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Bioaccumulation and Ecological Risk Assessment of Heavy Metals in the Soil and Wild Rats in Riyadh, Saudi Arabia

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Abstract

The present study aimed to analyze the impact of environmental contamination on metal accumulation and oxidative stress in wild rats, *Rattus rattus*, collected from metal-polluted areas in the vicinity of Riyadh Region, Saudi Arabia.Soil samples and wild rats were collected from four locations (Dhurma, Kharj Road, Al Muzahmiyah and Laban Valley) that differ in their extent of pollution load. However, Laban Valley was taken as a reference site in this study . High concentrations of heavy metals (cadmium, lead and zinc) were recorded in soil and tissues (liver, kidney and hair) of animals collected from Dhurma , the site with high anthropogenic pressure. Moreover, a significant increase in the level of liver malondialdeyhde (MDA) coupled with an inhibition of the activity of superoxide dismutase (SOD), catalase (CAT), Glutathione Peroxidase(GPx), Glutathione Reductase (GR) and Glutathione-S-Transferase (GST) were recorded in Dhurma followed by Kharj Road and Al Muzahmiyah compared to the reference site.From these results , it could be concluded that that the selected biomarkers are useful for the assessment of pollution impacts in natural environments and the small wild animal *R. rattus* can be used as a bioindicator model for metal toxicity in an arid environment.

Keywords: Riyadh, *Rattus rattus*, Soil, Heavy metals, Pollution, Antioxidants, Oxidative stress.

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1. Introduction

The rapid growth of Riyadh, the capital city of Saudi Arabia, has created numerous environmental problems common to most cities worldwide [1]. The accelerated industrialization process in combination with the rapid population growth and agricultural activities have brought the risk of increasing the pollution index in natural environments such as water, soil, air, etc [2, 3]. For their multipurpose usage, persistence in the environment, bioaccumulation and high toxicity, heavy metals are considered as one of the most serious pollutants in the environment. Heavy metals are involved in various industrial processes, agricultural activities, domestic waste and vehicle emissions. On the other hand, heavy metals that originate from anthropogenic sources could be found in all components of the environment [4-6]. Heavy metal poisoning is the toxic accumulation of heavy metals in soft tissues. A metal's toxic effect manifested in an organ is mainly a function of concentration and exposure time, because many toxicants tend to bioaccumulate. When they occur at certain levels, even essential elements that are critical for life, may lead to loss of organ function or death [7]. Metals can appear in different forms in the environment and metal toxicity is related to their oxidative state and reactivity with other compounds [8, 9]. The literature is replete with the reports on metal induced oxidative stress that has been recentlyimplicated in the pathogenesis of metal toxicities. It is established that metals can generate reactive oxygen species (ROS) which in turn overwhelms the cell's innate antioxidant defenses leading to oxidative stress [10].

Oxidative stress is a condition where there is an imbalance between antioxidant defense and the production of reactive oxygen species, antioxidant is overcome by radical formation causing oxidative damage to biomolecules [11]. Indicators of oxidative stress include changes in antioxidant enzyme activity**,** damaged DNA bases, protein oxidation and lipid peroxidation products [12]. Antioxidants play a major role in maintaining good health and can prevent or slow the rate of oxidative damage (too much oxygen) to body. They act as scavengers, preventing and repairing damage done by free radicals, unstable molecules (having an unpaired electron) that are created as part of the normal metabolic process or from exposure to environmental toxins. Antioxidant system consists of enzymatic and non-enzymatic compounds [13, 14]. The most important antioxidant defense systems include antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GR) as well as nonenzymatic antioxidants (e.g. glutathione(GSH)), which have been extensively used as biomarkers of oxidative stress [15-19].In Saudi Arabia, bioaccumulation of metals via aquatic food webs has been extensively studied. Because parallel information on terrestrial systems is still lacking, we targeted here a local small mammal species [20] small mammals respond to larger areas because of their high mobility [21]. Therefore, rodents are usually used as indicators of pollution, with elements being determined in either whole body or in specific organs, generally the liver [22].The present work was carried out to investigate the pollutants levels including the content of some heavy metals in some polluted locations in the vicinity of Riyadh City and their accumulation in the organs of *Rattus rattus*. Also , this study aimed to reveal the oxidative stress response in the liver of *R.rattus* and to evaluate the appropriateness of employing *R.rattus* as a bioindicator model for metal toxicity in an arid environment.

2. Materials and Methods

2.1. Study Area and Sampling Locations

Riyadh lies in the center of the Arabian Peninsula at a latitude 34 - 38° north and longitude 46 – 43° east 600 m above sea level.

All sampling locations for this study are in the vicinity of Riyadh city.Samples were collected from four locations (Fig 1). The first sampling site is Dhurma, which is considered as the most polluted location. Dhurma Area is a small town located 60 kilometres by road northwest on Riyadh. It includes the central plant of marble stone, fiber glass and cement block. The second polluted sampling location is Kharj Road District. Key sources of pollution into this site are a petroleum refinery, a cement plant and vehicle maintenance workshops and road traffic. The third location is situated at Al Muzahmiyah area which is about 45 km West of Riyadh City. Al Muzahmiyah is located between Tuwaiq Mountains on the east and Nfood on the West. Despite being a province famous for agriculture, it includes the many factories such as cement block and plastic. The reference site issituatedamidst Laban Valley which is one of the main valleys in the City of Riyadh. It is located about 25 Km southwest of Riyadh City, far enough from most sources of polluting activities; hence was selected as a clean reference site.

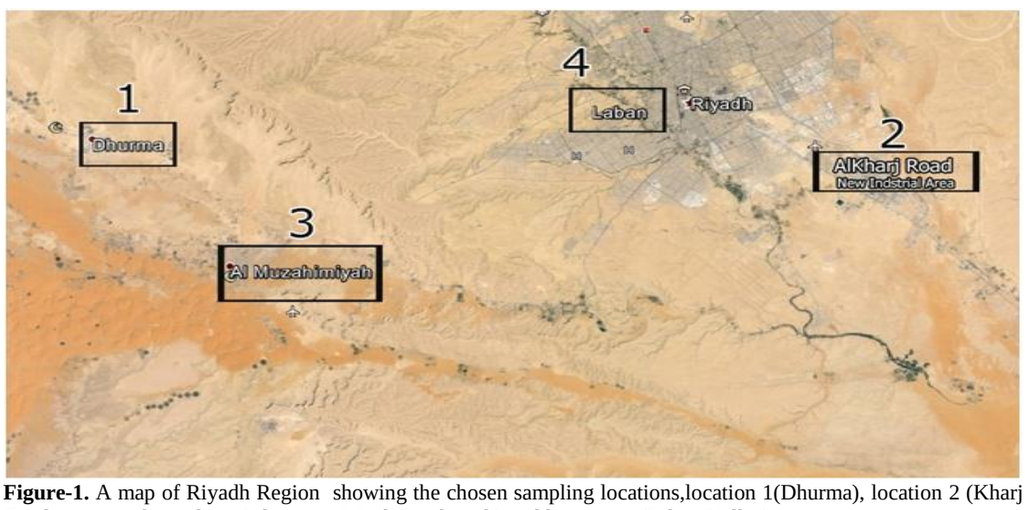

Figure-1. A map of Riyadh Region showing the chosen sampling locations,location 1(Dhurma), location 2 (Kharj Road in new industrial area), location 3 (AlMuzahmiyh) and location 4 (Laban Velley).

2.2. Sampling of Soil and Animals

Soil and rats *Rattus rattus* were collected from polluted and reference sites at three monthly intervals during a period extending from September to November 2012. From each sampling location, soil was randomly collected at 5 different spots for accuracy of the results. For each sample, the uppermost surface layer (2 cm down the soil surface) was carefully removed and used for metal analysis.

A total number of 45 adult live-trapped samples of rats, of almost uniform body length (12-18 cm) and weight (150- 250 g), were used for the present study. The trapped animals were then taken to the laboratory, where they were identified for maturity and general health status. They were then slightly anaesthetized , killed and the selected organs (liver, kidney and hair) were excised and stored in clean plastic containers at-20°C until later prepared for metal analysis.

2.3. Determination of Heavy Metals

Soil and animal tissues were digested according to USEPA Method 3052 [23] using a microwave (Ethos plus milestone, microwave laboratory systems, Italy).

Concentrations of lead, cadmium and zinc were determined in digested soil and animal tissues (liver, kidney and hair) using Varian AA-280 Zeeman atomic absorption spectrophotometer coupled to GTA-120 electrothermal atomizer and programmable sample dispenser (Varian Techtron Pty. Ltd., Australia). All metals were determined according to USEPA Method 2007 [24] under the recommended conditions and the detection limits (DL) in the manual for each metal; and all were expressed as µg /g dry weight.

2.4. Determination of Oxidative Stress Parameters

Tissue homogenates of liver from collected animals were prepared for determination of lipid peroxidation and antioxidant enzymes activities (Catalase, Superoxide dismutase, Glutathione reductase, Glutathione *S*-transferases and Glutathione peroxidase) in accordance with the protocol provided with the enzyme assay kits (Cayman Chemicals, USA).

Cayman's TBARS assay kit was used to measure lipid peroxidation (LPO) in terms of malondialdehyde (MDA) following the method of Ohkhawa, et al. [25].

Cayman's catalase assay kit was used to measure the Catalaze (CAT) activity according to the method of Cohen, et al. [26]. Glutathione Peroxidase(GPx)activity was estimated according to method of Forstrom, et al. [27]. The level of Glutathione Reductase (GR) was measured according to the method of Carlberg and Mannervik [28] Cayman'sGlutathione-S-TransferaseAssay Kit was used to measured GST activityfollowing the method of Morgenstern, et al. [29].

2.5. Statistical Analysis

Differences between obtained values (mean ± SEM, n = 5) were carried out by one-way analysis of variance (ANOVA) followed by the Tukey-Kramer multiple comparison test.

3. Results

Average concentrations of heavy metals in the soil are presented in Table 1. The concentration of metals in soil was found in the range of 0.014 - 0.072 µg/g for Cd, 0.224 - 2.66 µg/g for Pb and 0.712 -2.892 µg/g for Zn

Table-1. Concentrations (in micrograms per gram dry weight) of heavy metals in soil from locations 1-4.

Metals	Location 4 (LabanValley, Reference site)	Location 3 (Al Muzahmiyah)	Location 2 (Kharj Road)	Location 1 (Dhurma)
Cd	$0.014 \pm 0.002^{c.\ d.e}$	$0.04 \pm 0.003^{b,e}$	$0.043 \pm 0.005^{a,d}$	$0.072 \pm 0.007^{a,b,c}$
Pb	$0.224 \pm 0.067^{c,e}$	$0.896 \pm 0.142^{b,d}$	$1.788 \pm 0.339^{a,d,e}$	$2.66 \pm 0.195^{a,b,c}$
Zn	$0.712 \pm 0.099^{c,d}$	1.278 ± 0.243^{b}	$1.862 \pm 0.213^{a,d}$	$2.892 \pm 0.358^{a,b,c}$

Cd: cadmium, Pb: lead and Zn: zinc. Data are presented as means ± standard error
(n = 5). Different superscripts in the same row identify significant differences (LSD, $p < 0.05$) among sites.

Average concentrations of heavy metals in the liver of rats are presented in Table 2. Regardless of the site, the concentration of metals in rats was found in the range of 0.05 - 0.13 µg/g for Cd, 0.684- 2.534 µg/g for Pb and 3.07 - 7.17 µg/g for Zn. Average concentrations of heavy metals in the kidneys of rats are presented in Table 3. Regardless of the site, the concentration of metals in rats was found in the range of 0.053 - 0.156 µg/g for Cd, 0.62- 2.49 µg/g for Pb and 2.64 - 6.72 µg/g for Zn. Average concentrations of heavy metals in the hair of rats are presented in Table 4. Regardless of the site, the concentration of metals in rats was found in the range of 0.018 - 0.046 µg/g for Cd, 0.246 - 0.692 µg/g for Pb and 1.27- 2.93 µg/g for Zn.Metal accumulation among the different sites was ranked as follow: Dhurma (location 1) > the Kharj Road (location 2) > the Al Muzahmiyah (location 3) > the Laban Valley (location 4).

Table-2. Concentrations (in micrograms per gram dry weight) of heavy metals in liver of rats from the four sampling sites.

Metals	Location 4 (LabanValley, Reference site)	Location 3 (Al Muzahmiyah)	Location 2 (Kharj Road)	Location 1 (Dhurma)
Cd	$0.05 \pm 0.005^{d,c}$	0.07 ± 0.005^{b}	$0.08 \pm 0.06^{a,d}$	$0.13 \pm 0.007^{a,b,c}$
Pb	$0.684 \pm 0.11^{c,e,f}$	$1.342 \pm 0.13^{b,d,f}$	$1.945 \pm 0.16^{a,d,e}$	$2.534 \pm 0.19^{a,b,c}$
Zn	$3.07 \pm 0.39^{c,\ d}$	4.15 ± 0.46^{b}	$5.47 \pm 0.41^{a,\ d}$	$7.17 \pm 0.44^{a,\ b,c}$

Table-3. Concentrations (in micrograms per gram dry weight) of heavy metals in kidneys of rats from the four sampling sites.

Metals	Location 4 (LabanValley, Reference site)	Location 3 (Al Muzahmiyah)	Location 2 (Kharj Road)	Location 1 (Dhurma)
Cd	$0.053 \pm 0.006^{c,d,e}$	$0.085 \pm 0.007^{b,e}$	$0.104 \pm 0.007^{a,d}$	$0.156 \pm 0.011^{a,b,c}$
Pb	$0.62 \pm 0.13^{c,e}$	$1.06 \pm 0.09^{b,d}$	$1.75 \pm 0.20^{a,d,e}$	$2.49 \pm 0.19^{a,b,c}$
Zn	$2.64 \pm 0.36^{d,c}$	3.85 ± 0.43^{b}	$4.62 \pm 0.42^{a,d}$	$6.72 \pm 0.60^{a,b,c}$

Cd: cadmium, Pb: lead and Zn: zinc. Data are presented as means ± standard error
(n = 5). Different superscripts in the same row identify significant differences (LSD, $p < 0.05$) among sites.

Table-4. Concentrations (in micrograms per gram dry weight) of heavy metals in hair of rats from the four sampling sites.

Metals	Location 4 (LabanValley, Reference site)	Location 3 (Al Muzahmiyah)	Location 2 (Kharj Road)	Location 1 (Dhurma)
Cd	0.018 ± 0.003^{c}	0.024 ± 0.004^{b}	0.029 ± 0.004^{a}	$0.046 \pm 0.003^{a,b,c}$
Pb	$0.246 \pm 0.07^{c,b}$	0.417 ± 0.06^{a}	0.642 ± 0.06^{c}	$0.692 \pm 0.09^{a,b}$
Zn	$1.27 \pm 0.09^{d,c}$	1.66 ± 0.16^{b}	$2.12 \pm 0.17^{a,d}$	$2.93 \pm 0.29^{a,b,c}$

Cd: cadmium, Pb: lead and Zn: zinc. Data are presented as means ± standard error
(n = 5). Different superscripts in the same row identify significant differences (LSD, $p < 0.05$) among sites.

MDA and endogenous antioxidants in rats are presented in Table 5. Levels of MDA in the liver of rats captured at the location 1, location 2 and location 3 always significantly higher than those registered atthe reference site.In contrast, the activities of CAT, SOD, GR, GST and GPx were significantly lower in the location 1, location 2 and location 3than the reference site.

Table-5. Concentration of MDA and antioxidants in the liver of rats from the four sampling sites.

Parameters	Location 4 (LabanValley, Reference site)	Location 3 (Al Muzahmiyah)	Location 2 (Kharj Road)	Location 1 (Dhurma)
MDA (µM/g of tissue)	$1.01 \pm 0.15^{c,e,f}$	$1.87 \pm 0.25^{b,d,f}$	$2.82 \pm 0.22^{a,d,e}$	$3.84 \pm 0.18^{a,b,c}$
CAT (nmol/min/ml/g of tissue)	$12.08 \pm 0.81^{c,e}$	$10.66 \pm 0.81^{b,d}$	$7.83 \pm 0.60^{a,d,e}$	$5.17 \pm 0.35^{a,b,c}$
SOD (U/ml/g of tissue)	$6.33 \pm 0.41^{c,e,f}$	$4.57 \pm 0.49^{b,d,f}$	$2.77 \pm 0.38^{a,d,e}$	$1.088 \pm 0.26^{a,b,c}$
GPx (nmol/min/ml/g of tissue)	0.156 ± 0.014^{a}	0.118 ± 0.008	0.114 ± 0.007	0.106 ± 0.011^{a}
GR (nmol/min/ml/g of tissue)	$77.64 \pm 6.06^{a,b,c}$	42.94 ± 2.47^{c}	37.14 ± 3.38^{b}	33.68 ± 2.10^{a}
GST (nmol/min/ml/g of tissue)	43.80 ± 2.94^{b}	41.58 ± 2.48^{a}	36.08 ± 2.98	$27.42 \pm 1.86^{a,b}$

MDA: Malondialdehyde, CAT: catalase; SOD: superoxide dismutase; GPx: glutathione peroxidase; GR: glutathione reductase; GST :Glutathione *S*-transferases. Data are presented as means+ standard error (n = 5). Different superscripts in the same row identify significant differences (LSD, $p < 0.05$) among sites.

4. Discussion

The rapid growth of Riyadh, the capital city of Saudi Arabia, has created numerous environmental problems common to most cities worldwide. In addition to heavy metals released with industrial wastes, such big cities suffer from emissions from road ways and automobiles as major sources of heavy metals. Zinc, copper and lead are three of the most common heavy metals released from road travel, accounting for at least 90% of the total metals in road runoff. Smaller amounts of many other metals, such as nickel and cadmium, are also found in road runoff and exhaust [30].

In the present study, the elevated levels of most metals tested, in three locations versus reference site, are thought to be attributed to anthropogenic sources, given that every industry virtually discharges heavy metals into ecosystems [30]. In addition to emissions from road traffic, polluted sites are located within potential sources of heavy metals (Cd, Pb and Zn) including coal and oil combustion in electric power stations, heating and industrial petrochemical plants, and kiln operations in cement plants. Previous reports showed that Cd, Pb, and Zn had higher concentrations in the vicinity of industrials, vehicles and anthropogenic activities. Liu, et al. [31] determined heavy metals (Cd, Zn, Pb,Cu and Hg) and organic contamination in soil from waste region (industrials area) in South China. They reported that Cd, Cu and Hg were the most abundant metals in soil from region contamination. Asada, et al. [32] observed increased levels and accumulation of Zn concentrations in soil due to anthropogenic activity such as the use of compost in soil.

In the current study, heavy metal accumulated mostly in the liver of *R. rattus* followed by the kidney and hair collected from almost all polluted sites. On the other hand, the present data indicated that kidney and liver were the main target organs for Cd and Pb. Both kidney and liver have been mentioned as the preferential organs for Cd bioaccumulation [33, 34]. Similarly, Adham, et al. [35] also found increased levels in tissue Cd in the kidney and the liver in the Libyan jird *meriones lybicus* collected from a polluted site in Riyadh City compared to a reference site. The increase in the tissue concentration of Cd, Pb and Zn could be due to higher bioavailability of the metal of rats in most polluted area than other sites. Metals are transported through membranes by protein-carriers or protein-channels. At high external concentrations, availability of these carriers may become limited and uptake rates will decrease. Therefore, lipid layer resistance for influx via absorption is defined as a function of the exposure concentration.

In agreement with the results of the present study, Sato and Nagai [36] reported that although Cd administration resulted in Zn accumulation in both the liver and kidney, the increase in hepatic Zn content was more outstanding in Cd- treated rats. Also, metallothionein, which is a low molecular weight protein with high cysteine content and a high affinity for Zn, Cd and Cu, is suggested to play an important role in the concentration of these

elements in the liver and kidney [37]. The similarities and the differences in the biochemical behavior of Zn, Cd and Pb toward metallothionein may help to elucidate the distribution pattern of these elements.

In this study, increase levels of Cd, Pb and Zn in the hair was recorded in most polluted site which could be due the affinity of metals for SH- groups. Akesson and Vahter [38] reported that, cadmium is likely to accumulate in keratin-rich tissues like nails and hair. This might be attributed to the incorporation of elements into the keratin structure of hair which takes place by binding to the sulfhydryl groups that are present in the follicular protein [39]. Pereira, et al. [40] also reported that, exposure of wild rats and Algerian mice, living in an abandoned mining area, to a mixture of heavy metals showed a significant increase in the Cd concentration in hair of these animals. Koranteng-Addo, et al. [41] mentioned that, Pb and Zn levels showed significant increase in the hair of occupationally exposed workers in Cape Coast, Ghana than control.

Kidneys and liver are important sites for metal toxicity in vertebrates and can be used to characterize the risk of adverse effects and the potential for injury [42-44].

The prime manifestation of the oxidative cell damage induced by metal exposure is the increased lipid peroxidation in the cells. A study carried out by Yiin, et al. [45] demonstrated that the administration of Cd at various doses significantly increased the thiobarbituric acid reactive–substances (TBARS), a well known indicator of lipid peroxidation, in rat adrenals glands. Hussein, et al. [46] repoted that (Cd, Zn and Pb) concentration and bioaccumulation in tissues of chicken from some areas in the province of Mecca Almokaramah, KSA, showed a significant increase in the MDA level of liver and kidney. The present results showed that, rats collected from most polluted location induced oxidative stress which was manifested as increased lipid peroxidation in the cells. The levels of MDA were significantly higher in liver of rats from polluted locations. This is in consensus with the previous findings of experimental studies on metal–induced oxidative stress in rats [47, 48] in poultry [49, 50]. The increased levels of MDA (malondialdehyde), in the liver of rats collected from most polluted location is mainly due to the degeneration of the lipids caused by the formed hydrogen peroxide .

Metal-induced modulation in the endogenous anti-oxidant enzyme activity has been extensively reported in the literature. Enzymatic antioxidants present in the body include SOD, CAT and GPx that act as the body's first line of defense against ROS by catalyzing their conversion to less reactive or inert species. These agents either scavenge the ROS directly or prevent the production of ROS through sequestration of redox active metals like iron and copper [51]. The decreased CAT activity and SOD activity in the present study in liver of rats from polluted sites could also be attributed to direct binding of the metal to the active sites of the enzymes or due to their increased usage in scavenging free radicals induced by the metal thus causing irreversible inhibition in their activities [10]. Toplan, et al. [52] reported increase lipid peroxidation with decreased SOD activity in rats after Cd (20µg Cd/ml as cadmium sulfate) addition to their drinking water .

SOD and CAT are two important enzymatic antioxidants that act against toxic oxygen free radicals such as superoxide ($O^{\cdot 2}$) and hydroxyl ions ($^{\cdot}OH$) in biological systems. SOD catalyzes the destruction of superoxide radical by dismutation and H_2O_2 formation which explains a parallel increase in the lipid peroxidation with a decrease in the SOD activity in the liver of rats in most polluted sites. Catalase plays a role in the decomposition of H_2O_2 to water and oxygen. Reduced activity of this enzyme results in the accumulation of hydrogen peroxide, increasing the production of hydroxyl radical via Fenton's [53] which explains the associated oxidative stress. The low levels of CAT could be related to high production of superoxide anion radicals, which has been reported to inhibit CAT activity in case of excess production [54].

Also in this study GPx activity was significantly decreased in the liver of rats collected from most polluted site (location 1). The decreased GPx activity in present study might be attributed to increased utilization for elimination of H_2O_2 and organic hydroperoxides [55]. GPx is an enzyme containing four selenium cofactors that catalyzes the breakdown of H_2O_2 and organic hydroperoxides.

The current data showed decline in the activities of GR .This could be due to the involvement of this enzyme in the detoxification and possibly repair mechanism in liver and the antioxidant constituents which can scavenge free radicals [56]. GR is a glutathione regenerating enzyme that permits the conversion of oxidized glutathione (GSSG) to reduced glutathione (GSH) by the oxidation of NADPH to $NADP^+$.

In conclusion, this work reinforces the suitability of oxidative stress biomarkers as tools for assessing pollution impacts in situations where mixtures of contaminants are found. It is recommended that inhabitants of urban areas should avoid living in the vicinity of industrial areas. There is a need to establish more environmental friendly industries and restructure the traditional industries conforming to the standards emphasized by the Environmental Agencies worldwide.

5. Acknowledgement

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References

[1] United States Environmental Protection Agency USEPA, "Review of the national ambient air quality standards for lead: Policy assessment of scientific and technical information. OAQPS Staff Paper- First Draft, Office of Air Quality Planning and Standards, Research Triangle Park, EPA-452/P-06-002, 2006. C.F: K. Adham ,N. El- Eisa, M. Farhood, Risk assessment of heavy metal contamination in soil and wild libyan jird, Meriones libycus in Riyadh, Saudi Arabia," *J. Environ. Biol.*, vol. 32, pp. 813-819, 2011.

[2] R. Hassanzadeh, A. Abbasnejad, and M. A. Hamzeh, "Assessment of groundwater pollution in Kerman urban areas," *J. Environ. Stud.*, vol. 36, pp. 101-110, 2011.

[3] R. A. Mandour and Y. A. Azab, "Toxic levels of some heavy metals in drinking groundwater in Dakahlyia governorate, Egypt in the year," *Int. J. Occup. Environ. Med.*, vol. 2, pp. 7-112, 2011.

[4] A. M. Idris, "Combining multivariate analysis and geochemical approaches for assessing heavy metal level in sediments from Sudanese harbors along the red sea coast," *Microchem. J.*, vol. 90, pp. 2-159, 2008.

[5] A. B. Idris, M. A. H. Eltayeb, S. S. Potgieter-Vermaak, R. V. Grieken, and J. H. Potgieter, "Assessment of heavy metals pollution in Sudanese harbors along the red sea coast," *Microchem. J.*, vol. 87, pp. 104-112, 2007.

[6] F. E. Ayni, S. Cherif, A. Jrad, and M. Trabelsi-Ayadi, "Impact of treated wastewater reuse on agriculture and aquifer recharge in a coastal area: Korba case study," *Water. Res. Manag.*, vol. 25, p. 2251, 2011.

[7] R. A. Goyer, *Toxic effects of metals. In: Casarett and Doull's toxicology: Basic science of poisons Ed: C.D. Klaassen*. New York: McGraw- Hill, 1996.

[8] C. H. Walker, "Biochemical biomarkers in ecotoxicology--some recent developments," *Sci. Total Environ.*, vol. 171, pp. 189-195, 1995.

[9] M. Valko, H. Morris, and M. T. D. Cronin, "Metals, toxicity and oxidative stress," *Curr. Med. Chem.*, vol. 12, pp. 1161-1208, 2005.

[10] M. Waisberg, P. Joseph, and B. Hale, "Molecular and cellular mechanisms of cadmium carcinogenesis," *J. Toxicol.*, vol. 192, pp. 95–117, 2003.

[11] B. Halliwell and J. Gutteridge, *Free radicals in biology and medicine*, 4th ed. New York: Oxford University Press, 2007.

[12] V. J. Thannickal and B. L. Fanburg, "Reactive oxygen species in cell signaling," *Am. J. Physiol. Lung. Cell. Mol. Physiol.*, vol. 279, pp. L1005-1028, 2000.

[13] R. Mittler, "Oxidative stress, antioxidants, and stress tolerance," *Trends. Plant. Sci.*, vol. 7, pp. 405-410, 2002.

[14] N. Candan and L. Tarhan, "The correlation between antioxidant enzyme activities and lipid peroxidation levels in mentha pulegium organs grown in Ca2+, Mg2+, Cu2+, Zn2+ and Mn2+ stress conditions," *Plant Sci.*, vol. 163, pp. 769-779, 2003.

[15] A. M. Abdel-Moneim, A. M. Abu El-Saad, and H. K. Hussein, "Gill oxidative stress and histopathological bio- markers of pollution impacts in tilapia oreochromis niloticus from Mariut and Edku Lakes (Egypt)," *J. Aquat. Anim. Health*, vol. 24, pp. 148–160, 2012a.

[16] E. O. Farombi, O. A. Adelowo, and Y. R. Ajimoko, "Bio markers of oxidative stress and heavy metal levels as indicators of environmental pollution in African cat fish (Clarias Gariepinus) from Nigeria Ogun river," *Int. J. Environ. Res. Publ. Health*, vol. 4, pp. 158–165, 2007.

[17] S. Pandey, S. Parvez, and I. Sayeed, "Biomarkers of oxidative stress: A comparative study of river Yamuna fish Wallago Attu Bl. & Schn," *Science of the Total Environment*, vol. 20, pp. 105–115, 2003.

[18] C. B. G. Ruas, C. S. Carvalho, and H. S. S. De Araujo, "Oxi-dative stress biomarkers of exposure in the blood of cichlid species from a metal-contaminated river," *Ecotoxicol. Environ. Saf.*, vol. 71, pp. 86–93, 2008.

[19] A. Sahan, E. B. Kurutas, and T. Altun, "The determination of biochemical indicators (Biomarkers) in the com mon carp (Cyprinus Carpio) to the physico-chemical parameters of the Ceyhan river (Adana-Turkey)," *Eko. loji*, vol. 19, pp. 8–14, 2010.

[20] S. Aulagnier, P. Haffner, A. J. Mitchell-Jones, F. Moutou, and J. Zima, *Guide des mammifères d'Europe, d'Afrique du Nord et du Moyen- orient*. Paris: Delachaux Et Niestlé, 2008.

[21] J. Mertens, S. Luyssayert, S. Verbeeren, P. Vervaeke, and N. Lust, "Cd and Zn concentrations in small mammals and willow leaves on disposal facilities for dregded material," *Environ. Pollut.*, vol. 115, pp. 17–22, 2001.

[22] S. S. Talmage and B. T. Walton, "Small mammals as monitors of environmental contaminants," *Rev. Environ. Contam.*, vol. 119, pp. 47–145, 1991.

[23] United States Environmental Protection Agency USEPA, "Microwave a ssisted acid digestion of siliceou s and organically based matrices, EPA Method 3052,1996. C.F: K. Adham ,N. El- Eisa, M. Farhood, Risk assessment of heavy metal contamination in soil and wild libyan jird, Meriones libycus in Riyadh, Saudi Arabia," *J. Environ. Biol.*, vol. 32, pp. 813-819, 2011.

[24] United States Environmental Protection Agency USEPA, "Trace elements in water, solids, and biosolids by in ductively coupled plasma-atomic emission spectrometry, EPA Method 2007,1998. C.F: K. Adham ,N. El- Eisa, M. Farhood, Risk assessment of heavy metal contamination in soil and wild libyan jird, Meriones libycus in Riyadh, Saudi Arabia," *J. Environ. Biol.*, vol. 32, pp. 813-819, 1998.

[25] H. Ohkhawa, N. Ohishi, and K. Yagi, "Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction," *Anal. Biochem.*, vol. 95, pp. 351-358, 1979.

[26] G. Cohen, D. Dembiec, and J. Marcus, "Measurment of catalse activity in tissue extracts," *Anal. Biochem.*, vol. 34, pp. 30-38, 1970.

[27] J. W. Forstrom, J. J. Zakowski, and A. L. Tappel, "Identification of the catalytic site of rat liver glutathione peroxidase as selenocysteine," *Biochemistry*, vol. 17, pp. 2639-2644, 1978.

[28] I. Carlberg and B. Mannervik, "Glutathione reductase," *Methods Enzymol.*, vol. 113, pp. 484-490, 1985.

[29] R. Morgenstern, J. W. DePierre, and L. Ernster, "Activation of microsomal glutathione S-transferase activity by sulfhydryl reagents," *Biochem. Biophys. Res. Commun.*, vol. 87, pp. 657-663, 1979.

[30] J. M. Pacyna and E. G. Pacyna, "An assessmen t of global and regio nal emissions of trace metals to the atmosphere from anthropogenic sources worldwide," *Environ. Rev.*, vol. 9, pp. 269-298, 2001.

[31] M. Liu, B. Huang, X. Bi, Z. Ren, G. Sheng, and J. Fu, "Heavy metals and organic compounds contamination in soil from an e-waste region in South China," *Environ. Sci.*, vol. 15, pp. 919-929, 2013.

[32] K. Asada, K. Toyota, T. Nishimura, J. Ikeda, and K. Hori, "Accumulation and mobility of zinc in soil amended with different levels of pig-manure compost," *J. Environ. Sci. Health B.*, vol. 45, pp. 285-92, 2010.

[33] L. Leita, G. Enne, M. De Nobili, M. Baldini, and P. Sequi, "Heavy metal bioaccumulation in lamb and sheep bred in smelting and mining areas of S.W. Sardinia Italy," *Bull. Environ. Contam. Tox.*, vol. 46, pp. 887-893, 1991.

[34] M. L. Alonso, J. L. Benedito, M. Miranda, C. Castillo, J. Herna Ndez, and R. F. Shore, "Interactions between toxic and essential trace metals in cattle from a region with low levels of pollution," *Arch. Emerg. Med.*, vol. 42, pp. 165-172, 2002.

[35] K. G. Adham, N. A. Al- Eisa, and M. H. Farhood, "Risk assessment of heavy metal contamination in soil and wild Libyan jird, meriones libycus in Riyadh, Saudi Arabia," *J. Environ. Biol.*, vol. 32, pp. 813-819, 2011.

[36] M. Sato and Y. Nagai, "Mode of existence of cadmium in rat liver and kidney after prolonged subcutaneous administration," *Toxicol. Appl. Pharmacol.*, vol. 54, pp. 90-99, 1980.

[37] S. Saito, "Role of metallothionein on Zn, Cd and Cu accumulation in liver of heavy metal-injected rats. Metal Ions in Biology and Medicine, Eds. Ph. Collery, P. Br.tter, V. Negretti De Br.tter, L. Khassanova, JC Etenne," *John Libbey Eurotext, Paris*, vol. 5, pp. 258-261, 1998.

[38] A. Akesson and M. Vahter, *Health effecte of cadmium in Sweden*. Sweden: Institute of Environmental Medicine (IEM), 2011.

[39] J. Buchancova, M. Vrlik, M. Knizkova, D. Mesco, and L. Holko, "Levels of selected Goyer. Toxic and essential metal interactions," *Annu. Rev. Nutr.*, vol. 17, pp. 37-50, 1993.

[40] R. Pereira, M. L. Pereira, R. Ribeiro, and F. Gonc Alves, "Tissues and hair residues and histopathology in wild rats (Rattus Rattus L.) and Algerian mice (Mus Spretus Lataste) from an abandoned mine area Southeast Portugal," *Environ. Pollut.*, vol. 139, pp. 561-575, 2006.

[41] J. Koranteng-Addo, E. Owusu-Ansah, L. Boamponsem, J. Koka, and R. Agjei, "Analyses of lead and zinc levels in human scalp hair in occupationally exposed workers in Cape Coast, Ghana," *J. Chem. Pharm.*, vol. 2, pp. 384-391, 2010.

[42] K. G. Adham, "Metallic micropollutants in the harvest of oreochromis niloticus (Linnaeus, 1757) from polluted waters: Wildlife and human concerns," *Pak. J. Biol. Sci.*, vol. 4, pp. 1550-1558, 2001.

[43] K. D. Reynolds, M. S. Schwarz, C. A. McFarland, T. McBride, B. Adair, R. E. Strauss, G. P. Cobb, M. J. Hooper, and S. T. McMurry, "Northern pocket gophers (Thomomys Talpoides) as biomonitors of environmental metal contamination," *Environ. Toxicol. Chem.*, vol. 25, pp. 458-469, 2006.

[44] A. Sánchez-Chardi and J. Nadal, "Bioaccumulation of metals and effects of landfill pollution in small mammals. Part I. The greater white-toothed shrew, crocidura russula," *Chemosphere*, vol. 68, pp. 703-711, 2007.

[45] S. J. Yiin, J. Y. Sheu, and T. H. Lin, "Lipid peroxidation in rat adrenal glands after administration of cadmium and role of essential metals," *J. Toxicol. Environ. Health Part A*, vol. 62, pp. 47-56, 2001.

[46] H. K. Hussein, O. A. Abu-Zinadah, H. A. El Rabey, and M. F. Meerasahib, "Environmental assessment of ground water pollution by heavy metals and bioaccumulation of mercury residues in chicken tissues," *Afr. J. Biotechnol.,* vol. 10, pp. 16089-16100, 2011.
[47] Z. A. Shaikh, T. T. Vu, and K. Zaman, "Oxidative stress as a mechanism of chronic cadmium-induced hepatotoxicity and renal toxicity and protection by antioxidants," *Toxicol. Appl. Pharmacol.,* vol. 154, pp. 256-263, 1999.
[48] B. I. Ognjanović, S. D. Markovic, S. Z. Pavlovic, R. V. Žikic, A. Š. Štajn, and Z. S. Saicic, "Effect of chronic cadmium exposure on antioxidant defense system in some tissues of rats: Protective effect of selenium," *Physiol. Res.,* vol. 57, pp. 403-411, 2008.
[49] K. Bharavi, A. G. Reddy, G. S. Rao, P. Ravikumar, A. R. Reddy, and S. V. Rama Rao, "Reversal of cadmium induced oxidative stress and its bio-accumulation by culniray herbs murraya koenigii and allium sativum," *Resf. Pharmacol.,* vol. 4, pp. 60-65, 2010.
[50] V. Kant, M. Mehta, C. Varshneya, and S. Chauhan, "Induction of oxidative stress by subacute oral exposure of c admium sulphate in adult poultry," *Braz. J. Vet. Pathol.,* vol. 4, pp. 117-121, 2011.
[51] J. M. Mates, "Effects of antioxidant enzymes in the molecular control of reactive oxygen species," *Toxicology,* vol. 153, pp. 83-104, 2000.
[52] S. Toplan, D. Ozcelik, and M. C. N. Dariyerli, "Akyolcu oxidant and antioxidant status of cadmium administered rats," *J. Phys.,* vol. 107, pp. 1309-1312, 2003.
[53] E. Padmini, M. Usha Rani, and B. Vijaya Geetha, "Studies on antioxidant status in mugil cephalus in response to heavy metal pollution at Ennore estuary," *Environ. Monit. Assess.,* vol. 155, pp. 215–225, 2009.
[54] Y. Kono and I. Fridovich, "Superoxide radicals inhibit cat- alase," *J. Biol. Chem.,* vol. 257, pp. 5751–5754, 1982.
[55] T. Ramesh, C. Sureka, S. Bhuvana, and B. V. Hazeena, "Sesbania grandifloar diminishes oxidative stress and ameliorates antioxidant capacity in liver and kidney of rats exposed to cigarette smoke," *J. Physiol. Pharmacol.,* vol. 61, pp. 467-476, 2010.
[56] P. Das, N. Raghuramulu, and K. C. Rao, "Determination of in vitro availability of iron from common foods," *J .Hum. Ecol.,* vol. 18, pp. 13-20, 2005.