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RESEARCH ARTICLE

A study on the concentration of heavy metals and histopathological changes in Persian jirds (Mammals; Rodentia), affected by mining activities in an iron ore mine in Iran

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Abstract

Mining activity constitutes a potential source of heavy metal pollution in the environment. Long-term exposure to heavy metals (e.g., cadmium) has adverse health effects. Rodents frequently serve as bioindicators to monitor the levels of heavy metals in the environment. In the present study, concentrations of 10 heavy metals (Cd, Co, Cr, Cu, Fe, Mo, Ni, Pb, Sb, and Zn) in kidney, liver, and muscle tissue of the Persian jird (*Meriones persicus*) were evaluated. This is the first study to examine the histopathological changes in Persian jird tissues caused by the bioaccumulation heavy metals. The samples were taken at location that surrounded by Sangan Iron Ore Mine (SIOM) mining activities, in northeastern Iran. The results show that the highest concentrations for the metals were observed in kidney and liver, whereas lowest concentrations were found in muscle of Persian jirds. The concentration of Pb was below the limit of detection. Sex and age were two factors that could explain the different levels of heavy metal bioaccumulation, which affects the concentration of some metals. Adults had significantly higher Cu and Cd levels compared to juveniles. Males bioaccumulated more Zn in their kidneys than females, whereas females bioaccumulated more Fe in their livers. As expected, heavy metals affected various organs of the studied specimens. Hyperemia, hemorrhage, necrosis, and degenerative damage to the epithelial cells of the tubules, the presence of hyaline casts, and in one case, mononuclear leukocyte infiltration, were observed in samples of renal tissue. Hemorrhage and hepatocyte vacuolization were the most common histopathological changes found in samples of hepatic tissue. These effects and the concentrations of heavy metals in the studied specimens indicate the need for monitoring and frequent sampling to evaluate long-term persistent pollutants.

Keywords Iron ore mine · Pollution · Rodents · Heavy metals · Histopathology

Introduction

Mining activities are widely regarded as having severe consequences on the environment, both in terms of their magnitude and their impact on biodiversity. Industrial activities such as mining operations release heavy metals into the environment (Nagajyoti et al. 2010; Shahsavari and Akbari 2018). Some of these metals (e.g., lead and cadmium) are toxic to living

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organisms even at low concentrations. Others (e.g., copper and iron) are harmful at high concentrations. When toxic heavy metals are released into the environment, they can be absorbed and transferred through the food chain and accumulate in the body of predator species (Sánchez-Chardi et al. 2009).

Bioindicators, alternatively referred to as biomonitors, are organisms that accumulate pollutants in their tissues. Bioindicator species, native species that reflect environmental quality, can be studied for monitoring the bioavailability of pollutants in ecosystems (Viarengo and Canesi 1991; Rainbow and Phillipsm 1993). The use of biomonitors has increased due to the realization that many laboratory models show weak correlations with natural ecosystems (Betty et al. 1990; Levengood and Heske 2008; Phelps and McBee 2008; Tovar-Sánchez et al. 2012). Many reports have documented the direct and indirect effects of heavy metal pollution on different organisms (Ma et al. 1991; Świergosz-Kowalewska et al. 2005; Sánchez-Chardi et al. 2007a, 2007b, 2007c; Sánchez-Chardi and Nadal 2007; Sánchez-Chardi et al. 2009; Schleich et al. 2010; Khazaee et al. 2015; Zarrintab and Mirzaei 2017).

Small mammals, especially rodents, have been used as bioindicator species for years (Clark et al. 1992; Quinn 2010; Milton et al. 2004; Topolska et al. 2004; Damek-Poprawa and Sawicka-Kapusta 2003; Beernaert et al. 2007). Rodents living within mining areas are a means to investigate metal exposure since they are in close contact with soil and air pollutants and studying them can provide a realistic approach to the composition and concentration of metals in the environment. It is reported that rodents accumulate high concentrations of metals in their tissues (Damek-Poprawa 2002; Mažeikytė and Balčiauskas 2003; Sumbera et al. 2003). Thus, in addition to playing an important role in terrestrial ecosystems, rodents can provide useful information for pollutant risk assessment regarding human health and health of the environment (Ware 2000; García-Sevillano et al. 2014).

Persian jird (*Meriones persicus*, Blanford 1875) is a good bioindicator of heavy metal pollution (Khazaee et al. 2015). This species lives on the slopes of mountainous areas and is distributed from Transcaucasia and Turkey to the eastern part of the Iranian Plateau (Tabatabaei Yazdi and Adriaens 2011). This species is present in most of Sangan Iron Ore Mine's (SIOM) site. They are nocturnal and feed on seeds, leaves, and green plants (Firouz 2005; Ziaie 2008). At present, this species is not on the International Union for Conservation of Nature's (IUCN) Red List of threatened species and is classified in the least concern category. Moreover, the abundance of the Persian jird and its key role in the food chain have made it one of the most suitable species for monitoring programs (Ziaie 2008).

Exposure to doses of heavy metals exceeding a critical threshold may lead to changes in the functioning of organisms (Zarrintab and Mirzaei 2017). Some heavy metals may cause mutations, apoptosis (Pereira et al. 2006; Jadhav et al. 2007), or necrosis (Bragadin et al. 2003; Leonard et al. 2004). Heavy metals accumulate in various tissues depending on the element (Szyczewski et al. 2009). Being the main detoxification organs in the vertebrate body, liver and kidneys are the target organs for metal accumulation and are thus commonly used as bioindicator organs for studies of long-term heavy metal pollution in various ecosystems (Christopher 1986; Williams and Iatropoulos 2002; Faroon et al. 2012). Recently, Khazaee et al. (2015) studied the environmental impacts of copper mining on Persian jirds in terms of heavy metal accumulation and concluded that it is a suitable species for biomonitoring programs. Thus, in this study, Persian jird was used as the bioindicator. Our study is the first to assess the histopathological changes in Persian jird tissues caused by the bioaccumulation of heavy metals. It is also the first study to use this species to measure environmental impacts felt beyond this large open-pit mine.

Sangan Iron Ore Mine (SIOM) is the largest open-pit iron ore mine in Iran and one of the richest iron ore mines in the Middle East (Fig. 1). Mining activities in this area have exposed minerals containing heavy metals and increased heavy metal pollution in the ecosystem. Iron ore mining, either by surface or underground methods, has adverse effects on the environment. Iron ore mines are responsible for the introduction of metals, both essential elements such as Fe and non-essential metals such as Cd into the environment (Zabowski et al. 2001; Pereira et al. 2008). Since very few studies have investigated the environmental impacts of this large open-pit mine on rodents, a database is required for evaluating the effects of heavy metal pollution on Persian jirds.

This study has provided new data on the concentrations of selected heavy metals (Cd, Co, Cr, Cu, Fe, Mo, Ni, Pb, Sb, and Zn) in different organs and tissues (liver, kidney, and muscle) of Persian jirds in SIOM in Iran. In the present biomonitoring research, metal concentrations were compared between the sexes and age classes, as well. Moreover, the histopathological changes due to the accumulation of these metals in liver and kidney of the species were investigated (see "Material and methods").

Material and methods

Study area and sampling

The study was carried out in the Sangan opencast iron ore mine located in the southeast of Khorasan Razavi Province, in northeastern Iran, at 60° 22′ 28″ E and 34° 27′ 22″ N (Fig. 1). The size of the study area was about 1 ha, surrounded by iron ore mining activities. The area used for mining activities spanned tens of hectares. During July and August 2017, 18 specimens of Persian

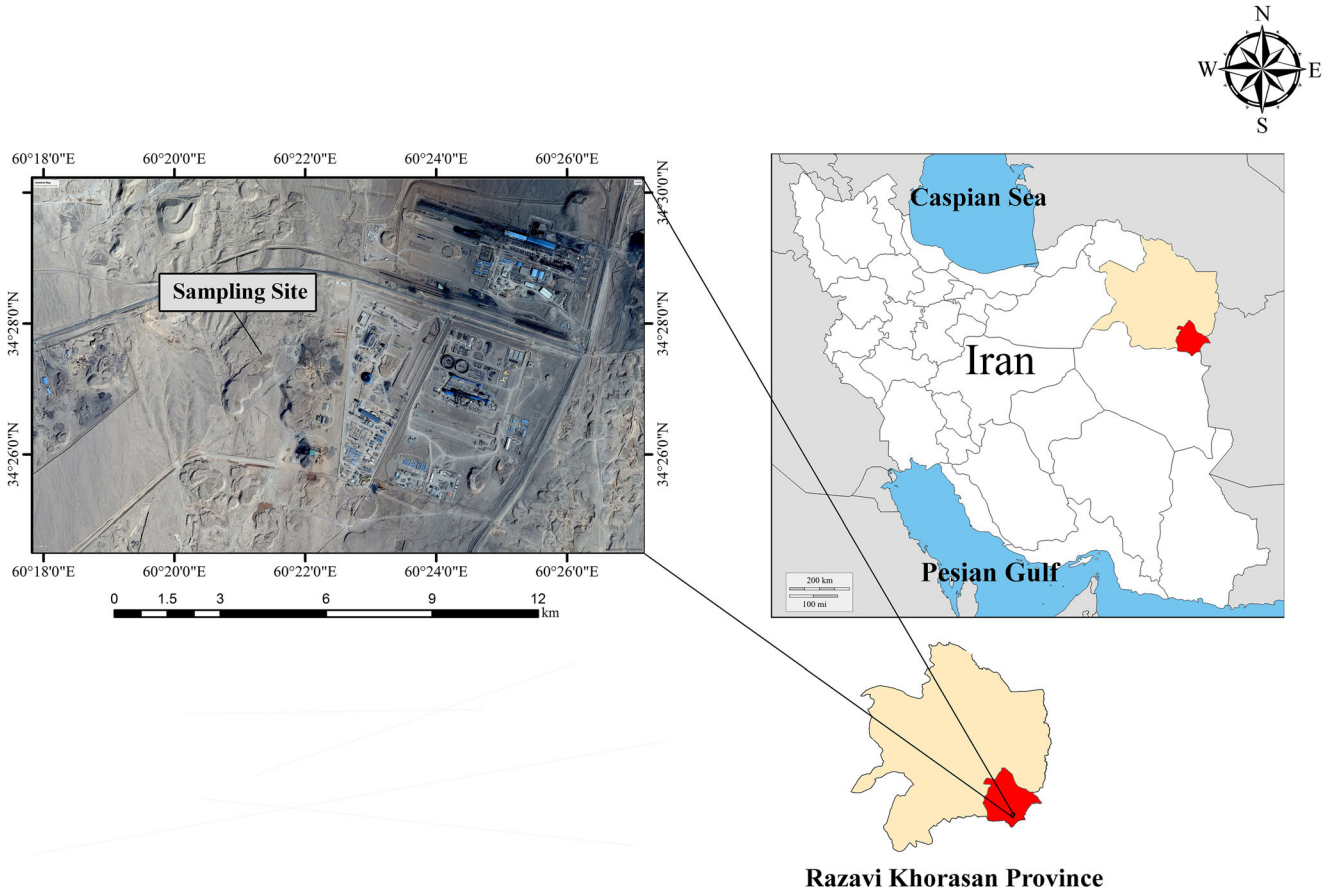

Fig. 1 Map showing the geographical location of study area (SIOM)

jird, *Meriones persicus* (10 adults and 8 juveniles, 10 of which were male and 8 female) were trapped. The specimens were identified based on identification keys for jirds using external and cranial data available (Chaworth-Musters and Ellerman 1947; Darvish 2011). All collected animals were caught in an area affected by the iron ore mining activities. Traps were placed 5–10 m apart in a 300-m transect running along the study area. For this study, trapping was carried out using live box traps, set in the evening and collected the next morning. Traps were baited with pieces of apple and fresh cucumber. Thirty traps were placed in the area every night. Trapped jirds were transported to the laboratory, anesthetized, and killed with chloroform. Dissection of rodents was performed with stainless steel instruments. Internal organs such as kidney, liver, and muscle were removed and then weighted. Right kidney and liver and muscle samples of all specimens were placed in polystyrene tubes and stored at .20 °C until chemical analysis. Left kidneys and liver samples of all the specimens were fixed in 10% buffered formalin for histopathological analyses. Sex of trapped rodents was determined during dissection. Rodents were divided into two relative age groups (i.e., adults and juveniles) on the basis of the degree of tooth wear (Millán et al. 2008; Sánchez-Chardi et al. 2009; Sengupta 2013). Body weights of specimens ranged between 50 and 125 g for males and 48 and 128 g for females.

To evaluate soil pollution, soil samples were collected at the mining site. Samples were taken with a stainless steel trowel at a depth of 10 cm from the soil surface after the organic layer was removed. Soil samples were stored in polyethylene jars at room temperature. Afterwards, samples were air-dried and passed through a 2 mm sieve to remove rocks, roots, and other large particles.

Chemical analyses

Soil samples were dried at 60 °C for 24 h. The dried soil sample of 0.25 g was digested. About 1000 mg of the liver, right kidney, and muscle from each specimen was dissected, dried at 60 °C for 48 h, and weighted. Soil and tissue samples were placed in Teflon digestion vessels with 5 ml of 67% nitric acid HNO3 (ultra-pure Merck, Darmstadt, Germany) and 15 ml of 37% hydrochloric acid HCl (Aqua Regia). Digestion was completed by heating the samples in a microwave (Mileston Ethos Plus). In each step of digestion, a blank was added with samples for quality assurance and was

included in the metal detection procedure. Ten heavy metals (Fe, Pb, Cd, Cr, Cu, Ni, Sb, Co, Mo, and Zn) were measured using an Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES, PerkinElmer: Optima 2100 DV). Metal concentrations were expressed in μg g^{-1} (ppm) on a dry weight (DW) basis. All chemical analyses were performed at the central laboratory of the Ferdowsi University of Mashhad, Iran.

Histopathological analyses

For microscopic evaluation, collected tissue specimens were fixed in neutral 10% buffered formalin; after the tissue was dehydrated in alcohol and cleared in xylol, it was embedded with paraffin wax. Then, 4- to 5-μm-thick paraffin sections were prepared, subsequently stained with hematoxylin and eosin (Bancroft and Stevens 1996). Microscopic slides were used for histopathological examination and were observed under a light microscope. Finally, the slides were photographed and lesions were graded into four levels based on the severity of injury being: severe (+++), moderate (++), slight (+), and absence of lesions or normal (−), as described in Table 1.

Statistical analyses

Since some of the data for heavy metals were not normally distributed and their variance was not homogeneous, some data had to be transformed into a normal distribution. After the transformation, the Shapiro-Wilk test for normality of data was performed. The p value for the majority of variables was less than 0.05, but some variables, such as the concentration of cadmium, were not normally distributed in samples from the kidney, liver, and muscle. Variables which were not normally distributed later underwent log transformation (log10). To test the homogeneity of variance, the Berlet test was performed, followed by a one-way analysis of variance (ANOVA) to compare metal concentrations in different organs. Moreover, an independent t test was used to analyze metal levels in selected samples from different age and sex groups (juvenile vs. adult). The juvenile specimens were identified based on the molars' eruption (Pavlinov 2008) and having no signs of sexual activities. All statistical procedures were conducted using RStudio-1.0.136.

Results

Heavy metal content in the soil

The total organic carbon (TOC), pH, EC, and metal concentrations in the soil samples from the study site are given in Table 2. In the iron mining area, higher concentrations of Fe (39,724.03 μg g^{-1} DW), Cr (502.87 μg g^{-1} DW), Ni (215.24 μg g^{-1} DW), Zn (61.19 μg g^{-1} day DW), and Cu (32.02 μg g^{-1} DW) were observed. Cd concentrations were below the limit of detection.

Heavy metal concentrations in different organs

The mean concentration of metals (Fe, Pb, Cd, Cr, Cu, Ni, Sb, Co, Mo, and Zn) in kidney, liver, and muscle samples from each animal is presented in Table 2. For most elements, the highest concentrations were observed in the liver and the kidneys. The highest levels of Cd, Cr, Co, Sb, and Ni were observed in kidney samples, while the highest concentration of Cu, Mo, Fe, and Zn was found in the liver. In general, the highest concentrations of most essential metals occur in the liver and kidney tissue and the lowest concentrations are observed in muscle tissue. Based on the results, heavy metal accumulation in the liver of specimens showed the following pattern: Fe > Zn > Cu > Mo. The mean concentrations of Cu, Mo, Fe, and Zn in hepatic tissue of Persian jirds from the mining site are higher than in other tissues ($p < 0.001$). For all of the metals, except Sb, concentration in muscle was lower than concentrations in kidney and liver samples.

The kidneys showed significantly higher bioaccumulation of Cd and Cr ($p < 0.001$). The highest concentration in the sampling site was detected for Fe in the liver. Since the study area was an iron ore mine, this result was expected. Higher concentrations of Fe were recorded in the liver, kidney, and muscle of rodents from the mining area ($p < 0.001$). The highest concentration of Co, Cr, and Ni was observed in renal tissue samples, followed by hepatic tissue samples. Pb was undetectable in the kidney, liver, and muscle in most samples. There were no significant differences between the concentration of Sb in the liver and muscle samples ($p > 0.05$). A statistically significant difference in the concentration of Sb between kidney samples was observed ($p < 0.05$).

Table 1 Description of the grades of lesion severity

Description	Grading of lesion
No lesion	−(normal)
Lesion in 25% studied microscopic domains	+(slight)
Lesion in 25% to 75% studied microscopic domains	++(moderate)
Lesion in more than 75% studied microscopic domains	+++(severe)

Table 2 Heavy metal concentrations (μg g^{-1}) in the soil and in different organs of *M. persicus* expressed as mean ± standard deviation (SD) on top and range of them in bellow (μg g^{-1} dry weight)

Metal	Soil (PH 8.10, TOC:0.18,7, EC(dS/m): 2.71)	Organs		
		Muscle	Kidney	Liver
Cd	ND	0.027 ± 0.004^{C}	0.071 ± 0.023^{A}	0.033 ± 0.008^{B}
		0.02–0.034	0.03–0.112	0.023–0.044
Co	9.84	0.0324 ± 0.030^{B}	0.0865 ± 0.063^{A}	0.045 ± 0.036^{B}
		0.001–0.078	0.023–0.245	0.004–0.118
Cr	502.87	16.657 ± 4.687^{B}	31.120 ± 7.813^{A}	17.901 ± 5.052^{B}
		9.148–25.539	15.251–45.802	10.789–26.972
Cu	32.02	1.4263 ± 0.314^{C}	4.6985 ± 0.952^{B}	5.457 ± 1.104^{A}
		0.656–1.891	2.914–6.692	3.250–7.325
Fe	39,724.03	93.1 ± 21.834^{C}	205.735 ± 65.041^{B}	232.619 ± 86.060^{A}
		55.671–141.901	81.540–330.991	141.628–422.363
Mo	0.29	0.063 ± 0.052^{C}	0.65 ± 0.158^{B}	1.16 ± 0.245^{A}
		0.021–0.224	0.390–0.964	0.749–1.679
Ni	215.24	6.408 ± 1.256^{C}	12.5835 ± 3.154^{A}	6.8431 ± 1.399^{B}
		4.229–8.960	6.700–18.216	4.564–9.138
Pb	11.03	ND	ND	ND
		ND	ND	ND
Sb	1.1	0.108 ± 0.028^{AB}	0.257 ± 0.127^{A}	0.093 ± 0.030^{AB}
		0.076–0.176	0.064–0.5	0.061–0.171
Zn	61.19	9.047 ± 2.400^{C}	16.942 ± 3.916^{B}	28.78 ± 5.508^{A}
		5.239–13.166	10.959–24.624	19.453–36.563

A > AB. ($p < 0.001$) A > B > C; ($p < 0.05$)

*Different letters in each row express significant difference concentration of heavy metals between organs ($p < 0.05$; $p < 0.001$)

ND not detected

Concentrations of metals in samples from different sexes

The results of the analysis of bioaccumulated heavy metals in samples from specimens of different sexes are reported in Table 3. In Persian jirds, sex had an effect on the mean concentration of some heavy metals (Table 3). In general, females had higher levels of most metals in their livers in comparison with males. However, sex had no statistically significant effect on toxic metal concentrations. Except for cobalt (Co), female jirds bioaccumulate higher levels of heavy metals in their livers. The only significant difference in terms of heavy metal concentration between sexes was observed for Fe and Zn. Results indicate that females had significantly higher levels of Fe in their livers ($p < 0.05$). However, males had higher levels of Zn in their kidneys ($p < 0.05$). Nearly all metals (with the exception of Cd) were found at higher levels in the kidneys of males; yet, these differences were not statistically significant ($p > 0.05$). No significant difference in the levels of Cd, Cu, Ni, Cr, and Mo between females and males was observed in muscle tissue ($p > 0.05$).

Concentrations of metals in adults and juveniles

The concentration of the majority of heavy metals varied with age (Table 4). The levels of heavy metals in the kidney, liver, and muscle of Persian jirds tend to increase with age. High levels of most metals were measured in the livers of adults. However, there were no significant differences in the levels of Co, Cr, Fe, Ni, Sb, and Zn between the two age classes ($p > 0.05$). Only the concentration of Cd and Cu in liver samples and the concentration of Cu in muscle samples were significantly different ($p < 0.05$) with respect to age. The concentration of Cd and Cu in the livers of adults was significantly higher than the concentration of these metals in the livers of juveniles ($p < 0.05$). Adults had higher Co, Cr, Cu, Fe, Mo, Ni, Sb, and Zn concentrations in their muscle tissue compared to juveniles, whereas the concentrations of Sb and Ni in kidney samples, Zn in liver samples, and Cd in muscle samples tend to be higher in juveniles.

Histopathological study

Histopathology is the science of diagnosing and studying diseases of tissues, which typically involves examination

Table 3 Concentrations of metals expressed as mean ± standard deviation (SD) (μg g^{-1} dry weight) in organs of different genders of *M. persicus*

Metal	Gender	Organs		
		Muscle	Kidney	Liver
Cd	Male	0.027 ± 0.004	0.069 ± 0.026	0.031 ± 007
		0.02–0.034	0.030–0.112	0.023–0.044
	Female	0.027 ± 0.004	0.074 ± 0.018	0.033 ± 0.005
		0.021–0.034	0.049–0.100	0.027–0.041
Co	Male	0.034 ± 0.033	0.109 ± 0.068	0.048 ± 0.043
		0.002–0.078	0.032–0.245	0.004–0.118
	Female	0.030 ± 0.029	0.0528 ± 0.039	0.042 ± 0.031
		0.001–0.074	0.023–0.111	0.006–0.096
Cr	Male	16.259 ± 5.17	36.802 ± 14.899	16.329 ± 5.319
		9.148–25.539	15.25–45.8	10.789–26.972
	Female	17.320 ± 4.116	28.775 ± 3.690	20.386 ± 3.834
		12.461–22.129	24.93–34.700	13.345–25.043
Cu	Male	1.411 ± 0.346	4.962 ± 0.387	5.57 ± 1.166
		0.656–1.891	2.914–6.692	4.205–7.325
	Female	1.450 ± 0.281	4.284 ± 1.119	5.618 ± 0.623
		0.903–1.733	3.813–4.936	4.571–6.499
Fe	Male	90.687 ± 29.009	217.905 ± 79.97	198.689 ± 77.938 [B]
		40.603–141.901	81.54–330.991	141.628–422.363
	Female	81.545 ± 24.191	186.61 ± 25.068	285.938 ± 73.712 [A]
		38.158–115.396	160.336–232.617	179.762–372.422
Mo	Male	0.049 ± 0.016	0.7439 ± 0.1924	1.136 ± 0.212
		0.028–0.079	0.390–1.274	0.749–1.470
	Female	0.052 ± 0.036	0.624 ± 0.103	1.231 ± 0.305
		0.021–0.096	0.473–0.756	0.929–1.679
Ni	Male	6.376 ± 1.419	14.992 ± 5.253	6.499 ± 1.542
		4.229–8.960	6.7–25.69	4.564–9.138
	Female	7.307 ± 2.264	11.155 ± 1.783	7.359 ± 1.069
		5.576–11.514	8.482–13.654	5.892–8.506
Pb	Male	ND	ND	ND
		ND	ND	ND
	Female	ND	ND	ND
		ND	ND	ND
Sb	Male	0.114 ± 0.029	0.328 ± 0.097	0.099 ± 0.058
		0.080–0.176	0.123–0.707	0.043–0.242
	Female	0.083 ± 0.029	0.244 ± 0.258	0.102 ± 0.041
		0.044–0.127	0.150–0.500	0.069–0.171
Zn	Male	9.236 ± 2.469	20.7707 ± 7.532 [A]	27.816 ± 5.569
		5.298–13.166	12.281–39.442	19.453–36.027
	Female	8.750 ± 2.460	14.687 ± 2.695 [B]	30.295 ± 5.469
		5.239–11.751	10.959–18.557	23.489–36.563

*Different letters express statistically significant differences ($P < 0.05$) between genders in heavy metal concentrations in every organ (A > B)

ND not detected

of tissue or cells under a microscope. In this study, bioaccumulation of heavy metals and the effects of the iron ore mine on individuals of Persian jirds were examined. During the study of kidney and liver samples under a light microscope, lesions were observed. The most commonly observed pathological changes in the kidney of

Table 4 Concentrations of metals, expressed as mean ± standard deviation (SD) (μg g^{-1} dry weight) in organs of different age of *M. persicus*

Metal	Age	Organs		
		Muscle	Kidney	Liver
Cd	Adult	0.0274 ± 0.006	0.080 ± 0.02156	*0.034 ± 0.006 A
		0.021–0.04	0.049–0.112	0.025–0.044
	Juvenile	0.0277 ± 0.004	0.05975 ± 0.02061	0.028 ± 0.004 B
		0.02–0.034	0.03–0.084	0.023–0.034
Co	Adult	0.037 ± 0.030	0.1002 ± 0.072	0.0577 ± 0.041
		0.005–0.078	0.024–0.245	0.008–0.118
	Juvenile	0.042 ± 0.031	0.066 ± 0.046	0.028 ± 0.020
		0.012–0.074	0.023–0.146	0.004–0.058
Cr	Adult	17.506 ± 4.731	33.451 ± 7.664	18.679 ± 5.111
		9.148–25.539	24.929–45.802	11.149–26.972
	Juvenile	15.565 ± 4.755	28.011 ± 7.496	16.786 ± 5.273
		10.819–22.129	15.251–35.948	10.789–25.043
Cu	Adult	1.575 ± 0.1997 A	4.7490 ± 0.246342	6.0149 ± 0.8531 A
		1.277–1.891	3.783–6.102	4.423–7.325
	Juvenile	1.240 ± 0.342 B	4.635 ± 0.411	4.760 ± 1.011 B
		0.656–1.648	2.914–6.692	3.25–6.602
Fe	Adult	93.052 ± 28.498	206.936 ± 46.245	246.619 ± 90.363
		38.158–141.901	124.653–263.283	141.628–422.363
	Juvenile	79.732 ± 24.454	204.233 ± 86.715	215.119 ± 82.816
		40.603–115.396	81.54–330.991	142.062–372.422
Mo	Adult	0.052 ± 0.024	0.692 ± 0.168	1.212 ± 0.230
		0.023–0.087	0.473–0.964	0.749–1.679
	Juvenile	0.042 ± 0.029	0.614 ± 0.269	1.108 ± 0.155
		0.006–0.224	0.390–0.777	0.929–1.325
Ni	Adult	6.785 ± 1.166	13.098 ± 3.367	7.185 ± 1.522
		5.576–8.960	8.482–18.216	4.564–9.138
	Juvenile	6.706 ± 2.425	13.868 ± 5.889	6.452 ± 1.237
		4.229–11.514	6.700–25.690	4.781–8.392
Pb	Adult	ND	ND	ND
		ND	ND	ND
	Juvenile	ND	ND	ND
		ND	ND	ND
Sb	Adult	0.116 ± 0.033	0.285 ± 0.178	0.103 ± 0.035
		0.076–0.176	0.123–0.707	0.064–0.171
	Juvenile	0.091 ± 0.026	0.312 ± 0.143	0.072 ± 0.019
		0.044–0.127	0.161–0.500	0.043–0.099
Zn	Adult	9.828 ± 1.961	18.027 ± 4.490	28.458 ± 6.133
		7.587–13.166	10.959–24.624	19.453–36.001
	Juvenile	8.070 ± 2.670	15.133 ± 1.858	29.1832 ± 5.001
		5.239–12.417	12.281–17.012	23.489–36.563

*Different letters express statistically significant differences ($P < 0.05$) between ages in heavy metal concentrations in every organ (A > B)

ND not detected

M. persicus collected from mining area included hyperemia and hemorrhage, degeneration of tubular epithelial cells, necrosis, the presence of hyaline casts, and in one case, mononuclear leukocyte infiltration. Also, hyperemia and congestion, hemorrhage, necrosis, and vacuolization of hepatocytes were observed in liver samples (Fig. 2).

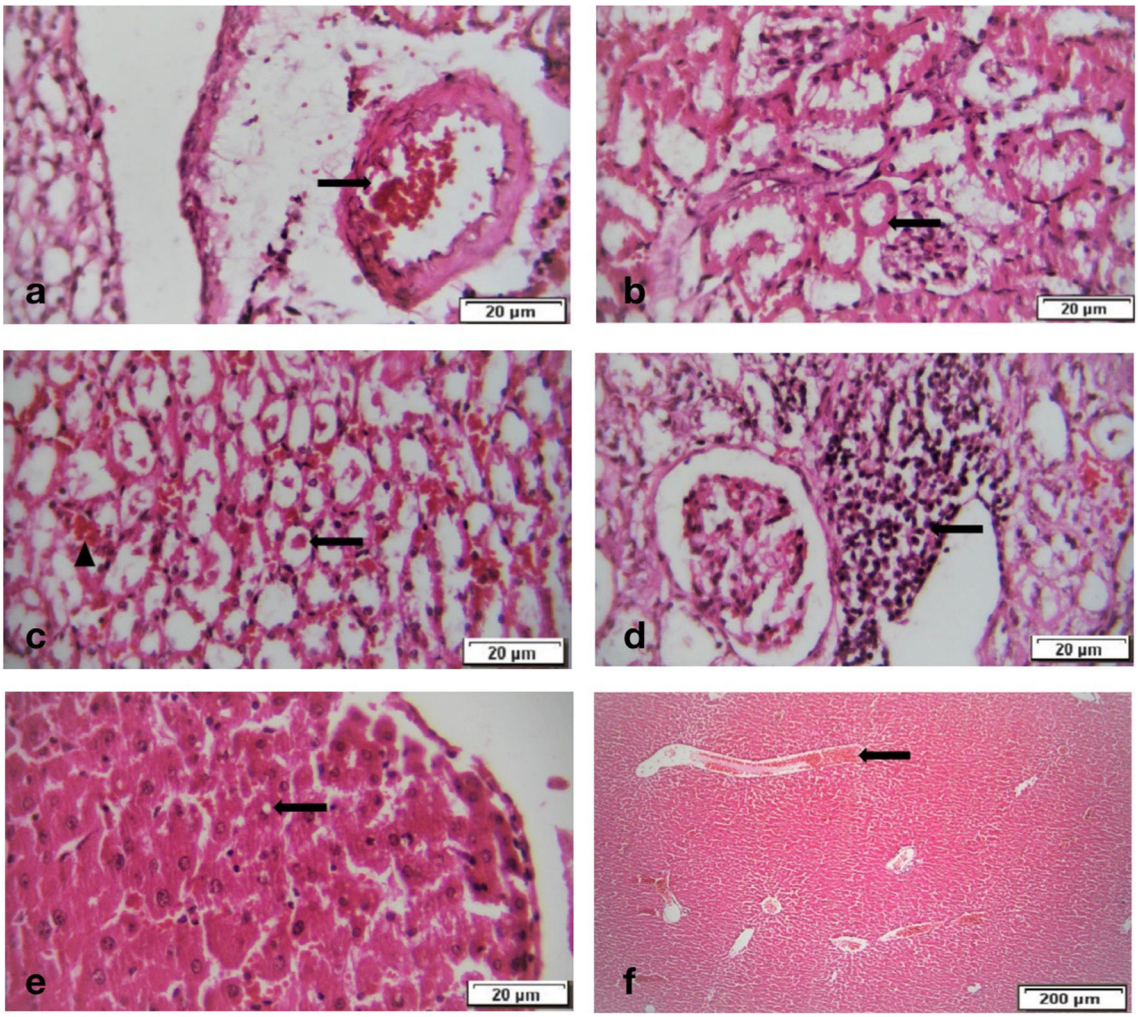

Fig. 2 Photomicrographs of kidney (**a**–**d**) and liver (**e**, **f**) histological structure in Persian jirds collected from SIOM. **a** Hyperemia (black arrow) in an artery. **b** Necrosis of epithelial cells in renal tubules (black arrow). **c** Hemorrhage (head pointer) and hyaline cast (pointer) in renal tubules. **d** Infiltration of mononuclear leukocytes in interstitial tissue. **e** Hepatocyte vacuolization (black arrow). **f** Hyperemia and congestion (black arrow); hematoxylin and eosin staining

Severity of lesions in kidney and liver tissues and frequency of observation is shown in Table 5.

Discussion

Heavy metal concentrations in different organs of *M. persicus*

Persian jirds trapped in the mining area accumulated high levels of Fe, Cr, Ni, Mo, Co, Cu, Zn, Cd, and Sb in their bodies. Although several researchers have assessed the exposure to pollutants in wild animals, e.g., experiments by Ma et al. (1991), Salamat et al. (2014), Sánchez-Chardi et al. (2007a), and Marques et al. (2007), there is little information on exposure to heavy metals for Persian jirds. In many studies, high concentrations of heavy metals have been reported in wild mammals living in contaminated areas. Under normal conditions, the concentration of heavy metals in small mammals follows a certain pattern (Fe > Ni > Cu), with some variation depending on the lifestyle of the species (Sumbera et al. 2003; Damek-Poprawa and Sawicka-Kapusta 2004; Schleich et al. 2010; Khazaee et al. 2015). This observation is consistent with the results of our study. Regardless of the presence of heavy metals in diet or the environment, heavy metal concentration in animal tissues depends on several factors such as age, physiological mechanisms to decrease toxicity, reaction between elements, function and half-life of metals in soft tissues, time of exposure, and individual hemostatic mechanisms (Alonso et al. 2002; Sánchez-Chardi et al. 2007c; Schleich et al. 2010; Khazaee et al. 2015). In our study, the accumulation of most metals follows the following order: liver > kidney > muscle. As a general rule, elemental turnover rates are higher in liver and kidney than muscle tissue (Kojadinovic et al. 2007). Based on the results of many studies, the liver is the main target organ for several elements (Beardsley et al.

Table 5 Frequency of observation for each grade of lesion severity in liver and kidney tissue (shown as percentages)

Severity (grades)	Kidney					Liver		
	Hyperemia	Hemorrhage	Degeneration and necrosis	Hyaline cast	Inflammation	Hyperemia and congestion	hepatocyte vacuolization and necrosis	Hemorrhage
−	33.3	33.3	55.5	44.4	88.8	44.4	88.8	72.2
+	27.7	33.3	27.7	33.3	0	44.4	0	27.7
++	22.2	16.6	11.1	16.6	11.1	11.1	11.1	0
+++	16.6	16.6	5.5	5.5	0	0	0	0

1978; Alonso et al. 2002; Pereira et al. 2006). In our study, the liver of Persian jirds was the main target organ for most of the elements (Fe, Cu, Mo, and Zn), and the concentration of these elements in the liver is significantly higher than in kidney and muscle tissue ($p < 0.001$). According to our results, the average concentration of Fe, Cu, Zn, and Mo in liver samples was 232.62, 5.45, 1.16, and 28.78 μg g^{-1} dry weight, respectively. Compared to other metals, Fe is present in the internal organs of Persian jirds in higher concentrations. This may be due to soil and plant pollution with Fe as a result of emissions from iron mining activity in Sangan. Fe, Cu, and Zn are essential elements for normal growth and function of the animal body (Wintz et al. 2002; Nagajyoti et al. 2010). High levels of different essential elements, particularly Fe, in the livers of Persian jirds in a mining area have also been reported by Khazaee et al. (2015), consistent with the role of this organ in hemostasis. Also, the highest levels of Fe, Cu, and Zn were recorded in the livers of Persian jirds from our study area, similar to the results reported by Lopes et al. (2002) and Pereira et al. (2006) for the Algerian mice (*Mus spretus Lataste*(and by Damek-Poprawa and Sawicka-Kapusta (2004) for bank voles. The difference in the concentration of heavy metals in the hepatic tissue compared with other tissues is the result of various regulating mechanisms. The liver is the most important detoxification organ and plays a vital role in food conversion, biotransformation of xenobiotic molecules, and vitellogenesis for reproductive purposes (Sánchez-Chardi et al. 2009). Bioavailability of Cu is also influenced by the presence of other metals. For example, Zn, Mo, and some other metals reduce the absorption of Cu, while some amino acids increase its absorption (EPA 1997). In addition, Cu and Zn participate in detoxification mechanisms in the body, as well as being parts of the antioxidant enzymes that prevent the formation of active oxygen species, which can be one of the reasons behind the presence of significant amounts of Zn and Cu in the liver (Okati and Rezaee 2013).

Our study showed that the highest concentration of Cd was found in kidney, followed by liver, and muscle. In other studies, both the kidneys and liver are similarly reported as the main target organs for Cd accumulation (Christopher 1986; Pereira et al. 2006; Leita et al. 1991; Talmage and Walton 1991; Alonso et al. 2002; Zarrintab and Mirzaei 2017). Also, in other vertebrates such as birds, mammals, and turtles, kidneys are the main target organ for Cd accumulation (Furness et al. 1993; Storelli et al. 2005; Kojadinovic et al. 2007).

Our results showed the highest level of Ni in kidney samples, followed by liver, and muscle samples. Compared to Cd, information on the concentration of Ni in the liver is scarce, especially for Persian jirds. In contaminated areas, the levels of nickel were 0.00–0.64 μg g^{-1} dry weight in common shrews (*Sorex araneus*), 3.39 μg g^{-1} dry weight in pygmy shrews (*Sorex minutus*), and 0.13–0.25 μg g^{-1} dry weight in moles (*Talpa europaea*) (Pankakoski et al. 1993; 1994). In our study, the average concentration of Ni in the liver of Persian jirds was 6.84 μg g^{-1} dry weight, which is relatively high. Nickel is a ubiquitous element, easily transferred throughout the food chain (Marques et al. 2007). Inhalation is one of the major routes of entry for Ni and Cr (ATSDR 1997). The most important adverse health effects of exposure to nickel in humans include pulmonary fibrosis, lung cancer, and severe renal damage (Kong et al. 2014; ATSDR 1997).

Cr had one of the highest concentrations in hepatic, renal, and muscle tissue of Persian jirds in SIOM. This result shows increased bioavailability of this metal in SIOM. The average Cd concentration in hepatic, renal, and muscle tissue of Persian jirds was 0.071, 0.033, and 0.0268 μg g^{-1} dry weight, respectively. In blood, Cd is transmitted through red blood cells and proteins with high molecular weights, such as albumin. Cd is widely distributed in the body but mainly accumulates in the kidneys and liver (Goyer 1991). The kidneys are key organs for detoxification of water-soluble metabolites, including metals and xenobiotics, due to the strong interaction between the kidneys and blood (García-Sevillano et al. 2014).

Exposure in mining areas may occur via a variety of pathways, including inhalation of dust in the air, ingestion of contaminated water or soil, or via the food chain (Khazaee et al. 2015). In mammals, the main route of exposure to contaminants is through diet. Rogival et al. (2007) report that non-essential metals are transferred through the food chain more than essential metals. Previous studies have reported that there is a positive relationship between the concentration of heavy

metals in plants and the concentration of metals in tissues of mammalian species (Hunter et al. 1989; Ma et al. 1991; Mažeikytė and Balčiauskas 2003; Sánchez-Chardi et al. 2009). As mentioned earlier, leaves, seeds, and other plant parts are eaten by Persian jirds (Ziaie 2008). Consequently, Persian jirds can be exposed through feeding metal-contaminated seeds, leaves, and green vegetation. Also, a complementary pathway for the acquisition of pollutants in Persian jirds is the accidental ingestion of sediment particles with food. When cadmium is ingested, the target organs are the kidneys (Goyer 1991). The concentration of heavy metals in various tissues of animals is directly proportional to the heavy metal content of their food. However, several other studies suggested that concentrations of certain metals in mammalian tissues are following the concentration metals in the soil (e.g., Zarrintab and Mirzaei 2017; Beernaert et al. 2007; McLean et al. 2009); it should be noted that this relationship is not always observed (e.g., Komarnicki 2000).

Heavy metals are significant contaminants in soil environments. Human activities have caused a considerable increase in the release of these metals into soils (Selim and Sparks 2001). In the study area, a positive relationship was demonstrated between the concentration of the majority of metals (with the exception of Cd and Pb) in soil and their levels in *M. persicus* tissues. Sangan soils are contaminated, showing high levels of Fe, Sn, Co, Cu, Sb, S, and Cd (Dabiri et al. 2017). In our study area, heavy metals (except for Cd) were observed in the soil (Table 2). Although Cd concentration in the soil sample was below the limit of detection, it was observed in *M. persicus* tissue samples. The occurrence of Fe, Co, Cu, Sb, and Cd is predominately related to anthropogenic sources and mining activities, whereas Pb, Cr, and Zn are mainly released by geological processes in the area (Dabiri et al. 2017). Fe, Cr, Ni, Zn, and Cu levels in animal tissues reflected the Fe, Cr, Ni, Zn, and Cu concentrations that were measured in the soil. Presence of Pb was clearly observed in soil samples but was not observed in liver, kidney, or muscle samples. Detection of low levels of lead in tissues of Persian jirds can be explained by low bioavailability, which could be due to the low mobility of Pb in less acidic soils (Kraus and Wiegand 2006). It should be noted that more than 90% of total body burden of Pb accumulates in bones, with an average half-life of about 10 years (Vahter et al. 2007; Talmage and Walton 1991). SIOM has alkaline soil with a pH greater than 8 (Table 2). Low Mo and Sb concentration in the liver and kidneys of Persian Jirds and their low concentrations in muscle may reflect the low concentration of these metals in soil. Comparison of heavy metal concentrations in soil and organisms can provide insight into the movement of elements in the environment, their accumulation, and their potential toxicological impact (García-Sevillano et al. 2014).

Metal bioaccumulation by sex

The results of our study indicated that sex affects the average concentration of some heavy metals in Persian jirds (*M. persicus*). Sex differences could have a significant impact on uptake and accumulation of metals in various internal organs (Beernaert et al. 2007). Generally, female Persian jirds showed higher levels of essential elements in their livers compared to males, while the concentrations of all heavy metals in the kidneys of males tended to be higher, except for cadmium. In females, the levels of Cd in kidney, liver, and muscle samples were higher. In contrast, the results showed that males had significantly higher levels of Zn in their kidneys ($p < 0.05$).

Many recent studies have investigated the effect of sex on bioaccumulation of metals, reporting various and sometimes contradictory results. For example, Beernaert et al. (2007) reported that male wood mice showed a higher Cd concentration in their kidneys, compared to females. Also, Zarrintab and Mirzaei (2017) showed that male rats had higher levels Cd in their liver. Pankakoski et al. (1993) reported that the adult females of *Talpa europaea* had lower levels of Cu in their livers, while Sánchez-Chardi et al. (2007c) stated that there was no notable difference between sexes in samples from wood mice with respect to the concentration of heavy metals. Also, Cooke et al. (1990) did not observe any differences between male and female wood mice (*Apodemus sylvaticus* L.) in levels of Zn in the liver and kidneys.

There are several factors that lead to the different concentrations of essential elements between sexes. These factors include different nutritional requirements, interactions between elements, different metal dynamics, intake or uptake rate of elements, the activity of sex hormones, and the metabolic profile of elements (Sánchez-Chardi et al. 2009; Millán et al. 2008; Khazaee et al. 2015). The sex with a higher metabolic rate would be more susceptible to chemical-induced toxicity. One of the main reasons that may be considered for the differences reported is hormonal effects due to higher levels of xenobiotic metabolism, which may be the result of the effects of female sex hormones (Mugford and Kedderis 1998). In Iran, the female *M. persicus* usually has three litters per annum, each with about seven pups. Therefore, females consume more energy and have a higher metabolic rate to support reproduction and offspring care (Ziaie 2008; Khazaee et al. 2015).

Metal bioaccumulation by age

Age can be considered analogous to the duration of exposure of living organisms to a pollutant in the environment. Some metals tend to accumulate in tissues over time (Gonzalez et al. 2008; Mar'quez-Ferrando et al. 2009). In the present study, in most of the samples, concentrations of metals from adult Persian jirds tend to increase with age. Our results revealed

differences between the two age categories in the levels of Cd, Co, Cr, Cu, Fe, Mo, and Zn in renal tissue samples and Cd, Co, Cr, Cu, Fe, Mo, Sb, and Ni in hepatic tissue samples. The amount of accumulated essential elements that are physiologically well-regulated in mammals tends to increase with age (Table 4). The concentration of metals is more closely related to the metabolic requirements of adults and interferences with non-essential elements (Goyer 1997; Bellés et al. 2002). Age-dependent bioaccumulation of Cd is related to detoxification mechanisms, namely the formation of cadmium-metallothionein (Cd-MT) in hepatic tissue, which is transported through blood and then stored mainly in renal cortex (Bonilla-Valverde et al. 2004; Sánchez-Chardi et al. 2009).

Several authors have reported an age-dependent variation of heavy metal concentrations in rodents. High concentrations of Fe, Cu, and Zn in the liver and some other organs of mammals have been found in several contaminated areas (Sánchez-Chardi et al. 2007d; Sánchez-Chardi et al. 2009). As stated by Sánchez-Chardi et al. (2007d), concentrations of Fe, Mo, and Cu in tissue samples from shrews living in polluted and unpolluted areas show age-dependent patterns. Based on the results of our study, compared to adults, juveniles exhibited a higher concentration of Ni and Sb in their kidneys, higher levels of Zn in their livers, and higher concentrations of Cd in their muscle. Higher concentrations of Ni and Sb in the kidneys of juvenile Persian jirds may be related to high intake and incorporation during their growth period. Furthermore, greater energy requirements of juveniles, implying higher uptake of food, may explain increased Zn levels observed in the livers of these specimens.

Histopathological study

Chemical analyses of heavy metals alone do not provide sufficient information on their environmental risk. Because absolute heavy metal concentrations do not reflect the degree of injury affecting organs and tissues, histopathological studies could be one of the fundamental techniques which can assist in recognizing target organs of heavy metal toxicity. This method may also be utilized to determine mechanisms of action for human risk assessment using rodents (Wester et al. 2002).

Kidney

The present study provides evidence that exposure to heavy metals due to mining activities caused toxicity in Persian jirds. Histopathological examination showed hyperemia, hemorrhage, degeneration of tubular epithelial cells, necrosis, presence of hyaline casts, and in one case mononuclear leukocyte infiltration in the kidney samples obtained from *M. persicus*. The occurrence of necrosis and apoptosis in kidney samples from individuals chronically exposed to high concentrations of Cd, Fe, Cr, and Co corroborates the effects reported in the laboratory. Moreover, the nuclear alterations observed in this study are further evidence that the uptake and high bioaccumulation of toxic heavy metals in the kidneys of *M. persicus*. Cadmium concentration in the liver and kidney samples from the Persian jirds which were exposed to this metal, as well as other metals, causes significant changes in the histology and functioning of the liver and the kidneys. Cd accumulates in the liver through binding to metallothionein (a Cd-inducible protein). Then, these low molecular weight proteins are transported to the kidneys, Cd's final and main storage organ, where it causes serious damage (Järup et al. 1998; Bonilla-Valverde et al. 2004; Nordberg et al. 2007; Bernard 2008). Therefore, early histopathological changes can be observed in this organ (S'wiergosz-Kowalewska 2001). Prolonged exposure to airborne cadmium initially affects the kidneys, which can cause renal tubular proteininosis (Goyer 1991). Cadmium can be stored for a long time in renal tissue, which can lead to tubular necrosis (Orlowski and Piotrowski 2003; Rehman et al. 2018). Kidney glomerulus then filters the Cd-MT complex, which is finally reabsorbed into the proximal tubules. The stored amount of cadmium in renal tubules increases steadily in organisms chronically exposed to Cd, especially in those living in polluted areas (Rehman et al. 2018).

Average Cd level in kidney samples was 0.071 μg g^{-1} dry weight. Several studies on rodents have shown that accumulation of cadmium can cause renal tissue damage. The concentrations of 350, 105, and 56.7 μg g^{-1} dry weight of cadmium (lowest observable adverse effect level) cause kidney tubular dysfunction in rodents and other small mammals (Shore and Douben 1994; Sánchez-Chardi and Nadal 2007; Zarrintab and Mirzaei 2017). The concentration of 110–260 μg g^{-1} dry weight of cadmium can have nephrotoxic effects in mice. Also in another study, it was found that in bank voles, a cadmium concentration of 15.1 μg g^{-1} in the liver and 17.0 μg g^{-1} in the kidney can cause tissue damage (*Clethrionomys glareolus*) (Wlostowski et al. 2003). While, in another study by Friberg (1950), rabbits developed proteinuria after a 4-month inhalation exposure to cadmium dust at 4 mg/m^3 (0.004 μg g^{-1} dry weight) for 3 h/day, 21 days/month, histologic lesions were only observed after an extra 3–4 months of exposure. Friberg (1950) reported that a 4-h exposure to cadmium dust at 28.4 mg Cd/m^3 (0.0284 μg g^{-1} dry weight) resulted in the death of rabbits. Barrett et al. (1947) also reported LC_{50} values of 46.7 mg/m^3 (0.0467 μg g^{-1} dry weight) for cadmium for a 15-min exposure in mice. Gumbleton (1988) reported that hexavalent chromium (Cr^{+6}) can cause renal damage in rats.

Liver

The liver is one of the primary organs exposed to toxic metals if these metals enter the body through inhalation or ingestion

(Pereira et al. 2006; Williams and Iatropoulos 2002; Haschek and Rousseaux 1998). The most common pathological changes observed in the livers of *M. persicus* collected from the mining area included hemorrhage and hepatocyte vacuolation. Hepatocyte necrosis is a common finding after exposure to chemicals. Hepatocyte vacuolization is indirectly linked to inhibited protein synthesis, cell discharge, and microtubule isolation. Large vacuole in hepatocytes can move the nucleus to the margins of the cell (Salamat et al. 2014).

As mentioned above, the highest concentrations of Cd were recorded in kidney and liver samples, and the lowest concentration was observed in muscle tissue. The Cd concentration (0.033 μg g^{-1} DW) observed in Persian jirds from the mining site caused hepatic damage to specimens, but the concentration was much lower than the concentration that had led to hepatic changes in bank voles (15.1 μg g^{-1} DW) (Wlostowski et al. 2003). Also, this concentration (0.033 μg g^{-1} DW) is far below 20.32 μg g^{-1} (lowest observable adverse effect level) for cadmium in rodents (Sánchez-Chardi and Nadal 2007). Cadmium and zinc have similar oxidation steps (Świergosz-Kowalewska et al. 2005; Szyczewski et al. 2009; Jaishankar et al. 2014). Therefore, Cd can replace Zn in metallothionein, thus preventing its action in protecting the cell against free radicals. Cadmium concentration can increase up to 3000 times when Cd is bound to a cysteine-rich protein such as metallothionein. In the liver, the synthesis of cysteine-pholythionin causes liver toxicity which later passes through the bloodstream to the kidneys and accumulates there, causing nephrotoxicity (Jaishankar et al. 2014).

Based on results of a study by Brzóska et al. (2003), after rats were exposed to 50 mg/L of cadmium, the trabecular structure of the liver was seriously affected within 12 weeks. Cytoplasm of the liver cells was bright, foamy, and full of vacuoles, and cells were large. In general, the critical concentration of Cd in the liver is 20–30 μg g^{-1} dry weight in mammals (Marques et al. 2007). Zinc exhibits the same pattern as other essential metals: the highest values were observed in the liver and the kidneys and lowest values were observed in muscle tissues. The critical point for zinc concentration in the kidneys and liver for mammals is 465 and 274 μg g^{-1} dry weight, respectively (Świergosz-Kowalewska et al. 2005). In the present study, Zn concentration (28.78 μg g^{-1} DW) in the liver of Persian jirds is less than the reported average. As mentioned earlier, among all the metals, the highest concentration belonged to Fe in liver samples (232.619 μg g^{-1} DW). When absorbed iron is not attached to a protein, it can significantly affect iron concentrations in cells and biological fluids. In high concentrations, iron can form a broad range of harmful free radicals. These free radicals penetrate into cells in the liver, heart, and brain (Jaishankar et al. 2014).

In Persian jirds, the concentration of molybdenum in liver tissue was higher than kidney and muscle samples. Studies on rats showed that copper, especially dietary copper, can help with the detoxification of molybdenum and possibly reduce its toxicity. Exposure to high concentrations of copper reduces molybdenum toxicity. In lab animals, the liver does not appear to be sensitive to molybdenum toxicity. The information available from laboratory animals indicates that the kidneys are a target organ in molybdenum toxicity (ATSDR 2015). Similar to heavy metal accumulation damage in rodent tissues, health of other animals in the habitat, especially predators of rodents, as well as the people who are living in cities and villages around the iron ore mine is threatened by heavy metal pollution.

Conclusion

The iron ore mine in Sangan is a significant source of heavy metals. Mining activities increase the concentration of iron, nickel, cadmium, copper, zinc, and chromium in the tissues of Persian jirds. In light of the low Pb levels in Persian jirds, it appears that iron mining activity in Sangan has no measurable effect on the selected tissues regarding Pb levels. We concluded that the difference in metal concentrations in Persian jirds depends on the tissue being analyzed, as well as the sex and age of the animal. The levels of most metals (except for Pb and Cd) in soil were found to correlate positively with the levels found in the bodies of Persian jirds. The results showed that the concentration of most essential and non-essential metals in the liver and kidneys are significantly higher than in muscle tissue. Therefore, the kidneys and liver are useful indicator organs to measure the concentration of heavy metals. The results indicated that heavy metal pollution from mining activity causes damage to the liver and kidneys. The Persian jirds has proved to be a strong bioaccumulator of essential and non-essential metals, so this species can be an appropriate species for monitoring this type of contamination, accurately reflecting the presence and quantity of heavy metals in a polluted area. However, given the severity of iron ore mining activity, a continuous monitoring scheme is necessary. Monitoring environmental pollution through wildlife is critical to assessing the quality of the environment and improving our knowledge regarding the capacity of natural responders for contamination.

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