Bull. Environ. Contam. Toxicol. (1997) 58:945-952

Bulletin of Environmental Contamination and Toxicology

Heavy Metal Contamination in a Lizard, *Agama stellio stellio,* Compared in Urban, High Altitude and Agricultural, Low Altitude Areas of North Greece

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Received: 30 September 1996/Accepted: 17 February 1997

Contamination from heavy metals is a serious problem recognised in most countries of the world. Heavy metals from mining industry, fertilisers, motorcar exhaust, are all factors contributing to global pollution in natural resources and the environment generally and, eventually, human health.

Direct and indirect methods have been developed to detect heavy metals quantitatively and qualitatively and to evaluate the level of pollution in an area. Direct methods are applied to soil and water; indirect methods use plants and animals as bioindicators: the most usual animal bioindicators are invertebrates (Marigomez et al,1991; Terhivuo et al,1994) and vertebrates such as fishes (Schmitt and Brumbaugh, 1990), mammals (Way and Schroder,1982), and less frequently birds (Husain and Kaphalia, 1990).

Reptiles on the other hand have not been used as bioindictors of pollution for various reasons,including difficulty in sampling large enough numbers, an ancient fear, their little economic importance, difficulty in acclimatising them in the laboratory etc., although, certain groups are near to the top of the food chain (crocodiles, snakes) and with a low metabolic rate, they could be used as such. Furthermore. since invertebrates are the prey of most lizards, the uptake of metals through invertebrates is an important pathway by which metals enter in the body of lizards. Heavy metals thus accumulate through the ingestion of contaminated food, and also by the incidental ingestion of soil (small stones are often found in the stomach of the lizards).In the few studies using reptiles, especially turtles (Albers et al,1986), accumulation of metals to high concentration, suggests that reptiles would be good pollution bioindicators. Reptiles have recently started to be used as bioindicators of pesticides entering the environment(Lambert,1993), with great success.

As in the environment globally, the environment in Greece has a burden of heavy metals , resulting from the use of fertilisers, pesticides with a heavy metal content (Cu,Zn) in their formula, and moreover as a by-product in mining and smeltering industries, and also the result of motorcar exhaustion (Pb,Mn) etc.

The main purpose of this contribution has to ascertain whether reptiles, especially lizards, can be used as bioindicators of heavy metal pollution by comparing indirectly the level of pollution of two areas differing to some extent ecologically in the vicinity of a large city and in the vicinity of cultivated areas.The bioindicator species used, was the lizard Agama steliio stellio. which lives in both areas . This is the first time that the levels of so many metals have been studied in a lizard species, even exceeding those in intensely studied mammals and fishes.. Moreover, there have been few studies on heavy metals in carcass and liver.

MATERIALS AND METHODS

Adult *Agama stellio stellio* were collected from two areas under study by a usual method of pulling them with a large pair of forceps from the crevices . Once returned to the laboratory, they were sexed and anesthetized with ether, each was individually weighed to the nearest mg and snout-vent length was recorded to the nearest millimeter.The liver was quickly removed and the liver and the carcass (the rest of the body minus the digestive tract), were weighed to the nearest 0.1 mg. The tissues were placed into plastic bags, deep frozen, and they were taken in dry ice to the geochemical laboratory Department of Earth Sciences of the University of Greenwich, United Kingdom for heavy metal analysis.

For heavy metal determinations, the liver and the carcass were cut in small pieces, dried in an oven at 80° C for about 48h (to constant weight) and were turned into powder by a mortar and pestle. About 0.5 gr. of the carcass and liver was used. Glassware was cleaned with 1% Nitric acid, rinsed thoroughly with deionized water and dried in an oven.Tissues were digested in 10 ml HNO_3 at about 120-150° C under a reflux cap and heavy metals were analysed using an ICP-MS spectrophotometer. The carrier gas was Argon and the internal standard Rhodium. Heavy metal concentration was expressed as μg/gr. dry weight. Analyses for fourteen heavy metals were performed.

The first of the two areas chosen for the study; the T area, lies at the edge and 4-5Km east of the centre of Thessaloniki (1.000.000 people), altitude 500m. The second area (the K area) near the village Kolhiko, lies about 25km NE of Thessaloniki, altitude 50m. There are cultivated areas at about 1km from the study site. The main industrial area of Thessaloniki was equidistant from both study areas, the three forming a triange.

All statistical comparisons were performed by Mann-Whitney non- parametric test and Spearman's rank correlation (r) at 0.05 level of significance were applied.

RESULTS AND DISCUSSION

The mean and standard deviation of the fourteen metals were estimated in the liver and carcass of lizards (table 1) . Results for both sexes were pooled, due to the lack of any statistically significant difference between them in any of the heavy metals analysed.

The livers of the lizards collected from T area contained mean copper, molybdenum and cadmium concentrations that were significantly higher (P<0.05) than those of the carcass. On the contrary, the carcass contained mean aluminium,chromium, nickel, barium and strontium that were significantly higher (P<0.05) than those of the liver. There was no statistically significant difference in the rest of the 6 metals. The same observations were recorded for the area K lizards, except from the fact that the concentration of the lead was significantly higher in the carcass.

A strong positive correlation was observed between liver and carcass of site T lizards for rubidium (r=O.94).ln site K lizards, strong positive correlations were observed for cobalt (r=0.71), rubidium (r=0.99), aluminium (r=0.77) and barium (r=0.84).

Strong positive correlations were observed between aluminium and chromium (r=0.97) in T lizard carcassess and between aluminium-cobalt (r=0.84) in livers of the same animals. Similarly, between aluminium and cobalt (r=0.80), and chromium and cobalt (r=0.74) in K lizard carcasses and aluminium and cobalt (r=0.75) and nickel and copper (r=0.77) in their livers. Strong negative correlations were observed between nickel and zinc (r=-0.72) of T carcasses and manganese and cobalt (r=-0.76) in the livers , and between nickel and rubidium (r=-0.90) and copper and rubidium (r=-0.77) in carcasses and copper and rubidium (r=-0.78) in livers of K site lizards.

Concentration of heavy metals compared in the livers of lizards of both sites revealed statistically significant differences in chromium, cobalt, nickel, copper, zinc, strontium, cadmium , barium and lead ($p<0.05$). Livers of the site K lizards always contained higher concentrations of the above metals (table 1), while there were no statistically significant differences observed in the remaining metals. Similarly, comparison of lizard carcasses from both sites showed statistically significant differences for chromium, manganese, cobalt, nickel, strontium and barium ($p<0.05$). As in liver, site K carcasses contained higher concentrations of the above metals.

The lack of sufficient data for comparison, makes discussion difficult, forcing thus for comparison with turtles and other vertebrates, since there are no data from other reptiles. However, for some metals such as rubidium, caesium, strontium, molybdenum and barium it was impossible to find data for comparison, even from the intensely studied fishes and mammals.

Mean values of cadmium, lead, chromium and nickel in the liver and carcass in lizards of the two areas compared to those found in other vertebrates, correspond to the moderately contaminated areas. Thus, Stone et al (1980), found on average 17 µg/gr. Cd expressed as wet weight in four livers of snapping turtles *Chelydra serpentina* on one side of the Hudson river. About 73% of the liver consists of water, and so this value expressed in dry weight corresponds to about 65 µgr/gr. Small insectivorous mammals, such as *Sorex araneus,* living near mining sites, contained 236 ppm in their liver (Talmage and Walton,1991). Studies on fishes showed that concentrations of about l8ppm whole body wet weight, are considered as toxic. The liver represents about 5% of the whole body (water about 73%) and the concentrations for the lizard after the appropriate conversions were estimated to be about 0.5 ppm for the site T and about 1.5 ppm for the site K lizards ; these are much lower numbers, than those recorded in fish.

The mean lead concentration of respectively 6.79 and 13.32 ppm dry weight in the T and K sites were much lower than 21.60 ppm wet wt (about 75 ppm dry wt) reported for the liver of the box turtle(Beresford et al, 1981) near a lead smelter, but higher than that about 0.4ppm found in the snapping turtle Chelydra serpentina (Albers et al, 1986).Studies on the liver of small mammals (Talmage and Walton,1991) gave 0.5-12 ppm dry weight for reference sites and 9.5-13 ppm for contaminated sites. In another study on small mammals (Pankakoski et al, 1995), lead concentration in the liver of juvenile *Sorex araneus* around a lead smelter gave a median of 15.55 ppm dry wt.

In both populations of lizards in this study chromium concentration in the liver is much lower than that found in the snapping turtle *Chelydra serpentina* (Albers et al 1986) . In Mexican birds, concentrations of 1.7-4.6 ppm wet wt (about 6.5-17 ppm dry wt) were considered to be below the thresholds for biological effects(Mora and Anderson, 1995). In fishes, chromium concentrations higher than 3.5-4 ppm whole body wet wt. were representative of areas with evidence of chromium contamination (Wiener et al, 1984 ; Elsler, 1986).

The nickel concentration in tissues of site T lizards is as high as in the liver of turtles of an area of Maryland (Albers et al 1986), while that of K site exceeds it. Jenkis (1980), noted that freshwater fish from uncontaminated areas throughout the world may contain NI at concentrations up to about 7 ppm dry wt. The concentrations of NI measured in *Agama stellio stellio,* converted to whole body weight, were about 8 and 9 ppm. The area therefore is regarded as moderately polluted.

Table 1. Heavy metals present in the lizard *Agama stellio stellio* compared in a urban, high altitude and an agricultural, low altitude area. Values (± standard deviation) are expressed in ppm dry wt and are compared by Mann-Whitney U test. NS=non significance.

Heavy metal	Site	Liver	Carcass	U test
Aluminium Al	T	119.98±71.38	769.49±758.45	P=0.0027
	K	132.99±71.10	1601.70±563.60	P=0.009
	U test	NS	NS	
Chromium Cr	T	2.37±0.59	4.16±1.45	P=0.0088
	K	1.35±0.77	5.57±1.27	P=0.009
	U test	NS	NS	
Manganese Mn	T	41.02±28.36	40.71±24.81	NS
	K	52.03±29.71	61.09±5.94	NS
	U test	NS	NS	
Cobalt Co	T	3.50±2.39	2.53±0.63	NS
	K	5.08±1.44	3.62±0.62	NS
	U test	N S	P=O.O3	
Nickel Ni	T	3.6O±3.11	33.83±22.12	P=0.002
	K	7.33±5.07	47.52±14.87	P=0.009
	U test	NS	NS	
Copper Cu	T	139.67±37.71	27.44±6.06	P=0.002
	K	209.09±122.23	27.14±4.30	P=0.009
	U test	NS	NS	
Zinc Zn	T	614.55±165.26	608.99±106.64	NS
	K	794.14±178.03	643.47±52.03	NS
	U test	NS	NS	
Rubidium Rb	T	33.92+8.63	30.96±5.38	NS
	K	35.05±15.81	33.73±12.81	NS
	U test	NS	NS	
Strontium Sr	T	16.48±11.86	16.,23±91.58	P=0.002
	K	40.24±12.72	396.12±63.11	P=0.009
	U test	P=0.02	P=0.004	
Molybdenum Mo	T	8.32±2.62	1.24±O.43	P=0.002
	K	7.51±2.42	1.39±0.83	P=0.009
	U test	NS	NS	
Caesium Cs	T	0.48±0.34	0.26±0.20	NS
	K	0.98±1.17	0.16±0.009	NS
	U test	NS	NS	
Cadmium Cd	T	9.87±4.71	1.01±0.54	P=0.002
	K	27.18±12.59	1.36±0.61	P=0.009
	U test	P=O.O2	NS	
Barium Ba	T	13.04±7.30	49.76±l9.79	P=0.002
	K	22.77±11.13	122.88±48.05	P=0.009
	U test	NS	P=O.O1	
Lead Pb	T	6.79±2.82	12.81±3.76	P=0.006
	K	13.32±5.03	15.65±5.51	NS
	U test	P=0.02	NS	

In lizards of both areas, Zn at very high concentrations of >600ppm, perhaps the highest recorded in vertebrates, exceeded three times levels observed in the whole body of snapping turtles (Albers et al, 1986). In mammals, concentrations are reported of 120-169 ppm for small mammals of the Wisconsin (Smith and Rongstad, 1981), and 130-160 ppm for the Finnish Elk (Noemi et al, 1993). In the liver of a number of mammals, (Talmage and Walton, 1991), average concentration ranged from 113 to 199 ppm , while at a zinc smelter site, concentrations of 377 ppm for shrews and 192 ppm for mice were recorded. A mean concentration ranging from 23.3 to 28.8 ppm was recorded in the liver of a number of birds from the Mexicali valley in Mexico (Mora and Anderson, 1995), an area considered to be unpolluted.

This high concentration of zinc in lizard bodies of both areas is difficult to explain. A possibility is that areas are contaminated by an unknown pollutant, or that this Zn is bound to metallothioneins, which, are known to have critical role in detoxification, and Zn and Cu homeostasis .According lo Lance et al (1995), lizards and snakes exhibit zinc plasma levels 5 to 50fold above those seen in higher vertebrates, concentrations which are toxic in mammals, birds and crocodilians. They suggest that these reptiles possess a plasma protein with a strong affinity to zinc. It is probably, the reason that they con accumulate high concentrations of this element.

Copper concentration is at high levels in the liver and carcass lizards of both areas. In snapping turtle, Albers et al (1986), found concentrations ranging from 4.5 to 40ppm. and in the liver of Finnish Elk Niemi et al (1993) reported values of 100-120ppm and in small mammals Smith and Rongsted (1981) l5-80ppm. In three fishes, Carpene et al (1990) recorded 1ppm for the gold fish, 6.9ppm for the sardine and 50.2ppm for the trout, all expressed as whole body wet weight. The latter value nearly equals the site K sample. As in the case of the zinc, copper may be bound to metallothioneins for detoxification and homeostasis purposes.

A metal found in high concentrations is aluminium. Although found in high concentrations in the earth's crust, there are not many studies related to its concentration in animal tissues. Many studies have been performed on the relationship between wafer pH and aluminium in amphibian larvae(Brady and Griffiths,1995). Low water pH together with high concentration of aluminium is fatal. In some fishes, a concentration ranging from 2.92 to 460ppm was recorded (Saiki and Palawski,1990).

Winger et al(1990), indicated that cobalt concentrations ranging from 0.43 to 1.56ppm wet weight did not appear lo be unduly high for the fishes of the Savannah river. These concentrations are close to those observed in this study. From the data presented by Elsler (cited by E.I. Hamilton, 1995), marine molluscs contain about 4ppm and crustacea contain about 22ppm Co dry wt in their body soft parts.

Experiments by E.I. Hamilton (1995), showed that cobalt becomes adsorbed to manganese oxide in the soil and, once fixed, the Co is unavailable for plant uptake. Taking the trophic chain plants®insects® lizards into account, this might explain the negative correlation between manganese and cobalt in the liver of site T lizards.

Another element which seems to be in somewhat high concentrations in both areas, is manganese. Values detected correspond to moderately polluted fishes stations(Winger et al,1990). Similarly, compared to the fish *Limnothrissa niadon* (Berg et al, 1995), levels are nearly at the same. Levels 3-4 times lower, have been observed in the pigeon Columba livia (Loranger et al,1995). According to these authors, a source of manganese pollution is the combustion of unleaded petrol by the motorcars, since this petrol contains methyl-cyclo-pentadienyl-manganeset tricarbonyl (MMT) as an antiknock agent (Loranger et al, 1994). In Greece, about a quarter of the cars combust unleaded gasoline, thus contributing to manganese pollution It is of course premature to associate manganese pollution of an area with combustion of MMT, but there could be a contribution to some extent. Other sources also possibly contribute to pollution of the two areas, especially alloys found in the industrial area.

Less studied elements are molybdenum, rubidium, strontium and caesium. In the liver of rabbit foetuses, a mean rubidium concentration of 8.85ppm wet weight was found (Palavinskas et al,1984), a value close to that found in the liver and carcass.

It is of note that there was a strong positive correlation between carcass and liver of both sites lizards for the rubidium. It seems likely that this element activates fructokinase in the liver and renin in the kidney of the rat foetus (Palavinskas et al, 1984) and its distribution follows that of potassium(Schulten et al, 1983b).

The relationship between aluminium, cobalt and chromium is also of interest. All three of these metals exhibit a strong positive correlation between them, suggesting that, the three metals act synergistically. Somewhat different is the relationship between nickel, copper and rubidium. The first two elements show a strong positive correlation between them and, both show a strong negative correlation with rubidium. Nickel and copper probably act synergistically, while acting antagonistically with rubidium.

Based on the statistically significant differences found between liver and carcass of the two sites lizards, it is obvious that a number of metals (aluminium, chromium nickel, strontium, and barium) accumulate in significantly higher concentrations in the carcass, while others (copper, molybdenum and cadmium) accumulate more in the liver, and the remainder (manganese, cobalt, zinc, rubidium, caesium and lead) show similar concentrations in the liver and carcass. This separation is useful, and should be taken into account in further studies.

The difference in concentrations of heavy metals in the tissues of lizards of both areas is characteristic and interesting. Although site T was near a big city with a large number of cars, it seems to be less polluted than K. A possible explanation is that T lies at a higher altitude of about 500m than K and a high percentage of pollutants tend to accumulate at lower altitudes. Moreover, the direction of prevailing winds is such, that heavy metals are pushed to the K-area, since the main industrial area lies between the two. Another explanation could be the use of pesticides with a heavy metal in their formula (Cu,Zn,Mn), as well as the use of fertilisers, which, as it is known, contain heavy metals.

The present study showed that lizards accumulate heavy metals and can be used as bioindicators of pollution, since a clear difference was revealed in the body load of the two areas lizards, which probably reflects different degrees of pollution in each.

Acknowledgments. This study was partly supported by the European Environmental Research Organization (EERO). Many thanks are due to Dr David Wray of the University of Greenwich for helping in preparing the samples and providing all the needed facilities. Many thanks are also due to Dr MRK Lambert, of the Natural Resources Institute (NRI), England, for critically reading an early draft of this manuscript and correcting it especially linguistically and phraseologically.

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