ACCUMULATION OF HEAVY METAL POLLUTANTS IN THE COMPONENTS OF PETROLEUM POLLUTED ARID ECOSYSTEM

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ABSTRACT

The coast of Arabian Gulf is considered among the highest oil impacted regions in Saudi Arabia. Heavy metals contamination in coastal and marine environments is becoming an increasingly serious threat to both the naturally stressed marine ecosystems and humans relying on marine resources for food, industry and recreation. The heavy metal concentration in soil, plants and rodents were measured. The bioaccumulation of five heavy metals, *viz*., sulfur (S), vanadium (V), nickel (Ni), cadmium (Cd) and lead (Pb) in the soil, plant, the wild Libyan Jird *Meriones libycus* and the wield mouse *Mus musclus* were estimated. In the jird and mouse, the heavy metals were measured in fur, liver, kidney. The results showed that the bioaccumulation in polluted sites reached five times that in the reference unpolluted site. The mean values of the liver enzymes activities increased in the animals collected from contaminated sites. The bioaccumulation of heavy metals decreases the reduced glutathione (GSH) and increases the lipid peroxidation and nitric oxide (NO), and produced histopathological changes in liver and kidneys of jird and mouse in the polluted sites.

Keywords: Soil, Plants, *Meriones libycus*, *Mus musclus*, Liver, Kidney, Fur

1. Introduction

The emission of pollutants to the environment from the petroleum industries has been considerably affecting the flora and fauna which accumulate large amounts of associated heavy metals (Grzesiak and Sieradzki, 2000). Marine and terrestrial pollution leads to dispersion of heavy metals, which circulate in trophic chains and accumulate in bodies of living organisms (Merian, 1991). The heavy metals V, Ni, Cd and Pb do not play a functional role in the organism's metabolism, however, some metals like sulfur are physiologically essential, but they may also alter the function of organisms when the exposure dose exceeds a critical threshold. Similarly, Nickel is considered as a metal with biological function in small concentrations and a toxic metal at high concentrations. The influence of heavy metals is species-specific, and depends on age, sex, reproductive state and physiological condition of the organism (Shore and Rattner, 2001). Chronic exposure of animals to heavy metals causes renal dysfunction (Nolan and Shaikh, 1992), liver damage (BolognaniFantin *et al*., 1992), and decreased fertility in males and increased spontaneous abortion in females (Friberg *et al*., 1986). The metal pollutants accumulate mainly in kidney, liver and affect the re-absorption functions of the proximal tubules (Roels *et al*., 1993).

Most data concerning the influence of heavy metals on the structure and function of organisms have been derived from laboratory experiments (Shore and Rattner, 2001). It has been suggested that one of the mechanisms involved in heavy metal toxicity is the induction of reactive oxygen species (ROS) (Ercal *et al*., 2001); highly reactive oxygen-containing molecules produced in oxidation–reduction reactions (Dowling and Simmons, 2009). This ROS formation results in metal-related oxidative stress, a state of imbalance between antioxidant defense and ROS production (Valavanidis *et al*., 2006; Halliwell and Gutteridge, 2007).

Previous studies on histopathological changes in tissues of rodents fed on a diet containing high amounts of lead and/or cadmium. Damages to the structure of the internal organs of the test rodents due to excessive accumulation of Pb and Cd in the animal tissues (Abu Tawell *et al*., 2013). Due to the scarcity of studies on heavy metal concentrations and cycling in terrestrial ecosystems in oil producing countries, particularly in the Middle East, this study aims at assessment of the accumulation of the five heavy metals V, Ni, Pb, Cd and S in soil, common wild plants and rodents in the eastern arid desert of Saudi Arabia.

2. Materials and methods

2.1. Samples collection

Total of 15 replicate samples for each of soil, the two common plant species *Launeae mucronata* (Forssk.) Muschl., from two polluted sites and *Rhazya stricta* Decne. from the reference site were collected in April and May 2014. Two common wild rodents, *Mus musculus* and Libyan Jird *Meriones libycus* were collected from the same sites. The two polluted sites are coastal site (MPS) in Abu Ali island subjected to marine oil pollution sources (located at N 27º18′54.8", E 49º38′05.6") and the second site located in the industrial zone of Al-Jubail (IPS) where pollution sources comes from oil industrialization activities (located at N 27º00′00.0", E 49º34′57.3"). The reference materials (RS) were collected from Rodaht Khoraim site about 80 km from Riyadh city (located at N 25º22′19.4", E 47º17′10.9"). By using rat traps, adult male wild mice and wild Libyan Jirds were collected and transferred to the lab for further investigations. The soil was collected from the top 20 cm of soil profile. The experimental protocols and investigations comply with the *Guide for Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996) and was approved by the Ethics Committee for Animal Experimentation at King Saud University (Permit Number: PT 983).

2.2. Determination of heavy metals

The analytical determination of the heavy metals V, Ni, Cd, P band S in soil, plant, liver, kidney and fur samples was carried out according to Shah *et al*. (2013) by ICP-MS (Inductively Coupled Plasma Mass Spectrometer): ELAN 9000 (Perkin Elmer Sciex Instruments, Concord, Ontario, Canada). For details of the biochemical assays see Allam *et al*. (2015).

2.3. Biochemical assays

The adult males of both mice and Jirds, 15 replicate samples each, were collected from the study sites and transferred to the laboratory in the same day for investigation (*Cf.* Allam *et al*. 2015). Blood was withdrawn from the heart after dissection. The liver, kidney and fur were extracted rapidly from the animals. About 0.5g of liver from each animal was homogenized in 5ml cold 0.1 M HClO4 containing 0.05% EDTA. The homogenate was centrifuged at 10,000rpm for 10 min at 4 °C and the clear supernatant collected in a microfuge tube (0.5ml each) and stored for one week at -40 °C until used. The rest of organs were rapidly preserved at -80 for further studies.

Lipid peroxidation was determined by assaying thiobarbituric acid-reactive substances (TBARS) according to the method of Preuss *et al*. (1998). GSH content was determined according to the procedure of Beutler *et al*. (1963). Nitric oxide (NO) activity was determined according to the method of Berkels *et al*. (2004).

Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP) activities were determined by monitoring the concentrations of pyruvate and oxaloacetate, respectively, according to the method of Reitman and Frankel (1957). Assays were performed using reagent kits from Diamond Diagnostics Chemical Company. Creatinine was determined by the method of (Bertine and Goldberg 1971)using assay kit from Scico Diagnostics Chemical Company. For details of the biochemical assays see Allam *et al*. (2015).

2.4. Histological preparations

For the histological preparations, liver and kidney of five animals of both mice and Jird were immediately extracted, cutting into small longitudinal sections and fixed in 10% phosphate buffer formalin for 24 hours. The tissues were washed to remove the excess fixative and then dehydrated in ascending grades (70, 80, 90 and 95%) of ethyl alcohol for 45 min each, then in two changes of absolute ethyl alcohol for 30 min each. This was followed by two changes of xylene for 30 min each. The tissues were then impregnated with paraplast plus (three changes) at 60 ºC for three hours and then embedded in paraplast plus. Sections (4 to 5 µm) were prepared with a microtome, de-waxed, hydrated and stained in Mayer's haemalum solution for 3 min. The sections were stained in Eosin for one min, washed in tap water and dehydrated in ethanol as described above according to the method of Mallory (1988).

The Statistical Package for the Social Sciences (SPSS for windows version 11.0; SPSS Inc, Chicago) was used for the statistical analyses. Comparative analyses were conducted by using the general linear models procedure (SPSS, Inc). Also, the data were analyzed using one-way and two-way analysis of variance (ANOVA) followed by LSD computations to compare the various groups. Results were expressed as mean±SD. The level of significance was expressed as significant at $P<0.05$ and highly significant at $P<0.01$.

2.5. Results and discussion

The mean concentrations of the most hazardous heavy metals Cd, Pb and Ni in liver and kidney of wild mouse and Jird collected from polluted sites attained significantly ($P<0.01$) higher values than those for animals collected from the reference unpolluted site. The highest values of Cd, Ni and Pb in liver and kidney were found in the Jirds collect from MPS which reached 51 ng/gm, 9738 ng/gm, 384 ng/gm in the liver and 233 ng/gm, 2493 ng/gm and 733 ng/gm in the kidney, respectively (Table 1). The elevation of V in the liver and kidney of mice collected from polluted sites was insignificant ($P>0.05$) while reached significant values ($P<0.05$) in Jirds liver and kidney higher than the animals in RS. The sulfur metal is essential element to the body but it is one of the most prominent petroleum pollutants (Grzesiak and Sieradzki, 2000). The significant ($P<0.001$) elevations of S in liver and kidneyof Jirds and mice collected from RS seems to be related to the high content of protein metabolism in their cells (Allam *et al*., 2010). The highest value of S was found in mice liver in RS that reached 29579 ng/gm (Table 1).The accumulation of the current hazardous metals in liver and kidney tissues is known to induce tissues and cells damage (Abu Tawell *et al*., 2013). The damage mechanisms may be due to the increased oxidative stress produced by heavy metals accumulations in the tissues (Faisal *et al*., 2013).

The results shown in Table 2 indicate the significant ($P<0.05$) reduction GSH and elevation of NO and TBARS in the liver of both wild Jirds and mice collected from MPS and IPS. Srivastava *et al*. (1983) have shown that enhancement of TBARS and NO are a consequence of glutathione (GSH) depletion to a certain critical levels. This delicate balance between the production and the catabolism of oxidants is critical for the maintenance of biological functions (Sridevi *et al*. 1998; Allam *et al*., 2010).Metals act as oxidizing agent, a reactive epoxide, and undergo conjugation reaction with GSH (Dybing and Sanner 2003). Metals also interacts with other vital cellular nucleophiles possessing –SH, –NH2 or –OH and forms glutathione-Conjugates, which is the initial step in the biotransformation of electrophiles into mercapturic acids (Awad *et al*. 1998). Glutathione is an important reducing agent

and cellular antioxidant in mammalian cells (Tong *et al*. 2004). The disturbance in oxidative stress may be produced from the accumulation of hazardous metals in the liver. The detected increase in the oxidative stress may induce tissue damages as appear in liver and kidney of the animals collected from the polluted sites (Fig 1).

The heavy metals concentration in the animal's fur is lower than the values detected in liver and kidney, except for S values which attained the highest values in the reference mice and Jirds (Table 1) The detected heavy metals in the fur reflect the animal's ability to get rid from excessive amounts of these metals.

The common plant species in the two polluted sites is *Launeae mucronata*, while in unpolluted site is *Rhazya stricta*. The concentration of the five heavy metals in *L. mucronata* is significantly higher ($P<0.001$) than the concentration in *R. stricta* (Table 1). Although the estimated high values of the metals concentration in the liver and kidney of Jirds in the polluted sites, it still lower than those in plant samples of the polluted sites. The lowest values of the metals concentration are found in soil samples from reference and polluted sites where the highest value of Cd, Pb, Ni and V are 1.5 ng/gm, 5.05 ng/gm, 92 ng/gm and 37.9 ng/gm in MPS, respectively.

The histological sections of liver and kidney of *M. libycus* and *M musclus* showed some abnormal changes in the animals collected from MPS and IPS sites. The changes appear more sever in the liver and kidney of *M. musclus* (Fig 1G-L) than in *M. libycus*, although the high accumulation of the hazardous metals is in *M. libycus* liver and kidney (Table 1). The liver distortions represented by the appearance of kuffer cells and chromatolysis in the hepatic lobules of *M. libycus* and hepatic cirrhosis in *M. musclus* while the distortions in the kidney are the degeneration of glomeruli (Fig 1). The histological changes in the liver and kidney of the animal collected from polluted sites may lead to failure in the organ functions (Abu Tawell *et al*., 2013). The beginning of liver function failure was representing in the elevation of liver enzymes in the blood (Table 3). The elevation of ALT and ALP in the serum of both animals may be evidence of hepatic cells degenerations due to the heavy metals accumulation (Bolognani Fantin *et al*., 1992). The increased serum ALT and ALP enzyme activities is reflected cellular damage that results in the release of proteins and enzymes into the circulatory fluid (e.g., serum) when cell membrane integrity is damaged as a result of toxemia (Rawi 1995; Kim and Mahan 2001). The elevated levels of these enzymes may be ascribed to the increased hepatic enzymes synthesis (Feilleux-Duche *et al*. 1994; Allam *et al*., 2010). The elevation of these clinically important aminotransferases has been reported in hepatic toxicity (Gad and Abd El- Twab 2006). Although the detected damage in the kidney glomeruli of the study animals in the two polluted sites, there is insignificant ($P>0.05$) increase in the creatinine level in the serum i.e., the functional glomeruli and kidney nephrons still regulating the kidney function.

In conclusion, the accumulation of the most hazardous prominent oil pollution heavy metals in the soil attained the least concentration followed by the plants which attained the highest concentration in the marine polluted site. As for animals the metals concentrations reached the highest values in Jirds kidney. The accumulation of the metals in liver and kidney increased the oxidative stress; produced the tissue damage, induced disturbance in organ functions and finally the organ failure where the pollution severity in marine contaminated site was associated by hepatic fibroses and glomeruli degeneration in the kidney of the collected mice and Jirds. The liver and kidney aberrations produced an elevation in ALT, AST activities and Creatinine in the animal blood.

Table 1: The mean concentration level of Cd, Pb, V, Ni and S (ng) in the liver, kidney, fur of *M.libycus* and *M. musculus* as well as plant and soil collected from reference site (RS), marine polluted site (MPS) and industrial polluted site (IPS).

	Cd			**Pb**			**V**			**Ni**			**S**		
	RS	MPS	IPS	RS	MPS	IPS	RS	MPS	IPS	RS	MPS	IPS	RS	MPS	**IPS**
Liver mouse	18.6 (2.63)	32.14** (6.30)	31.25** (11.65)	693 (85)	2253** (104)	2160** (155)	193 (4.66)	202 (41)	203 (36)	937 (103)	1758 ** (6.66)	1534** (208)	29579 (739)	14314** *(1311)	13994*** (926)
Liver Jird	17.7 (3.7)	51** (12)	44** (12.3)	668 (239)	9738** (453)	2282** (165)	104 (12)	384* (32)	211* (26)	731 (49)	2803** (426)	2118*** (212)	26566 (1125)	14309** *(1311)	23043** (2895)
Kidney mouse	46 (15)	136** (22)	127** (9.89)	1076 (125)	5874** (209)	3514* (148)	326 (50)	480 (53)	425 (42)	815 (32)	2692*** (555)	1508* (117)	26819 (374)	19901* (1470)	12229** (374)
Kidney Jird	99.4 (31.1)	233** (76.6)	202.4** (66.6)	852 (123)	2493* (418)	2050* (560)	301 (10.5)	733* (28.7)	472* (32.3)	749 (35.3)	4667** (301)	1816* (142)	26884 (698)	11912** (578)	21944 (3124)
Fur mouse	12.1 (2.16)	16.7 (0.64)	14.38 (3.76)	1596 (361)	3886* (410)	1958 (446)	285 (42)	497 (115)	346 (64)	1404 (127)	1695* (178)	1508*** (200)	51298 (1811)	20705** (1068)	19186** (750)
Fur Jird	9 (1.1)	26.5* (9.58)	24.7* (7.4)	834 (297)	9048* (473)	1154 (289)	355 (33)	734* (128)	403 (104)	1206 (108)	2859*** (195)	2230** (269)	46675 (2662)	30828** (2313)	32985* (4755)
Plant	9.20 (0.03)	228.1*** (7.07)	155.2*** (6.87)	507.1 (10.72)	2885*** (16.16)	2354** (17.7)	545 (6.93)	7843* **(21)	5992* **(44)	3390 (4.95)	3807*** (4.19)	2147*** (17.9)	61599 (70.7)	93585** *(18.5)	106726*** (77.5)
Soil	0.17 (0.05)	1.5* (0.474)	0.69** (0.051)	2.07 (0.44)	5.05 (0.28)	2.68 * (0.78)	16.2 (0.56)	92** (1.54)	86** (1.41)	7.75 (0.67)	37.9*** (2.135)	35.35*** (5.6)	1307 (70.37)	1880*** (96.9)	1901.4*** (18.67)

Data are expressed as a mean ±SE (N =15). Values significantly compared to the control animals; p*≤0.05, p**≤0.01and p***≤0.001. Plant sample in RS is *Rhazya stricta* in MPS and IPS *Launeae mucronata*

Table 2: The mean values of GSH, No and MDA in the testes of collected *M. libycus* and *M. musculus.*

	GSH (µg/gm)			NO (u/gm)			TBARS (nmol/gm)		
animal	RS	MPS	IPS	RS	MPS	IPS	RS	MPS	IPS
M. libcus	21.6 (5.4)	10.1* (1.1)	16.8* (1.5)	3.46 (0.5)	4.58* (0.3)	5.04* (0.3)	68.6 (6.9)	192* (29.3)	107* (24)
M. musculus	18.8 (4.2)	8.6* (1.9)	14.5* (2.4)	4.21 (0.8)	5.81* (1.2)	5.21* (1.1)	82 (13)	157* (26.5)	141* (19.9)

Data are expressed as a mean± SE (N =15). Values significantly compared to the control animals; p*≤0.05, p**≤0.01and p***≤0.001.

Table 3: shows the mean values of AST, ALP and creatinine in the serum of collected *M. libycus* and *M. musculus.*

	AST (50-90) u/l			ALP (60-130) u/l			Creatinine (0.2-0.8) mg/dl		
animal	RS	MPS	IPS	RS	MPS	IPS	RS	MPS	IPS
M. libcus	85 (12)	387** (42)	256* (23)	111 (18)	131 (21)	182* (22)	0.22 (0.02)	0.72 (0.21)	0.64 (0.09)
M. musculus	89 (15)	405** (39)	298* (27)	119 (18)	128 (32)	195* (29)	0.4 (0.04)	0.84 (0.23)	0.67 (0.08)

Data are expressed as a mean±SE (N =15). Values significantly compared to the control newborns; p*≤0.05, p**≤0.01and p***≤0.001.

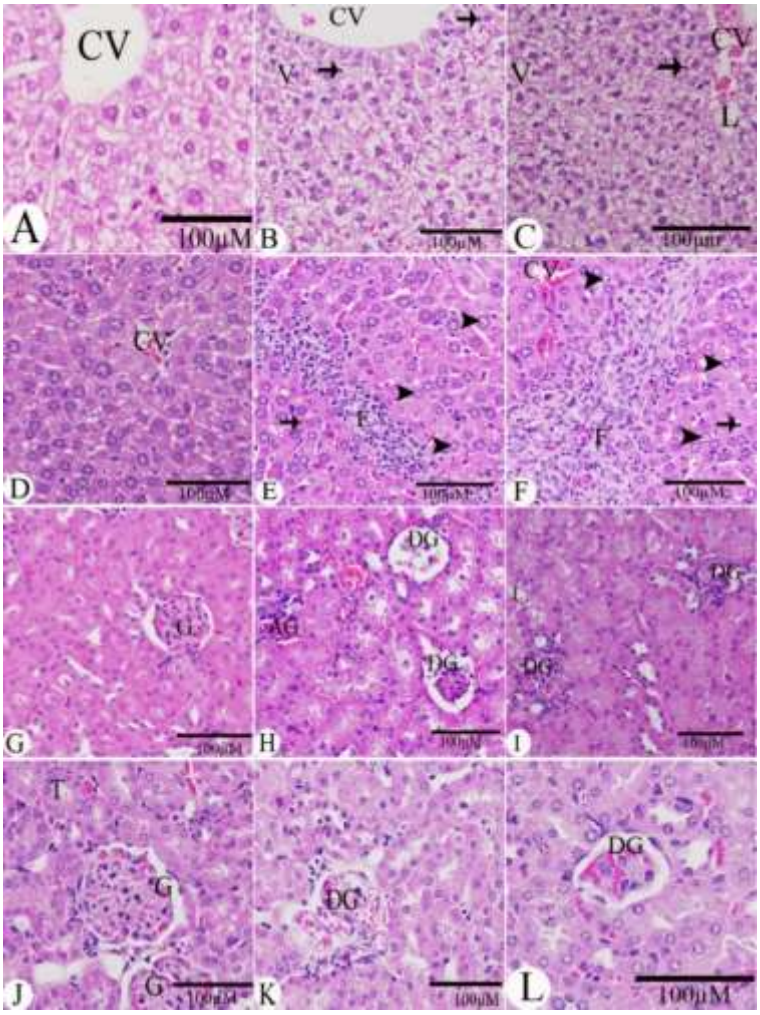

Figure 1: Sections of liver and kidney of collected animals show central vein (CV), lipid droplet (L) vacuoles (V), fibroses (F), chromatolysis (arrow head), kupffer cells (arrow), glomerulus (G) and damaged glomerulus (DG). The A, B and C liver sections of *M. libycus* collected from reference, marine polluted and industrial polluted sites, respectively. The D, E and F liver sections of *M. musculus* collected from reference, marine polluted and industrial polluted sites, respectively. The G, H and I kidney sections of *M. libycus* collected from reference, marine polluted and industrial polluted sites, respectively. The J, K and L kidney sections of *M. musculus* collected from reference, marine polluted and industrial polluted sites, respectively.

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