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ASSESSING THE CONCENTRATIONS OF SOME HEAVY METALS IN BLOOD SAMPLES OF CHILDREN DIAGNOSED WITH AUTISM SPECTRUM DISORDERS IN BAGHDAD CITY

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ABSTRACT
Autism is caused by a variety of factors, including environmental factors. The study investigated the concentrations of lead, cadmium, and mercury in a sample of autistic children in Baghdad City. Blood serum samples were collected from 60 patients with autism spectrum disorder and 35 healthy controls. The samples were analyzed using flame atomic absorption spectrometry and a direct mercury analyzer. The results showed that the concentration of lead in ASD 54 µg/dl and the concentration of cadmium was 2 µg/dl and the concentration of mercury was 304 µg/dl all of heavy metals was higher than acceptable limit according to the WHO and CDC .research has shown that children with ASD have a decreased capacity to remove harmful metals from their bodies, which causes the metals to accumulate and exacerbate the symptoms of autism. A deeper comprehension of the significance of trace elements as environmental variables in the etiology of ASD is made possible by extensive metallomic research. Even though ASD is known to have a mineral imbalance, relevant testing, and the development of reference values of trace elements as prospective biomarkers helpful in ASD diagnosis, prevention, and therapy are still anticipated.

Keywords: Autism, Heavy metals, lead, cadmium, mercury, methylmercury

INTRODUCTION
It is a heterogeneous set of neurodevelopmental disorders characterized by several symptoms, the most important of which are: impaired social interaction, expressive communication, and stereotypical behavior. ASD features vary individually, including difficulty in understanding direct speech and ambiguous speech, as well as deficits in nonverbal communication skills and discourse, childhood onset behavior such as loneliness, delayed pronunciation, elaborate repetitive routines, and loss of emotional connection(1), Recent studies have shown that the pathogenesis of ASD would be multi-factorial, with genetic, biophysiological, and environmental factors (such as heavy metals exposure) jointly involved. Environmental factors (including neurotoxic heavy metals exposure) play a critical role in the occurrence and progression of ASD. Several studies discover that among the risk factors for ASD, environmental factors are more important than genetic factors (2). Heavy metals and their compounds exist widely in the natural environment. These substances are difficult to be metabolized due to their stable chemical properties and can accumulate in the food chain. Previous epidemiological investigations show that heavy metal exposure plays a significant role in the pathogenesis of ASD. Exposure to these toxic substances induces the release of cytotoxic substances, immune response, neuronal inflammation, and generation of reactive oxygen species (ROS), and subsequently causes irreversible damage to the brain development of ASD individuals (3) ,lead (Pb) is an indigenous metal that often creates lead compounds by its combination with two or more elements. Lead undergoes reactions with air and water to produce lead sulfate, lead carbonates, or lead oxide. While lead naturally occurs in the environment, it is mostly human activities that have been identified as the main cause of the rising levels of lead (4, 5). Lead is released into the air from the mining of lead, factories utilizing lead compounds, alloys, vehicle exhaust, and burning of fossil fuels (6). Rainwater washing away these soil particles allows lead to find its way into lakes or other bodies of water. As a result, lead enters the soil, water, and air and is subsequently taken up by plants and animals (7, 8). Lead poisoning or toxicity can occur when lead accumulates in the human body system (9).

Cadmium (Cd) typically occurs in nature as zinc sulfide compounds. Most of the Cadmium (Cd) is primarily utilized in the production of batteries, accounting for 83% of its usage. The remaining portion is allocated for applications such as alloys, coatings, plating, and serving as a stabilizer for plastics (10), Cadmium is a pollutant introduced into the environment because of the rapid development of industries and modern technologies (11, 12). Cd may occur especially in areas near factories or mines (13). low doses of cd affect both human male and female reproduction and affect pregnancy or its outcome (14) Mercury (Hg) is a widespread contaminant with harmful effects on the environment and human health due to its toxicity and detrimental impact (15). This pervasive pollutant is present in several forms, including organic, inorganic, and elemental. It is widely distributed in the atmosphere and can be found in terrestrial and aquatic environments (16). Mercury is a very volatile substance that easily turns into vapor. Anthropogenic sources of mercury include industrial activities, combustion of fossil fuels, mining activities, extraction of minerals, use of pesticides and fertilizers containing mercury, and the release of waste materials, In 2015, UNEP (United Nations Environment Programmer) reported that human activities led to the release of 220 tons of mercury into the atmosphere (17). Methylmercury (MeHg) is a potent toxic substance that accumulates in living organisms and poses a significant risk to public health. It is found in both natural and human-made environments, such as soil erosion and biomass burning. MeHg is formed when inorganic mercury, present in the environment, dissolves in freshwater and seawater. The study aimed to measure the concentrations of some heavy metals such as lead, cadmium, and mercury in the blood samples of autistic children from Baghdad City as potential factors for developing this disease.

MATERIALS AND METHODS

The participants in this study with autism spectrum disorder (ASD) were selected from the Medical City Hospital for Mental and Psychological Diseases in Baghdad, a specialized center, as well as private clinics. The study comprised patients living in Baghdad/ Iraq, who were diagnosed by consultants specializing in psychiatry and neurology. Their ages varied from 3 to 15 years. All the patients had a cognitive evaluation using psychological assessments in the hospital's psychological counseling section. In addition to generating a consent document for every patient's involvement in the research. Patients who had mental, neurological, or inflammatory illnesses, or a medical history of immunological or malignant diseases, were not included. Furthermore, individuals who were taking medications containing psychotropic chemicals, had a history of psychosis, or had a family history of psychosis were also excluded from the study. The control group included individuals in good health, with ages spanning from 3 to 15 years. The chosen youngsters did not have any prior familial record of ASD or any mental or neurological disorders. Similarly, the laboratory tests conducted on the patients were also conducted on the participants to facilitate result comparison.

Collection of blood samples

Blood samples were collected from 60 patients and 35 healthy children. Five to ten milliliters of blood samples were obtained by vein puncture for each person using a disposable syringe of 10 ml. The blood samples were placed in gel tubes of 6 ml and allowed to clot at room temperature. The serum was then separated from the blood cells by centrifugation at 3000 rpm for 10 minutes. The serum was then transferred to airtight plain tubes and stored in the freezer at -20°C until it was used to measure the concentrations of lead, cadmium, and mercury [18].

Measurement of lead, and cadmium in the blood serum

Before injection into the flame atomic absorption spectrometer, the standard solutions were prepared to evaluate the concentration of heavy metals in the blood serum samples. These standard solutions were matrix modifier solutions and calibration solutions which also were used for dilution and dissolution [19,20]. Serum samples were collected from 66 patients with autism spectrum disorder and 39 healthy controls that were pre-screened and frozen to measure the concentration of lead and cadmium. Lead and cadmium were measured using flame atomic absorption spectrometry (NOV-AA 800/ Analytik Jena /Germany). There is more than one method for estimating the flame atomic absorption spectrometry of the elements, and they differ according to the type and concentration of the element to be estimated in the sample [21,22]. Lead and cadmium concentration measurement was

conducted in the laboratories of the Quality Control Department/ Ministry of Trade, the wavelength that used to detect the element 213.9 to 228 nm .

Measurement of mercury in the blood serum

Direct Mercury Analysis (DMA-80) uses the principles of thermal decomposition, amalgamation, and atomic absorption. A solid or liquid sample is weighed into a quartz or metal boat, and the sample weight is transferred from the analytical balance to the DMA-80. Depending on the stated working procedure of the manufacturer. Sample boats were loaded onto the instrument auto-sampler. Samples were first dried and then thermally decomposed in an oxygen-rich furnace. Mercury and other combustion products are released from the sample and carried to the catalyst section of the furnace, where nitrogen and sulfur oxides, as well as halogens and other interfering compounds, are eliminated. Mercury is selectively trapped in a separate furnace, through gold amalgamation. Combustion by-products are flushed off, the amalgamation furnace is heated, and mercury is rapidly released. Mercury is flown via the carrier gas into a unique block with a dual-cell or tri-cell arrangement, positioned along the optical path of the spectrophotometer.

The Statistical Analysis System- SAS program was used to detect the effect of different groups (patients and control) on study parameters [23]. To clarify differences between the means, the least significant differences (LSD) values were calculated at $p \leq 0.01$ and the data were expressed as mean and standard error (Mean ± S.E).

Ethical Clearance

Consent was obtained from the legal guardians or parents of all participating children. A detailed explanation of the study, its purpose, and benefits, and consent was obtained from the guardians or parents before any data collection. Also, the participation in the study was entirely voluntary, and the children were not subjected to any coercion or undue influence. Parents or guardians were given the freedom to withdraw their children from the study at any point without consequences.

RESULTS AND DISCUSSION

1. Measurement of the concentration of lead in **blood specimens**

In general, the concentration of lead in the blood serum was evaluated in autism disease syndrome patients and healthy controls to establish its bioavailability. Although it measures the total dosage absorbed, it does not assess the toxicity of the substance. In table (1), the mean values data for lead in autism spectrum disorders patients are presented and compared with the controls.

Table (1): The lead concentration of autism spectrum disorders blood compared with the control

Groups	**Lead concentration (µg/dl)**		
Patients	**Min.-Max. value**	**Mean ± SE**	**Acceptable limit(µg/dl)**
	1.5- 54	22.626±2.051	3.5
Control	1.2- 18	3.737±0.716	
LSD $_{P\leq 0.05}$	0.000 S*		
	Significant		

In this study, the ASD patients were different from the control group in that they had higher levels of lead concentrations in their blood sampling than the control P-value ≤ 0.01,the results showed that the highest value of lead in the blood reached 54 µg/dl and the lowest value was 1.5 µg/dl recorded in ASD patients, The highest value was 18 µg/dl and the lowest was 1.2 µg/dl in the control group. The lead mean value of lead was 22.626±2.051 µg/dl and 3.737±0.716. µg/dl in ASD patients and control groups. The statistical analysis of mean

values was carried out as shown in.1. The results of the analysis showed high statistical significance (P≤ 0.05) between the groups (Figure 1).

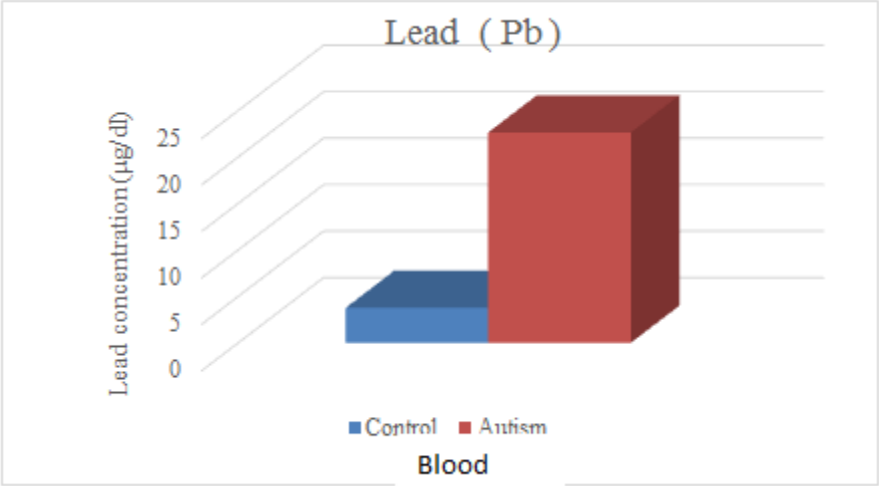


Figure (1) Comparison between the concentration of lead in the blood of autism spectrum disorders patients and control groups

The acceptable limits for lead in the blood of children vary depending on the country and the specific guidelines provided by relevant health authorities. The Centers for Disease Control and Prevention (CDC) has established a reference value for lead in the blood of children. The reference value is 3.5 µg/dl. This means that if a child's blood lead level exceeds 3.5 µg/dl, it is considered elevated and may warrant further evaluation and intervention (24).

2.Measurement concentration of cadmium in blood specimens for patients and healthy control

Cadmium and its compounds are toxic to humans and animals and are classified as "Group 1" human carcinogens once absorbed into the human body by the International Agency for Research on Cancer (IARC) (25).

Table (2) displays the mean values of cadmium concentrations in the blood of ADS patients compared to the control group. In ADS patients, the lowest mean value recorded for cadmium concentration in the blood serum was 0.11 µg/dl, and the highest value was 2 µg/dl, while the values 0.1 µg/dl to 1.1 µg/dl were the lowest and the highest levels of cadmium concentrations recorded in control.

Table (2): The cadmium concentration of autism spectrum disorders blood samples compared with the control

Groups	**Cadmium concentration (µg/dl)**		
Patients	**Min.- Max. value**	**Mean ± SE**	**Acceptable limit**
	0.1- 2	0.734±0.067	≤ 0.04
Control	0.1-1.1	0.663±0.057	
LSD P≤ 0.05	0.4244 NS		
	NS: Non-significant		

The mean values were 0.734±0.067 µg/dl and 0.663±0.057 µg/dl for patients and healthy controls, respectively Figure (2) reveals a comparison between patients and control groups in Cd. The highest level that records cadmium concentration in the blood of patients was 2 µg/dl compared to healthy controls which was 1.1 µg/dl.

Both results were higher than the acceptable limit proposed by CDC (2012) which recommended less than 0.04 µg/dl for cadmium concentration in children's blood. The presence of high concentrations of cadmium in both children with autism and the control group may be due to exposure to cadmium resulting from both natural and anthropogenic activities, such as volcanic eruptions, soil erosion, smelting operations of metal ores, fuel combustion, tobacco smoking, and other various ways are also contributing significantly to the introduction of cadmium into the environment (27). Cadmium can be transmitted to the human body through polluted air, food, and water, where it accumulates in various vital organs and causes adverse health effects.

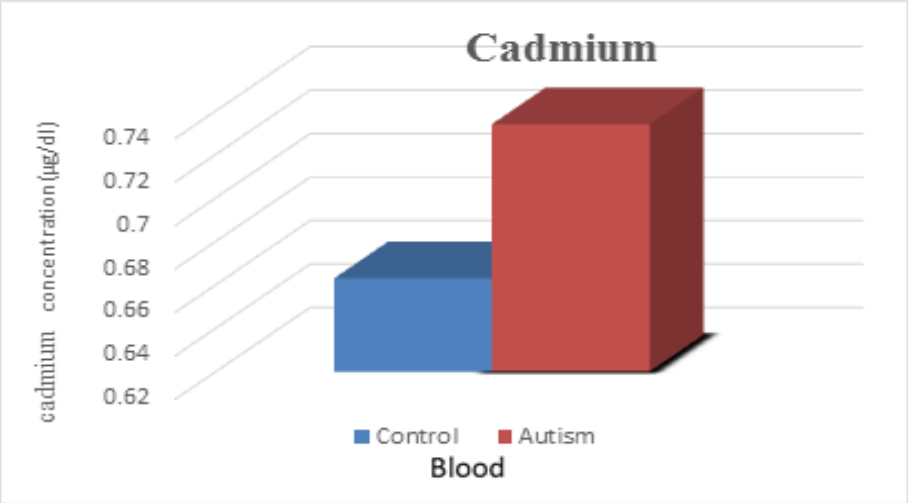


Figure (2) Comparison between the concentration of cadmium in the blood of autism spectrum disorders patients and the control groups

Prolonged exposure to elevated amounts of Cd can cause impairment to the kidneys, liver, skeletal system, cardiovascular system, and eyesight and hearing. Cadmium has strong teratogenic and mutagenic effects; in addition, at low levels, it has negative effects on human male and female reproductive and influences the course of or result from pregnancy (28). This results in abnormal methylation in both the placenta and the embryo and is caused by alterations in the expression of several genes in the embryo. Cadmium-induced epigenetic modification patterns have been associated with thiols' facile binding to thiols upon depletion of the methyl donor S-adenosyl methionine, which in turn causes methylome modifications and modifications to DNA methyltransferase activity. This might result in abnormalities in fetal and placental development (29). Research in Bangladesh indicated that cadmium contents in infants' urine were associated with quantities in maternal breast milk, saliva, and urine (30). More recently, Kippler et al. (2012) revealed that maternal cadmium exposure during pregnancy was inversely linked with infants' physical growth (birth weight and head circumference (31).

3. Measurement concentration of mercury in blood specimens for patients and healthy control

3.1 .The concentration of mercury in blood

Mercury concentration was measured in this study, as it was found that the highest value of mercury concentration in the blood of autism spectrum disorders blood patients was 304 ug/dl and the lowest value 29 µg/dl, in controle the high concentration of mercury in blood 34.4 while the lower 2.1 , the acceptable limit of mercury concentration in whole blood is usually lower than 0.5 µg/dl, but a value of 2 ug/dl or below is considered normal (32).

Table (3) The Mercury concentration of autism spectrum disorders blood compared with the control

Groups	Mercury concentration (µg/dl)		
Patients	Min.- Max. value	Mean ± SE	Acceptable limit
	29-304	132.333±16.483	< 10
Control	2.1-34.4	18.450±2.842	
LSD P≤ 0.05	0.000 S*		
	* Significant.		

The blood mercury concentration can rise to 35 µg/dl after long-term exposure to mercury vapor (34)(35). A statistical analysis of these values was carried out and shown in table (.3) the mean values for the level of mercury constrictions in ADS patients was 132.333±16.483µg/dl, the concentration of mercury records 18.450±2.842 µg/dl in healthy controls in Figure (3).

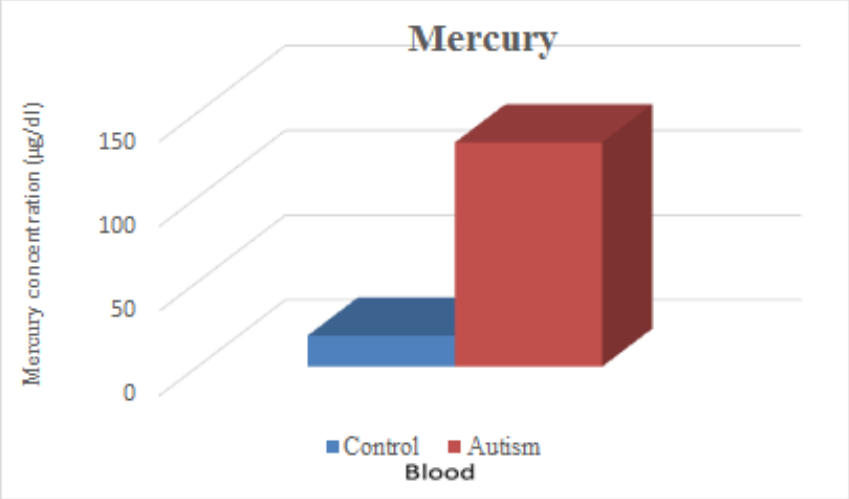


Figure (3) Comparison between the concentration of mercury in the blood of autism spectrum disorders patients and control groups

Mercury comes from both natural and man-made sources and is found in the environment all around the world. According to the US government's Agency for Toxic Substances and Disease Registry (ATSDR), mercury is the third most hazardous element to human health. It can be found in a variety of forms, including organic (like methyl- and ethyl-mercury) and inorganic (like mercuric chloride) (35). Each of these forms has a toxicity distinct to its species, which means that different countermeasures to prevent exposure are needed for varied effects on health surveillance. Mercury exposure can occur from a variety of sources, such as using skin creams and soaps containing mercury, eating fish when pregnant or as a child, receiving pediatric vaccines, and so on. The toxicity of mercury compounds varies depending on the developmental stage—whether it is prenatal or postnatal—dose, timing of exposure (acute or chronic), and the exposure pathway (ingestion, inhalation, transdermal, and transplacental absorption) (36). Nonetheless, exposure to various mercury compounds can happen simultaneously and frequently with other neurotoxic drugs, endangering the child's development (37)(38). Mercury is hazardous primarily because of its strong affinity for sulfur groups found in many biological proteins (39). It is hypothesized that variations in metabolism and delivery to the target tissues account for the variations in symptoms resulting from intoxication with different forms of mercury (40). One of the main targets of mercury exposure is the central nervous system (CNS), where exposure at both high and low doses can frequently result in substantial, long-term neurological damage (41).

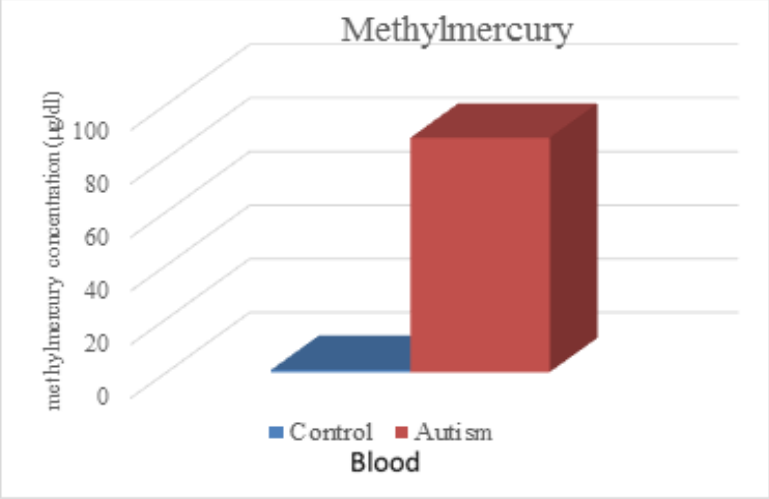
3.2.The concentration of methylmercury in the blood

Methylmercury (MeHg) is a potent neurotoxin that can have devastating effects on the developing nervous system. Exposure to MeHg can lead to a range of neurological impairments, including cognitive deficits, developmental delays, and motor dysfunction. In severe cases, MeHg poisoning can result in permanent neurological damage and even death (42). It is found in certain types of fish and shellfish and can also be released into the environment from industrial pollution (43).

Table (4): The methylmercury concentration of autism spectrum disorders blood compared with the control

Groups	**Methylmercury concentration (µg/dl)**			
Patients	**Min.-Max. value**	**Mean ± SE**	**Acceptable limit**	**References**
	10- 180	87.500±22.177	0.58	(EPA, 2000)
Control	0.1- 4.5	0.871±0.607		
LSD P≤ 0.05	0.008 S*			
	* Significant.			

The mean value for the level of methylmercury constrictions in autism spectrum disorders blood patients was 87.500±22.177, while it records a concentration of methylmercury 0.871±0.607 µg/dl in µg/dl healthy controls Figure (4).



CONCLUSION

The ASD group's concentrations of Cd, Pb, and Hg were greater than those of the healthy control groups, and the differences in Pb and Hg were statistically significant. Subgroup analysis suggested that there might be regional variations in the results. Research conducted in Asia and Europe revealed that children with ASD had greater levels of Pb, Hg, and Cd than the healthy controls, however research conducted in North America produced the opposite results regarding Hg and Cd. The cause is still unknown. Additionally, due to a decreased capacity for heavy metal elimination, children with ASD showed greater blood concentrations of heavy metals. (1) Biological causes of heavy metal exposure causing ASD should be the focus of future research. (2) The association's regional variation. In general, preventing ASD could benefit by limiting exposure to heavy metals and maintaining a healthy diet

REFERENCES

1. Jiang CC, Lin LS, Long S, Ke XY, Fukunaga K, Lu YM, et al. Signalling pathways in autism spectrum disorder: mechanisms and therapeutic implications. *Signal Transduct Target Ther*. (2022) 7(1):229. doi: 10.1038/s41392-022-01081-0
2. Zeidan J, Fombonne E, Scorah J, Ibrahim A, Durkin MS, Saxena S, et al. Global prevalence of autism: a systematic review update. *Autism Res*. (2022) 15(5):778–90. doi: 10.1002/aur.2696
3. Pugsley K, Scherer SW, Bellgrove MA, Hawi Z. Environmental exposures associated with elevated risk for autism spectrum disorder may augment the burden of deleterious de novo mutations among probands. *Mol Psychiatry*. (2022) 27(1):710–30. doi: 10.1038/s41380-021-01142-w.
4. Shahid, M., D. D. Westhouse, M. B. A. Khan, H. M. Rajput, A. A. Ali, M. Usman, and F. M. Munir. 2015. "Bioaccumulation of Lead (Pb) and Its Effects on Humans: A Review." Environ Pollut 203: 250-259
5. Abdulhay, H.S. and Yonius, M.I. (2020). Effects of diseases and pests on honey bee (Apis mellifera) in different parts in Baghdad city, Iraq. Plant Archives, 20, pp. 220–223
6- Violante, N., P. C. Lombi, M. P. Balocchi, G. Chiarenzi, and A. P. Costantino. 2010. "Mobility and Bioavailability of Heavy Metals and Metalloids in Soil Environments." In Soil Biology, 19 (2): 243-265. Elsevier.
7. Samuel, M., Ekeanyanwu, C. R., & Adie, G. (2022). Lead contamination of soil, air, and water: A review of sources, pathways, and human health implications. Environmental Science and Pollution Research, 29(13), 9829-9850
8- Nafal, D.H. and Abdulhay, H.S. (2020). Bioremediation of petroleum polluted soils using consortium bacteria. Iraqi Journal of Science, 61(5), pp. 961–969.
9. Järup, L., Åkesson, A., Alfvén, H., Berglund, M., & Elinder, C. G. (2009). Cadmium exposure and health effects. In Handbook on the toxicology of metals (pp. 491-537). Academic Press
10. national Institute for Occupational Safety and Health (NIOSH) (2020) states that "Cadmium is used in alloys, coatings, and platings to protect metals from corrosion and wear."
11. Satarug, S. Cadmium Sources and Toxicity. Toxics 2019, 7(2), 25; doi:10.3390/toxics7020025
12. Atiya, L. R., & Abdulhay, H. S. (2022). DNA-damage in blood of welders occupationally exposed to welding fume using comet assay. Caspian Journal of Environmental Sciences, 20(3), 513–517.
13. Järup, L., Åkesson, A., Alfvén, H., Berglund, M., & Elinder, C. G. (2009). Cadmium exposure and health effects. In Handbook on the toxicology of metals (pp. 491-537). Academic Press
14. Kumar, V., et al. (2019). Cadmium exposure and its effect on human reproductive health: A review. Environmental Toxicology and Pharmacology, 70, 103197
15. Rice, C. M., Schoonmaker, E., & Gonzalez, A. (2014). Mercury pollution: A global problem with local sources. Current Opinion in Environmental Science & Health, 9(10), 1-6.
16. Gustin, M. S., Evers, D. C., Brunson, D. A., & Krabbenhoft, M. P. (2020). Advances in Mercury Research—Reviews of Recent Advances in Mercury Research and Understanding the Biogeochemical Cycle. Science of the Total Environment, 745, 139854. doi:10.1016/j.scitotenv.2020.139854
17. Teng, M. M., Lin, Z., & Wang, X. (2020). Biogeochemical cycle of mercury and its health risks. Frontiers in Environmental Science, 8, 570805. doi:10.3389/fenvs.2020.570805

18. **Arun, P., et al. (2023). Trends in mercury emissions from anthropogenic sources. Environmental Science & Technology, 57(2), 701-713.**

19. **World Health Organization. (2017). Mercury and health. Retrieved from https://www.who.int/en/news-room/fact-sheets/detail/mercury-and-health**

20. Sobecka, M., Szymczak, W., & Skwierawski, R. (2022). The influence of storage conditions on the determination of lead, cadmium, and nickel in human serum. Biological Trace Element Research. doi:10.1007/s12011-022-02924-w

21. Subramanian, V. (1987). Heavy metal determination in blood serum by inductively coupled plasma

22. Centers for Disease Control and Prevention. (1991). Manual of analytical methods for lead in blood. Atlanta, GA: Author.

23. Resano, M., Flórez, M. R., & García-Ruiz, E. (2013). Progress in the determination of metalloids and non-metals by means of high-resolution continuum source atomic or molecular absorption spectrometry. A critical review. Analytical and Bioanalytical Chemistry, 406(9-10), 2239–2259. doi:10.1007/s00216-013-7522-9.

24. Garcia, E., & Báez, J. (2012). Atomic absorption spectrometry (AAS). In Encyclopedia of Analytical Chemistry (pp. 1-16). John Wiley & Sons, Ltd

25. SAS. 2018. Statistical Analysis System, User's Guide. Statistical. Version 9.6th ed. SAS. Inst. Inc. Cary. N.C. USA

26. Centers for Disease Control and Prevention (CDC). (2019). Recommendations for blood lead screening in children: Update 2019. MMWR Recommendations and Reports, 68(4), 1-15.

27 **.World Health Organization (WHO). (2022). Cadmium and inorganic cadmium compounds. In Handbook on the toxicology of metals (pp. 461-512). Academic Press.**

28. Taylor, A., et al. (2016). Cadmium and its potential role in autism spectrum disorder: A systematic review of the literature. Environmental Toxicology and Pharmacology, 48, 244-251.

29. Kumar, V., et al. (2019). Cadmium exposure and its effect on human reproductive health: A review. Environmental Toxicology and Pharmacology, 70, 103197

30. Geng, X., et al. (2019). Cadmium exposure and epigenetic alterations in placental development and diseases. Frontiers in Toxicology, 11, 587583.

31. Kippler, M., et al. (2010). Burden of cadmium in early childhood: longitudinal assessment of urinary cadmium in rural Bangladesh. Environmental Health Perspectives, 118(10), 1429-1434.

32. Kippler, M., et al. (2012). Maternal cadmium exposure and infant growth in rural Bangladesh: A longitudinal cohort study. Environmental Health Perspectives, 120(2), 259-264.

33. World Health Organization (WHO). (2021). Mercury and health. Retrieved from https://www.who.int/en/news-room/fact-sheets/detail/mercury-and-health:

34. Klaassen, C. D. (2007). Casarett and Doull's toxicology: The basic science of poisons (7th ed.). McGraw-Hill.

35. Jin Ye, S., et al. (2016). Association between blood mercury levels and cognitive function in mercury-exposed workers. Environmental Health Perspectives, 124(11), 1754-1760

36 . ATSDR. (1999). Toxicological profile for mercury. Agency for Toxic Substances and Disease Registry

37. Ruggieri, L., et al. (2017). Mercury toxicity: A review of current knowledge. Journal of Toxicology and Environmental Health, Part B: Critical Reviews, 20(3), 205-232

38. Dórea, J. G., et al. (2013). Neurotoxicity of developmental exposure to mercury and other environmental toxicants: A review with emphasis on recent advances. Journal of Toxicology and Environmental Health, Part B: Critical Reviews, 16(3), 123-145.

39. Grandjean, P., et al. (2017). Adverse effects of methylmercury on human brain development and function. Journal of Toxicology and Environmental Health, Part B: Critical Reviews, 20(3), 233-272.

40 . Clarkson, T. W. (2002). Mercury: An overview. In Handbook on the toxicology of metals (pp. 37-57). Academic Press.

41. Castoldi, A. F., et al. (2001). The differential toxicity of mercury species. Toxicology Letters, 121(3), 311-317.

42 . Johansson, P., et al. (2007). Mercury exposure and central nervous system disorders. Journal of Toxicology and Environmental Health, Part B: Critical Reviews, 10(8), 407-449

43. National Center for Biotechnology Information (NCBI). (2014). Methylmercury. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3514465/

44. **United States Environmental Protection Agency (EPA).** (2022). Methylmercury. Retrieved from https://www.epa.gov/mercury