule in the terrestrial food webs. Arthropods, amphibians and lizards may be suitable species to assess environmental quality and act as an early warning of pollution. A significant increase in liver mass and relative liver mass of the Norway rat from the polluted site was observed. Our results emphasise the urgent requirement for continued research concerning the trace metal concentrations in domesticated meat animals from the same areas.

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