ASSESSMENT OF HEAVY METALS ACCUMULATION IN THE ECOSYSTEM AND HAZARDOUS EFFECTS ON THE TESTES AND EMBRYO OF WILD LIBYAN JIRD *MERIONES LIBYCUS* COLLECTED FROM PETROLEUM OIL POLLUTED ARID REGION

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ABSTRACT

The accumulation and cycling of heavy metals in the food chain as a result of crude petroleum oil or oil industries in eastern Saudi Arabia were assessed. The concentrations of heavy metals in soil, two plant species (*Launaea mucronata and Calotropis procera*) and the wild Libyan Jird *Meriones libycus* were measured. Five heavy metals, *viz*., sulfur (S), vanadium (V), nickel (Ni), cadmium (Cd) and lead (Pb) were assessed in the soil, plants and the Jird blood, testes and embryos. By using rat traps, the Jird was collected randomly from two polluted sites and one unpolluted site as a reference. The collected Jird were anaesthetized and dissected by decapitation. The blood and testes of adult male and full formed embryos in pregnant females were preserved for further histological and biochemical examinations. The concentration of S, V, Ni, Cd and Pb in adult Jird blood reached higher values than in embryos and testes in the polluted sites than in the reference site. In the Jird testes, the disturbances in the oxidative stress were measured in glutathione reduced (GSH), nitric oxide (NO) and lipid peroxidation (TBARS). The deleterious changes were recorded in complete blood count (CBC) and in the testes histological sections in relation to heavy metal concentration. Oil contamination induces the accumulation of the hazardous heavy metals in the testes and developed prenatal embryos which may be lead to teratogenicity and fertility problems in the wild Libyan Jird.

Keywords: Aridland soil, *Launaea mucronata*, *Calotropis procera*, Histoarchitecture, Lipid peroxidation, Fertility, embryo, testes

1. Introduction

Petroleum oil related pollution is a major threat to ecosystems and environmental health in oil producing countries in the Middle East. As a result of crude oil and oil industrialization, heavy metals have become increasingly prevalent in the environment. Petroleum mining, refining, utilization and disposal activities have increased both occupational and non-occupational exposure to numerous heavy metals (Hanna, *et al*., 1997). Many heavy metals were reported in the ecosystem components and are bioactive at some level of exposure (Domingo, 1994). While some metals are nutritionally essential for organisms, all can be toxic at certain concentrations. Previous studies on human estimated that approximately 50% of all human conception are lost prior to implantation (Kline, 1989). The imbalances in metal concentrations in maternal plasma, oviductal and/or uterine fluids may result in embryo lethality and/or abnormal morphogenesis (Keen, 1996). Hanna *et al*. (1997) suggest that the observed high frequency of early human embryonic losses is due, at times, to high concentrations of essential, as well as non-essential metals.

Toxicological impacts of heavy metals are far reaching, and the generation of oxidative stress has been regarded as the base of certain disorders, including the productive system diseases. Studies on reproductive toxicity have showed that oxidative stress affected male fertility, which were concerned of damaging sperm membranes, increasing polyunsaturated fatty acids, and adding the reactive oxygen species (ROS) generation (Tremellen, 2008). The productions of the low molecular weight thiols and protein thiol groups (SH) may be transformed when the oxidation of cysteine residues occurs (Valko *et al*., 2007). Also, it may influence the redox status, and even sometimes it leads to cellular redox imbalance.

The threat of heavy metal poisoning on organisms in the ecosystem often seems to be overlooked; however, many of the negative effects of heavy metals on humans apply to organisms within the surrounding ecosystems. Heavy metals, such Cd, Pb, V, Ni and S, can all act as poisons or teratogens, causing deformities or deaths in many organisms (Jezierska *et al*., 2009). The effect of heavy metals on early development, particularly on early embryonic stages, can be detrimental to a species that occupies a habitat into which heavy metals are introduced (Thompson and Bannigan, 2008).

The differences in the effect of heavy metals are expressed not only in the extent of toxicity, but also in the pattern of change in toxicity as related to concentration. This is more pronounced at earlier stages of development, more vulnerable (sensitive) to toxicants. The estimation of toxicity of a substance by the pathological changes induced in embryos is one of the most sensitive methods, which allows not only to determine the extent of toxicity, but also to give prognosis of its possible effect on the population (Mironova and Andronikov, 1992). Testicular changes due to Cd toxicity have been seen in a variety of animal models at different stages of growth and maturity. Gonadal development in mouse embryos exposed to Cd in early organogenesis was studied early by Tam and Liu (1985). Genital ridge size was reduced in exposed animals, with retarded germ cell migration into the ridges, resulting in depleted populations of germ cells, defective maturation of gametes and subfertility in male offspring.

Previous studies showed that preimplantation embryo culture models provide insights into the vulnerability of reproduction to metals toxicities (Peters *et al*., 1995; levengood and Heske, 2008). The teratogenicity of many metals has been described *in vivo* and *in vitro* on a variety of species in the laboratory models (Leonard and Gerber, 1994; levengood and Heske, 2008). In the present study, the heavy metals accumulation in soil, plants and the Libyan wild Jird rat were estimated in sites subjected to pollution from petroleum oil related activities in the eastern coast of Saudi Arabia. The relative toxicity produced due to accumulation of Cd, Ni, V, Pb and S was investigated in the Jird. The prenatal embryonic and adult testes oxidative stress as well as the histoarchitecture of the adult Jird testes was assessed.

2. Materials and methods

2.1. Samples collection

A number of 15 samples of soil, plants and the wild Libyan Jird *Meriones libycus* (males and pregnant females) were collected from two sites in the industrial Al-Jubail city region on the Arabian Gulf, Eastern Saudi Arabia. One coastal site (MPS) in Abu Ali island subjected to marine oil pollution sources (located at N 27º18′54.8", E 49º38′05.6") and 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 and female wild Libyan Jird was collected and transferred to the lab for further investigations. The soil was collected from the top 20 cm of soil profile and the most common plant species in every site was collected for heavy metal determination. The plant species *Launeae mucronata* (Forssk.) Muschl., which was collected from MPS and IPS sites, while *Calotropis procera* (Ait.) R. Br., collected from RS site. 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. Heavy metals determination

The analytical determination of the heavy metals V, Ni, Cd, Pb and S in soil, plant, blood, testes and embryos samples was carried out according to Shah *et al*. (2013) by ICP-MS (Inductively Coupled Plasma Mass Spectrometer): ELAN 9000 (Perkin Elmer Sciex Instrumento, Concord, Ontario, Canada).

Samples were prepared by accurately weighing 200mg of embryos and testes, 5 gm of plant and 20 gm of soil samples into a dry and clean Teflon digestion beaker, 6 ml of HNO3, 2ml HCl and 2 ml HF were added to the Teflon beaker. Samples were digested on the hot plate at 120-150 °C for approximately 40 minutes .The resulting digest was not clear ,so it was filtered through whatman filtered paper no 42. The filtered digest was transferred to a 50 ml plastic volumetric flask and made up to mark using deionized water. The estimation was done three times in each sample and the mean of them has been used as a single value.

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 $P<0.01$.

2.3. Biochemical assays

In the laboratory, the adult male and female Jirds were separated. Females with advanced pregnancy (fully formed embryos) and adult male were anesthetized by light ether and sacrificed by decapitation. The blood of males and females was collected from the ventricle. Complete blood count (CBC) for males was performed on an automated hematology analyzer (Beckman Coulter AC T). Male testes and female embryos were extracted rapidly and preserved at -80 for further analysis. Exact 0.5g of testes from each male was homogenized in 5ml of cold 0.1 M HClO4 containing 0.05% EDTA. The homogenate was centrifuged at 10,000 rpm for 10 min at 4 °C and the clear supernatant collected in a 0.5 ml microfuge tube and stored at -40 °C until used.

Lipid peroxidation was determined by assaying thiobarbituric acid-reactive substances (TBARS) according to the method of Preuss *et al*. (1998). Briefly, 1.0 ml supernatant was precipitated with 2 ml 7.5 % trichloroacetic acid and centrifuged at 1000 rpm for 10 min. Clear supernatant was mixed with 1 ml 0.70% thiobarbituric acid, incubated at 80 °C and the absorbance measured at 532 nm. Tetramethoxypropane was used as the standard.

GSH content was determined following Beutler *et al*. (1963). Briefly, 0.20 ml of the brain tissue supernatant was mixed with 1.5 ml precipitating solution containing 1.67% glacial metaphosphoric acid, 0.20% Na-EDTA and 30% NaCl. The mixture was allowed to stand for 5 min at room temperature and centrifuged 1000 rpm for 5 min. One ml clear supernatant was mixed with 4 ml 0.30 M Na_2HPO_4 and 0.50 ml DTNB reagent. A blank sample was similarly prepared by using 0.20 ml water. The color absorbance was measured spectrophotometrically at 412 nm.

Nitrite/nitrate concentrations were determined via the formation of nitrous acid diazotise sulphanilamide in an acidic medium and the products were coupled with N-(1–naphthyl) ethylenediamine. The resulting azo dye has a bright reddish – purple color which can be measured at 540 nm (Berkels *et al*. 2004).

2.4. Histological preparations

The left testes of 5 male Jird were immediately fixed in 10% phosphate buffer formalin for 24 hours. The tissues were washed to remove the excess of the fixative and then dehydrated in ascending grades (70, 80, 90 and 95%) of ethyl alcohol for 45 minutes (m), then in two changes of absolute ethyl alcohol for 30 m. This was followed by two changes of xylene for 30 m. 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 m. The sections were stained in Eosin for one minute, washed in tap water and dehydrated in ethanol according to the method of Mallory (1988).

3. Results and discussion

The study of heavy metals accumulation in the ecosystem components and its concentration in specific plant and animal organs or tissues is essential for understanding its cycle in the food chain. In this study, plants accumulated higher amounts of heavy metals than values measured in the soil and in the organs and embryo of the wild Libyan Jird rat (Table 1), indicating the plant's role in remediation of polluted soils (Khan, 2005). The highest value of the heavy metals concentration is attained in the plant *Launaea macronata* in the polluted sites MPS and IPS, and in *Calotropis procera* in RS site and the lowest values record in the soil samples. In general, the heavy metals concentration in samples collected from MPS site were higher than values in IPS and RS sites (Table 1).

The concentration of Cd in the Jird embryo attained the lowest values of the 1.61, 3.51 and 2.02 ng/g in RS, MPS and IPS sites, respectively. The levels of Pb, V, Ni and S concentrations in the embryos of Jird were significantly ($P<0.01$) higher in the two polluted sites than in the reference site. The low values of the current metals in the developed embryo tissue as compared to the other tissues may be due to the placental barriers which protect the embryo against hazardous material which may harm the developed embryo (Thompson and Bannigan, 2008). This accumulation of heavy metals in the tissues of the developed embryos may cause malformed organs and teratogenicity (Abu-Taweel *et al*., 2013). In the current case, the metals act as teratogens or environmental Toxic substances that are capable of causing structural congenital abnormalities (Allam *et al*., 2011). teratogens are agents extrinsic to embryo or fetus which exert deleterious effects leading to increased risk of malformation, carcinogenesis, mutagenesis, altered function, deficient growth or pregnancy wastage (Shirish and Sudip, 2011).

In the collected adult Jirds testes, Cd, Pb and V concentrations in MPS and IPS are significantly ($P<0.01$ and $P<0.05$, respectively) higher than their concentration in RS while Ni and S are significantly ($P<0.01$) high in both polluted sites (Table 1). The heavy metals bioaccumulation in testes may be the main reason of the increased oxidative stress (Valko *et al*., 2007). As shown in Table 2, the depletion of GSH ($P<0.01$) and increased amounts of NO ($P<0.01$) and TBARS ($P<0.01$) in the testes tissues of the Jird rat collected from the two polluted sites. The elevation in TBARS in the tissues reflects the increasing of lipid peroxidation and tissues damage (Allam *et al*., 2010, 2011; Abu-Taweel *et al*., 2013). The abnormalities in the oxidative stress state of the cell induce and initiate cell death (Faisal *et al*., 2013). Studies on reproductive toxicity have showed that oxidative stress affected male fertility, due to damaging of sperm membranes, increasing polyunsaturated fatty acids, and adding the reactive oxygen species (ROS) generation (Valko *et al*., 2007; Tremellen, 2008).

The histological sections in the testes of the Jird rat collected from the polluted sites showed significant the aberrations in the cellular structures of seminiferous tubule where pyknotic nuclei and vacuoles are observed especially in the Jirds collected from MPS site (Figure 1). Many of the Jird epididymis of the Jird collected from polluted sites appear empty and lack of spermatozoa which reflects the abnormalities in the spermatogenesis and thus the fertilities of the animals in the polluted sites. The anomalies which appear in the testes and epididymis sections agree with the present disturbance observed in the oxidative stress of the testes tissues. The disturbance in the Jird oxidative stress in the polluted sites may be attributed to the bioaccumulation of the heavy metals in

the testes tissue (Zeneli *et al*., 2015). As reported by Thompson and Bannigan (2008), the exposure to Cd causes malformations in the spermatozoa and harms testicular cells.

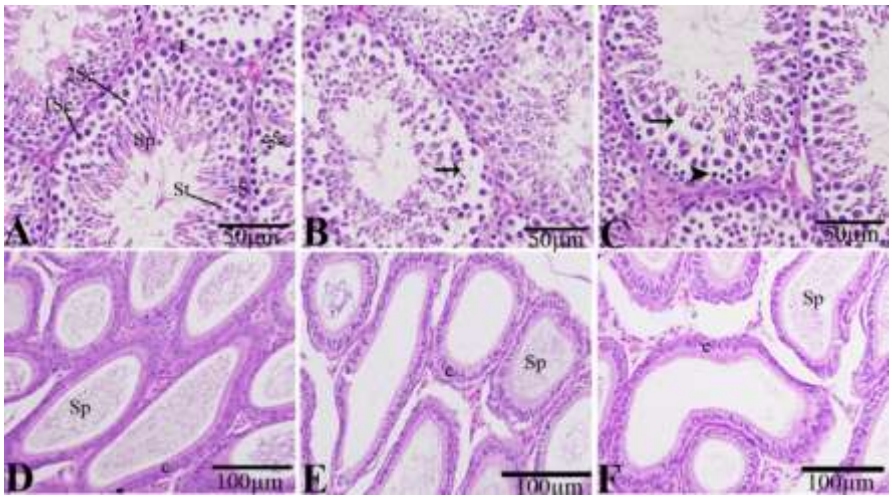

Figure 1: sections of *M. libycus* testes and epididymis collected from reference site (A& D), industrial polluted site (B& E) and marine polluted site (B& F) show spermatozoa (Sp), sertoli cells (S), spermatogonia (Sg), primary spermatocytes (1Sc), secondary spermatocytes (2Sc), tunica albuginea, pyknosis nuclei (arrow head), empty area (arrow) and epididymis columnar cells (c).

The gradient in the concentration of the most hazardous metal Cd in the embryo tissues, testes and blood is 3.51, 17.2 and 36.5 ng/g in MPS site, respectively. The high accumulation of Cd and the other current metals in the blood may be due to the exposure to heavy metals in the environment and accumulation through food chain or respiratory inhalations (Qu *et al*., 2012). The significantly ($P<0.05$) high concentrations of the heavy metals in the Jird blood collected from the polluted sites (Table 1) seems to be responsible for the abnormalities observed in the CBC (Table 3) as mentioned by Shumakova *et al*. (2014) . The measurements of metal concentration in the blood are good bioindicators of recent exposure (Shlosberg *et al*., 2012). The main abnormal parameter in the CBC is the significant ($P<0.01$) increasing in the number of white blood corpuscles (WBC). The increased WBC in animal's blood may be attributed to several reasons; most important is the toxicity effect (Levengood and Heske, 2008). The highest values of the heavy metals bioaccumulation was in blood of the Jirds collected from polluted sites in comparing to tests and embryos (Table 1).

Table 1: The concentration of the heavy metals Cd, Pb, V, Ni and S (Nano gram per gram) in soil, plants and in the embryo, testes and blood of the wild Libyan Jird *Meriones libycus* 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**
Embryo (ng/g)	1.61 (0.17)	3.51* (0.16)	2.02 (0.46)	182 (6.81)	234** (5.08)	235** (2.28)	45.2 (12.0)	177** (11.9)	142** (8.6)	242 (30.0)	1365** (17.0)	680** (254)	2261 (374)	8640** (28.0)	5614** (333)
Testes (ng/g)	2.3 (0.28)	17.2** (4.11)	7.2* (1.16)	354 (46)	2051** (132)	1498* (315)	136 (29)	659** (46)	238* (169)	539 (29)	3577** (94)	1483** (106)	3814 (471)	18216** (5739)	20534** (3461)
Blood (ng/ml)	14.2 (2.47)	36.5* (9.4)	33.2* (8.1)	1553 (151)	1772* (452)	1624* (234)	3688 (365)	18066* *(1213)	15211* *(1012)	1520 (603)	2228* (507)	2054* (421)	69105 (3817)	66547 (387)	58975 (425)
Plant (ng/g)	9 (0.03)	228*** (7.07)	155*** (7.07)	507 (0.72)	2885** *(6.16)	2354*** (7.07)	545 (6.93)	7843** *(7.07)	5992*** (4.44)	3390 (4.95)	3807*** (4.19)	2147*** (7.07)	61599* **(7071)	93585*** (1846)	106726* **(7071)
Soil (ng/g)	0.17 (0.05)	1.495* (0.47)	0.67*** (0.05)	2.07 (0.44)	2.62 (0.28)	5.05* (0.78)	16.2 (0.56)	86.0*** (1.54)	92.0*** (1.41)	7.75 (0.67)	35.90*** (2.135)	37.35*** (0.4)	307 (7.37)	1880*** (96.9)	1901*** (18.67)

Data are expressed as mean±SE (N =15). Values between brackets in the second row are the standard error. Values significantly compared to the control Jird; p*≤0.05, p**≤0.01and p***≤0.001. Plant species in RS site is *Calotropis procera* and in MPS and IPS sites is *Launaea mucronata.*

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

GSH (µg/gm)			NO u/gm			TBARS (nmol/gm)		
RS	MPS	IPS	RS	MPS	IPS	RS	MPS	IPS
11.4 (0.31)	7.03*** (0.29)	4.98*** (0.83)	0.69 (0.22)	3.42*** (0.24)	1.75** (0.01)	64.1 (5.9)	140** (24.6)	90.77* (24.5)

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

Table 3: The complete blood count (CBC) of *Meriones libycus*.

	WBC (x10^3/ul)	**RBC(x 10^3/ul)**	**HB (g/dl)**	**Hematocrit (%)**	**MCV(fl)**	**MCH (pg)**	**Platelets (x10^3/ul)**	**MPV(fl)**	**Neutrophil (%)**
RS	3.12 (0.5)	7.4 (0.2)	13.1 (0.4)	38 (3.268)	52 (0.7)	15 (0.23)	689 (52)	5.07 (0.13)	28.04 (3.32)
MPS	10.22** (0.5)	7.4 (0.5)	10* (0.7)	40 (3.268)	50 (0.9)	12* (0.54)	224** (24)	5.07 (0.15)	58.15** (5.65)
IPS	11.95** (1.2)	8.2 (0.3)	11 (0.6)	42.07 (1.59)	50 (0.93)	13* (0.19)	386** (45)	5.52 (0.18)	44.34* (4.20)

Data are expressed as a mean ±SE (N =15). Values significantly compared to the control Jird; $p^{*}\leq0.05$, $p^{**}\leq0.01$and $p^{***}\leq0.001$.

REFERENCES

1. Abu-Taweel G.M., Ajarem J.S. and Ahmad M. (2013), Protective Effect of Curcumin on Anxiety, Learning Behavior, Neuromuscular Activities, Brain Neurotransmitters and Oxidative Stress Enzymes in Cadmium Intoxicated Mice. Journal of Behavioral and Brain Science, **3**, 74-84.
2. Allam A.A., El-Ghareeb A.A., Abdul-Hamid M., Bkry A. and Sabri I. (2011), Prenatal And Perinatal Acrylamide Disrupts The Development Of Cerebellum In Rat: Biochemical And Morphological Studies.Toxicol Ind Health, **27(4)**, 291-306.
3. Allam A.A., El-Ghareeb A.A., Abdul-Hamid M., Gad M.A. and Sabri I. (2010), Effect Of Prenatal And Perinatal Acrylamide On The Biochemical And Morphological Changes In Liver Of Developing Albino Rat. Arch Toxicol., **84(2)**,129-41.
4. Berkels R., Purol-Schnabel S. and Roesen, R. (2004), Measurement of nitric oxide by reconversion of nitrate/nitrite to NO.Methods Mol. Biol., **279**: 1-8.
5. Beutler E., Duron O. and Kelly B.M. (1963), Improved method for determination of blood glutathione. J. Lab. Clin. Med., 61, 882-888.
6. Domingo J.L. (1994), Metal-induced developmental toxicity in mammals: a review. J. Toxicol. Environ. Health, **42**, 123-141.
7. Faisal M., Saquib Q., Alatar A.A., Al-Khedhairy A.A., Hegazy A.K. and Musarrat J. (2013), Phytotoxic hazards of NiO-nanoparticles in tomato: a study on mechanism of cell death. J. Hazard Mater., **15**; 250-251:318-32.
8. Hanna L.A., Petersb J.M., Wileyb L.M., Clegg M.S. and Keen C.L. (1997), Comparative effects of essential and nonessential metals on preimplantation mouse embryo development in vitro. Toxicol., **116**, 123-131.
9. Jezierska B., Lugowska K. and Witeska M. (2009), The effects of heavy metals on embryonic development of fish (a review). Fish Physiol Biochem., **35(4)**, 625-40.
10. Keen C.L. (1996), Teratogenic effects of essential metal deficiencies and excesses. In: L.W. Chang (Ed), Toxicology of Metals, CRC Press, West Palm Beach.
11. Khan A.G. (2005), Role of soil microbes in the rhizospheres of plants growing on trace metal contaminated soils in phytoremediation. Journal of Trace Elements in Medicine and Biology, **18 (4),** 355-364.
12. Kline J. (1989), Conception to birth epidemiology of prenatal development. Oxford University Press, New York.
13. Leonard A. and Gerber G.B. (1994), Mutagenicity, carcinogenicity and teratogenicity of vanadium compounds. Mutat. Res., **317**, 81-88.

14. Levengood J.M. and Heske E.J. (2008), Heavy metal exposure, reproductive activity, and demographic patterns in white-footed mice (Peromyscus leucopus) inhabiting a contaminated floodplain wetland. Sci Total Environ., **389(2-3)**, 320-8.
15. Mallory F.B. (1988), Pathological techénique. W. B. Saunders, Philadelphia.
16. Mironova A.P. and Andronikov V.B. (1992) The effect of heavy metal salts on the embryonic development of the common frog. Tsitologiia., **34(8)**:96-101.
17. Peters J.M., Duncan J.R., Wiley L.M. and Keen C.L. (1995), Influence of antioxidants on cadmium toxicity of mouse preimplantation embryos in vitro. Toxicology, **99**, 11- 18.
18. Preuss H.G., Jarrel S.T., Scheckenbach R., Lieberman S. and Anderson R.A. (1998), Comparative effects of chromium, vanadium and Gymnema sylvestre on sugar-induced blood pressure elevations in SHR. J. Am. Coll. Nutr., **17(2)**,116-123.
19. Qu C.S., Ma Z.W., Yang J., Liu Y., Bi J. and Huang L. (2012), Human exposure pathways of heavy metals in a lead-zinc mining area, Jiangsu Province, China. PLoS One., **7(11),** 46793.
20. Shirish D. and Sudip C. (2011), Manual of Obstetrics, 3rd Edition. Elsevier. pp. 38-41.
21. Shlosberg A., Wu Q., Rumbeiha W.K., Lehner A., Cuneah O., King R., Hatzofe O., Kannan K. and Johnson M. (2012), Examination of Eurasian griffon vultures (Gyps fulvus fulvus) in Israel for exposure to environmental toxicants using dried blood spots. Arch Environ Contam Toxicol., **62(3)**:502-11.
22. Shumakova A.A., Trushina É.N., Mustaphina O.K., Soto S.K., Gmoshinskiĭ I.V. and Khotimchenko S.A. (2014), Influence of titanium dioxide and silica nanoparticles on accumulation and toxicity of lead in experiments with intragastric co-admini. Vopr Pitan, **83(2)**, 57-63.
23. Tam P.P. and Liu W.K. (1985), Gonadal development and fertility of mice treated prenatally with cadmium during the early organogenesis stages. Teratol., **32(3),**453-62.
24. Thompson J. and Bannigan J. (2008), Cadmium: toxic effects on the reproductive system and the embryo. Reprod Toxicol., **25(3)**, 304-315.
25. Tremellen K. (2008), Oxidative stress and male infertility a clinical perspective. Hum. Reprod., Update **14**, 243-258.
26. Valko M., Leibfrit D., Moncol J., Mark T.D., Cronin M.M. and Joshua T. (2007), Free radicals and antioxidants in normal physiological functions and human disease. Int. J. Biochem. Cell Biol., **39**, 44-84.
27. Wang Y., Fang J., Huan S. and Chen L. *et al* (2013), The chronic effects of low lead level on the expressions of Nrf2 and Mrp1 of the testes in the rats. Env. Toxcol. Pharmacol., **3(5)**,109-116.
28. Zeneli L., Sekovanić A., Ajvazi M., Kurti L. and Daci N. (2015), Alterations in antioxidant defense system of workers chronically exposed to arsenic, cadmium and mercury from coal flying ash. Environ Geochem Health. [Epub ahead of print]