Risk assessment of heavy metal contamination in soil and wild Libyan jird *Meriones libycus* in Riyadh, Saudi Arabia

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Abstract

This study was undertaken to document the impact of heavy metal pollution on the Libyan jird, *Meriones libycus* and to contribute to an environmental impact statement for the rapidly growing City of Riyadh. All metal concentrations in surface soil of a polluted site (within Riyadh City) were higher than those from a reference site (outside the city). Although Pb declined versus earlier reports on Riyadh soil, Cd (0.97 μg g^{-1}) and Hg (0.28 μg g^{-1}) were above some of the most stringent quality guidelines (0.07- 0.62 μg g^{-1} for Cd and 0.14- 0.18 μg g^{-1} for Hg). Metal distribution in *M. libycus* proved site-related and organ-specific, recognizing a higher affinity of most tested metals towards the kidneys, liver and brain than the lung and heart. The comparatively lower site-specific accumulation of Pb in soft tissues was attributed primarily to its major hypothetical accumulation in bones, whereas, the transition rate of Hg from the liver was suggested to be lower to the brain than to the kidneys. Although a non hazardous status was assumed for Cu (11.27-13.16 μg g^{-1}) and Hg (up to 0.207 μg g^{-1}) in tissues of *M. libycus*, a potential risk was imposed by mean tissue concentrations of Cd (up to 3.29 μg g^{-1}), Ni (up to 1.48 μg g^{-1}) and Pb (up to 1.94 μg g^{-1}). On the grounds of the significantly higher metal levels in polluted soft tissues versus reference subjects, Libyan jirds possess high exposure potential and can be useful biomonitors of environmental metal contamination.

Introduction

The rapid growth of Riyadh, the capital city of Saudi Arabia, has created numerous environmental problems common to most cities worldwide. In addition to heavy metals released with industrial wastes, such big cities suffer from emissions from roadways and automobiles as major sources of heavy metals. Zinc, copper and lead are three of the most common heavy metals released from road travel, accounting for at least 90% of the total metals in road runoff. Smaller amounts of many other metals, such as nickel and cadmium, are also found in road runoff and exhaust (USEPA, 2006). In addition, climatic conditions and mobile sands create particular problems to Riyadh with respect to dust, a common pollutant that represents an important source of toxic metals. The city suffers from heavy dust fall, averaging about 2 mg km^{-2} d^{-1} and suspended particles of about 500 μg m^{-3} (El- Shobokshy, 1984, 1985; El- Shobokshy *et al*., 1990).

Heavy metal poisoning is the toxic accumulation of heavy metals in soft tissues. A metal's toxic effect manifested in an organ is mainly a function of concentration and exposure time, because many toxicants tend to bioaccumulate. When they occur at certain levels, even essential elements that are critical for life, may lead to loss of organ function or death (Goyer, 1996).

Regulation, handling and bioremediation of hazardous materials require an assessment of the risk to some living species other than human being. The use of sentinel species can provide

data to monitor the quality of the environment of their biological habits for exposure to contaminants. Emphasis on the importance of the use of small mammals as sentinel and biomonitor species has been provided by several authors (Tataruch and Kierdorf, 2003; Reynolds *et al.*, 2006; Sánchez-Chardi *et al.*, 2007). In Saudi Arabia, bioaccumulation of metals via aquatic food webs has been extensively studied. Because parallel information on terrestrial systems is still lacking, we targeted here a local small mammal species, the Libyan jird, *Meriones libycus,* for the present biomonitoring study. Libyan jirds are mainly found in sandy plains with patches of bushy vegetation, where they dig burrows in the firmer soil around roots of bushes as the foliage provides some cover. They frequently change burrows or even migrate if foraging conditions deteriorate, but they often return to the burrow to eat (Aulagnier *et al.*, 2008); hence, are pertinent sentinel species. Like other small mammals, Libyan jirds are ideal for monitoring environmental pollution as well as for evaluating the risk for human populations living in polluted areas because of their abundance, widespread distribution, short migration/ dispersal distance, generalized food habits, short life span, high reproductive rate and susceptibility to capture (Tataruch and Kierdorf, 2003).

The objectives of this study were (1) to assess the soil metal content of a polluted location (in Riyadh City) versus a reference location (outside the city); (2) to determine heavy metal accumulation in wild *M. libycus*; and (3) to evaluate the appropriateness of employing *M. libycus* as a bioindicator model for metal toxicity in an arid environment.

Materials and Methods

Study area and sampling locations: The City of Riyadh is extending over 1600 km^2 on a relatively flat plateau, at 24° 40' N, 46° 41' E and an altitude of 600 m. It is situated in an extremely arid area with an average annual rain fall of 3.5% annum^{-1}. Riyadh is surrounded by sandy hills, sand dunes and vast deserts that cause serious dust contamination. Two large industrial complexes were established in the vicinity of Riyadh city. The first of them, with a total area of 451 000 m^2 and 59 industrial units, is located in close proximity to city centre. The second, with an approximate total area of 12 000 000 m^2 and 497 industrial units, is situated 17.5 km southeast of the city core.

A location at An Nazim district was selected as the polluted site (P) (Fig. 1). Key sources of pollution into this site include illegal dumping of raw sewage into public use areas, road traffic, two nearby industrial quarters, a petroleum refinery, a cement plant and vehicle maintenance workshops. The reference site (R) is situated amidst Al Khafs area (a meadowland refuge and local tourist resort). Site R is a sandy smooth plain land overlooking Aarma mountains from the east and surrounded by sand from the west. Site R is located about 70 km northwest of Riyadh city (Fig. 1), far enough from most sources of polluting activities; hence was selected as a clean reference site.

Sampling and analysis: Soil and rodent animals were collected from the selected trapping locations (polluted, P and reference R) at three monthly intervals during a period extending from March to May, 2007. From each sampling location, soil was randomly collected at 5 different spots for accuracy of the results. For each sample, the uppermost surface layer (2 cm down the soil surface) was carefully removed, collected in plastic bags and transported to the laboratory for metal analysis. A total number of 45 adult live-trapped samples of Libyan jirds, *M. libycus,* of almost uniform body length (27-28 cm including the tail area) and weight (184 -200 g), were used for the present assays. Jirds were captured using traps placed randomly in each sampling location. The trapped animals were then taken alive to the laboratory, where they were identified for sex, maturity and general health status. Animals were then allocated in cages (1 animal cage^{-1}), left for 2-4 hr to acclimate to laboratory conditions prior to sampling. They were then slightly anaesthetized by inhalation of diethylether for 1 to 2 min to avoid the effects of handling stress reactions. The anaesthetized animals were killed and the selected organs (liver, kidney, brain, heart, and lung) were excised and stored in clean plastic containers at -20°C until later prepared for metal analysis.

Soil and animal tissues were digested according to USEPA Method 3052 (1996) using a microwave (Ethos plus milestone, microwave laboratory systems, Italy) method. Acids used were all of optima grade. Soil samples were dried overnight at 60°C and ground and sieved through a 2 mm- pore sieve. Soil samples (0.05 g from lead, copper and nickel and 0.1 g from cadmium and mercury) were weighed into teflon vessels. In a fume hood, 9 ml nitric acid and 3 ml hydrofluoric acid (Suprapure grade; Merck, Darmstadt, Germany) were added to each vessel. The samples were then transferred to cool at room temperature; the digestate was then centrifuged at 3000 to 5000 xg for 10 min and the clear supernatant was transferred to polypropylene tubes and was diluted to 20 ml with deionised water.

Concentrations of lead (Pb), cadmium (Cd), nickel (Ni) and copper (Cu) were determined in digested soil and animal tissue (liver, kidney, brain, heart, and lung) using Varian AA-280 Zeeman atomic absorption spectrophotometer coupled to GTA-120 electrothermal atomizer and programmable sample dispenser (Varian Techtron Pty. Ltd., Australia); for mercury, Varian AA-880 with Zeeman background correction coupled to a vapor generation accessory VGA-76 (Varian Techtron Pty. Ltd., Australia) was used. All metals were determined according to USEPA Method 2007 (1998) under the recommended conditions and the detection limits (DL) in the manual for each metal; and all were expressed as µg g^{-1} dry weight.

For Cd and Pb, one volume of digestate (usually 150 µl) was mixed with one volume of 1% (w/v) ammonium dihydrogen phosphate modifier; for Ni, 1% ascorbic acid (modifier 1) and 500 µg ml^{-1} palladium (modifier 2) were added instead of ammonium dihydrogen phosphate modifier (in case of Cd and Pb).

Statistical analysis: All data are expressed as mean ± standard error (SE) and significance limits of (p<0.001, p<0.01 and p<0.05) using Student 't' Test. Pearson correlation coefficients (r) were also

calculated (Snedecor and Cocharan, 1994). All statistical procedures were performed with SPSS (version 11.5 for Windows).

Results and Discussion

Concentrations in soil: All metals (Cd, Cu, Hg, Ni and Pb) in soil samples reported higher concentrations in P site than in R site (Table 1). Interesting results were drawn by total and differential metal calculation. In site P, the sum of means of all metals tested (Cd, Cu, Hg, Ni and Cd) was almost 61% higher than that of site R; whereas that for toxic metals only (Cd, Hg and Pb) was much higher (69%), which justifies using the total and differential soil content of metals as key indices to estimate the degree of soil exposure to heavy metal pollution (Kuo *et al.*, 1983). In soil, a significant rise (P < 0.05) was shown in the soil of P site compared to R site for all metals tested. In a respective order, Cd showed the highest difference (0.31 vs 0.17 µg g⁻¹), followed by Ni (14.37 vs 8.33 µg g⁻¹), Cu (53.64 vs 32.12 µg g⁻¹), and Pb (5.223 vs 2.586 µg g⁻¹). In fact, Hg exhibited the least significant difference between locations among other metals tested (0.24 vs 0.28 µg g⁻¹).

Concentrations in animal tissue: The effects of exposure to a polluted habitat is shown in the drastic changes outlined in the mean total concentrations of all metals (Cd, Cu, Hg, Ni, Pb) in tissues of *M. libycus* captured on the polluted site relative to reference subjects (Fig. 2a-e). No significant differences were found regarding the sex factor for all metals in all organs, and hence were ignored. Tissue wise, Cd accumulated most in the kidneys and liver (p<0.01) and to a less extent in the brain (p<0.01, p<0.05). Also, significant Cd accumulation was reported in lung and heart tissues (p<0.05). As for Cu, the only observed accumulation was displayed as a slight insignificant rise in lungs and kidney of male and female P jirds.

As a general rule for Hg, kidney and brain tissues showed the highest (p<0.05) accumulation in P jirds. A peak and a second highest Hg mean concentration values were recorded in P female kidney and P male brain, respectively (0.207 and 0.160 µg g⁻¹, respectively). Location wise, a significant (p<0.05) increase in Hg concentrations was reported in brain and kidney of P jirds relative to parallel ones. Changes in Ni mean values were displayed here in the significant (p< 0.05) rise in Ni concentrations in male and female P jirds in the lung (up to 1.48 µg g⁻¹) and kidney (up to 1.22 µg g⁻¹) versus parallel R lung (up to 0.85 µg g⁻¹) and kidney (up to 0.77 µg g⁻¹). Changes in other organs were, however, insignificant.

Regardless of the sex factor, Pb mean concentration values exhibited a significant (p<0.05) elevation in the liver (up to 1.94 µg g⁻¹), kidney (up to 1.91 µg g⁻¹), brain (up to 1.42 µg g⁻¹) and heart (up to 1.32 µg g⁻¹) in P jirds versus parallel R values of the liver, kidney, brain and heart (1.13, 1.01, 1.11 and 0.93 µg g⁻¹, respectively). To the best of our knowledge the present data constitute the first measurements in surface soil and wild *M. libycus* in the locations specified.

Data on metal content in soil authenticated site selection criteria. Concentrations of all metals in soil (Cd, Cu, Hg, Ni and Pb) were higher (by 2.3, 1.7, 1.2, 1.7 and 1.4 orders of magnitude, respectively) in P than in R site. Slight differences were noted between the current mean values of Cd and Pb in Riyadh soil (location P) (0.97 and 6.22 µg g⁻¹, respectively) and those reported in an earlier study by Hashem in 1993 (0.9 and 8.8 for Cd, Cu and Pb, respectively). The current decline of Pb mean concentration (6.22 µg g⁻¹) versus that of Hashem (1993) (8.8 µg g⁻¹) is mostly a cumulative effect arising from discontinuing leaded gasoline in the City of Riyadh few decades ago. When the present data are compared to the most stringent soil quality guidelines for Cd and Hg (0.35 and 0.1, respectively) (BKH Consulting Engineers, 1995) (Table 1), the frequency of increment occurs in the order Cd > Hg for P site and Hg > Cd for R site. Generally speaking, Cd in P site was above the average of Hashem (1993), the Dutch target value of the Netherlands and the environmental quality standard of China (Chen *et al.*, 1997). In both R and P trapping sites, Cd was above the most stringent soil quality guidelines (BKH Consulting Engineers, 1995) and soil remediation goals for sensitive land use of Sweden (Chen *et al.*, 1997).

The elevated levels of most metals tested, in P versus R site, are thought to be attributed to anthropogenic sources, given that every industry virtually discharges heavy metals into ecosystems (Pacyna and Pacyna, 2001). Raw sewage is a point-source pollutant that contains Hg, Cd, Pb and Cu (Khatri and Dhankhar, 2003). In addition to emissions from road traffic, P site is located within potential sources of heavy metals (Cd, Cu, Hg, Ni and Pb) including coal and oil combustion in electric power stations, heating and industrial plants, gasoline combustion, oil refineries, petrochemical plants, and kiln operations in cement plants. Yet based on the slight difference in soil content of Hg between P (0.28 µg g⁻¹) and R (0.24 µg g⁻¹) sites, and supported by the hypothesis that biogenic deposition of Hg accounts for over 50% of the deposited

Table - 1: Mean values of heavy metal concentrations in the soil of the reference and polluted locations (R and P, respectively) compared to previous report on Riyadh soil (Hashem, 1993)

Element	Cd	Cu	Hg	Ni	Pb
Reference R	0.42	32.12	0.24	8.33	4.59
Polluted P	0.97	53.64	0.28	14.37	6.22
Guidelines					
Previous report on Riyadh soil	0.9	7.1	N.A.	N.A.	8.8
Increment of R site over to previous report	N.A.	25.02	N.A.	N.A.	……..
Increment of P site over to previous report	0.07	46.54	N.A.	N.A.	……..

All concentrations are expressed as parts per million (ppm: x 10⁻⁶, mg kg⁻¹, µg g⁻¹) on a dry weight basis, NA = Not available or analysed
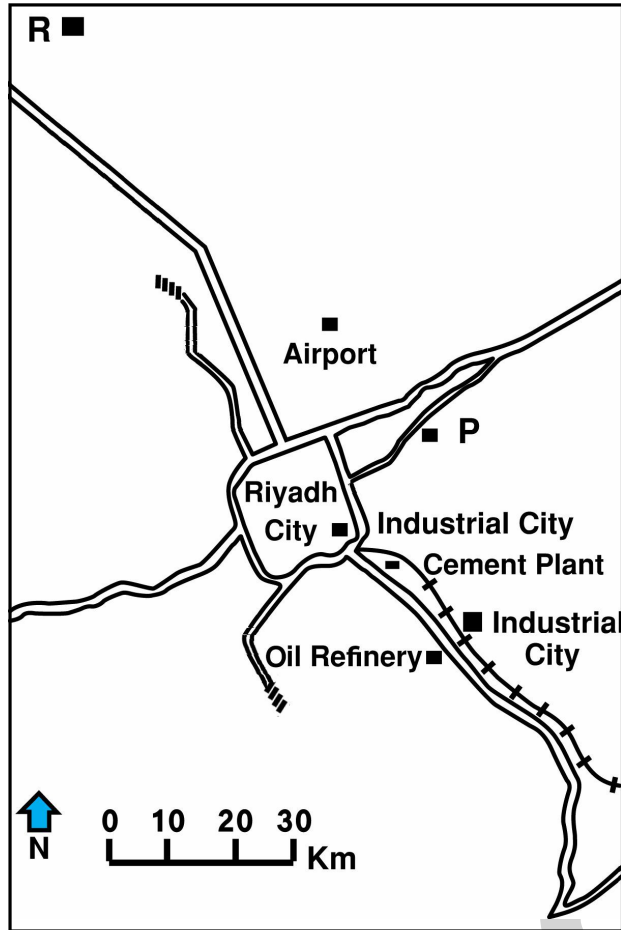
Fig. 1: A schematic illustration of Riyadh and neighbourhood showing the relative position of the reference (R) site from the polluted (P) site within the eastern section of Riyadh city

Hg (Pacyna and Pacyna, 2001), it is thought that a great part of the current record of Hg could have been derived from natural rather than anthropogenic sources.

In some instances, comparisons with earlier literature showed a non hazardous status of Cd in tissues of *M. libycus*. For example, the current polluted- reference ranges of Cd in the liver (0.74 -1.79 µg g^{-1}) and kidneys (0.72-3.29 µg g^{-1}) lie between the lowest (reference) and highest (near zinc smelters) values reported by Damek-Poprawa and Sawicka-Kapusta (2003) in the liver (0.169 - 8.669 µg g^{-1}) and kidneys (1.169- 23.589 µg g^{-1}) of the yellow-necked *mice Apodemus flavicollis.* Also, the frequently cited "critical concentration" (200 µg g>^{1}) of Cd for kidney toxicity (ATSDR, 1993) has not been reached in *M. libycus*. In other instances, these comparisons pointed at a questionable non hazardous status of the current tissue Cd levels that were shown to be higher in P (3.29 µg g^{-1}) and R (0.72 µg g^{-1}) kidneys of *M. libycus* than their parallels (0.7 µg g^{-1} vs 0.2 µg g^{-1}, respectively) in the rodent *Peromyscus leucopus* (Anthony and Kozlowski, 1982). Therefore, the formerly cited "critical concentration" has to be reconsidered in view of the disparity of tissue Cd levels and the consequent Cd-related toxicity that was shown not to be necessarily related, in small mammals, to a threshold tissue concentration (Chapman *et al.*, 2003).

Essential metals displayed quite different accumulation results in the tissues of *M. libycus*. The range of kidney content of Cu in *M. libycus* caught on both R and P sites (11.27-13.16 µg g^{-1}) fitted well with the Cu mean concentration (10.8 µg g^{-1}) in kidneys of the reference grey-brown vole, *Microtus agrestis*, referring to a non hazardous status of Cu in tissues of *M. libycus*. Nonetheless, the mean concentration of Ni in the liver of polluted shrews, *Crocidura russula* (0.95 µg g^{-1}) collected in spring from a mine area in Portugal (Sánchez- Chardi *et al.*, 2007) is almost similar to the current index (0.87 µg g^{-1}) in *M. libycus* collected from P site (polluted with several anthropogenic activities) during a related season (March- May), which points at a potential risk of Ni contamination in *M. libycus*.

Average values of Hg in kidneys and liver of *M. libycus* (up to 0.207 µg g^{-1}) lie below a threshold value (1.1 µg g^{-1}), the concentrations above which are suggested to pose an environmental Hg threat in wild mammals (Eisler, 1987); and also below kidney content of Hg in several species of small mammals used as monitors of heavy metals (Talmage and Walton, 1993). The current Hg content in kidneys and brain are also close to corresponding values in kidneys (0.667-4.64 µg g^{-1}) and brain (0.383-2.90 µg g^{-1}) of the rabbit *Pentalagus furnessi* collected from an isolated island in Japan (Horai *et al.*, 2006), emphasizing the non hazardous status of Hg that could be attributable to a normally high Hg-specific excretion rate in *M. libycus* preventing its bioaccumulation.

Mean concentrations of Pb in the liver (1.94 µg g^{-1}) and kidneys (1.91 µg g^{-1}) of *M. libycus*, caught from P site, were lower for the liver but higher for the kidneys than those of northern pocket gophers inhabiting a contaminated smelter area (0.945 and 5.32 µg g^{-1}, respectively) (Reynolds *et al.*, 2006). Positioning these liver concentrations of Pb in the context of studies linking toxic effects to similar Pb concentrations (Reynolds *et al.*, 2006), suggests that *M. libycus* living on the polluted P site could be experiencing some Pb toxic effects. However, liver and kidney content of Pb in *M. libycus* are below the critical renal Pb level (15 µg g^{-1}) and the concentrations (10 in liver and 25 µg g^{-1} in kidney) reported to be harmful to the common shrew (Ma, 1996). However, mean concentrations of Pb and Cd in the liver (0.18 and 0.34 µg g^{-1}, respectively) and kidneys (0.44 and 1.19 µg g^{-1}, respectively) of the yellow-necked mice *Apodemus flavicollis*, exposed to dust fall (including 24.2 and 0.627 mg m^{-2} of Pb and Cd, respectively) (Damek-Poprawa and Sawicka-Kapusta, 2003), are far less than parallel values in *M. libycus* in the liver (1.94 and 1.79, respectively) and kidneys (1.91 and 3.29 µg g^{-1}, respectively). Therefore the above threshold values seem overstated, given that there is insufficient evidence to indicate a hazard to small mammals from airborne Cd and Pb.

Except for Cu, all metals showed higher mean concentrations in jirds trapped from the polluted P rather than the reference R site.
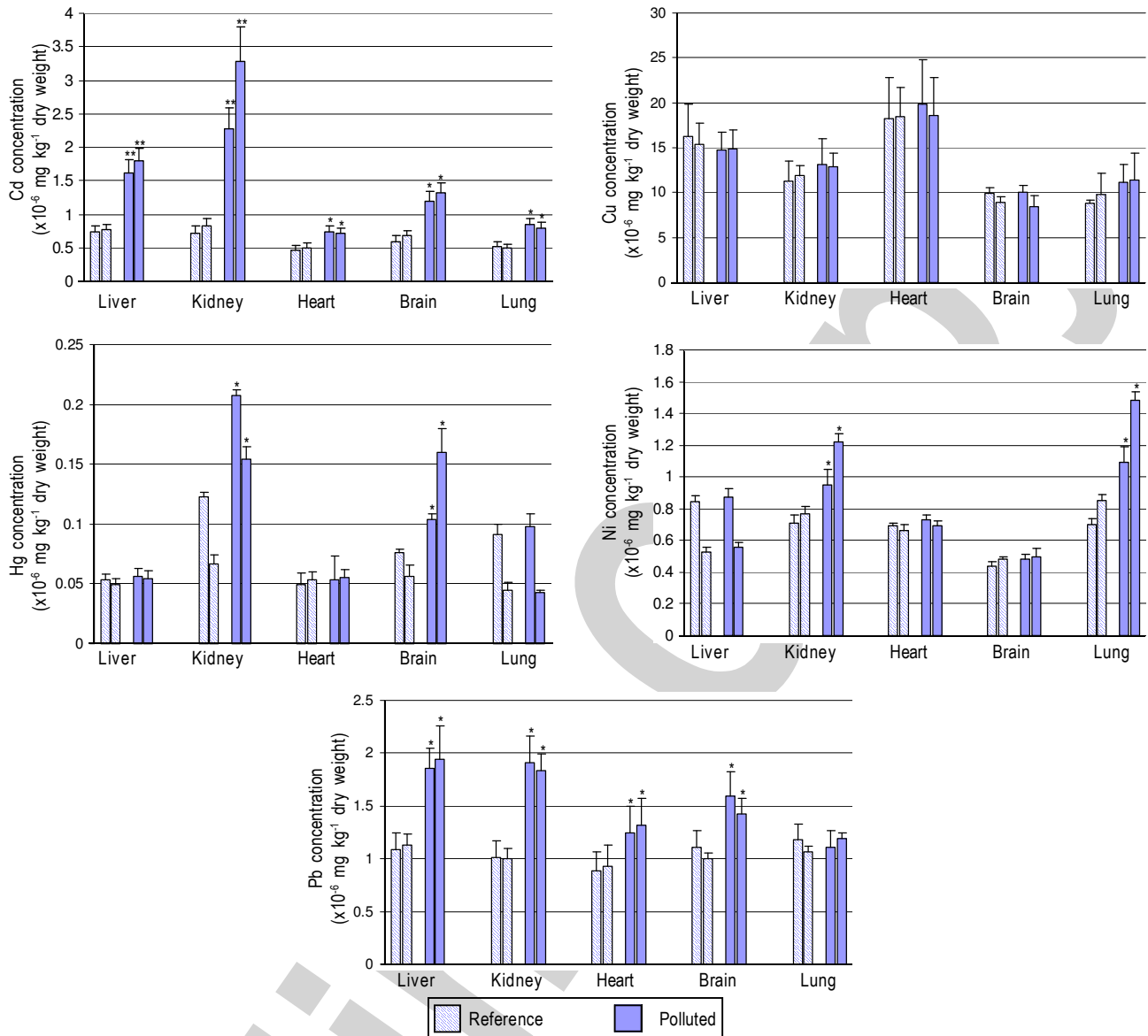
Fig. 2: Values are mean ± SE of 10 analyses for Cd , Cu, Hg, Ni and Pb in selected tissues of male and female *M. lybicus.* Male values are situated left followed by female ones. Asterisks (*)denote significant differences (*p < 0.05 and **p< 0.01) between locations.

The current findings, on the absence of significant site-specific changes of Cu in tissues of *M. libycus* and their presence in soil, suggest that Libyan jirds possess a strong ability to homeostatically regulate Cu in their soft tissues. Copper is an essential metal internally regulated within mammalian systems and is required by all organisms as a vital constituent of cofactors, enzymes and proteins required for a wide range of metabolic processes. In the meantime, Cu is involved in toxicological interactions with cellular components, mainly originating from its ability to produce toxic hydroxyl radicals (Schumann *et al*., 2002). Probably, as a consequence of this double biological role, intracellular and tissue concentrations of Cu are homeostatically regulated in many animal species including mammals (Thiele, 2003), and hence accounting for the insignificant changes.

Among nonessential toxic metals, the most prominent accumulation statistics in *M. libycus* for P versus R animals were drawn by Cd that accumulated most in the kidneys (p< 0.01) (up to 4.0 folds), followed by the liver (up to 2.3 folds) (p< 0.01), and then brain (up to 2.0 folds) (p<0.05). An organ-specific pattern of Pb distribution showing a higher tendency to accumulate in the kidneys and liver (up to 2 orders of magnitude) than other organs (up to 1.4 folds) agrees with the main results of Pb distribution in tissue of small mammals. While some authors (Pankakoski *et al*., 1994; Milton *et al*., 2003; Sánchez-Chardi and Nadal, 2007) showed higher Pb concentrations in the kidneys of some small mammal species (*Peromyscus leucopus, Blarina brevicauda, Microtus pennsylvanicus, Clethrionomys glareolus* and *Crocidura russula*),

others (Topashka-Ancheva and Metcheva, 1999; Sánchez-Chardi *et al.*, 2007) reported higher levels in the livers of *Mus musculus* and *Apodemus sylvaticus*. Based on the controversy about the hazardous status of Pb in tissues of *M. libycus*, the lower site-specific accumulation of Pb (up to 1.7 orders of magnitude) than Cd (up to 4.0 orders of magnitude) and Hg (up to 2.3 orders of magnitude) in soft tissues of *M. libycus* could be due to a hypothesis speculating a primary accumulation of Pb in bones (a tissue not targeted in this study) (Wijnhoven et al., 2008), or to another hypothesis assuming that non-toxic Pb concentrations do not trigger storage in soft tissues (Wijnhoven *et al.*, 2008).

Nickel had a different distribution dynamics pattern in *M. libycus*, showing a mere uptake by the lungs (up to 1.7 folds) and kidneys (up to 1.6 folds). Comparatively elevated Ni concentrations were also reported in human lung, brain, kidney and liver after inhalation of Ni compounds and in the kidney, lung, and liver of rodents following parenteral administration of Ni salts (ATSDR. 1997). According to ATSDR (1997), among the toxicologically important routes of entry for Ni are inhalation and ingestion assigning the lung as one of the main organs, which are affected by exposure to Ni.

Mercury followed the general distribution pattern in *M. libycus*, showing the highest differential uptake by the kidneys (up to 2.3 folds), followed by the brain (up to 2.0 folds) and then other organs. This finding might indicate that the transition rate of Hg from the liver was lower to the brain than to the kidneys as suggested by Horai *et al.* (2006). A similar pattern of Hg distribution was displayed small mammal species (*P. leucopus, S. hispidus*, and *B. brevicauda*) that showed a major differential uptake of Hg (Talmage and Walton, 1993).

Kidneys and liver are important sites for metal toxicity in vertebrates (Adham, 2001; Reynolds *et al.*, 2006; Sánchez-Chardi *et al.*, 2007). Therefore, data on metal residues in kidney and liver can be used to characterize the risk of adverse effects and the potential for injury. Based on the major differential uptake of most metals in kidney tissues compared to the liver, it is suggested that metals accumulate initially in the liver where they form a complex with one or more metal-binding proteins, such as metallothionein (Reynolds *et al.*, 2006), until an accumulation threshold is reached, hence, the metal–protein complex is transported via the plasma to the kidney, where they accumulate over time.

By virtue of the fossorial characteristics of Libyan jirds and their position in food webs, and based on the significantly higher metal levels in their polluted soft tissues versus reference subjects, they are entitled for a substantial role in metal biomonitoring.

Acknowledgments

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