Assessment of Heavy Metals and Related Impacts on Antioxidants and Physiological Parameters in Oil Refinery Workers in Iraq

Mohammed A. Ajeel,1 Akram A. Ajeel,2 Aws Maseer Nejres,1 Riyam Ameen Salih3

1 College of Pharmacy, University of Mosul, Iraq
2 Ministry of Education/Salah El-Din Education Directorate, Iraq
3 Al-Dour Technical Institute, Northern Technical University, Iraq

Corresponding author
Aws Maseer Nejres
aws.m.nejres@uomosul.edu.iq

Introduction

Heavy metals can be harmful in elevated doses to human health such as zinc (Zn) and magnesium (Mg), while others such as lead (Pb), cadmium (Cd), mercury (Hg), and copper (Cu) have harmful consequences to health even in small doses.1–3 Several studies have shown that accumulated heavy metals in the body can damage nerves, kidney, liver, blood composition and other organ systems.1,2 Heavy metals and additives are incorporated into crude oil to enhance performance.3,4,5 Crude oil is well known to contain heavy metals like Cu, Cd, Ni and Pb.6,7

Objective. The current study aimed to assess levels of heavy metals and the impact of these metals on antioxidant levels and physiological variables in the serum of oil refinery workers in Iraq.

Methods. Heavy metals such as Pb, Cd, Hg, Zn, Cu, and Mg were assessed in the serum of a sample of refinery workers (N=40) and a control group (N=20) using atomic absorption spectrometry (AAS). Additionally, levels of malondialdehyde (MDA), δ-aminolaevulinic acid dehydratase (ALAD), and total antioxidant capacity (TAC), and physiological variables such as blood urea, serum creatinine, glutamate-oxaloacetic transaminase (GOT), glutamate-pyruvic transaminase (GPT), and gamma-glutamyl transferase (GGT) were measured to assess impact of these heavy metals.

Results. Mercury, Cd, and Pb were significantly elevated in the refinery worker group in comparison with the control group, while the levels of Zn, Cu, and Mg were significantly lower in the refinery worker group compared to the control group. There was a significant difference between the control group and the worker group for most of the antioxidants and functional variables. Total antioxidant capacity (TAC), δ-aminolevulinic acid dehydratase (ALAD), and malondialdehyde (MDA) were significantly lower in the worker group while blood urea, serum creatinine, glutamate pyruvate transaminase (GPT), and gamma-glutamyl transferase (GGT) showed a significant elevation in the workers’ group. Glutamic oxaloacetic transaminase (GOT) showed a significant elevation in the workers’ group. Oxidative stress is an imbalance between free radical production and capacity and antioxidants.7 Cell damage resulting from free radicals plays a key role in the progression of disease and aging processes.10 The

Conclusion. Refinery workers are at increased risk of having higher serum levels of Pb, Cd, and Hg compared to controls which can lead to an increase in oxidative stress, decrease in TAC, and decrease in the essential trace elements Zn, Cu and Mg.

Participant Consent. Obtained
Ethics Approval. This study was approved by the ethics committee within the Nineveh Health Department, Mosul, Iraq.

Competing Interests. The authors declare no competing financial interests.

Keywords. heavy metals, antioxidants, physiological parameters, refinery workers, atomic absorption spectrometry.

Received August 11, 2020. Accepted April 26, 2021.
J Health Pollution 31: (210907) 2021
© Pure Earth
negative effects of oxidative stress affect several structures of the cells, such as lipids, proteins, lipoproteins, membranes, and deoxyribonucleic acid (DNA). \cite{13,14,15,16} Oxidative stress leads to the formation of conjugated diene compound and malondialdehyde (MDA), which are known for their mutagenic and cytotoxic activity. Proteins may undergo conformational changes under the oxidative stress effect that may cause impairment and/or loss of their activity. \cite{17,18}

Lipid peroxidation spreads by a radical chain reaction, affecting a large number of lipidic molecules in the cell. \cite{19} The DNA and epigenetic information are prone to the lesions of oxidative stress. \cite{20,21} Oxidative damage to DNA could contribute to tumor onset when a certain amount of modifications on the DNA structure happens, such as strand breaks, DNA-protein cross-links, sugar and base lesions, and base-free sites. \cite{22,23,24} Many factors can lead to increased exposure to free radicals, including exposure to heavy metals. \cite{25} The current study aimed to assess levels of some heavy metals in blood, and to evaluate the impact of these metals on antioxidant levels and physiological variables in the serum of the oil refinery workers.

### Methods

The study was conducted in Bayji, a major industrial city in northern Iraq (Figure 1). Sampling was performed at the Bayji oil refinery. The refinery is far from industrial activities or agricultural activities involving the use of heavy metal-containing fertilizers, suggesting that heavy metal distributions are solely attributed to refinery activities. The city of Bayji is located about 34 miles from Tikrit. Tikrit is the center of Salah-Addin province and is characterized by higher levels of anthropogenic activities that may increase accumulation of heavy metals. Baiji is nearly the same size as Tikrit, yet most of its area is rural.

Non-smoking refinery workers (n = 40 males) were selected through convenience sampling. After consent was taken, the questionnaire was delivered to each volunteer and blood samples were taken.

All of the workers working in direct contact with the refining process were males, so all of the study participants were male as a result. Twenty subjects were recruited from urban areas away from any industrial activity or highways as a control group. The control group resembled the refinery workers group in age and gender.

A questionnaire (Supplemental Material) included data on health problems, age, daily working hours, industry working experience, and personal protective equipment. \cite{26} The questionnaire data was collected by the staff of the Baiji refinery health center, trained by the researchers in questionnaire administration. Ethics approval for the study was obtained from the Ethics Committee of the Nineveh Health Department, Mosul, Iraq. The study objective was explained to all participants and written consent was obtained from all study subjects. Samples were divided into two blue EDTA tubes (ethylendiaminetetraacetic acid). One EDTA tube (4 ml) was used for assessing heavy metals while the other EDTA tube was centrifuged and blood serum was isolated. The EDTA tubes were inverted 8–10 times to prevent clotting, then each tube was labeled and kept at 4°C. \cite{27}

### Blood serum digestion

For digestion of blood samples, 15 ml of (10%) Triton X-100, 5 ml of ammonium dihydrogen phosphate ((NH₄)₂(H₂PO₄)) (20%), and 1 ml of concentrated nitric acid (HNO₃) was mixed in a 500-ml volumetric flask. \cite{28} The volume was completed to 500 ml with deionized water. Next, 9 ml of the solution was mixed with 4 ml of each blood sample. After 15-20 minutes of incubation, the mixture was heated and evaporated to near dryness. The incubation, heating and evaporation process was then repeated. After cooling, 2 ml of sodium hydroxide NaOH was added for pH neutralization.

### Determination of metal concentrations

Standard solutions for magnesium (Mg), copper (Cu), zinc (Zn), mercury (Hg), cadmium (Cd), and lead (Pb) were used for atomic absorption spectrophotometry (AAS) (AA-6300, Shimadzu, Japan.) Quality assurance/quality control for AAS was performed.

### Abbreviations

| Abbreviation | Description |
|--------------|-------------|
| ALAD         | δ-aminolevulinic acid dehydratase |
| TAC          | Total antioxidant capacity |
| MDA          | Malondialdehyde |
according to the manufacturer’s instructions manual. Standard solutions were prepared from a stock solution of 1 g/L for each of the metals through several successive dilutions by distilled water. The results were used for instrument calibration. Then, heavy metals in each digested blood sample were measured and recorded for the current study analysis.

**Physiological variables**

Blood urea and serum creatinine were used to evaluate the functional state of the kidney. Glutamate-pyruvic transaminase (GPT), glutamate-oxaloacetic transaminase (GOT), and gamma-glutamyl transferase (GGT) were used as indicators of liver function. All tests were performed using a semi-auto biochemical analyzer (SK3002b).

**Antioxidant variables**

A double beam spectrophotometer (Fisher Scientific, Jenway™ 6850/230V) was used for analysis of antioxidant variables. For quality assurance/quality control of the spectrophotometry, a standard stock solution was made using potassium permanganate (KMnO₄) 0.072g in a 250 ml standard flask. A series of concentrations (1, 2, 4, 6, 8, 10, 20, 40 mg/dm³) working solutions were prepared of the stock solution of potassium permanganate using an appropriate amount of deionized water.

The absorbance of each solution was measured at 480, 526, and 580 nm using the spectrophotometer. To determine the maximum absorption wavelength at the different concentrations, the spectrum of KMnO₄ was performed by plotting
the data of absorbance versus wavelength. The calibration curve was made by fitting the absorbance versus concentration at the maximum absorption wavelength. Ringbom–Ayre’s plot was performed by fitting transmittance versus concentration and transmittance versus the logarithm of concentration. An R² value close to 1 considered to be an indication of the instrument functionality and the accuracy of the results.

Total antioxidant capacity (TAC) measurement

The ferric-reducing ability of plasma (FRAP) procedure, proposed by Koracevic et al.,23 was utilized to measure total antioxidant capacity (TAC). A standardized iron (Fe)–EDTA solution reacts with hydrogen peroxide by a Fenton-type reaction, leading to the hydroxyl radicals formation. Then thiobarbituric acid (TBA), a reactive acid, was released as a result of reactive oxygen from the Fenton reaction degrade benzoate. Antioxidants from the serum sample cause TBA production to be suppressed which leads to decreasing color intensity. Absorption at wavelength of 532 nm will then be reduced as a result of decreased color intensity.

Malondialdehyde measurement

Malondialdehyde (MDA) level was measured using Buege and Aust’s method.26 In this method, biological samples (e.g., plasma, erythrocytes or other tissues) are heated with TBA in a trichloroacetic–hydrochloric acid (TCA–HCl) mixture for 15 min. Colored products were measured at 535 nm using a double beam spectrophotometer.

Blood δ-aminolevulinic acid dehydratase measurement

Blood δ-aminolevulinic acid dehydratase (ALAD) activity was assessed according to Berlin and Schaller’s procedure27 which incubates ALAD with an excess amount of δ-aminolevulinic acid. The formed porphobilinogen was mixed with modified Ehrlich’s reagent then measured at 532 nm using a double beam spectrophotometer.

Statistical analysis

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) version 23. The results were expressed in the tables as mean ± standard deviation (SD). Mean values of refinery workers and the control group were compared using Student’s t-test.

Results

Table 1 presents characteristics of the study participants (refinery workers and control group), health status and working profiles. Personal protective equipment included safety glasses and respirators and face masks. Self-reported health problems related to work activities in the study population included eye and respiratory symptoms, especially allergic symptoms of the eye, occupational asthma and allergic rhinitis.

Table 2 shows the mean concentration of heavy metals in the blood of the study participants. Half of the measured heavy metals (Hg, Cd and Pb) in the refinery worker group showed a significant increase compared with the control group, while the other half (Zn, Cu, and Mg) were significantly higher in the control group.
Table 3 shows the mean concentrations of antioxidants and functional variables of studied refinery workers and controls. There was a significant difference between the control group and the refinery worker group for most of the antioxidants and functional variables. Total antioxidant capacity, ALAD, and MDA were significantly lower in the refinery worker group, while blood urea, serum creatinine, GPT, and GGT showed a significant elevation in the refinery worker group. Glutamic oxaloacetic transaminase showed no significant difference between the control group and the refinery worker group.

### Discussion

The results of the current study demonstrated a correlation between increased serum concentrations of Pb, Hg, Cd and altered TAC in refinery workers. These heavy metals have a unique affinity for electron-sharing that could form covalent attachments. These covalent attachments are mainly formed between sulphydryl groups of proteins and heavy metals. Metal interaction with glutathione (GSH) metabolism is a critical step in the metal’s toxic responses.

Previous studies have indicated that heavy metal toxicity in workers could be clearly seen as an ROS increase that increases MDA levels and lowers TAC levels. The increase in ROS production leads to increases in lipid peroxidation and the production of MDA; which is a good oxidative stress indicator. Zinc is an essential component of...
antioxidant enzymes. The antioxidant properties of zinc can be presented by two mechanisms. One involves reducing the protein’s essential thiol group susceptibility to oxidation and the other is competing for binding sites with Cu, Fe and other pro-oxidant metals. As shown in Table 2, there is a low serum Zn level which might be the cause of reduced levels of antioxidant enzymes.

The low concentration of Cu in the serum of refinery workers may be due to the hydroquinone interaction, a benzene metabolite, with Zn and Cu, which are cofactors for the SOD enzyme. Therefore, it displaces Cu from the enzyme. The ROS generated as a result of Cu reaction with other compounds results in the lipid oxidation chain reaction initiation. Copper deficiency thus increases the peroxidation processes by two-fold.

Oxidative stress is induced through free radical increases and/or disturbance of the redox system among refinery workers. It is possible that a low Mg level simply reflects excessive sweating, low Mg intake, or is due to Mg interaction with other heavy metals. A decreased magnesium level can be also justified by the high utilization rate of this metal, as this metal is utilized for protection toward oxidative stress.

The ALAD is an enzyme that contains thiol groups that are susceptible to oxidation. Inhibition of ALAD activity is one of the most sensitive indicators of lead exposure. In this study, ALAD levels in the whole blood of refinery workers were significantly decreased. Therefore, the results of whole blood ALAD activity were decreased as a result of the sulphydryl group oxidation of the ALAD enzyme by ROS and other oxidants which are generated in refinery workers. The decline in ALAD level among the refinery workers could be due to sulphydryl group oxidation of this enzyme by Hg, or Hg might be competing with the Zn within the ALAD leading to its inactivation.

The results clearly showed that levels of trace elements Cu, Zn, and Mg were significantly lower in refinery workers compared with controls. Low levels of Cu, Zn, and Mg in the refinery workers could be a result of an adaptive response to oxidative stress increment and free radical generation as a result of exposure to gasoline and heavy metals in gasoline. Mercury is known to change some trace element function and metabolism, such as Cu, Mg, Se, and Zn through competing for the binding sites of these trace elements within the human body. Therefore, interference with the absorption of some essential elements in the digestive system could be the reason for the deficiency of these essential elements.

The levels of heavy metals in the present study were lower than those found in many other studies in Iraq. Several studies have reported that heavy metals levels are very elevated in the Iraqi population and the effect of this elevation can appear as serotonin imbalance, thyroid gland function imbalance and even cancer in some cases.

The present study showed a significant increase in the functional variables of the liver and the kidney (Table 3). Most heavy metals exhibit harmful effects at high concentrations on several biological systems such as energy metabolism-related organs, ion transporters, the respiratory system, reproductive system, central nervous system (CNS), cardiovascular system, and other vital organs like the kidney, liver, and lungs.

Oxidative stress results from heavy metal exposure (such as Hg and Pb) and a decrease in the serum level of essential metals (such as Zn and Se). As a result, the redox status of the cell can be shifted, damaging biomolecules like nucleic acid, lipids, and proteins as well as some organs like the kidney, the liver, and the CNS.

The liver contains a divalent transporter protein system that transports some trace elements like Fe, Zn, Pb, and Cu. The level of this transporter protein decreases in the liver when some of the transported element levels are low, giving some sort of protection to the liver, but will be increased in the cells of the kidney as an excretion mechanism. This mechanism could harm the kidney, and consequently increase the level of kidney functional variables.

When heavy metals enter the body, they compete with essential trace elements and spend their nephrotoxic consequence through oxidative stress induction, apoptosis mediated by Ca, and ROS, as well as mitochondrial dysfunction. Heavy metals (except arsenic) alter the permeability and absorption of epithelial cells in the kidney, causing kidney dysfunction and proteinuria. The antioxidant characteristics of Zn could be potent in alleviating the acute effect of heavy metal exposure.

The present study has some limitations. All of the workers involved in the refining process that are in contact with petroleum refining processes were male because of the social and geographical habits in the Middle East generally, and in Iraq specifically. However, this should not affect the generalizability of the results, as heavy metals (at a certain levels) negatively impact all biological systems.
Conclusions

The present study found evidence for a strong correlation between increased serum concentrations of Pb, Hg, Cd and altered TAC and decreased serum level of the essential trace elements Zn, Cu, and Mg, adversely affecting the renal and liver functional states of refinery workers.

Acknowledgments

This study was funded as part of employment. Sincere appreciation to all those who have directly or indirectly contributed to this work, especially to the University of Mosul/College of Pharmacy for their time and effort.

Copyright Policy

This is an Open Access article distributed in accordance with Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0/).

References

1. Bachanek T, Staroslawska E, Wolanska E, Jarmolinska K. Heavy metal poisoning in glass worker characterised by severe. Annals of Agricultural and Environmental Medicine. 2000;7(1):51-53. http://www.iaem.pl/Keyword-heavy++metal++poisoning/126647
2. Mortada WI, Sobh MA, El-Defrawy MM, Farahat SE. Study of lead exposure from automobile exhaust as a risk for nephrotoxicity among traffic policemen. Am J Nephrol. 2001;21(4):274-279. https://doi.org/10.1159/0000646261
3. Ahmad H, Tsafe AI, Zuru AA, Shehu RA, Atiku FA, Itodo AU. Physicochemical and heavy metals values of Nigerian crude oil samples. Int J Nat Appl Sci. 2010;6(1):10-15. https://www.ajol.info/index.php/ijonas/article/view/76696
4. Onunkwo B, Dosumu O, Odakoya OO, Arowolo T, Ademuyiwa O. Biomarkers of lead exposure in petrol station attendants and auto-mechanics in Abeokuta, Nigeria: effect of 2-week ascorbic acid supplementation. Environ Toxicol Pharmacol. 2004;17(3):169-176. https://doi.org/10.1016/j.etap.2004.04.003
5. Mehmel MA. Dangerous properties of petroleum-refining products: Carcinogenicity of motor fuels (Gasoline). Teratog Carcinog Mutagen. 1990;10(5):399-408. https://doi.org/10.1002/tcm.1770100505
6. Kim Y, Kim NY, Park SY, Lee D, Lee JH. Classification and individualization of used engine oils using elemental composition and discriminant analysis. Forensic Sci Int. 2013;230(1-3):58-67. https://doi.org/10.1016/j.forsciint.2013.01.013
7. Osuji LC, Onojake CM. Trace heavy metals associated with crude oil: A case study of Ebocha-8 Oil-spill-polluted site in Niger Delta, Nigeria. Chem Biodivers. 2004;1(11):1708-1715. https://doi.org/10.1002/cbvd.20040129
8. O.Y.A, S.A.O. Determination of Heavy Metal Contents in Refined Petroleum Products. IOSR J Appl Chem. 2014. https://doi.org/10.9790/5736-07610102
9. Hong Y-C, Park E-Y, Park M-S, et al. Community level exposure to chemicals and oxidative stress in adult population. Toxicol Lett. 2009;184(2):139-144. https://doi.org/10.1016/j.toxlet.2008.11.001
10. Pham-Huy LA, He H, Pham-Huy C. Free radicals, antioxidants in disease and health. Int J Biomed Sci JBS. 2008;4(2):89. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3614697/
11. Drige W. Free radicals in the physiological control of cell function. Physiol Rev. Published online 2002. https://doi.org/10.1152/physrev.00018.2001
12. Pacher P, Beckman JS, Liandet L. Nitric oxide and peroxynitrite in health and disease. Physiol Rev. 2007;87(1):315-424. https://doi.org/10.1152/physrev.00029.2006
13. Halliwell B. Biochemistry of oxidative stress. Biochem Soc Trans. 2007;35(5):1147-1150. https://doi.org/10.1042/BST0351147
14. Young IS, Woodsid J V. Antioxidants in health and disease. J Clin Pathol. 2001;54(3):176-186. https://doi.org/10.1136/jcp.54.3.176
15. Frei B. Reactive oxygen species and antioxidant vitamins: mechanisms of action. Am J Med. 1994;97(3):55-513. https://doi.org/10.1016/0002-9343(94)90292-5
16. Nishida N, Arizumi T, Takita M, et al. Reactive oxygen species induce epigenetic instability through the formation of 8-hydroxydeoxyguanosine in human hepatocarcinogenesis. Dig Dis. 2013;31(5-6):459-466. https://doi.org/10.1159/000355245
17. Yasui M, Kanemaru Y, Kamoshita N, Suzuki T, Arakawa T, Honma M. Tracing the fates of site-specifically introduced DNA adducts in the human genome. DNA Repair (Amst). 2014; 15:11-20. https://doi.org/10.1016/j.dnarep.2014.01.003
18. Valko M, Izakovic M, Mazur M, Rhodes CJ, Telzer J. Role of oxygen radicals in DNA damage and cancer incidence. Mol Cell Biochem. 2004;266(1):37-56. https://doi.org/10.1023/B:MCBI.0000049134.69131.89
19. Willcox JK, Ash SL, Catignani GL. Antioxidants and prevention of chronic disease. Crit Rev Food Sci Nutr. 2004;44(4):275-295. https://doi.org/10.1080/10408690490468489
20. Pizzino G, Bitto A, Interdonato M, et al. Oxidative stress and DNA repair and detoxification gene expression in adolescents exposed to heavy metals living in the Milazzo-Valle del Mela area (Sicily, Italy). Redox Biol. 2014;2:686-693. https://doi.org/10.1016/j.redox.2014.05.003
21. Polkis A. Death resulting from gasoline "sniffing": A case report. J Forensic Sci Soc. 1976;16(1):43-46. https://doi.org/10.1016/S0015-7368(76)71024-7
22. Boecx RL, Postl B, Coodin FJ. Gasoline sniffing and tetraethyl lead poisoning in children. Pediatrics. 1977;60(2):140-145. https://pediatrics.aappublications.org/content/60/2/140
23. Koracevic D, Koracevic G, Djordjevic V, Andrejevic S, Cosic V. Method for the measurement of antioxidant activity in human fluids. J Clin Patol. 2001;54(5):356-361. https://doi.org/10.1136/jcp.54.5.356
24. Xiao M, Huang Z, Cai J, et al. Comparison of different sample preparation methods for platinum determination in cultured cells by graphite furnace atomic absorption spectrometry. PeerJ. Published online 2017. https://doi.org/10.7717/peerj.2873
25. AdeeinwO CE, Okorie NN, Idowu GO. Basic calibration of UV/visible spectrophotometer. Int J Sci Technol. 2013;2(3):247-251. https://www.academia.edu/10084337/Basic_Calibration_of_UV_Visible_Spectrophotometer
26. Bucie JA, Aust SD, [30] Microsomal lipid peroxidation. In: Methods in Enzymology, Vol 52. Elsevier; 1978:302-310. https://doi.org/10.1016/S0076-6879(78)52032-6
27. Berlin A, Schaller KH. European standardized method for the determination of delta-aminolevulinic acid dehydratase activity in blood. Z klin Chem klin Biochem.
Biochem. 1974;12(8):389-390. https://doi.org/10.1015/cclm.1974.12.8.389

28. Ercal, Nuram, Hande Gurer-Orhan, and Nukhet Aykin-Burns. “Toxic metals and oxidative stress part I: mechanisms involved in metal-induced oxidative damage.” Current topics in medicinal chemistry 1.6 (2001): 529-539. https://doi.org/10.2174/1568026013394831

29. Quiq D. Cysteine metabolism and metal toxicity. Altern Med Rev. 1998; 3:262-270. https://pubmed.ncbi.nlm.nih.gov/9727078/

30. Hultberg B, Andersson A, Isaksson A. Interaction of metals and thiol in cell damage and glutathione distribution: potentiation of mercury toxicity by dithiothreitol. Toxicology. 2001;156(2-3):93-100. https://doi.org/10.1016/S0300-483X(00)00331-0

31. Belch JJ, Bridges AB, Scott N, Chopra M. Oxygen free radicals and congestive heart failure. Heart. 1991;65(3):245-248. https://doi.org/10.1136/hrt.65.3.245

32. Hussain S, Atkinson A, Thompson SJ, Khan AT. Accumulation of mercury and its effect on antioxidant enzymes in brain, liver, and kidneys of mice. J Environ Sci Heal Part B. 1999;34(4):645-660. https://doi.org/10.1080/03601239909373219

33. Whaley-Connell A, McCullough PA, Sowers JR. The role of oxidative stress in the metabolic syndrome. Rev Cardiovasc Med. 2019;12(1):21-29. https://pubmed.ncbi.nlm.nih.gov/19106443/

34. Lockitch G, Godolphin W, Pendray M. Serum zinc, copper, retinol-binding prealbumin and ceruloplasmin concentrations in infant receiving intravenous zinc and copper supplementation. J Pediatr. 1983; 102:305. https://doi.org/10.1016/S0022-3476(83)80548-4

35. Al-Fartosy, Adnan JM, Sanaa K. Shanan, and Kalar Area, Kurdistan Region, Iraq. Niger J Clin Pract. Published online 2018. https://doi.org/10.4103/jncp.jncp_384_16

36. García JDD, Arceo E. Daño renal asociado a la toxicidad por plomo y la función de antioxidantes: estudio de la toxicidad por plomo y la función de antioxidantes. Rev Colomb Nefrol. 2017;5(1):43-53. https://doi.org/10.22265/acnel.5.2.254

37. Li Y, Kuppusamy P, Zweir JL, Trush MA. Assessment of Risk Behaviors and Toxic Heavy Metals Exposure of Car Dye Workers in Repairing Services in Erbil City, Kurdistan Region, Iraq. ZANCO J PURE Appl Sci. Published online 2020. https://doi.org/10.21271/zipas.32.6.1

38. Nejres et al. Heavy metal induced oxidative stress & its possible reversal by chelation therapy. Indian J Med Res. 2008;128(4):501. https://pubmed.ncbi.nlm.nih.gov/19106443/

39. Jawad AH. Impact of Some Heavy Metals and BPA Resulting from Terrorist Operations in Three Regions of Baghdad, Iraq on Thyroid Function. J Al-Nahrain Univ Sci. 2018;21.2.02

40. Machado MF, Quig D. Biological effects resulting from the of Cu/Zn-superoxide dismutase in xenobiotic exposure. II. Biological effects resulting from the Cu/Zn-superoxide dismutase-oxidant interaction of the benzene metabolite 1,4-dihydroquinone. Mol Pharmacol. 1996;49(3):412-421. https://pubmed.ncbi.nlm.nih.gov/8643080/

41. Hochschild R, Regelson W, Marott SF. Intervention in the Aging Process, Part A: Quantitation, Epidemiology and Clinical Research. New York Alan R Liss. Published online 1983:113-125. https://doi.org/10.7326/0003-191-101-6-894_1

42. Ramírez DCR, Contreras YA, Alfaro MM. Biochemical and molecular bases of lead-induced toxicity in mammalian systems and possible mitigations. Chem Res Toxicol. 2018;31(10):1009-1021. https://doi.org/10.1021/acs.chemrestox.8b00193

43. Singh N, Kumar A, Gupta VK, Sharma B. Biochemical and molecular targets of heavy metals and their actions. In: Biomedical Applications of Metals. Springer; 2018:297-319. https://doi.org/10.1007/978-3-319-74814-6_14

44. Salazar-Lugo R, Lozada U, Rosales M, et al. Heavy metals and its relationships with biomarkers of oxidative stress in chronic smokers. Rev SALUD Ambient. 2015;15(2):88-95.