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The Nile monitor (*Varanus niloticus*; Squamata: Varanidae) as a sentinel species for lead and cadmium contamination in sub-Saharan wetlands

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ABSTRACT

Wetland pollution is a matter of concern in sub-Saharan Africa. Though regularly exploited, the Nile monitor (*Varanus niloticus*), a large amphibious lizard, is not threatened. This work aims at assessing the value of this varanid as a sentinel species in surveys of environmental contamination by metals. Lead and cadmium quantifications were performed by graphite furnace-atomic absorption spectrophotometry in bone, intestine, kidney, liver and muscle in 71 monitors from three unevenly polluted sites in Mali and Niger, plus a reference site. The effects of sex, size and fat reserves as well as factors related to the sampling strategy (tissue sampled, sampling site) were studied with a mixed linear model. Metal contamination is moderate at the four sites but clear differences nevertheless occur. Lead levels are generally maximal in bone, with a gender-independent median value 320 $ng.g^{-1}$. Median cadmium concentrations never exceed 70.2 $ng.g^{-1}$ in females (kidney) and 57.5 $ng.g^{-1}$ in males (intestine). Such levels should have no detrimental effects on the monitors. Lead and cadmium levels in muscles are generally below 200 and 20 $ng.g^{-1}$, respectively, and should provoke no health hazard to occasional consumers of monitor meat. Metal organotropisms are consistent with those observed in other studies about Squamates: for lead: bone>[kidney, intestine, liver]>muscle in males and [bone, kidney]>[intestine, liver]>muscle in females; for cadmium: [liver, intestine, kidney]>[bone, muscle] for both genders. Females are more contaminated, especially in their kidneys. In this tissue, median values in $ng.g^{-1}$ are 129.7 and 344.0 for lead and 43.0 and 70.2 for cadmium, for males and females, respectively. Nile monitors can reveal subtle differences in local pollution by metals; moreover, the spatial resolution of the pollution indication that they give seems to be very sharp. The practical relevance of this new tool is thus validated.

1. Introduction

Environmental contamination by metals in sub-Saharan wetlands is a matter of concern. On a global scale, inland waters are presumably less contaminated in Africa than in industrialized countries; however this essential resource is threatened by fast urbanization and economic activities (Biney et al., 1994; Brönmark and Hansson, 2002; Calamari, 1985; N'Riagu, 1992; Yabe et al., 2010). The lack of information on the ecotoxicological status of freshwater in southern developing countries is considerable (Karlsson, 2002; Karlsson et al., 2007; Lacher and Goldstein, 1997). For these reasons, monitoring wetland pollution by metals and metalloids is especially needed in the sub-Saharan region. However, efficient procedures for characterizing precisely the nature and importance of local pollutions are scarce and tools suited to the economic and cultural context of Africa remain to be developed.

Several procedures for assessing local environmental pollution have already been used or are potentially usable. Some of these methods are focused either on the pathogenic effects of the pollutants on individual organisms, or on the consequences of pollution on biological communities. Examples of such approaches in Africa are given by *e.g.*, Lambert (2005) in sub-Saharan reptiles, Sowunmi (2005) in Nigerian fishes or Ochieng et al. (2008) in macro-invertebrates from Uganda (see also Ramade, 2010, for an extensive methodological review including methods referring to biomarkers). A common feature of these methods is that they are based on species that are not top chain predators. Therefore, they fail to accurately reflect biomagnification processes in food webs. Moreover, they are not aimed at identifying and quantifying chemical elements, a restriction that precludes straightforward designation of the main pollution sources. In brief, these methods give only gross indications on the ecotoxicological status of an area.

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In African continental waters, metallic contaminant quantifications were almost exclusively conducted in the southern and eastern parts of the continent, in Maghreb and in Nigeria (review in Yabe et al., 2010). These studies dealt mainly with contamination by lead, cadmium and/or mercury in inorganic matrices (sediment, water, suspended matters), plants or animals ranking at low levels of food chains (see *e.g.*, Ak'Habuhaya and Lodenius, 1988, for several metal quantifications in Tanzanian river sediments, vegetation, arthropods and molluscs; Greichus et al., 1977, 1978a, b, for quantifications of chlorinated pollutants and metals in several elements of freshwater ecosystems in South Africa, Kenya and Zimbabwe). Although metal quantifications were recently implemented in surface waters in Chad and Niger (Zairi, 2008), data are extremely scarce for Sahelian Africa (Yabe et al., 2010). Again, such studies can provide general information on local pollution but fail to indicate the concentration of pollutants within the highest elements of food chains.

The standardized use of an amphibious top chain predator found throughout the continent as a sentinel species could help achieve this goal. In Africa, Nile crocodiles were considered in this respect (Almli et al., 2005, in Zambia; Phelps et al., 1986, in Zimbabwe; Swanepoel, 1998; Swanepoel et al., 2000, in South Africa; see also Campbell, 2003 for other *Crocodylia* out of Africa). However, crocodilian populations are considerably depleted and most are strictly protected (CITES, 2011; Ross, 1998); therefore they became unsuited for routine or large-scale surveys. Today, the most relevant candidate to play this role could well be the Nile monitor, *Varanus niloticus*, a species ranking just below crocodiles in soft water food chains. A previous work (Berny et al., 2006) already used this species as an indicator of organochlorine contamination in Chad. Moreover, several metals (Al, As, Br, Cr, Cu, Fe, Hg, Mn, Pb, Rb, Se, Sr and Zn) were positively evidenced in some monitor species (Asian and Oceanian ones, as well as African savannah forms) by Boman et al. (2001), Lance et al. (1995) and Yoshinaga et al. (1992). These three studies used monitors opportunistically; they were not specifically aimed at developing a new tool for environmental pollution surveys.

The ecological and physiological traits of the Nile monitor suggest that this species could be excellent for assessing environmental contamination by metals and metalloids. It indeed meets several of the characteristics considered by some authors (Beeby, 2001; Elder and Collins, 1991; Peakall and Burger, 2003; Ramade, 2010) as defining a good sentinel species.

Nile monitors are large opportunistic carnivorous lizards feeding along the banks of rivers, lakes and ponds as well as in water. Their distribution spreads over most sub-Saharan Africa, in wetlands located in both remote areas and densely populated cities (Bayless, 2002; Lenz, 2004). Their diet is initially composed of arthropods, annelids, molluscs and amphibians, but they progressively add other prey, such as fishes, reptiles, birds and small mammals. They also appreciate eggs and carrion, and can occasionally prey on conspecifics (Bennett, 2002; Cissé, 1972; Cowles, 1936; Lenz, 1995; Luiselli et al., 1999). Contrary to crocodilians, varanids are highly active and voracious predators with an activity metabolism higher than that of most other reptiles (Thompson, 1999). They explore their territories during most of the day (Lenz, 1995) and can eat considerable amounts of prey (Cissé, 1972; pers. obs.) that are swallowed whole, with shells and bones. For this reason the bio-accumulation process is likely to be intense in monitors: a trait predisposing them to be good indicators in ecotoxicology, especially for osteotropic element accumulation. In addition Nile monitors are long living animals, with a life span commonly exceeding ten years in the wild (de Buffrénil et al., 1994; de Buffrénil and Hémery, 2002). Though very mobile, they are sedentary, with adult home ranges averaging 5 ha in males (Lenz, 1995). Contamination data given by monitor samples would thus refer precisely to the places where they were captured. This excellent spatial resolution is a crucial advantage that migratory species lack (Berny et al., 2002; Prudente et al., 2005; Ramade, 2010). Nile monitors display some other important advantages such as a large size (enough tissue can be sampled for several analyses), ease to identify (they are morphologically different enough from other African monitors to avoid confusion) and precision of individual aging by skeletochronology (de Buffrénil and Castanet, 2000).

The objective of this study is to further document the value of the Nile monitor as a sentinel species in wetlands. With this aim, we tested its capability to indicate heavy metal contamination in selected areas of the Sahelian region of Africa located in Mali and Niger. Special attention was paid to the propensity of different tissues for metal concentration and to the influence of some individual traits (*i.e.* sex, snout-vent length and proportion of fat in the organism) on the concentrations observed. Lead and cadmium occur in the lists of chemicals of environmental importance (CEPA, 1999; EC, 2001a; USEPA, 2007) and show the highest degree of ecotoxicological relevance (Sparling et al., 2010). This is why attention was focused on these elements.

2. Materials and methods

2.1. Remarks on Nile monitor populations

Nile monitors have long been exploited for the international skin trade in Mali, Chad, Cameroon and Sudan (de Buffrénil and Hémery, 2007a,b) under the control of CITES (Washington convention on the international trade in wildlife). Although annual CITES quotas for skin exports were fixed at 180 000 in 2010, 80 000 in 2011, 70 000 in 2010 and 150 000 in 2001 for these four countries, respectively (CITES, 2011), actual gross skin exports have collapsed dramatically during the last ten years. According to latest reports in CITES trade database (http://www.unep-wcmc-apps.org/citestrade/report.cfm, year 2011) no skins were officially exported from Mali, Chad, Cameroon or Sudan in 2010, a trend that obviously reflects a change in the demands of European and Japanese leather industries. The places where Nile monitors are most intensively hunted are restricted to Lake Chad (Chad, Cameroon and Nigeria) and the inner delta of the Niger River (Mali), where teams of specialized hunters have for decades been capturing several tens of thousands of monitors each year. In spite of this intense harvest, local monitor populations seem to resist, although slight accommodative modifications in their life history traits (longevity, growth rates, female fecundity, *etc.*) may occur (de Buffrénil and Hémery, 2007b). Intensive monitor harvesting is now bound to cease as the demand for skins from main importing countries decreases. Elsewhere in Africa, Nile monitors are moderately but constantly hunted for food purposes. They nevertheless remain common near water stretches (de Buffrénil and Hémery, 2007a,b).

V. niloticus is not considered globally threatened. It is classified in CITES Appendix II (trade permitted under control) and is not included in the IUCN Red List of Threatened Species. In addition, local authorities tolerate its exploitation provided that it is conducted with traditional techniques exclusively (de Buffrénil, 1993).

It is thus possible to use the by-products of hunting opportunistically for pollution control. For this work we accompanied fishermen during their ordinary activities. When they caught a monitor (in general with large hooks baited with frogs) as described by de Buffrénil (1993) and sacrificed it according to their traditional practices, samples were collected from various tissues (see Section 2.2) in presence of, and in cooperation with, local competent authorities. The monitors were then returned to their owners who generally consumed the meat.

2.2. Sampling sites, dates and sample collection

In Niger, specimens were collected from October to November 2009 near the cities of Diffa and Niamey, the capital. They were collected in Mali (November to December 2008) in two regions: on the one hand, the adjacent cities of Niono and Molodo and on the other hand, the village of Flabougou (Fig. 1). In each site, the areas elicited for captures did not exceed 5 km^2. The four sites were selected in order to compare three supposedly different situations of environmental contamination (an urban site, two agricultural sites and a control site) and consequently assess the sensitivity of our biological indicator. Apart from the work of Zairi (2008), no data related to environmental contamination by metals were found in any of the four study areas. This author reported the concentrations of 16 metals or metalloids in the surface waters of the Komadougou-Yobé River less than 50 km away from Diffa. Lead levels were very low ($\leq$0.04 mg.l^{-1}). To our knowledge, no specific study of metal pollution in aquatic biota (plants and animals) has ever been conducted at any of these sites.

Diffa is a small city located in the easternmost part of south Niger. Nile monitors are common around Diffa in the fields irrigated by the waters of the Komadougou-Yobé River and in the reservoirs used for stocking water all year long. Agricultural activities (mainly vegetable culture) are very ancient at that site. The Malian towns Niono and Molodo are two medium-sized cities (some 5000 [2004 estimation] inhabitants) situated along the Sahel Channel in an important rice culture zone. Nile monitors are frequently encountered in the rice fields and in the irrigation channel network. In Diffa and Niono/Molodo, metal pollution sources of anthropogenic origin are limited. Cadmium accumulation may result from the use of phosphatic fertilizers (de Meeûs et al., 2002; Pérez and Anderson, 2009; Taylor, 1997) and we can assume that lead levels in the environment around these places are slightly higher than the background levels due to a number of activities: car use, burning of fuel wood and agricultural waste, metallic solid waste piling, incineration of combustible material from dumpsites, *etc*. (N'Riagu, 1992) Natural lead deposition from aerial dusts originating from the Sahara desert is supposed to be substantial in this area (N'Riagu, 1992; WMO, 1989). Niamey is a large city (675 000 inhabitants in 2002) crossed by the Niger River. Nile monitors were captured in or around the irrigated fields located near the only bridge that was in use at the time of sampling. Industrial activities in Niamey are limited to small businesses, but road traffic on the bridge is very dense most of the time. Road traffic is a potential source of local lead pollution; the vicinity of the bridge is supposed to be the most heavily polluted of our four sites. Finally the small village of Flabougou is located near the "*Boucle du Baoulé*" national park along the "*Nid du Serpent*", a large but temporary tributary of the Baoulé River. To our knowledge, this place is void of any significant anthropogenic pollution source and is considered here as the reference site.

A total of 71 Nile Monitors were captured, distributed as follows: 18 in Niono and Molodo, 32 in Diffa, 7 in Niamey and 14 at the reference site, Flabougou. All these specimens were caught by villagers and fishermen with their traditional hook-based technique. The large hooks in use (sea hooks, size no. 5 or no. 4) are not suited for catching small individuals. Since female Nile monitors are somewhat smaller than males (de Buffrénil et al., 1994), a slight bias towards males could be suspected to have occurred in the samples. This situation is nevertheless unlikely because previous studies of monitor demography based on the same capture technique revealed no significant difference between observed sex ratios and the theoretical ratio of 1:1 known to occur in monitor populations (de Buffrénil et al., 1994; see also King and Green, 1993).

Three measurements were made on each specimen: body mass (BM), snout-vent length (SVL) and mass of abdominal fat (MF). Two indices were calculated from these measurements: body condition index ($BC_i = BM/SVL$) and abdominal fat somatic index ($FS_i = MF/BM$). Median values (min. − max.) of BM, SVL, BC_i and FS_i are given in Table 1. The following tissues were sampled in each specimen: liver, kidney, proximal part of intestine, striated muscles of the thigh, one femur. Samples were stored in clean polypropylene pill-boxes and kept frozen until their analysis in the toxicology laboratory of VetAgro-Sup/National Veterinary School of Lyon, France.

2.3. Metal analyses

Liver, kidney, intestine and striated muscle samples were dried for 48 h at 70 °C, ground, homogenized, then digested and analyzed. An amount of 0.15 to 0.25 g of each tissue was introduced in overnight acid rinsed (65% nitric acid diluted 1:9) ceramic hearths and added 1 ml 96% sulphuric acid diluted 1:1. Samples were then oven incinerated, with a 10-hour rise in temperature from 20 to 700 °C,

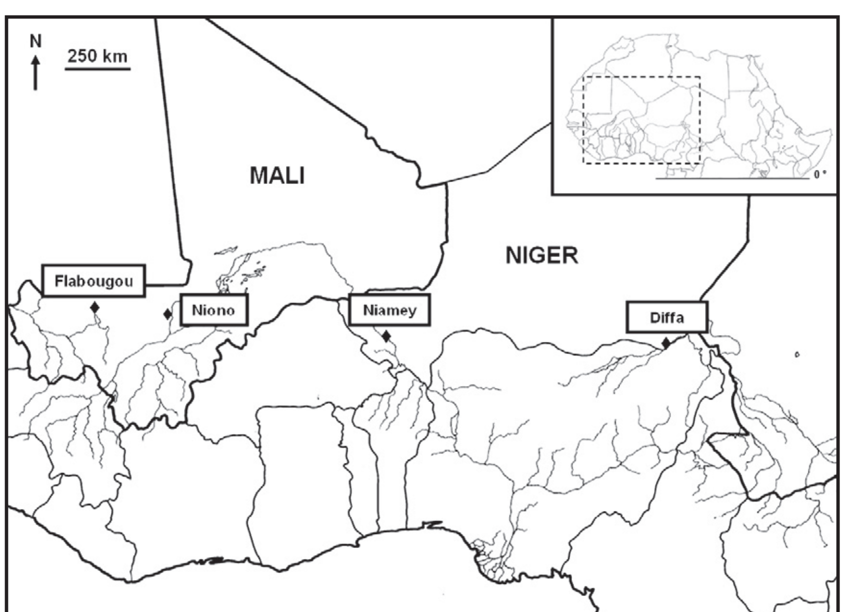

Fig. 1. Location of the four sampling sites: Flabougou, the reference site (14.463091, −8.884141) and Niono (14.252429, −5.976305) in Mali; Niamey (13.505513, 2.104869) and Diffa (13.3091, 12.62078) in Niger.

Table 1
Morphometric data in local samples and in the whole sample for female and male Nile monitors. Comparisons were made between sexes from Mann–Whitney–Wilcoxon non-parametric rank tests. Significant differences are represented for both sexes (*** p<0.001; * p<0.05).

	Female (n=30)				Male (n=41)			
	BM[a]	SVL[b]	BCi[c]	FSi[d]	BM[a]	SVL[b]	BCi[c]	FSi[d]
Flabougou (n=14)	1.50 (1.14–2.76)	45.8 (39.5–52.5)	0.033 (0.029–0.053)	0.027 (0.001–0.072)	2.24 (0.32–5.66)	49.2 (25.7–66.6)	0.045 (0.012–0.085)	0.044 (0.003–0.063)
Niono (n=18)	1.34 (0.49–1.60)	42.1 (31.0–45.2)	0.032 (0.016–0.035)	0.032 (0.009–0.043)	2.30 (0.78–4.18)	46.2 (32.0–58.4)	0.050 (0.024–0.072)	0.052 (0.017–0.082)
Diffa (n=32)	0.99 (0.61–1.98)	35.3 (31.8–48.1)	0.026 (0.018–0.042)	0.054 (0.014–0.095)	1.58 (0.34–3.44)	40.0 (27.2–55.7)	0.038 (0.013–0.062)	0.036 (<0.0005–0.109)
Niamey (n=7)	2.08 (1.18–3.01)	52.9 (42.5–58.0)	0.039 (0.028–0.052)	0.019 (0.007–0.023)	2.90 (0.64–4.80)	53.0 (32.1–67.2)	0.049 (0.020–0.071)	0.018 (0.002–0.031)
All four sites (n=71)	1.08*** (0.49–3.01)	39.5* (31.0–58.0)	0.028*** (0.016–0.053)	0.042 (0.001–0.095)	1.98*** (0.32–5.66)	45.7* (25.7–67.2)	0.043*** (0.012–0.085)	0.044 (<0.0005–0.109)

[a] Body Mass in kg; median value (minimum–maximum).
[b] Snout-vent length in cm; median value (minimum–maximum).
[c] Body condition index; median value (minimum–maximum).
[d] Fat somatic index; median value (minimum–maximum).

then a 6-hour plateau at 700 °C and a passive cooling. They were then added 2 ml 65% nitric acid diluted 1:1 and heated gently on hot plates until total evaporation. Once cooled down, 1 ml 65% nitric acid diluted 1:9 was used to retrieve metals from the hearths. Samples were then brought to a final volume of 10 ml with deionised water.

In each femur a 2.5 mm transversal section was cut with a diamond-edged circular saw at the level of the nutritive hole. This part of the bone is ontogenetically the oldest and the cortex in this region is both thick and absolutely void of inner, intracortical remodeling; however, it undergoes an extensive (but variably pronounced among individuals) process of perimedullar resorption (de Buffrénil and Castanet, 2000; de Buffrénil et al., 2008). The samples were dried and defatted with increasing concentrations drenching (70%, 95% and 100%) of Normapur ethanol (VWR Int., Strasbourg, France) and Pestanal acetone (Sigma, Saint-Quentin-Fallavier, France). Remnants of bone marrow could then be completely removed with a smooth paint brush. Clean bone sections (60 to 110 mg) were thoroughly acid washed, rinsed with deionised water, dried and weighed. High pressure microwave digestion (Ethos Plus 2 Microwave Labstation, Milestone s.r.l., Sorisole, Italy) was made in Teflon inserts in contact of 1 ml concentrated 65% nitric acid. The reactors contained 3 ml 35% hydrogen peroxide and 10 ml deionised water. Reaction timing was as follows: a 20 min rise from 20 to 180 °C, a 10 min plateau at 180 °C and a passive cooling. Samples were brought with deionised water to a final volume of 10 ml.

In each animal lead and cadmium quantifications were performed for the 5 tissues with a Unicam 989 QZ graphite furnace atomic absorption spectrophotometer (Thermo Optek, Trappes, France). For lead, a six points calibration line was established by least squares regression from a blank and daily prepared lead (II) nitrate solutions at 2, 5, 10, 15 and 20 μg.kg^{-1}; for cadmium a six points calibration curve was established by linear regression from a blank and daily prepared cadmium sulfate solutions at 0.5, 1, 2, 4 and 5 μg.kg^{-1}. In both cases the calibration was accepted when the correlation coefficient was ≥0.995. Concentrated calibration standard solutions of lead and cadmium were analyzed at the end of each analysis series in order to detect a possible drift in elemental detection. The values of these signal drifts ranged between −5.2% and +10.1% (lead) and between −10.0% and +6.8% (cadmium). Blanks and spikes were included in each sample batch analyzed. Recovery rates were always above 80% for lead (with one exception at 73.4%) and always above 80% for cadmium spiked liver, kidney, intestine and muscle samples with the dry digestion techniques. For bone analysis with the microwave digestion technique, positive controls consisted in NIST standard reference material no. 1400 for lead (recovery rates were always above 80% with one exception at 77.7%) and spiked samples for cadmium (recovery rates were always above 90%). Quantification limit was computed based on the lowest calibration point with a coefficient of variation (CV)<10% and taking into consideration a 10-fold dilution. Quantification limits were determined at 20 ng.g^{-1} and 5 ng.g^{-1} for lead and cadmium respectively, in all biological samples. For each metal, 10 blank samples spiked at the limit of quantification were tested to verify this value (with a CV<15%).

Deionised water used for all dilutions had a minimum resistivity of 18.2 MΩ.cm. The lead (II) nitrate, all acids and the hydrogen peroxide solution were supplied by Merck, Darmstadt, Germany, and were the purest quality available. Cadmium sulfate was supplied by Sigma Chemical Co., St Louis, MO, USA.

2.4. Statistical analyses

Potential imbalance in global and local *sex ratios* was examined with χ^2 tests. In the whole sample, Mann–Whitney–Wilcoxon non parametric rank tests were used to investigate possible sex based differences in individual characteristics (BM, SVL, BC_i and FS_i); and Kruskal–Wallis non-parametric identity test was employed to compare BM and SVL between the sampling sites, with implementation of the Bonferroni correction method to address the problem of the multiple comparisons. The correlation between the concentrations of lead and the concentrations of cadmium in the tissues was evaluated with Spearman non parametric correlation tests.

We tested the effects of five factors (sex, tissue, sampling site, SVL and FS_i) on lead and cadmium concentrations by an analysis of variance, using a mixed linear model analysis with the package *nlme* of the software R (R Development Core Team, 2010). Due to the distribution of the data, lead and cadmium concentrations as well as the variable snout-vent length underwent a decimal logarithm transformation before being included in the model. We considered in this model the fixed effect of three qualitative factors (sex, tissue and sampling site) and two quantitative variables (SVL and FS_i). Our model also took into account one random effect: the individual random effect (*i.e.* the model takes into account that the different tissues of a given monitor actually belong to a single individual). Additionally, we introduced in this model the first order interactions between the qualitative factors (except for the random one) and the interactions between each qualitative variable with each quantitative factor.

The inclusion of the variable *tissue* was justified by a possible propensity of pollutants to accumulate differentially in various organs and variable *sampling site* by potential differences that may occur in environmental pollution levels between local sites. *Sex* is recognized as a critical feature in organism contamination patterns, at least because females can remove certain contaminants from their tissues when gravid (Hall, 1980). Variables snout-vent length and body mass on the one hand, and variables FS_i and BC_i on the other, are correlated ($p<10^{-15}$ for both). Thus we chose to include only one variable of each pair in the model. In monitors, snout-vent length is closely related to age (de Buffrénil et al., 1994), making this characteristic essential when bioaccumulative processes are to be addressed. *Fat somatic index* reflects the amount of food that the monitors have consumed since the beginning of their annual activity period. Variation in this index may have substantial bearing on physiological or behavioral performances and thus affect the contamination processes.

When a result was below the quantification limit, it was set at the threshold, *i.e.* 20 $ng.g^{-1}$ for lead and 5 $ng.g^{-1}$ for cadmium. The normality hypothesis may therefore not always be respected. However no mixed linear model analysis integrating censored data is available. Additionally, a common alternative using non parametric tests would excessively simplify the analysis, especially ignoring potential interactions between factors and preventing a global analysis. Therefore, keeping this potential source of error in mind, the use of this model was maintained.

Although the analysis through the model considered the quantitative values, the quantitative variables were grouped into classes for clear visualization in graphical representations. For SVL, distinction was made between a) subadults (SVL<40 cm), b) adults (40 cm≤SVL<50 cm) and c) large adults (SVL≥50 cm) and for FS_i distinction was made between a) low ($FS_i<0.02$), b) intermediate ($0.02\leq FS_i<0.04$) and c) high ($FS_i\geq 0.04$) FS_i. Classical interaction plots (Chambers et al., 1992) were then used to represent and interpret the influence of the significant factors of the model on the logarithm of the values of metal concentrations.

3. Results

3.1. Morphometric data

The whole sample comprises of 41 males and 30 females. *Sex ratio* was calculated as n_{males}/N. Beyond remarkable (though not significant) local differences (*sex ratios* range from 0.47 in Diffa to 0.72 in Niono) most probably due to the small size of local samples, we could not find evidence of any significant imbalance in the overall *sex ratio* for the 71 individuals. Basically, the *sex ratio* in varanids is genetically determined and not different from the ratio 1:1 (*e.g.* de Buffrénil and Hémery, 2007a,b). We did not proceed to comparisons at local levels since the sizes of local samples are not large enough to perform meaningful statistical tests. Snout-vent length ranges from 25.7 to 67.2 cm and body mass from 320 to 5660 g. Males are significantly longer and heavier than females ($p<0.02$ and $p<10^{-3}$, respectively). The relative mass of abdominal fat, FS_i, is not significantly different between genders whereas BC_i is higher in males ($p<5.10^{-4}$), a difference that could possibly reflect a slight morphological dimorphism.

3.2. Metal quantifications

Because of the logarithmic pattern of data distributions, median values were considered here as the best expression of the central tendencies for lead and cadmium (non-transformed) concentrations in each tissue. Table 2 details the lead and cadmium median values detected in the tissues within the whole sample and for both sexes, with respect to sampling sites.

For each of the 71 monitors, quantifiable amounts of lead and cadmium could be detected at least in one tissue sample. Moreover the results also show that for the whole sample, lead levels are positively correlated to cadmium levels in every tissue (p always below 5.10^{-2}; $+0.30<\rho<+0.61$) with the exception of bone. Beyond the general occurrence of pollutants, there is a considerable variability in contamination frequencies and pollutant concentrations for each of the two metals.

Table 2
Lead and cadmium concentrations ($ng.g^{-1}$ dry weight) in male and female tissues (median value, min., max.).

	Lead					Cadmium				
	Bone	Intestine	Kidney	Liver	Muscle	Bone	Intestine	Kidney	Liver	Muscle
Mali–Flabougou										
♂ (n = 9)	106.7 (31.1–394.1)	32.1 (qt–667.6)	66.5 (28.3–101.1)	96.0 (qt–214.2)	qt (qt–53.6)	qt (qt–12.5)	20.2 (11.4–41.0)	12.2 (qt–52.9)	44.1 (19.0–161.0)	qt (qt–8.8)
♀ (n = 5)	241.8 (100.0–522.8)	61.6 (qt–128.6)	135.7 (100.9–178.0)	68.8 (59.9–91.5)	43.0 (qt–122.2)	6.8 (qt–11.5)	38.7 (12.0–51.1)	70.1 (16.9–111.9)	72.8 (40.2–148.9)	qt (qt–7.9)
Mali–Niono										
♂ (n = 13)	332.7 (50.2–1958.4)	116.4 (20–543.9)	76.7 (qt–172.4)	59.6 (qt–119.5)	qt (qt–104.1)	qt (qt–17.4)	48.0 (qt–245.5)	20.1 (10.5–98.5)	40.9 (20.1–237.8)	qt (qt–7.7)
♀ (n = 5)	258.0 (174.1–539.0)	88.9 (39.8–149.1)	175.6 (98.9–232.9)	97.1 (28.4–129.2)	qt (qt–191.9)	8.5 (qt–22.2)	60.2 (10.6–83.2)	49.1 (19.2–62.0)	70.5 (31.2–166.4)	qt (qt–qt)
Niger–Diffa										
♂ (n = 15)	445.1 (106.7–4672.5)	199.5 (qt–484.1)	292.3 (154.6–1279.3)	123.1 (78.1–639.0)	72.8 (32.9–258.3)	7.3 (qt–11.6)	96.9 (13.7–268.0)	63.1 (37.6–116.9)	81.6 (30.0–331.6)	qt (qt–14.0)
♀ (n = 17)	366.2 (72.6–1323.4)	156.7 (90.9–2600.5)	482.4 (243.9–1039.0)	112.3 (52.4–249.9)	89.6 (38.8–249.2)	8.5 (qt–17.1)	98.2 (12.6–249.9)	79.4 (53.6–214.2)	38.4 (19.2–340.0)	qt (qt–8.1)
Niger–Niamey										
♂ (n = 4)	329.9 (51.0–701.0)	92.2 (29.0–155.5)	226.8 (129.7–247.4)	173.3 (57.2–239.4)	114.8 (94.0–180.9)	qt (qt–7.0)	68.3 (26.4–110.1)	86.8 (54.3–122.9)	134.9 (15.4–437.2)	qt (qt–13.1)
♀ (n = 3)	118.0 (92.1–393.2)	82.3	320.0 (296.0–320.0	144.0 (103.4–203.6)	82.2 (77.1–84.9)	qt (qt–8.6)	181.0	253.0 (123.1–302.2)	302.5 (57.9–543.9)	qt
All four sites										
♂ (n = 41)	315.8 (31.1–4672.5)	123.1 (<qt–667.6)	129.7 (<qt–1279.3)	96.5 (<qt–639.0)	40.8 (<qt–258.3)	qt (<qt–17.4)	57.5 (<qt–268.0)	43.0 (<qt–122.9)	51.5 (15.4–437.2)	qt (<qt–14.0)
♀ (n = 30)	320.2 (72.6–1323.4)	138.9 (<qt–2600.5)	344.0 (98.9–1039.0)	108.6 (28.4–249.9)	79.6 (<qt–249.2)	7.6 (<qt–22.2)	59.1 (10.6–249.9)	70.2 (16.9–302.2)	57.8 (19.2–543.9)	qt (<qt–8.1)

3.2.1. Lead concentrations

Three explicative factors have a significant influence on lead concentrations (expressed in logarithmic values) when considered alone: sex ($p<0.0001$), tissue ($p<0.0001$) and sampling site ($p<0.0001$). Moreover, interactions between factors *i.e.* tissue-sex ($p<0.002$), tissue-sampling site ($p<0.0005$) and tissue-log_{10}SVL ($p<0.005$), also help to explain log[Pb]. Log_{10}SVL, which is not a significant factor when considered separately, becomes significant when associated with the factor tissue.

The effect of the significant interaction tissue-sex on the mean values of log[Pb] can be visualized in Fig. 2a. In males and females, log [Pb] is relatively higher in bone, lower in muscle and of intermediate level in liver and intestine. The logarithm of lead concentration in female tissues always appears to be higher than in males, a trend which is especially obvious in kidneys, in which log[Pb] is high in females (even higher than in bone) and moderate in males. The latter difference explains on its own the significance of the interaction tissue-sex. Fig. 2b represents the mean values of log[Pb] in local samples for the various tissues. The monitors from Flabougou always presented the lowest log[Pb] with the exception of the liver. Monitors had generally higher log[Pb] in their tissues when originating from Diffa and Niamey than from Niono and Flabougou. The monitors from Niger always exhibited higher log[Pb] than Malian ones, with the exception of the bones and intestines of the specimens from Niono. Fig. 2c shows the mean values of log[Pb] in the different tissues, according to size classes. Although subadult monitors always display higher log[Pb] than large adults, the shape of the graphic representations differs for each tissue: there is a regular decrease from subadults to adults and large adults in kidney, intestine and muscle. However, in bone and liver log[Pb] slightly increases from subadults to adults, before decreasing steeply from adults to large adults.

3.2.2. Cadmium concentrations

Tissue ($p<0.0001$), sex ($p<0.0005$), sampling site ($p<0.0001$), log_{10}SVL ($p<0.02$) and FS_i ($p<0.0001$) are all five significant for log [Cd] in monitors' tissues. The logarithm of cadmium concentration is also influenced by the three interactions already observed for lead: tissue-sex ($p<0.0001$), tissue-sampling site ($p<0.0001$) and tissue-log_{10}SVL ($p<0.0001$), as well as three other ones: sampling site-log_{10}SVL ($p<0.05$), sampling site-FS_i ($p<0.001$) and tissue-FS_i ($p<0.0001$).

Fig. 3a shows the effect of the significant interaction tissue-sex on the mean values of log[Cd]. In both genders, log[Cd] is far higher in liver, intestine and kidney, than in bone and muscle. As for lead, mean log[Cd] reaches a peak value in female kidneys and the considerable difference in log[Cd] in this tissue between males and females accounts for the significance of this interaction. The mean values of log[Cd] in the various tissues for the four local samples are represented in Fig. 3b. The least contaminated tissues, bone and muscle, display very low levels in all sites. When considering the three tissues that are consistently contaminated, *i.e.* liver, intestine and kidney, log[Cd] is generally higher in the specimens from Diffa and Niamey than in those from Niono and especially Flabougou where the values of log[Cd] are lowest. This situation was already observed for lead; however, liver contamination levels in the monitors from Diffa on the one hand, and from Niono and Flabougou on the other hand are very close to each other. The influence of the size classes on the mean values of log[Cd] in the four sampling sites can be observed in Fig. 3c. Log[Cd] always increases with increasing size in the monitors from Diffa, Niamey and Flabougou, whereas it is highest in subadults from Niono. Fig. 3d shows the influence of the interaction involving FS_i and the sampling sites on the mean values of log[Cd]. Opposite to size, increasing FS_i in monitors from Diffa, Niamey and Niono corresponds to a decrease in log[Cd]. The interaction between the tissues and log_{10}SVL can be visualized in Fig. 3e. The significance of this interaction can be explained by a substantial increase in liver log[Cd] with increasing size, whereas the logarithm of cadmium concentrations tends to remain at a steady value or even decrease during growth in other tissues. Fig. 3f shows the influence of the interaction between the tissues and the FS_i classes on the mean values of log[Cd]. The evolution of log[Cd] with increasing FS_i looks quite different when the tissues that have a feeble capacity for cadmium accumulation (bone and muscle) are compared with other tissues. Log[Cd] in kidney, intestine and more obviously liver decrease with

Fig. 2. a. Mean values of log[Pb] in male and female tissues. b. Mean values of log[Pb] in local samples in the various tissues. c. Mean values of log[Pb] in the different tissues according to size classes.

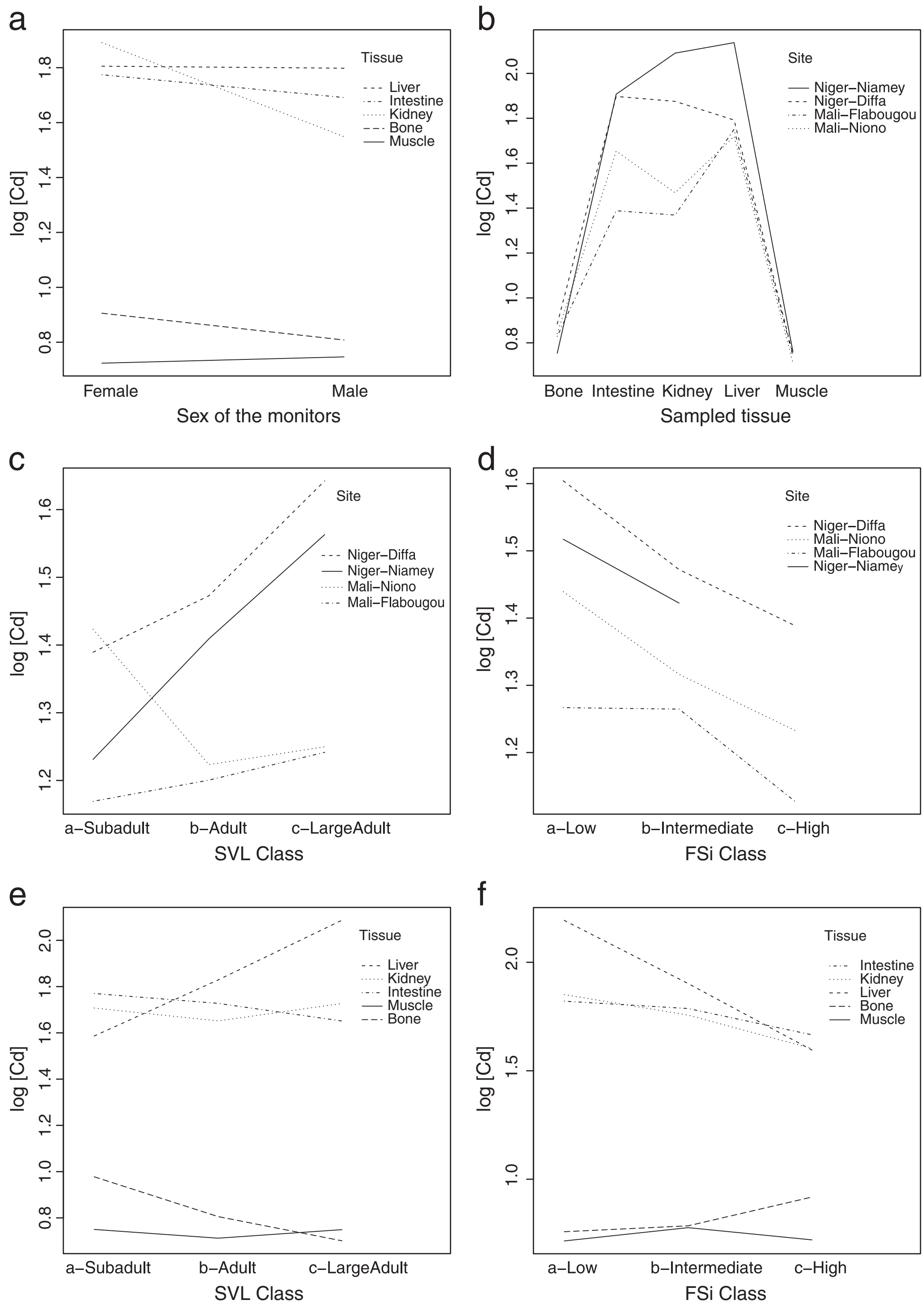

Fig. 3. a. Mean values of log[Cd] in male and female tissues. b. Mean values of log[Cd] in local samples in the various tissues. c. Mean values of log[Cd] in local samples according to size classes. d. Mean values of log[Cd] in local samples according to FSi classes. e. Mean values of log[Cd] in the different tissues according to SVL classes. e. Mean values of log[Cd] in the different tissues according to FSi classes.

increasing FS_i, whereas they increase or remain stable in bone and muscle. However, as regards the last two interactions mentioned, a possible variation in log[Cd] in muscle with increasing size or FS_i would not be necessarily observable since cadmium concentrations in this tissue often proved to be under the quantification limit.

4. Discussion

This study reveals that the four sampling sites are poorly contaminated in general; however, pronounced interindividual variability in pollutant loads was observed, even among specimens from the same site. The meaning of this variability, an important issue for the practical use of Nile monitors in pollution surveys, is discussed below. A part of this variability could possibly be explained by stochastic causes (see Section 4.1). Metal loads in the tissues of our monitors do not raise any obvious concern for the monitors themselves (Section 4.2). They should not represent a health hazard for occasional human consumers, like most villagers, but could become more problematic for regular consumers (Section 4.3). The contamination pattern of target tissues is globally in line with the organotropisms already evidenced in other sauropsids (Section 4.4). The differences observed between sites in the contaminations patterns displayed by the monitors are further considered below (Section 4.5). Lastly, the effects of individual parameters on metal concentrations will be discussed in part Section 4.6.

4.1. Indication of environmental contamination

To our knowledge, this study is the first investigation into metal contamination in the tissues of wild-caught Nile monitors; comparative data are thus scarce and indirect. Because sources of massive pollution by metals are very limited in Niger and Mali, it was hypothesized that contamination in monitor lizards should be close to background levels. The overall lead and cadmium levels observed in our specimens indeed proved to be low yet quantifiable. This result confirms the absence of important environmental contamination by these elements in the study sites. However, the correlation between lead and cadmium contamination levels in most of the tissues is unlikely to reflect a natural situation and thus suggests that the background contamination is of anthropogenic origin.

In this study, it was intentionally chosen to consider the median values of metal levels because they represent the central tendencies of our sample better than mean values would do. This choice tends to reduce the influence of extreme values on the global results, with the consequence of a lessening of assessed pollutant levels. Although overall levels are low, some individuals clearly display much higher contamination levels, at least for lead (several monitors have 700 $ng.g^{-1}$ in bone and one specimen has more than 4500 $ng.g^{-1}$). The explanation for a given contamination level in a single animal, whatever the tissue considered, is a complex combination of several factors. Intrinsic factors, such as specific or individual traits (*e.g.*, sex, size, age, reproductive status, body condition, importance of parasitism), as well as extrinsic parameters such as the contaminant levels in the different compartments (biotic and abiotic) of the ecosystem, the combined presence of several pollutants, or physico-chemical parameters of the environment can play a significant role (Peakall and Burger, 2003; Ramade, 2010). Moreover, additional stochastic events related to exposition history can also be determinant. As an example, a monitor may ingest a prey containing lead pellets and consequently display higher lead levels than similar individuals captured in the same area (see on this topic Lance et al., 2006). Such stochastic events may interfere with the overall contamination patterns prevailing in biological samples by skewing mean parameters towards higher values. This is why median values were considered more reliable and thus used in this study instead of mean values. Individual tissue concentrations therefore reflect the *risk* (*i.e.*, danger combined to exposition) associated to a given area. However, only median values resulting from measurements in a significant number of individuals can give information on the *environmental contamination* proper to a given area. This methodological constraint is relevant to all predator species. In this respect, the interest of the Nile monitor is all the most obvious since: i) this lizard is regularly exploited, a situation that allows rapid collections of large samples in order to reduce the consequences of individual variability and ii) it is sedentary (though very mobile), which tends to limit the occurrence of stochastic exposure events, as compared to migratory species with much larger home ranges.

In the Nile monitor, territories generally overlap and range from some tens of square meters in juveniles to an average of 5 ha in adult males (Lenz, 1995, 2004). The spatial resolution of the Nile monitor as a tool for characterizing pollution can thus be very sharp and reflect the pattern of environmental contamination on a small scale. In the present study, the spatial resolution of our data for each local sample corresponds to the total area covered by the individual territories of all the specimens in the sample, *i.e.* some 5 km^2 at most, as mentioned above.

4.2. Health hazard to the monitors

A possible important bias in our sampling strategy could have been that monitors heavily contaminated with metals are more likely to die and thus would not be included in the sample. However, the levels found in dorsal or leg muscle of apparently healthy Vietnamese *V. salvator* (14 $\mu g.g^{-1}$ dry weight) studied by Boman et al. (2001) demonstrate that other *Varanus* species, ecologically and morphologically very similar to the Nile monitor, can bear incomparably higher lead contamination without evident adverse effect. Moreover, in an experimental study dealing with *Podarcis sicula* (Lacertidae), Trinchella et al. (2006) showed that this species can withstand considerable acute or chronic cadmium exposures without survival being affected. Although this work deals with another lizard taxon than the Varanidae, it shows that a species of the same sub-order (Autarchoglossa) can undergo an important cadmium exposure without increased mortality. However, since closely related taxa can display very different responses to the same levels of cadmium (Burger, 2008), extrapolation to monitors must be considered with caution. In general, our results stand well below the values measured in other studies in similar tissues from free-ranging reptiles (see Grillitsch and Schiesari, 2010, for an extensive review of lead and cadmium contamination of target tissues in the three main orders of *Reptilia*). Of course, this by no means reflects an experimental failure in our dosages because recovery rates for spiked samples and reference material were all satisfactory. Finally, the comparison of our results to these data suggests no noticeable health hazard for the monitors themselves.

4.3. Hazard to human consumers

In our specimens, lead and cadmium levels in muscle samples never exceed 300 and 15 $ng.g^{-1}$, respectively. Since the consumption of monitor meat by humans is usually a minor, occasional food income, it would involve no evident health hazard related to lead or cadmium (UE maximum residue limits in meat are 100 and 50 $ng.g^{-1}$, respectively; EC, 2001b). However, some human populations, especially professional hunters or fishermen living near large water stretches, may consume monitor meat on a daily basis (at least seasonally; de Buffrénil, 1993). In these conditions the contamination levels occurring in monitor meat could actually represent a non negligible threat for the health of these populations. Indeed, comparable metal levels in the diet are known to provoke neurological and behavioral disturbances in mammals (Peakall and Burger, 2003).

4.4. Tissue contamination

Although most of the monitors examined in this study were exposed to relatively low pollution, the hierarchy of pollutant levels in their tissues reflects the pattern of organotropisms generally described by previous authors in diverse reptile taxa that were submitted to lead or cadmium (Almli et al., 2005; Beresford et al., 1981; Burger et al., 2007; Campbell et al., 2005; Caurant et al., 1999; Frías-Espericueta et al., 2006; Hopkins et al., 2001, 2002; Lance et al., 2006; Sakai et al., 2000; Storelli et al., 1998; Rie et al., 2001; Xu et al., 2006; see also Sparling et al., 2010, for an overview on reptile tissues contamination data). Important and long-lasting exposure to contaminants is considered to be a necessary condition for the emergence of well defined patterns of metal concentration among tissues (Burger et al., 2005; Grillitsch and Schiesari, 2010). The example of the Nile monitor suggests that this condition may not be of general necessity. For lead, the hierarchy that we observed in our sample is the following: in males, bone > [kidney, intestine, liver] > muscle; in females, [bone, kidney] > [intestine, liver] > muscle. For cadmium, this hierarchy is identical for both genders: [liver, intestine, kidney] > [bone, muscle]. These general trends diverge from previous data on two points only: i) According to most studies, it is very unusual to measure the highest lead level in a tissue other than bone; however, we surprisingly found the highest individual lead level in the intestine in females. ii) Although the maximum lead levels in bone were greater than in kidney in both genders and at any site, median lead values in the females from Niger were higher in kidney than in bone. The meaning of these peculiarities remains obscure. Moreover, important lead and cadmium levels in the intestine, as observed in our monitors, is particularly noteworthy since very few studies dealt with metal quantifications in this tissue in squamates. Though not addressing directly any temporal aspect, this result is in general agreement with the experimental works by Trinchella et al. (2006) and Mann et al. (2007) who showed in *Podarcis sp.* (Lacertidae) that cadmium preferentially accumulates in the intestine and remains for long periods in the liver at greater levels than in the kidneys. The latter would finally be the main target for long-term cadmium accumulation (Grillitsch and Schiesari, 2010). The ratio $[Cd]_{kidney} / [Cd]_{liver}$ in the Nile monitors is higher than in experimental *Podarcis* specimens, which could be explained by a mere problem of timing: since Nile monitors are long-living animals (*e.g.* de Buffrénil et al., 1994), cadmium level in their kidneys is likely to be the result of a longer accumulation process than in the shorter living *Podarcis* (Castanet and Roche, 1981). This process would substantiate the hypothesis proposed by Mann et al. (2007): with longer exposure duration, the ratio $[Cd]_{kidney} / [Cd]_{liver}$ tends to increase gradually until [Cd] in kidney becomes the greatest, as in most of the other taxa studied hitherto. Of course, additional experimental studies are wanted in order to further decipher the intriguing question of the kinetics of cadmium accumulation in Squamate tissues.

The question of gender-related differences in metal levels has already been addressed in sauropsids (review in Burger, 2007) and seems to vary depending on the animal species considered and the nature of the pollutant. The higher contamination in females, as compared to males, observed in our study would meet the observation by Burger et al. (2004) in *Anolis sagrei* (whole body). In our monitors, like in *A. sagrei* (Burger et al., *op. cit.*), this situation could possibly reflect differences in male and female diets. Soil, litter and sediment dwelling animals such as annelids, arthropods and molluscs are particularly prone to metal accumulation due to the contamination of their habitat (Brönmark and Hansson, 2002; Elder and Collins, 1991; Vahter et al., 2007). Since female Nile monitors grow slower (de Buffrénil et al., 1994; de Buffrénil and Hémery, 2007a,b) and thus feed on such prey for a longer period than males, they could progressively become more heavily contaminated. Another possible explanation would refer to sex-based differences in intestinal absorption. In humans, lead and especially cadmium uptake is greater when body iron stores are at low levels (Goyer, 1997; Järup et al., 1998), a phenomenon that could also occur in sauropsids. In monitors, both genders could indeed have contrasted levels in body iron stores, especially if males are compared to gravid females in which a substantial part of iron body reserves can be transferred into eggs (relative clutch mass is extremely elevated in Nile monitors; de Buffrénil and Rimblot-Baly, 1999). Such a process could finally result in higher contamination levels in females.

In our sample, kidneys display higher lead and cadmium levels in females than in males, a feature that greatly contributes to the differences in global contamination levels observed between genders, and could ultimately refer to physiological causes. The high affinity of cadmium for estrogen receptors has been evidenced in mice (Stoica et al., 2000). Two kinds of estrogen receptors occur in the murine kidney cortex: α and β receptors. According to Rogers et al.'s (2007) data, the relative abundance of α and β receptors in the kidney is uneven in male and female rats. Such discrepancies could finally explain differences in global cadmium affinity in the kidney cortex between genders. If a similar situation occurs also in monitors, this could explain why metal concentrations are higher in female kidneys, at least for cadmium. Attention should thus be called in the future to a potential risk of endocrine disruption in monitor populations due to pollution by metals (Vahter et al., 2007).

4.5. Site contamination

As expected, Flabougou, which was considered in this work as the reference site, proved to be the less contaminated. Our results indicate that monitors from Niger are more contaminated than those from Mali; particularly, the lead and cadmium loads in the specimens from Diffa are greater than in those from Niono. This situation could be due to a possible difference in the local use of agricultural chemicals containing metals. More probably however, it is likely to reflect the greater proportion of females caught at the two Nigerien sites: since females tend to be more contaminated than males their over-representation (that is not statistically significant in terms of sex-ratio) in some samples necessarily bears on the final results.

4.6. Influence of morphometric variables

As other variables, SVL and FS_i are to be considered in interaction with other factors. Surprisingly, lead does not appear to concentrate in monitors' tissues with increasing length (in other words with age, de Buffrénil et al., 1994) even in bone — a tissue commonly known as an excellent accumulator for that element (Aufderheide and Wittmers, 1992; Burger et al., 1992; O'Flaherty, 1992; Rabinowitz, 1991; Sparling et al., 2010). The most consistent hypothesis that could explain the paradoxical situation of the osseous tissue in our sample refers to the growth pattern of the femur shaft, in which bone samplings were made. Indeed, although the cortex of long bones in monitors (and other lepidosaurians) undergoes no Haversian remodeling or intracortical resorption, there is nevertheless an extensive, superficial resorption around the medullar cavity (de Buffrénil and Castanet, 2000; de Buffrénil and Francillon-Vieillot, 2001; de Buffrénil et al., 1994, 2008). This process, responsible for the maintenance of the inner architecture of the bones during growth, destroys the deepest cortical layers that formed early in ontogeny, when the monitors fed on more heavily contaminated prey (see Section 4.4). Therefore, when a monitor grows, there is a progressive elimination of the parts of the cortex where lead contamination is susceptible to be heaviest. Conversely, the cortical layers that form in relatively late ontogenetic stages are likely to be less contaminated because the prey of the monitor have then changed to include a greater proportion of supposedly less contaminated species (small to medium aquatic and terrestrial vertebrates; Cissé, 1972). In addition, the vascular supply of bone cortices decreases during growth in monitor lizards (de Buffrénil et al., 2008); as a consequence, late (superficial) cortical layers should receive fewer pollutants from the blood flow than earlier layers. The interesting question of a possible relationship between the morphogenetic processes occurring in the bones and the dynamics of their local contamination by metals remains very poorly studied. Future studies should address this question that obviously controls the strategy to be employed when sampling bone for lead quantification.

The only noticeable bearing of SVL on log[Cd] is the increase in cadmium concentration of the liver with size. Although this corresponds to a phenomenon already observed in reptiles (see Section 4.4), we also expected cadmium to accumulate in the kidneys and even at a greater rate than in the liver.

In the tissues most heavily contaminated by cadmium (*i.e.* intestine, kidney and liver), and especially in the liver, log[Cd] decreases with increasing FS_i. The latter factor reflects the recent foraging activity of the monitors (between their arousal from hibernation until their capture, *i.e.* 4 to 5 months later) and may be interpreted as an index of their current health status. Once again the precise reasons of the kinetics of cadmium in relation to FS_i remains unexplained and calls for further studies on larger biological samples.

5. Conclusions

The aim of this work was to develop a new ecological tool for characterizing contamination by heavy metals in African wetlands. As a first step, the practicability of this new tool was validated since lead and cadmium quantifications in monitor tissues enable detection of subtle differences between environmental contamination levels, even in lightly contaminated sites. For the time being, the sampling strategy can consider adult males and adult females indifferently provided the tissue used for lead quantification is bone, and for cadmium quantification, the intestine. This choice is justified by the fact that these tissues display relatively elevated metal levels, with results remaining approximately regular regardless of sex and size, but depending on the sampling site. The use of a significant number of monitors in the samples is necessary to avoid confusion due to the potential influence of stochastic events on individual contamination. The spatial resolution of the Nile monitor as a tool for characterizing pollution might well be very sharp and thus reflect the risks and the patterns of environmental contamination on a very small scale.

The lead levels measured in the tissues of our monitors are far below the concentrations reported for healthy varanids in other studies. Though cadmium measurements in varanids are lacking in literature, the concentrations observed in this study appear to be very low. Therefore, these contaminant levels do not justify any toxicological concern for the monitors themselves. Moreover, monitor meat does not present any special hazard for human populations, with the exception of the few fishermen and professional hunters who are accustomed to eat monitor meat each day during their long-term field operations. However, beyond the case of monitor lizards, this situation is common to most bush meat items.

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