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Assessing the use of small mammalians as bioindicators in northern Morocco (Oued Siad/Jbel Moussa) using heavy metal accumulation

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Absract

This study was conducted in the Mediterranean region of Oued Siad / Jebel Moussa in north of Morocco. It aimed at studying the diversity of rodents in this region, assessing the degree of contamination of their vital organs by adopting the morphometric analysis as well as the species identification. Four rodent species belonging to the family Muridae were caught. The concentrations (µg/g dry weight) of Cr, Pb, Cu, Fe and Zn in various organs (liver, kidney, and heart) of the analyzed species were determined by atomic absorption spectroscopy Varian AA 240 with graphite furnace. The highest concentrations of Pb, Cu, Fe and Zn were found in the liver of *Rattus norvegicus*, while those of Cr were detected in the kidneys of *Rattus rattus*. The medium and low contamination levels were detected in *Lemniscomys barbarus* and *Mus spretus,* respectively. The results suggest that the genus *Rattus* can be considered as a bio-indicator as it accumulates more Trace- metals than the genus *Mus* and *Lemniscomys*

1. Introduction

The Industrial Revolution that occurred during recent decades has led to tremendous upheavals in human society, which until then remained predominantly agrarian. The particularly intense extension, facing the industrial activity worldwide during this period, does not immediately take into account the environmental dimension. Many substances with unknown properties have been released into the water, air or soils. Some of them, such as heavy metals have unfortunately shown their potential toxicity during historical episodes of pollution. Yet, the metal pollution is particularly harmful because the traces-metal and metalloids are not biodegradable and can present at the same time a significant persistence in the environment and a proved eco-toxicity and, in extreme cases, lead to irretrievable loss of animal and plant species and irreversible damage to some ecosystems. It was also reported that these pollutants could be transferred to the organisms, where they could contaminate the entire food chain [1].

Ecological studies have demonstrated that, the rodents are sentinel species of environmental pollution by humans because of their ability to accumulate a wide range of pollutants that are present in the ecosystem [2]. The rodents have a natural geographical distribution that covers the worldwide [3]. This order is also the most diverse, if we must consider the morphological characteristics, physical abilities and the different environments occupied by these animals [4]. The classification of rodents is mainly based on morphological characters such as: body measurements, teething, shape, skull structure and other skin characteristics [5].

Many previous studies have used rodents as bio-indicators of worldwide pollution as in the Czech Republic [6], Slovak Republic [7] and France [8]. Thus, we chose to use, for the first time in Morocco, these species as pollution sentinels based on the assessment of different organs contamination in order to identify bioaccumulation of metal pollutants. Interestingly, the distribution model of risk factors and their levels in various rodents tissues are similar to those found in humans [9].

2. Materials and Methods

Twelve specimens, belonging to four species of the family Muridae including: *Lemniscomys barbarus* (LB), *Rattus norvegicus* (RN), *Rattus rattus* (RR) and *Mus spretus* (MS), were caught in grassy areas with bushy vegetation in the SIBE (Sites of biological and ecological interest) area of Oued Siad / Jebel Moussa (northern Morocco) located mainly in the semi-arid and sub-humid bioclimatic atmosphere (Fig.1). The soil is clay and limestone dominated by olive plantations and wheat fields with other spontaneous plants characterizing the forest areas. Two types of traps were used: Sherman traps and mesh traps with small grids. The majority of the rodents are nocturnal except for *Lemniscomys barbarous*. Traps were set the in afternoon, and checked early next morning. The various baited traps (bread, olive oil, peanut butter, dates) were placed in the vegetation at least 5 m from each other [10].

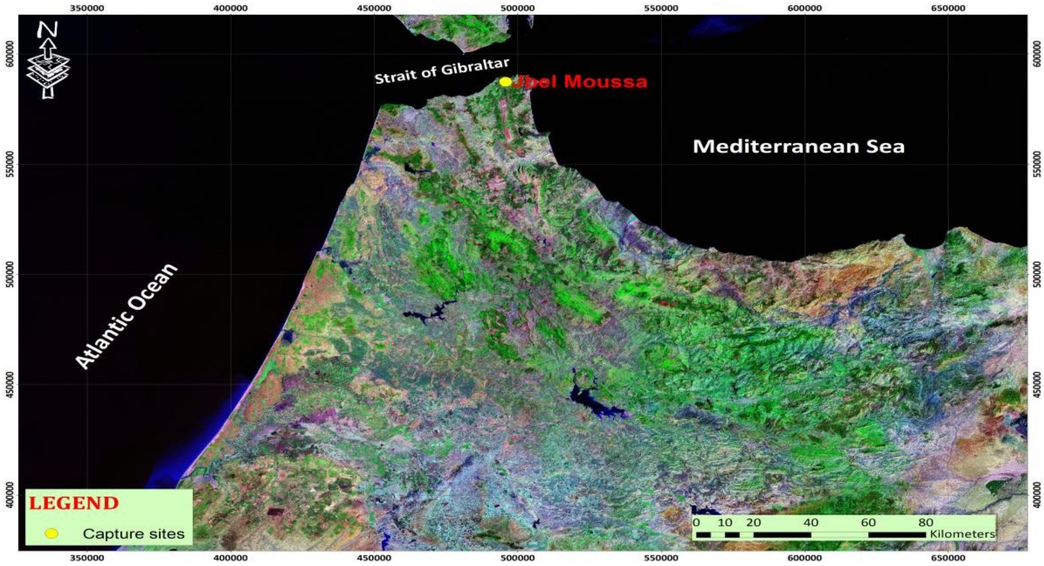

Figure 1: Location of capture sites.

By the end of the trapping mission, we captured 12 specimens which is low capture efficiency. This could be attributed to poor weather conditions during the trapping period (wind, cold) as well as to the limited number of traps and a strong traffic on the site.

After their capture, the animals were sacrificed with chloroform, sexed, weighed and measured (head and body length, tail length, length of the posterior legs, length of the ears) [11]. All caught specimens were adults, appeared to be in a good condition and without macroscopic lesions at autopsy (Table 1). Samples of kidney, liver and heart were maintained at -18 ° C until analysis. Afterward, three doses of 0.5 g of organ samples were mineralized at 120 ° C for 4 hours in the presence of 3 ml of nitric acid above pure (65% Merck), the metals assay were performed on mineralization diluted to 25 ml with bidistilled water[12].

The concentrations of Pb, Cu, and Cr in the different samples were performed by atomic absorption spectroscopy with graphite furnace (Varian AA 240 120 GTA Z) [13]. The background correction was carried out by a Zeeman effects while Zn and Fe were performed by atomic absorption spectroscopy with flame (VARIAN AA40 FS) logged at toxicology laboratory, National Institute of Health (Rabat, Morocco). To reduce the chemical interference and the volatility of Cr, Cu and Pb in an oven, a matrix modifier was used (mixture of PdCl2 and MgNO3). The calibration curve was made by the "MSA (standard addition method)", the validity of this method has been verified by internal control using standard samples (National Research Council Canada: DORM-2) and external control using inter calibration exercises (IAEA-MESL-TE-2014-01) using samples of blood doping after the linearity of the curve, the accuracy was verified by three successive readings for each sample, the mean of these measures would be considered if the RSD (RSD) was less than 10%.

Table 1: Average biometric measurements performed on the Muridae (Wt =Weight, HDL = head + body length, TL = tail length, PL = length of the posterior legs, CL = Claw, EL = length of the ears)

Species	Wt (g)	HDL (mm)	TL (mm)	PL (mm)	CL (mm)	EL(mm)
Lemniscomys barbarus	41.2	119.2	103	24.8	1,2	13.8
Rattus norvegicus	204.5	195.75	206.5	37.75	2.25	22.5
Rattus rattus	135	167	187.5	29	2	22
Mus spretus	10.7	76	65	11	0.5	12

3. Statistic analysis

The basic statistical parameters were calculated (mean, standard deviation, minimum, maximum, median) only for *Lemniscomys barbarus* (n = 5), *Rattus norvegicus* (n = 4) and *rattus rattus* (n = 2) because *Mus spretus* has only a one individual, using Excel and are presented in Annex 1. For the multiple comparison of the means, we used Duncan- Waller test implemented in SAS software (SAS version 9.0). Forthermore, we performed the principal component analysis (PCA) in order to group the individuals according to the accumulation of each metal in different tissues (liver, kidney, heart).

4. Results and discussion

The concentrations of Cr, Pb, Cu, Zn and Fe in the liver, kidneys and heart of the twelve specimens captured are listed in Table 2. The highest concentrations were recorded in the liver for Zn (5.91 ± 1.98) in the case *Lemniscomys barbarus*, for Fe (7.54 ± 0.82) and Cr (7.20 ± 0.45) in *Rattus norvegicus*, and for Cu (98 ± 6.00), and Pb (33.80 ± 4.80) in *Rattus rattus*. Comparing the heavy metal levels in the rodents species organs, shows that the Zn, Cu and Pb concentrations are not significantly different among the three organs (liver, kidney and heart). However, the Fe concentration in the liver is significantly higher from those in the kidney and the heart. Cr concentration in the liver and the kidney is significantly higher from that in the heart; but it did not differ between the kidney and the heart (Figure 2).

Table2: Mean ± SEM values for several metals (Zn, Fe, Cu, Pb and Cr) in Muridae species tissues in µg/g

		Lemniscomys barbarus (n=5)	*Rattus norvegicus*	*Mus spretus(n=1*	*Rattus rattus*
Liver	Zn	5.91 ± 1.98	5.74 ± 2.51	1.12	1.90 ± 0.09
	Fe	6.93 ± 0.79	7.54 ± 0.82	3.47	3.39 ± 0.83
	Cu	6.76 ± 1.48	67.42 ± 23.45	1.51	98.00 ± 6.00
	Pb	7.54 ± 2.24	26.48 ± 13.26	1.32	33.80 ± 4.80
	Cr	6.07 ± 0.60	7.20 ± 0.45	0.89	3.57 ± 0.03
Kidney	Zn	5.00 ± 1.80	5.23 ± 2.38	5.23	5.23 ± 0.52
	Fe	5.62 ± 0.62	5.11 ± 1.41	5.11	5.11 ± 0.32
	Cu	5.46 ± 1.19	45.81 ± 21.41	45.81	45.81 ± 2.45
	Pb	5.85 ± 2.28	22.94 ± 11.75	22.94	22.94 ± 2.25
	Cr	4.59 ± 0.58	5.43 ± 0.59	5.43	5.43 ± 4.40
Heart	Zn	3.01 ± 1.21	3.23 ± 0.65	3.23	3.23 ± 0.00
	Fe	3.07 ± 0.79	4.27 ± 1.64	4.27	4.27 ± 0.00
	Cu	3.70 ± 0.88	33.74 ± 17.69	33.74	33.74 ± 4.16
	Pb	3.59 ± 1.67	16.48 ± 9.50	16.48	16.48 ± 2.65
	Cr	2.60 ± 0.61	2.91 ± 0.65	2.91	2.91 ± 5.15

The Comparison of the accumulation levels of the different metals in each rodent species shows that the highest rate was recorded in the genus *Rattus* followed by *Lemniscomys* and *Mus* (Table 3). Indeed, except for

Zn where no significant difference was observed between the four species; the concentrations of other metals (Pb, Cu) differ significantly between the species *Rattus norvegicus* and *Rattus rattus* on one hand and the other species *Mus spretus* and *Lemniscomys barbarus* on the other hand. However, concerning the accumulation of Cr, a significant difference was observed between the species *Mus spretus* and the other species (*Rattus norvegicus, Rattus rattus* and *Lemniscomys barbarus*).

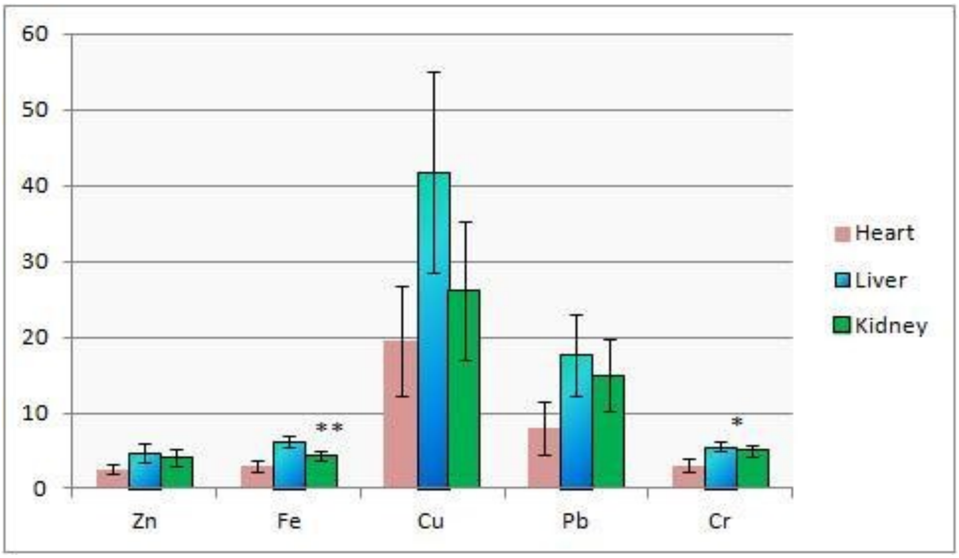

Figure 2: Mean ± SEM values for several metals (Zn, Fe, Cu, Pb and Cr) in liver, kidney and heart of Muridae ($* \ p \leq 0.05$; $** \ p \leq 0.01$).

Table 3: Comparison of mean concentration of heavy metals by Muridae species. Means with the same letter are not significantly different

Heavy metals / Species	Zn (µg/g)	Fe (µg/g)	Cu (µg/g)	Pb (µg/g)	Cr (µg/g)
Rattus norvegicus	4.733 a	5.639 a	48.99 a	21.966 a	5.178 a
Lemniscomys barbarus	4.641 a	5.209 a	5.31 b	5.659 ab	4.417 a
Rattus rattus	1.577 a	2.068 b	63.11 a	22.867 a	5.717 a
Mus spretus	0.833 a	1.710 b	0.91 b	0.867 b	0.590 b
LSD 0.05	5.091	2.351	28.69	18.002	2.833

In order to affirm these results, a PCA was performed. The first two factorial axes were selected as they represent 82.24% of the total variance (Figure 3). It appears from this analysis that the *Mus spretus* and young individuals of *Lemniscomys barbarus* form a group characterized by low metals concentrations. *Rattus norvegicus* and aged individuals of *Lemniscomys barbarus* are characterized by heavy and medium concentrations of Zn and Fe, respectively. As for *Rattus rattus* it is characterized by high levels of Pb and Cr. Therefore three groups can be distinguished:

- Group 1: includes the species *MS1* (19; 20; 21) which has a low contents of five trace-metals analyzed in the 3 organs and the species LB1 (1; 2; 3), LB2 (4; 5; 6) and LB4 (16; 17; 18) with medium contents of the trace metals in the 3 organs.
- Group 2: includes RN2 (10; 11; 12), LB3 (13; 14; 15), LB5 (22; 23; 24) which is characterized by high concentrations of Fe / Zn
- Group 3: defined by, RN4 (28; 29; 30), RN3 (25; 26; 27), RR1 (31; 32; 33), RR2 (34; 35; 36) that have a high concentrations of Pb / Cu. It is noted that RN1 leaves the group to form an isolated group characterized by a high concentration of Cr.

Considering these results, the PCA shows that the highest concentrations of metals were found in the liver and kidneys, while the lowest levels were detected in the heart. The significantly higher levels of Fe and Cu were observed in the liver and kidneys of *Rattus norvegicus*. The lowest concentrations of these elements were detected in the heart of *Mus spretus*. In the four species, the hierarchy of concentrations of Zn, Fe, Cu, and Pb is liver> kidney> heart, while for Cr is kidney> liver> heart. When, we compared the levels of heavy metals in four rodent species, we found that the genus *Rattus* accumulates higher rates followed by *Lemniscomys* and *Mus*.

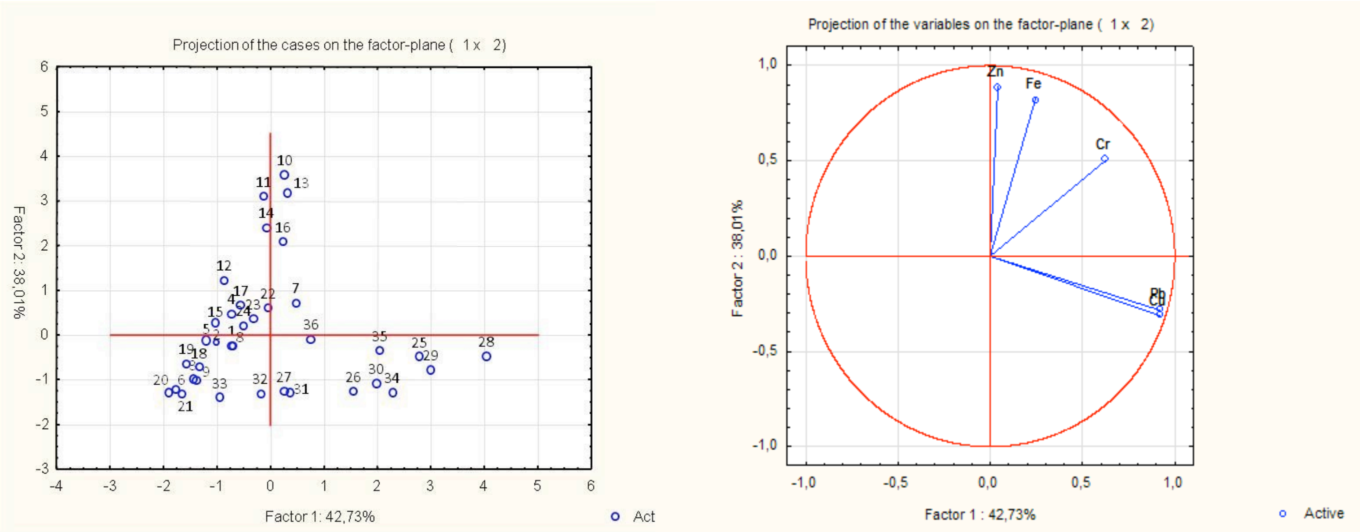

Figure 3: Principal components analysis applied to the concentrations of the trace-metals in the Muridae

Vital organs are good biomarkers of a long-term accumulation of various risk factors, including essential and non-essential metals. The essential metals are necessary for the proper functioning of living organisms; however, they can produce toxic effects when consumed in high concentrations [14]. It was reported that bioaccumulation of heavy metals is a process whose efficiency depends both on the level of pollution, the individual age and is also proportional to the rate of uptake [15]. However, differences in trace metals quantities may exist between males and females, particularly at the beginning of the reproduction period when the females eat more food and accumulate fat reserves [16]. In line with this, the increased accumulation of trace metals in the rodent organs studied herein can be used as an indication of the site contamination.

It is known that the application of agrochemical products can lead to a higher accumulation of specificelements, in particular Cu and Zn in the soil [16-17]. In addition, there is a heavy traffic near the capture location, which is also considered an important source of risk elements [18] as at least 90% of metals in water trickling from the road consist of Cu, Zn, and Pb. There is also a possibility of dust fallout transported into the air from large industrial areas like Tangier and Tetouan. This hypothesis can be supported by a study indicating the possibility of transporting various xenobiotics from a region to an other [19]. It is also known that the difference in metal concentration between species could result from the differences in the structure of the population [20]. In addition, they may be affected by the modified eating habits, the seasonal rains, the bioavailability aspect of food, the quality of the habitat and the food preferences of each species [21]. These parameters can create both large inter-individual variations in health indicators. Whatever the factors residing behind the metals accumulation in rodents tissues, our study showed that the genus *Rattus* is a better indicator of pollution than genus *Lemniscomys* and *Mus.*

Conclusion

This study illustrated the relevance of all species as bioindicators in the assessment of environmental quality. In addition, high levels of Cu and Zn in rodent organs are the result of pollution caused mainly by agricultural activities, while those of Pb by road traffic. The magnitude and spatial extent of trace metal accumulation in these species have been classified as "very serious" anthropogenic changes. Given the position of rodents in food webs, we can speculate on the accumulation of elements in higher trophic levels and assume a significant biomagnification scenario of potentially toxic elements. Finally, we note that our values are slightly average to the results found in the literature.

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Annex 1 Concentrations of Zinc (Zn), copper (Cu), iron (Fe), lead (Pb) and chromium (Cr) in the liver, kidneys and heart of Muridae caught in the Oued Siad region (Mediterranean, Morocco) obtained by AAS method

Species	Tissues	Statistical parameters	Zn (µg/g)	Fe (µg/g)	Cu (µg/g)	Pb (µg/g)	Cr
Lemniscomys barbarus (N=5)	Liver	Average	5.906	6.932	6.764	7.536	6.068
		Median	6.24	7,09	6.82	6.68	5.72
		Minimum	0.81	4.61	2.37	2,48	4.81
		Maximum	12.32	8.81	11.57	13.18	8.04
		Std.Dev.	4.438466	1.77122	3.308985	5.008062	1.334305
	Kidney	Average	5.002	5.62	5.458	5.848	4.588
		Median	5.86	4.99	5.71	4.52	4.09
		Minimum	0.72	4.34	2.45	0.96	3.76
		Maximum	10.21	7.28	9.52	12.69	6.88
		Std.Dev.	4.029767	1.389802	2.668533	5.105543	1.290298
	Heart	Average	3.014	3.074	3.702	3.594	2.596
		Median	2.3	2.69	3.44	2.16	2.27
		Minimum	0.32	1.54	1.7	0.34	1.19
		Maximum	6.33	6.07	5.87	9.63	4.11
		Std.Dev.	2.695984	1.764491	1.967186	3.729327	1.369226

Rattus norvegicus (N=4)	**Liver**	Average	5,74	7,5425	67,42	26,4825	7,2025
		Median	3,725	7,455	70,365	23,005	7,41
		Minimum	2,42	5,72	11,45	3,92	6,07
		Maximum	13,09	9,54	117,5	56	7,92
		Std.Dev.	5,01303	1,64654	46,90489	26,52696	0,89134
	Kidney	Average	5,2275	5,105	45,805	22,94	5,425
		Median	3,24	4,13	40,13	20,275	5,68
		Minimum	2,17	3,09	8,93	2,51	3,83
		Maximum	12,26	9,07	94,03	48,7	6,51
		Std.Dev.	4,76619	2,81688	42,81198	23,49913	1,17054
	Heart	Average	3,2325	4,27	33,7375	16,475	2,905
		Median	3,315	3,38	28,46	11,805	2,67
		Minimum	1,62	1,36	3,24	1,23	1,59
		Maximum	4,68	8,96	74,79	41,06	4,69
		Std.Dev.	1,29577	3,28814	35,37025	19,00758	1,29526

Rattus rattus (N=2)	**Liver**	Average	1,895	3,39	98	33,8	3,57
		Median	1,895	3,39	98	33,8	3,57
		Minimum	1,8	2,56	92	29	3,54
		Maximum	1,99	4,22	104	38,6	3,6
		Std.Dev.	0,13435	1,173797	8,485281	6,788225	0,042426
	Kidney	Average	1,3	1,73	51,45	28,75	7,385
		Median	1,3	1,73	51,45	28,75	7,385
		Minimum	0,78	1,41	49	26,5	2,99
		Maximum	1,82	2,05	53,9	31	11,78
		Std.Dev.	0,735391	0,452548	3,464823	3,181981	6,215469
	Heart	Average	1,535	1,085	39,865	6,05	6,195
		Median	1,535	1,085	39,865	6,05	6,195
		Minimum	1,53	1,08	35,7	3,4	1,05
		Maximum	1,54	1,09	44,03	8,7	11,34
		Std.Dev.	0,007071	0,007071	5,890199	3,747666	7,276129

Std. Dev. = Standard deviation

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