Evaluation of bioconcentration of trace elements in the Western Mediterranean mouse (Mus spretus Lataste, 1883) at two Moroccan wetland sites

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Abstract

Many micromammals were shown to be vulnerable to metallic trace elements and are therefore suitable for biomonitoring. The objective of our research is to assess the exposure of a small rodent, *Mus spretus*, as a bioindicator of pollution to trace elements. We have determined the concentrations of Cu, Pb, Cr, Zn and Fe in the liver, kidneys and heart of 71 individuals of *Mus spretus* in north-west Morocco in two areas: Merja Zerga and Dayet Erroumi. Tissue metal concentrations in this species varied widely with age, sex, sites and organs. The concentration of Cu in the liver was the highest among all the metals and was recorded in adult females of Merja Zerga with a maximum of 60 µg / g ; at Dayet Erroumi, it was around 14 µg / g. At the same time, we found a similar trend for Fe, Pb, Cr and Zn in the three organs in males and females at the two sites. Highly significant differences between females at the two sites were recorded for the variables weight (W) and head to body length (TL) ($t = -3.992$; $p < 0.001$ and $t = -2.242$, $p < 0.05$), however, males obtained equal values in the two sites. Spatiotemporal monitoring in small mammals must be studied, but requires the application of a non-lethal estimator.

Introduction

Metallic pollution occupies a prominent place among the various processes of anthropogenic disturbances that affect the normal course of living systems. Contrary to organic pollution, contamination by metallic trace elements poses a serious environmental problem because it accumulates and can persist for a long time in the soil (Alimohammad Kalhori *et al*, 2012; Ashraf et al, 2012; Okuku and Peter, 2012) which constitutes a real threat to human health (Fritsch et al 2010; Mzoughi and Chouba 2012; Ghaderi et al 2012; Slavka Stankovic et al, 2014; Benabbou et al, 2015). Living organisms need specific concentrations of essential metallic elements, such as iron (Fe) and zinc (Zn), but they can be toxic to the body in highlevels. Other non-essential metals such as lead (Pb), cadmium (Cd) and chromium (Cr) that exceed the allowed thresholds interfere with the normal functioning of biological processes. These metallic elements can enter the body of living organisms at all stages of development (Ogundiran *et al*, 2008; Serbaji et al, 2012).

Numerous studies have investigated the heavy metal contamination in micromammals. The measuring of heavy metals concentrations in the target organs of small wild mammals can reveal the high levels of these metals in the environment. In fact, that can be explained by environmental pollution, additionally metals residues in the soil and the organs or tissues of the body were noteworthy related (Tête et al, 2014). Indeed, studies made in different regions of several countries affirmed that small terrestrial mammals bioaccumulate heavy metals in their tissues (Metcheva et al, 2002; Ieradi et al, 2003; Blagojević *et al*, 2012; Tête et al, 2014) and several species have been proposed as promising indicators for estimating the risks of pollution by heavy metals.

The anthropized areas and agro-ecosystems in Morocco are occupied by various micromammals, especially Murid rodents from the genera *Gerbillus*, *Meriones*, *Rattus* and *Mus* (Bouarakia et al, 2019). Apart from the studies carried out by Tifarouine et al, (2018; 2019), no information concerning metal contamination of small mammals (rodents) in Moroccan agricultural fields is available. In addition to laboratory analysis, rodents constitute a real model to understand the effects of *in situ* metallic contaminants. Furthermore, the behavior and distribution of heavy metals in their tissues is similar to that of humans (Damek-Poprawa M *et al*, 2003).

The main objective of this study is to evaluate the bioconcentration of five trace elements (Cu, Pb, Cr, Fe and Zn) in rodents at two sites of major interest for Moroccan biodiversity; Dayet Erroumi and Merja Zerga that are threatened by the massive use of fertilizers. The North African Mouse, *Mus spretus Lataste* 1883, has been used as a bioindicator to assess the potential risk of exposure to heavy metals (Garcia-Sevillano M.A. *et al*, 2014; Quina A.S. *et al*, 2019). This small rodent is found in North Africa, as well as in the Iberian Peninsula and the south of France. In Morocco, it is present in all regions of plains and hills outside of the Sahara Desert; and it occupies a wide range of environments (crops, yards, juniperaies, mixed oak forests, etc.). It is a nocturnal and opportunist species, feeding mainly on grass seeds, fruits and sometimes insects (Aulagnier et al. 2017).

Material And Methods

Study sites

This study was realised in two areas (Fig.1) characterized by intense activities in terms of fishing, farming and strong urbanization:

Merja Zerga, located in the northwest of Morocco (34º47’N, 6º13'W), covers an area of 30 km^2, with a maximum length of 9 km and a maximum width of 5 km. It is divided into two areas of unequal surface area and importance (the Merja Kahla of 3 km^2 and Merja Zerga of 27km^2). In addition to its tidal inflow, the lagoon system receives drainage mainly from the Drader River to the east and from the Nador Canal to the south.

This paralic site is one of the first four sites on the Ramsar List, recognized world wide for its biological diversity (Benhoussa *et al*, 1999; Bazaïri *et al.* 2003; Maanane *et al*, 2013; Ainou, 2016; Touhami *et al*, 2018), it is an important wintering and migratory stopover site for waterbirds (Qninba *et al*, 2006; Cherkaoui and Lamrani 2007; Touhami *et al*, 2018). Agricultural activity is very developed on the site and uses a lot of fertilizers and phytosanitary products.

Dayet Erroumi is located 15 km from Khemisset (33º45'N and 6º12'W), classified as a site of biological and ecological interest of Morocco. It is a nearly 96 ha shallow lake (14 m maximum) characterized by a semi-arid climate and a Mediterranean rainfall pattern (Bounif I. *et al*, 2017). The lake is fed by groundwater and a stream from the southeast. To the northeast, a platform corresponding to the outlet of the lake makes the junction between the lake and a permanent stream (Oued Rehhou). At the northeast end of the lake, opens a drainage channel of a marshy depression located 1-2 km northeast of the lake.

Sampling sites

Sampling was done at night with Sherman traps, baited with a mixture of peanut butter, olive and bread. 100 traps are deposited along transects of six hectares. Catches were made in trapping sessions of five nights per site. A total of 71 specimens of *Mus spretus* were collected, including 25 males, 14 females captured at Merja Zerga and 12 males, 20 females sampled at Dayet Erroumi. The poor capture yield could be attributed to poor climatic conditions during trapping (wind, cold) as well as a limited number of traps and heavy site traffic. We note that these small mammals are herbivorous and have nocturnal activity. All captured individuals were weighed (W), measured head to body length (TL), ear length (E), and posterior leg length (PL)) and sacrificed by chloroform inhalation. After that, their organs (liver, kidney and heart) were extracted and maintained at -18C° until further analysis.

Metal analysis

The collected organs are weighed before being hot-mineralized at 120 °C for 4 hours according to a process of the accredited toxicology laboratory at the National Institute of Hygiene (INHRabat, Morocco). Metal elements contained in the three organs were found out by the mineralizations after dilution. The Pb, Cu and Cr concentrations were determined by atomic absorption spectroscopy with a graphite furnace (Varian AA 240 120 GTA Z) (Chiffoleau and Truquet, 1994), the background correction is made by a Zeeman effect. Fe and Zn contents were determined by flame atomic absorption spectroscopy (VARIAN AA40 FS). To reduce the chemical interference and volatility of Cr, Cu and Pb in an oven, a matrix modifier is used (mixture of PdCl2 and MgNO3). The calibration curve was generated by the "MSA (standard addition method)". The validity of this method is verified by internal control using standard samples (National Research Council of Canada: DORM-2) and by external control using intercalibration exercises (IAEA-MESL -2014-01-TE) which uses blood doping samples. After the linearity of the curve, the accuracy was checked by three successive readings for each sample, the average of these measurements would be taken into consideration if the RSD (relative standard deviation) was less than 10%.

Statistical methods

From each tissue, an overall measure of gender, age, and site effect on metal concentrations was obtained by a four-way multivariate analysis of variance (MANOVA). The normality and variances homogeneity of the aforementioned variables were checked. The differences were considered statistically significant at p-value≤0.05. All the statistical analyzes were performed by R software using FactoMiner, Factoextra, ggplot2 and corrplot packages.

Results

Morphological characterization

A total of 35 individuals were captured at Merja Zerga and 32 specimens at Dayet Erroumi. Males dominate at the Merja Zerga with 25 individuals, while females are more abundant at Dayet Erroumi (Table 1). It must also be noted that the proportions of juveniles are larger in the Merja Zerga sample than in the Dayet Erroumi sample, with respectively 46.15% and 21.87% males of the total catches in each site.

The comparative analysis of the morphometric characters (Weight (W), head to body length (TL), ear length (E), and posterior leg length (PL)) of the population of the Merja Zerga lagoon and that of Dayet Erroumi exhibits that the highest value for weight is recorded in Dayet Erroumi females with a maximum of 19.2g followed by Merja Zerga males with a value of 17g (Fig.2). The females of Dayet Erroumi have also larger sizes with TL values of around 95mm, followed closely by Merja Zerga females which recorded TL values of 92mm. The males of both sites have comparable sizes with TL values reaching 89mm. The other two characters, length of the ear (E) and length of the hind legs (PL) showed comparable values in both sexes of the two populations.

Females got higher wheight than males in Dayet Erroumi ($t = -3.315$ and $p <0.001$).When comparing females between the two sites, we found that Dayet Erroumi females got higher W and TL ($t = -3.992$; $p <0.001$ and $t = -2.242$, $p <0.05$), however, males got equal values in the sites. Males and females from Merja Zerga have equal morphological variables.

Elemental concentrations

The concentrations of several elements in the studied *Mus spretus* varied between tissues and sexes at each capture site (see Figure 3). In Dayet Erroumi, liver tissue had higher concentrations, followed by kidney and heart. The accumulation of metals in females was very high compared to males, with a maximum reaching 13.65 µg/g for the Cu, followed by the Pb at 12.34µg/g. In Merja Zerga, the highest levels of Cu and Cr liver were recorded in females with a maximum of 60µg / g and 28.68 µg / g successively. A similar trend was revealed for Fe, Pb and Zn in all three organs in both sexes.

In all the organs, males of Meja Zerga exceeded those of Dayet Erroumi for the majority of heavy metals. Females from both sites showed significant differences for Zn, Cu, and Fe. While the liver harbored the five element traces, the heart bioccumulates Zn, Cr, Cu, Pb and kidneys concentrated just Zn, Cr, and Cu.

To analyse the effect of sex, site, age and organs on metal concentrations, MANOVA for all data showed that Zn and Cu concentrations were significantly affected by all variables. Pb was affected only by organs and sex, Cr was affected by age and Fe was affected with all variables except site.

The pattern of metal accumulation by tissues was similar at both sites. The liver was the main target organ of the five trace elements examined (Zn, Cr, Cu, Fe and Pb). Indeed, the concentrations of their majority there were the highest, which implied that an examination of the liver was also important.

The second axis F2 (15.6% of total inertia) is determined by the concentration of Cr, as the dominant element, in the various organs analyzed and which follows the order of importance according to KCr> HCr> LZn.

On the scatter diagram of the first two axes of the PCA (Fig. 4.b) we can distinguish two main groups:

- Group 1: present on the positive side of the F1 axis the majority of adults of the species *Mus spretus* from the two areas studied. It is characterized by high contents of Zn, Fe and Cu followed by average contents of Pb and Cr, thus a large size (W, PL, TL and E). This confirms the existence of an increasing gradient in size and clear HMs concentrations demonstrated by the main component (PCA).

- Group 2: groups the juveniles of the species studied on the negative side. It indicates a weak presence of HMs in the three tissues analyzed and a small size.

The PCA dispersion diagram (Fig. 4.c) allows you to view four groups and indicates a well-defined accumulation by area and sex, as evidenced by the respective overlapping of the following groups:

- A and B, grouping the males and the females of Merja Zerga with significant contents in Cu, Cr and Pb.

- And groups C and D unify males and females of the Dayet Erroumi with a fairly large size in females and average concentrations of HMs (Fig.4.c).

We also verify the correlation between the various morphometric parameters and the rates of accumulation of HMs in their studied organs and we give a synthetic measure of the intensity of the relationship between these characters and of their senses (Fig. 5).

The correlation matrix indicates and confirms an accumulation in synergy between several couples, notably Zn/Cr, Zn-Fe and Cu-Zn. Strong significant correlations ($p < 0.05$) were observed between LZn-KZn, LZn-HZn, KZn-HZn, LFe-KFe, KFe-HFe and LFe-HFe ($0.76 < r < 0.94$) , means between KCr-HCr, LCu-KCu, KPb-HPb, LCu-HZn, LCu-KZn, LZn-LCu, LZn-KFe, TL-LZn and PL-E ($0.43 < r < 0.75$).

Discussion

Bioaccumulation of metals by site

Metal pollution is a growing environmental problem that requires constant attention (Nasrabadi *et al*, 2010; Sambo *et al*, 2014; Lai *et al*, 2010; Kargar et *al*, 2012). As one of the main groups of pollutants, they are not biodegradable and can persist for a long time in soil, water and sediment (Xiaoyu Li *et al*, 2012; Okuku and Peter, 2012). They can also bioaccumulate and biomagnify along the food chain (Farag et *al*, 2012). The results we obtained showed highly significant differences in the bioaccumulation of non-essential elements (Cu, Cr and Pb) by site. The *Musspretus* of the Merja Zerga have accumulated twice the Cu, Cr and Pb compared to those of the Dayet Erroumi. The high concentrations of trace elements reported at the Merja Zerga are the result of pollution whose origin is different. The concentrations are mainly due to the discharges of the exhausts of the vehicles and the wear of the tires containing the Lead (Blagojevic J *et al*, 2012), because this zone is known by a very dense road traffic (Tifarouine *et al*, 2019). Merja Zerga is positioned on two large agricultural perimeters: Gharb and Loukkos. The Gharb plain is one of the most important agricultural production areas in Morocco (ORMVAG 2010). Concurrently, Loukkos covers a large agricultural area nationally; principally it provides 80% of strawberry’s production, rice, peanuts (20%) and sugar crops (15%). Generally, these crops consume a lot of pesticides. According to ORMVAG 2007, 12 pesticide residues were recorded at the Gharb perimeter of water and soil resources, which may lead to a subsequent accumulation of specific elements, in particular Cu and Zn in the soil

(Abt KF, 1998). We recall also that measurements of the Pb concentration using a sampler placed directly in the middle of the fumes emerging from the chimneys of the city of Rabat, have shown Pb concentrations exceeding 1000 μg/m3 (El Abidi *et al*, 2000). Lee (2007) approved the long-range possibility of the fallout of airborne dust from large industrial cities, bordering the Merja Zerga such as Rabat, Kenitra, and Larache. Similarly, according to several authors, climatic conditions (temperature and wind) have a remarkable effect on seasonal variation in air pollution (Aunan *et al*, 2006; Ding *et al*, 2013; Wang *et al*, 2018). The low Dayet Erroumi levels are caused by the lack of a significant influence of the two origines of pollution mentioned above, since the sampling site is about 80 km away from these emissions.

Bioaccumulation of trace elements by age and sex

Micromammals are widely distributed in the world, which makes them a good model for studying pollution effects. The wide range of variation observed in the two areas can be explained in part by factors (biotic and abiotic) that may influence their level of exposure to xenobiotics such as the duration of exposure to pollutants, the properties of the element, the diet and, consequently, the individual response to the absorption and bioaccumulation of metals, the physiology of the population including size, sex and age is also added (Beernaert *et al*, 2008; Rautio *et al*, 2010). Generally, when a living organism is exposed to metals, it can penetrate into all stages of development (Ogundiran *et al*, 2008; Serbaji *et al*, 2012). In the case of variation in the concentration of essential metals, these may be related to the life cycle (growth requirements, gonad maturation and reproduction) (Lopes *et al*, 2002; Udroiu *et al,* 2008). Our results presented that heavy metals accumulation in tissues of adult females of *Mus spretus* approximately three times higher than those of female juveniles. The high Cu concentration was found in adult females from Merja Zerga with a maximum of 60μg/g; while at Dayet Erroumi, it is around 14μg/g. This metal is an essential element in humans and animals and is of paramount importance in the maintenance of biological processes, particularly for hemoglobin formation and the maturation of neutrophils. It is also known for its fungicidal effect on plants, is present in the receiving aquatic ecosystem and can cause disturbances in phytoplankton populations (Pesce S., 2006). At the same time, Cr and Pb increased with age in female *Mus spretus* with a maximum of 28.68 μg / g at Merja Zerga and 12.34 μg / g at Dayet Erroumi. The presence of higher trace element concentrations in adult females may be attributed to both elevated metabolic rate and disruption of toxic metal neutralization mechanisms (Kljaković Gašpić *et al*, 2002), therefore the nutritional status and physiological needs of individuals lead to intra-population variances in heavy metals accumulation. The increase in concentrations of the HMs in Merja Zerga matches the preceding studies reporting significant amounts of these elements in the soil (Mhamdi Alaoui *et al*, 2010 ; Maanane *et al*, 2015; Boutahar *et al,* 2019), in fish (Wariaghli *et al*, 2013) and in rodent tissues (Tifarouine *et al*, 2018). Tifarouine *et al*, 2019; reported that the effectiveness of the bioaccumulation process of *Apodemus sylvaticus* (*Muridae*) in Merja Zerga depends only on the individual's age whereas sex has no effect. Sanchez Cardi *et al*, 2007, demonstrated that mercury increase with age also occurs in another micromammal, *Crossidura russula* from a polluted wetland. In contrast, the present study has demonstrated that bioaccumulation of HMs in *Mus spretus* depends both on age and on sex.

Bioaccumulation of trace elements by tissues

Trace elements are necessary for terrestrial and aquatic organisms, such as Cu, Cr, Fe and Zn. For some organisms, minute amounts of these elements are essential for normal growth and development. However, at high levels of exposure and adsorption, these elements can be potentially harmful to most organisms (Yorulmaz *et al*, 2015). The kidneys and the liver are the key organs, when it comes to metabolic pathways involving metals, like bioaccumulation, biotransformation, excretion and detoxification (Dragun *et al*, 2009; Sunjog *et al*, 2012). Once micronutrients cross biological barriers and enter the bloodstream, they reach the liver and accumulate there. Our results on the distribution of metals in *Mus spretus* correspond to those reported for other mammalian species (Gdula-Argasińska *et al*, 2005; Jarić *et al*, 2011; Tête N *et al*, 2014; Prevendar Crnić *et al*, 2015; Gašparík *et al*, 2016; Zietara *et al*, 2018). The liver was the main accumulator of the five elements studied in both sites, followed by the kidney and heart. The hepatic concentration in females of Merja Zerga was higher than in males with a maximum of 60 µg / g for Cu and 28.68 µg / g for Cr. The same distribution was recorded at Dayet Erroumi with 13.65 µg / g for Cu and 12.34 µg / g for Pb. This tissue distribution corroborates the results obtained for *Apodemus sylvaticus* in Merja Zerga (Tifarouine *et al*. 2019), and *Crocidura leucodon* (Marques, 2007).

Conclusion

The present study provides original results on concentrations of numerous trace elements in the liver, kidney and heart of the rodent *Mus spretus* in Northern Morocco. We have found that the bioaccumulation of some metals in the organs is a process whose effectiveness depends on sex, individual age, and habitat. In Merja Zerga, concentrations of these elements in adults were two to three times higher than in Dayet Erroumi. A possible cause could be higher metal pollution levels in Merja Zerga. The accumulation process continues throughout their life, although these older micromammals have a lower rate of metabolism, so the accumulation of trace elements directly depends on the level of pollution. According to our results, a difference between the two localities can only be clearly observed in older individuals, so this category should be the target of further studies.

Declarations

Conflict of interest statement

On behalf of all authors, the corresponding author states that their is no conflict of interest.

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Author agreement statement & Authors Contributions

We would like to present our manuscript " The Western Mediterranean mouse (*Mus spretus: Lataste*): Capacity of accumulation and biomonitoring of trace elements in Atlantic wetlands.” It has not been published before and is not currently being considered for any publication elsewhere.

We confirm that this manuscript has been read and approved by all named authors. We would also like to confirm that the order of authors listed in the manuscript has been approved by all of us.

The main objective of this research was to assess the exposure of *Mus spretus* to the five metallic trace elements in their vital organs in two zones: Merja Zerga and Dayet Erroumi classified successively, Ramsar site and SIBE in northern Morocco. Tissue metal concentrations in this species varied significantly with age, sex, site and organ.

I have read positive comments on your review and the variety of fields and subjects it covers and publishes; particularly, in the area of ecotoxicology, environmental safety and bioremediation, as well as the conservation of biodiversity.

We have chosen: MOHAMED Abdel Daim, Muhammad Ajmal Shah and Jauad El Kharraz as international experts in ecotoxicology, conservation and biodiversity to evaluate and review our document.

Finally, we understand that the Corresponding Author is the sole contact for the Editorial process. He is responsible for communicating with the other authors about progress, submissions of revisions and final approval of proofs.

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Tables

Table 1: Distribution of captured specimens by age and sex (In brackets: males - females)

Site	Age		Total
	Juveniles	Adults	
Merja Zerga	18 (14-4)	21 (11,10)	39 (25,14)
Dayet Erroumi	7 (4,3)	25 (8,17)	32 (12,20)

Table 2: Student's t-test results for variation in tissue-specific metal concentrations for each site and comparison between the two sexes

Site / Organs/Metals	Merja Zerga(♂ ♀) t	p	Dayet Erroumi(♂ ♀) t	p	♂Merja Z. ♂Dayet E. t	p	♀Merja Z. ♀Dayet E. t	p
Liver								
Zn	0,987	-	-3,205	<0,01	-2,757	<0,01	-5,125	<0,001
Pb	-0,590	-	-2,24	<0,05	1,964	<0,05	1,042	-
Cr	0,805	-	-0,661	-	4,844	<0,001	1,583	-
Cu	0,422	-	-1,673	-	6,165	<0,001	2,7	<0,01
Fe	0,638	-	-3,172	<0,01	1,986	<0,05	-1,981	<0,05
Kidney								
Zn	1,66	-	-3,107	<0,01	-2,786	<0,01	-5,276	<0,001
Pb	-1,196	-	-2,264	<0,05	0,060	-	-0,134	-
Cr	1,980	<0,05	-1,042	-	3,743	<0,001	1,193	-
Cu	0,063	-	-1,604	-	4,752	<0,001	2,058	<0,05
Fe	0,409	-	-3,603	<0,001	1,564	-	-2,914	<0,01
Heart								
Zn	1,324	-	-3,003	<0,01	-2,969	<0,01	-5,04	<0,001
Pb	0,018	-	-1,622	-	-1,974	<0,05	-2,53	<0,01
Cr	1,484	-	-1,186	-	2,229	<0,05	-0,016	-
Cu	1,048	-	-1,584	-	2,977	<0,01	1,189	-
Fe	1,081	-	-3,423	<0,001	0,858	-	-3,805	<0,001

*p_0.05; **p_0.01; ***p_0.001

Figures

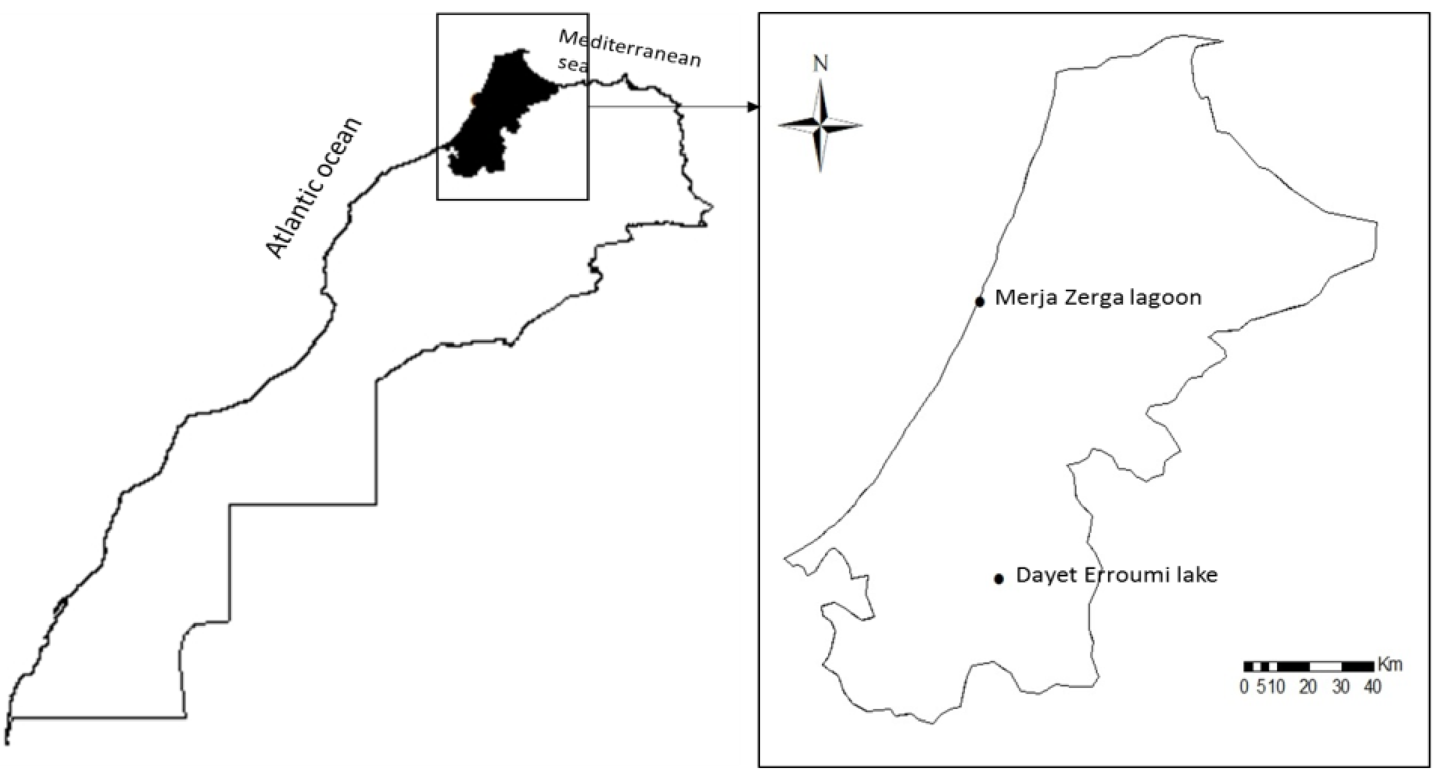

Figure 1

Localization of the study areas in North of Morocco

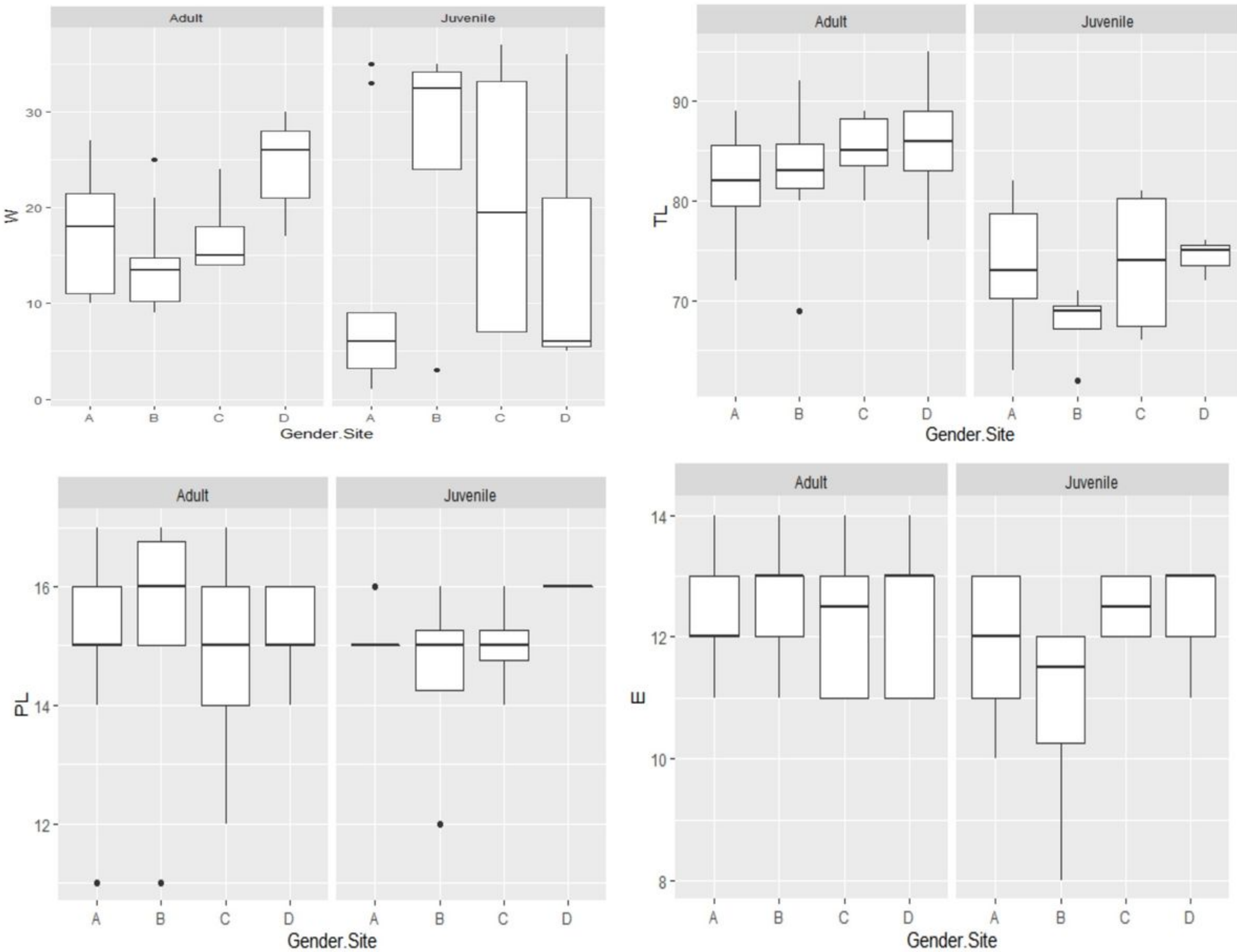

Figure 2

Body measurements (Weight: W, Head-Body Length: TL, Ear length: E, and Posterior Legs length: PL) of Mus sprteus captured in Merja Zerga and Dayet Erroumi (A: Male Merja Zerga and B: Female Merja Zerga; C: Male Dayet Erroumi and D: Female Dayet Erroumi)

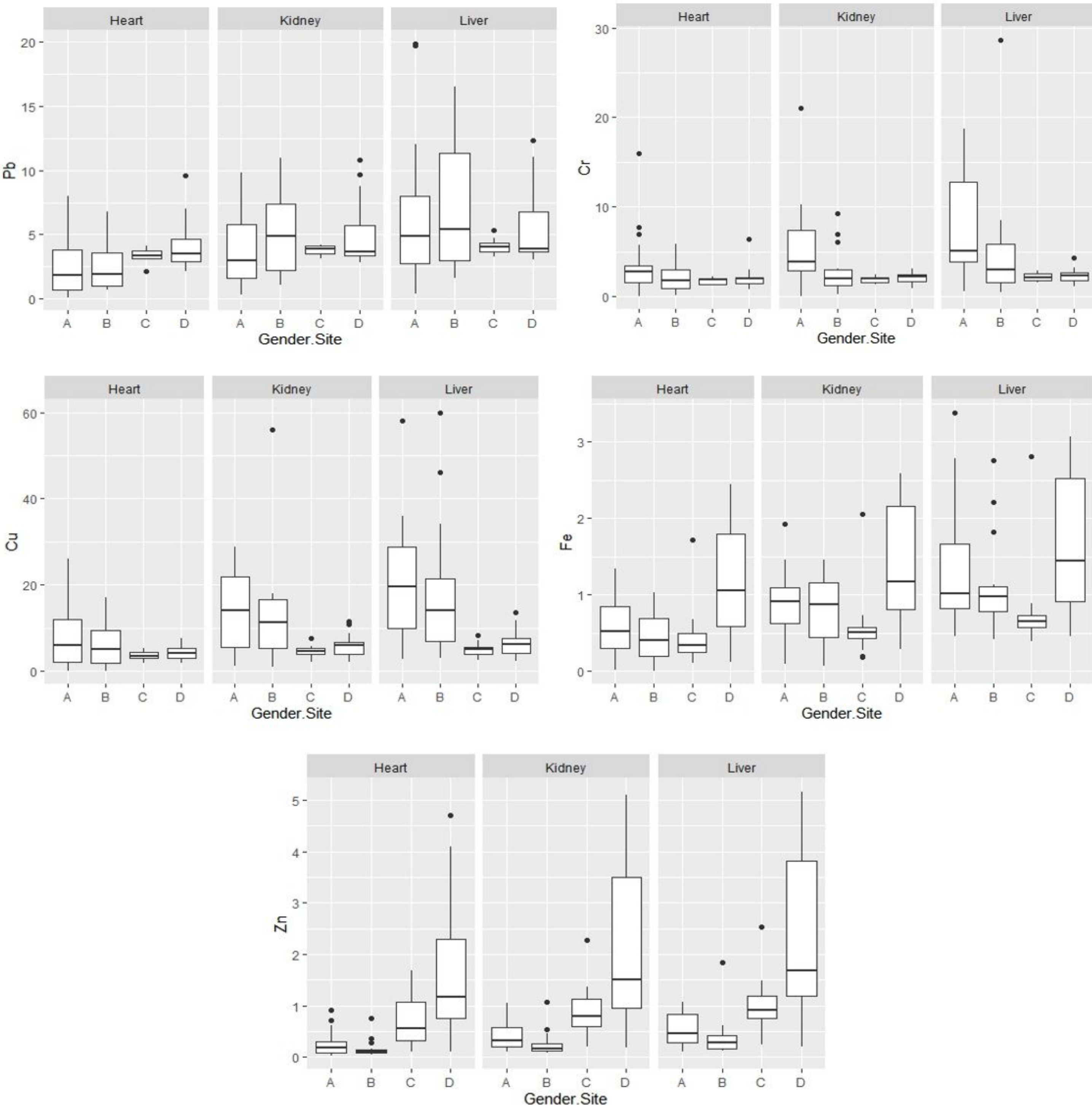

Figure 3

Concentrations of trace elements (µg / g) in the liver, kidney and heart of Mus spretus of Merja Zerga and Dayet Erroumi (A: Male Merja Zerga and B: Female Merja Zerga; C: Male Dayet Erroumi and D: Female Dayet Erroumi)

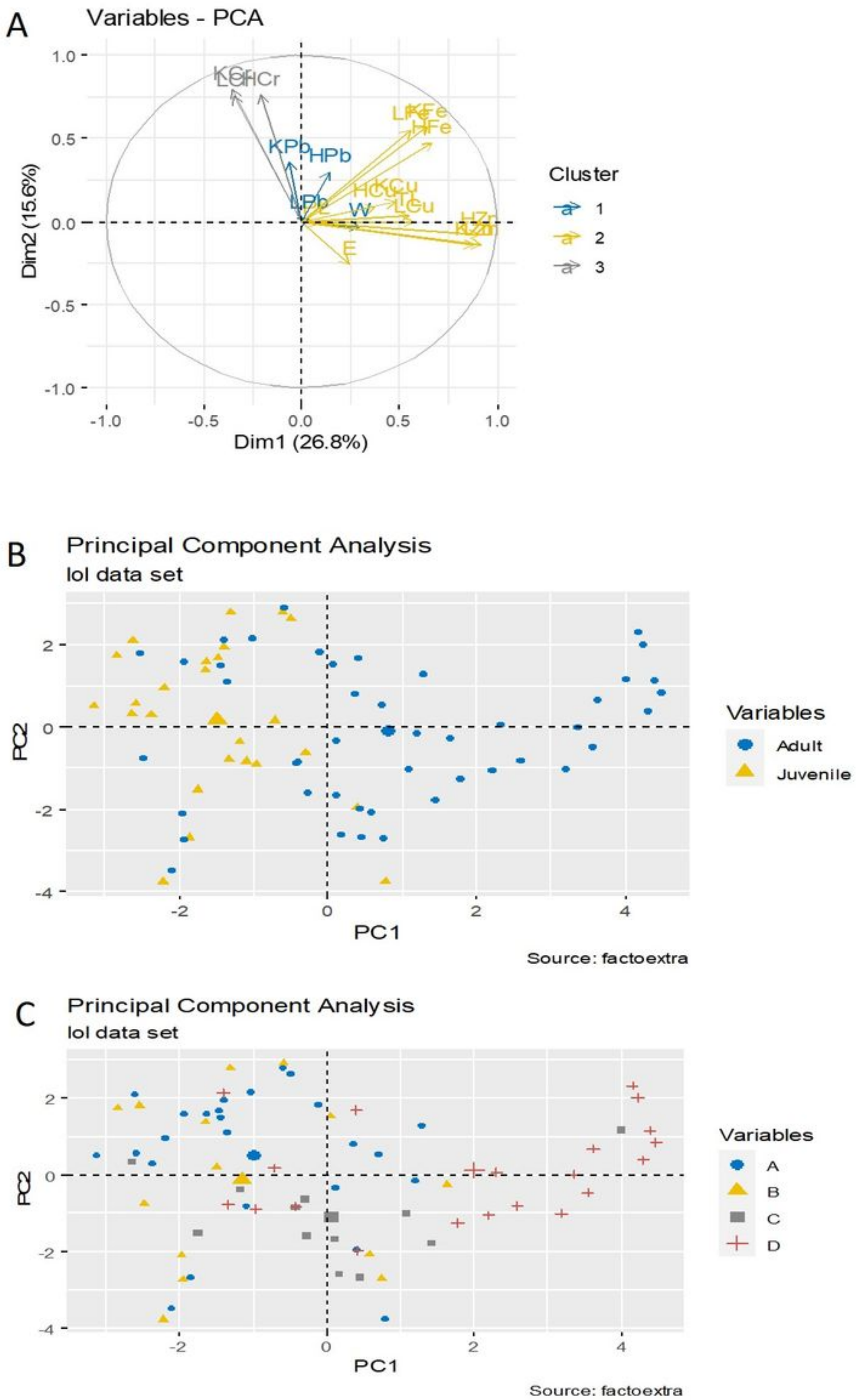

Figure 4

a: Correlation circle obtained by the analysis in principal components (PCA) of the morphometric variables and the concetrations of HMs in the organs of Mus spretus in the factor space of (F1 and F2) b : Factor loading plots for the first two principal components identified in the PCA of projection of individuals. The colors represent the age categories of the specimens (Adult: blue; Juvenile: mustard yellow) c : Factor loading plots for the first two principal components identified in the PCA of projection of individuals. The four colors represent the different groups according to sex and area (Male Merja Zerga: blue; female Merja Zerga: mustard yellow; Male Dayet Erroumi: gray and female Dayet Erroumi: bordeaux red)

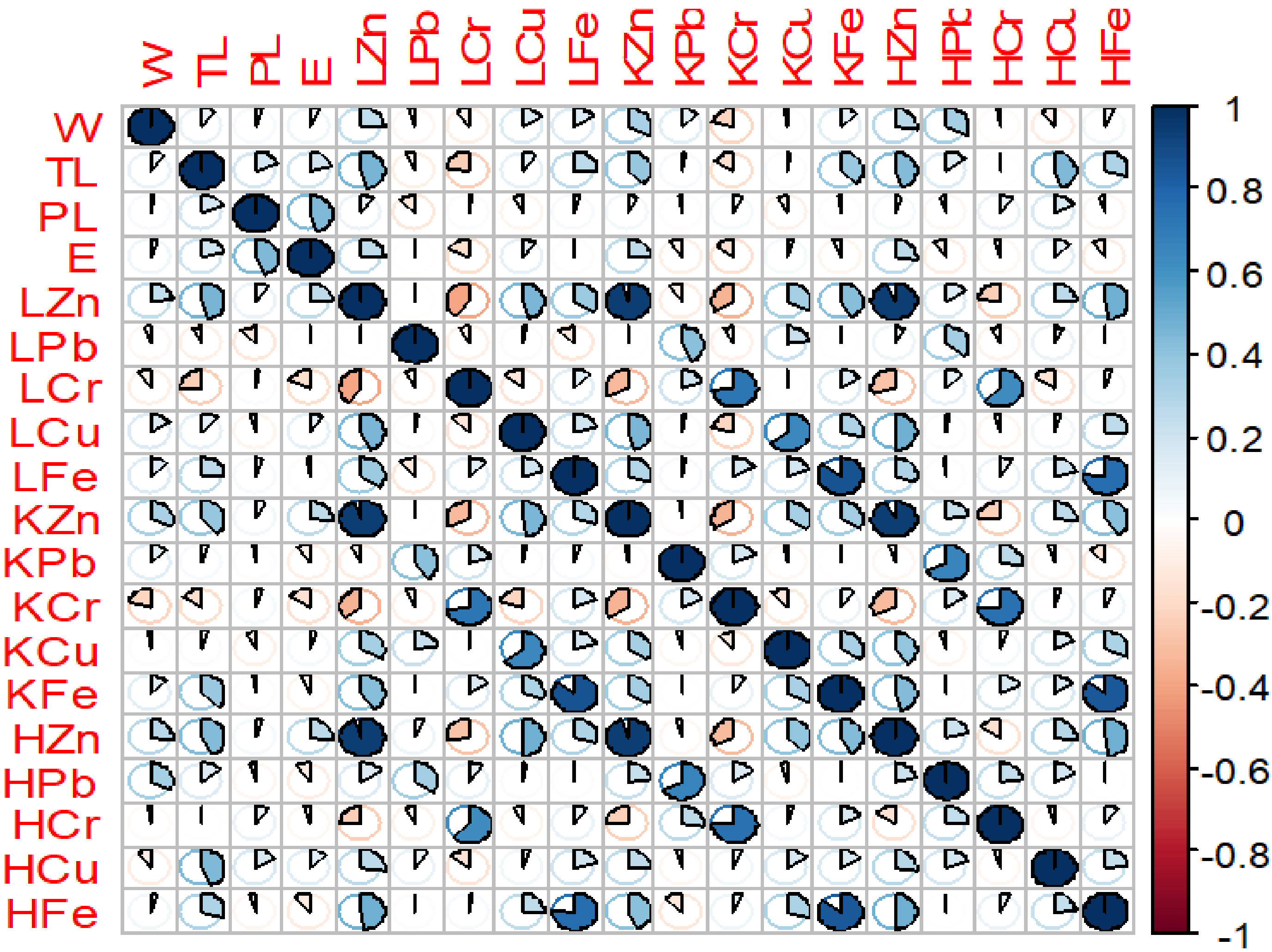

Figure 5

Correlations between the different variables: Morphometric (W, TL, PL, E) and heavy metals in the organs (Liver: LZn, LPb, LCr, LCu, LFe ; Kidney: KZn, KPb, KCr, KCu, KFe ; Heart: HZn, HPb, HCr, HCu, HFe)