

Determination of Heavy Metal Contamination of Some Commercially Available Herbal Preparations in Nigeria

Abstract

There has been reported increase in the use of herbal medicine preparations in the management of diseases in Africa over the past few decades. However, some of these preparations are contaminated with heavy metals which are toxic to both man and animals even at low concentration. Some of these heavy metals include cadmium, lead, zinc, chromium, arsenic and mercury. Availability of heavy metals in herbal medicine products may sometimes be due to plant materials used for production which are obtained from soils heavily contaminated or from the atmosphere. This study aimed to measure the levels of Pb, Cd, As, Cu and Hg in commercially available herbal products. Twenty products were purchased from traditional herbal medicine outlets in Lagos. The samples solutions were used to determine concentration of the heavy metals making use of Atomic Absorption Spectrophotometer (AAS). Results obtained indicated that some of the heavy metals were found in various percentages in the herbal products. Lead was found in 75% of the samples with concentrations range of 0.02 – 1.24ppm, cadmium (75%) with concentration range of 0.01 – 1.23ppm, arsenic (75%) with range between 0.01 – 0.08ppm, mercury (70%) with range between 0.08 – 1.32ppm and copper (80%) with range between 0.03 – 1.25ppm. The concentrations of heavy metals in few of the commercially available herbal remedies were well below the acceptable global recommendations, however our findings revealed that at present, the amount of heavy metals in most of the herbal preparations need to be reduced to acceptable limits so as to avoid heavy metal poisoning.

Keywords: Heavy metals, Herbal preparation, Herbal medicine, Plant materials, Diseases.

Introduction

Today, many medicinal plants are used in production of herbal medicine products. These medicinal plants have been reported to play significant roles in the prevention of human beings from various pathogenic microorganisms and diseases (1). According to the World Health Organization (2), close to 80% of the world population use herbal medicines for primary health care especially in South Asia, Latin America and Africa. Studies carried out indicate that phytochemicals in these medicinal plants help with building up the body's immunity and that they also have anti-inflammatory properties with fewer side effects (3). Majority of the people in the developing countries have been known to depend on herbal

medicine made from medicinal plants to treat and manage many diseases due to their wide availability and acceptability (4). There is a reported increase in the use of herbal medicine in European countries such as France and Germany (5,6). Though there is concern that not all herbal medicines are completely safe after all (7,8). Scientific knowledge on medicinal plants has provided us with the selection, preparation and application of herbal remedies (8,9). Therefore, there must be vigorous efforts in determining the effectiveness, safety and curative ability of the medicinal plant products (10). Because of the general acceptance of plant-based drugs, demand for medicinal plants has greatly increased (11). Interest shown by WHO in documenting the use of medicinal plants by various ethnic groups has further validated their uses and kept the people better informed about their effectiveness and safety (9). Regulations put in place by government have improved the production and quality of medicinal plant-based products (4).

Heavy metals such as copper, molybdenum, zinc, manganese, nickel and iron are essential micronutrients. They participate in natural growth and many other metabolic processes. It was reported that excessive amount of these heavy metals in soils cause metabolic disturbance and growth inhibition in most plant species (6). However, other heavy chemicals such as chromium, lead, cadmium and mercury have been reported to be toxic even at low concentrations (8, 12). It was reported by (6) that heavy metal pollution affects many countries around the world. Some human activities that lead to heavy metal contamination of medicinal plants are but not limited to industrial development, metal smelting, mining and consumption of chemical fertilizers. It is a known fact that heavy metals are hazardous to the environment and human health even in small concentration because they are known to be non-biodegradable (13). Though plants have been reported to have been used in the control of the pollution of heavy metals, but consumption of these plants have health implications such as cancer and organ failure (14,15).

The daily recommended intake of cadmium from food by a person in different countries is at the level of 10-35 μg per person (2,8). It has been implicated in many diseases such as myocardial infarction, diabetic nephropathy, hypertension and peripheral artery disease, (16). In an adult body of about 50 years of age, the cadmium content is about 15-30 mg and this increases with age which is due to the estimated long half-life of cadmium set at an average of about 20 years (17,18). Crops and plants grown for human consumption pick up cadmium through the atmosphere, human mining activities and the application of cadmium-containing fertilizers and sewage sludge on farm land (19). It was reported that

prolonged exposure to low doses of cadmium in most cases may result in the increased accumulation in the kidneys (20). Cadmium can also accumulate in the pancreas, lungs, central nervous system and testes in men (21). Cadmium also leads to demineralization and osteoporosis as it interferes with the metabolism of calcium, magnesium, iron, zinc and copper. When calcium ions are displaced by cadmium, this weakens the bones structure and is very often the cause of fractures, especially in children and women during menopause (22). Cadmium is a known carcinogenic heavy metal. It is reported to affect the DNA resulting in cell proliferation (23).

Heavy metals such as Lead (Pb) has been reported to be an environmental contaminant which can occur in organic and inorganic forms. It may be found both naturally and from diverse anthropogenic activities which when absorbed may affect almost every system in the body causing life-long adverse health effects. (24). Young children have been reported to be vulnerable to the toxic effects of lead which affects the development of the brain and nervous system (25). In adults, Lead is reported to cause long-term high blood pressure, cardiovascular problems and kidney damage while exposure of pregnant women has been reported to cause premature birth, stillbirth, miscarriage and low birth weight (26). Update by the World Health Organization in 2021 estimated that about 2 million lives were lost in 2019 due to Lead exposure (26). Lead exposure is estimated to account for 21.7 million years lost to disability and death (disability-adjusted life years, or DALYs) worldwide due to long-term effects on health, including 30% of the global burden of idiopathic intellectual disability, 4.6% of the global burden of cardiovascular disease and 3% of the global burden of chronic kidney diseases.

Arsenic is also a carcinogen. People have been reported to be exposed to elevated levels of inorganic arsenic through drinking contaminated water. Food preparation and irrigation of food crops with arsenic contaminated water also lead to arsenic poisoning (27). Acute arsenic poisoning may include diarrhoea, vomiting and abdominal pain which may be followed by muscle cramping and death, in extreme cases. Long-term exposure to arsenic are usually observed in the skin (28). The effect include pigmentation, skin lesions and hard patches usually found on the palms and soles of the feet. Also, there is reported cancer of the lung and bladder due to long term exposure to arsenic (29). In one study by (30), it was observed that diabetes, pulmonary disease and cardiovascular disease are other diseases associated with long-term ingestion of inorganic arsenic. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) in one of their report concluded that for certain regions of the world

where concentrations of inorganic arsenic in drinking-water exceed 50–100 µg/L, there is some evidence of adverse effects. (29).

Mercury is reported to be a neurotoxin and it is a heavy metal of known toxicity. The effects of mercury exposure can be very severe or subtle depending on the amount of mercury in the exposure. The age of the person exposed (unborn infants are the most vulnerable). The effect also depend on how long the exposure lasts, how the person is exposed either through breathing, eating, skin contact and the health of the person exposed (31). In a study, it was observed that very high doses of some forms of mercury have caused increases in several types of tumours in rats and mice (32). Some people who drink water containing inorganic mercury substantially in excess of the maximum contaminant level (MCL) for many years could experience kidney damage (33). Most human metallic mercury exposure comes from mercury vapour outgassing from amalgam fillings, at a rate of 2 to 28 micrograms per facet surface per day, of which about 80% is absorbed, according to the World Health Organization(2, 31).

Copper is a heavy metal and at the same time a very essential mineral which supports the human body in developing new blood vessels, balancing hormones that make nerve cells, forming enzymes that produces energy, building connective tissues and promoting healthy immune system functioning (34). The reported signs of copper toxicity includes nausea, vomiting, diarrhea, headache, weakness, dizziness and a metallic taste in her mouth. Excess copper induces not only oxidative stress but also DNA damage and reduced cell proliferation (34). In 2008, (35) reported the use of D-Penicillamine as the primary chelator in determining copper toxicity and concluded that and it may not be the treatment of choice in patients with neurologic symptoms. It was reported that when copper is in excess, it is cytotoxic and is capable of generating highly damaging free radicals such as hydroxyl radicals by Fenton or Haber-Weiss reaction, thereby contributing to oxidative stress. In addition, organelle dysfunction, lipid peroxidation, and formation of toxic alkenals such as 4-hydroxy nonenal, an inhibitor of pyruvate dehydrogenase and alpha-keto-glutarate dehydrogenase are damaging products of disrupted copper homeostasis (36). The main aim of this research is to determine the level of heavy metal poisoning in selected samples of some commercially available herbal preparations in Nigeria especially when they are built up in the body and to know whether or not they exceeded the recommended level.

Materials and methods

Sampling

Twenty products labelled A-T were purchased from Traditional medicine products outlets. Shown in Table 1 is the list of medicinal plants used in the manufacture of the products.

Table 1. Characteristics of herbal products investigated for heavy metals: Lead, Cadmium, Arsenic, Copper and Mercury.

Herbal medicine name	Pharmaceutical dosage form	Dosage quantity(mL)	Daily dosage	Medicinal plant composition	Claimed therapeutic properties
A	Solution	7.5	2	Ritchiea capparoidesvar.longipedicellata	Anthelmintic
B	Solution	2.5	3	Kalanchoe pinnata	Antimicrobial
C	Solution	5	2	Eugenia unifora	Contractile effect
D	Solution	5	3	Vitex doniana	Anti-hepatotoxic
E	Solution	7.5	1	Pilipostigmathonningii	Anti-inflammatory and antibacterial
F	Solution	5	2	Cassia occidentalis	Antimalarial
G	Solution	5	2	Carica papaya	Antiplasmodial
H	Solution	2.5	2	Ricinus communis	Anticonceptive
I	Solution	5	2	Parkia filicoidea	Antibacterial
J	Solution	5	2	Mucuna pruriens	Anti-snake
K	Solution	5	3	Plumbago zeylanica	Antibacterial
L	Solution	7.5	2	Mangifera indica	Antimalaria
M	Solution	5	3	Mitragynastipulosa	Muscle relaxant
N	Solution	5	3	Crossopteryx febrifugal	Hypotensive
O	Solution	5	2	Maeruaangolfensis	Analgesic

P	Solution	5	2	Mangifera indica	Antipyretic
Q	Solution	7.5	2	Hoslundiaopposita	Analgesic
R	Solution	7.5	2	Pericopsis laxiflora	Analgesic
S	Solution	5	2	Kyahagrandifoliola	Antimalarial
T	Solution	5	2	Sphenocentrum jollyanum	Analgesic

Sample analysis

The samples were analysed using Atomic Absorption Spectrometry using the recommended flame to measure their optical absorption. Standard solutions were prepared in five different concentrations for each metal separately to obtain calibration curve for quantitative analysis. Deuterium lamp was used for background correction.

Instrumentation

The digested samples were analyzed for the concentration of lead, cadmium, arsenic, copper and mercury by atomic absorption spectrophotometer at conditions recommended by the manufacturers. The metals were determined at the most sensitive spectral lines of the metals. Appropriate hollow cathode lamp corresponding to the metal to be determined was used. The AAS was calibrated using calibration standards reagents for the metal to be analyzed. The calibration curves were prepared separately for all metals by running different concentrations of standard solutions. The instrumental responses for the calibration working standard were used to plot the calibration curves for the metals automatically by the instrument and the concentration was extrapolated from the calibration graph. Each sample was then aspirated, and the results were recorded. Operational condition for the metal analysis is given in Table 2.

Table 2: Operational condition for metal analysis

Metal	Wavelength(nm)	Relative sensitivity	Detection limit
Pb	217.0	1.0	0.05
Cd	228.9	1.0	0.002
As	193.7	1.0	0.05
Cu	324.7	1.0	0.002
Hg	253.7	1.0	0.05

Preparation of blank

In order to account for possible contribution from acid or distilled water, a blank was prepared. 5mL of a mixture of concentrated Nitric acid and perchloric acid was measured and transferred into the digestion flask containing some mL of the distilled water, it was then digested and transferred into a 25mL standard flask and made up to the mark with distilled water.

Results and Discussion

The results of heavy metals determined in this study are presented in Tables 3. There has been several reports of heavy metals poisoning due to herbal preparation consumption(37). So many reasons have been adduced for the presence of heavy metals in herbal preparations which include condition of soils where the medicinal plants for production is obtained, hygienic condition of the machineries used for production, methods of extraction and possibly storage (38).Lead was found to be present in 15 out of 20 samples of the herbal preparation with concentration in the range of 0.02 – 1.24ppm. Lead may have contaminated the herbal preparation from a drinking water perspective considering the fact that tap water is the source for production of most of the herbal preparation. Lead is a cumulative general poison and associated with several health hazards such as anaemia, epilepsy, congenital paralysis, inhibition of hemoglobin synthesis, acute and chronic damage to the central and lateral nervous system (39). There is a wide range of lead gotten from this research work. Out of the 15 products, only 3 falls within the acceptable limit of 0.29 ppm. This implies that 20% of the products are safe while continuous consumption of the remaining products may lead to Lead poisoning. The results obtained for Cadmium indicated that 15 of the sample products contained the heavy metal with a range of 0.01 – 1.23 ppm. However, 7 of the products (46.67%) fall within the acceptable limit of 0.05 ppm for the metal. Though Cadmium accumulation in the human system has been reported to lead to demineralization and osteoporosis as it interferes with the metabolism of calcium, magnesium, iron, zinc and copper(21, 22). It is also a known carcinogenic heavy metal reported to affect the DNA resulting in cell proliferation (23). Arsenic was found in 15 out of the 20 sample products with a range between 0.01 – 1.02 ppm. Out of the 15 products, 4 (26.67%) were found to contain the amount of arsenic which fall within the acceptable limit of 0.01 ppm. Though (28) observed that Long-term exposure to arsenic may lead to major dermatological disorders such as melano-keratosis, melanosis, spotted and diffuse keratosis, leucomelanosis, and dorsal

keratosis. The major way to reduce arsenic poisoning is to be conscious of source of water used in the production of the herbal products.

Table 3: Results of heavy metals presence in the herbal medicines (N.B – All readings are in ppm)

Herbal medicine	Pb	Cd	As	Hg	Cu
A	0.42±0.02	ND	0.01±0.00	0.67±0.05	0.52±0.12
B	ND	0.08±0.03	0.03±0.01	ND	0.36±0.03
C	0.35±0.00	0.05±0.01	0.04±0.02	1.02±0.09	0.86±0.02
D	0.27±0.01	0.09±0.02	0.08±0.03	0.48±0.01	1.21±0.01
E	0.43±0.06	0.01±0.04	0.05±0.01	ND	0.82±0.020
F	0.62±0.05	0.02±0.02	ND	1.07±0.06	ND
G	0.45±0.01	ND	0.01±0.00	ND	0.80±0.05
H	ND	0.09±0.06	1.02±0.12	0.56±0.02	1.25±0.03
I	1.24±0.07	0.54±0.05	0.05±0.02	0.88±0.04	0.84±0.01
J	0.48±0.03	1.23±0.02	0.06±0.01	0.84±0.05	ND
K	0.85±0.02	ND	0.01±0.00	1.32±0.01	0.84±0.02
L	1.22±0.04	0.09±0.01	0.05±0.01	0.68±0.02	0.55±0.03
M	ND	0.07±0.07	ND	1.26±0.07	0.82±0.06
O	0.26±0.03	0.04±0.01	0.06±0.02	0.08±0.01	0.03±0.01
P	0.38±0.00	ND	0.01±0.00	ND	0.58±0.01
Q	0.02±0.08	0.08±0.02	ND	1.02±0.04	ND
R	0.35±0.01	0.04±0.05	ND	ND	0.83±0.02
S	ND	0.05±0.03	0.04±0.01	0.95±0.01	0.68±0.04
T	0.37±0.02	0.03±0.01	0.03±0.01	1.21±0.02	0.72±0.06

Values are mean ±SD

Mercury is contained in 14 (70%) sample products with concentration range between 0.08 - 1.32 ppm. It was also observed that 8 (57.14%) of the herbal products containing mercury fall within the acceptable limit of 1.00 ppm. Though reported to be a neurotoxin, mercury exposure can be very severe or subtle depending on the amount of mercury contamination (31, 33). Out of the 20 herbal products tested, 16 (80%) were contaminated with traces of copper with range between 0.03 1.25 ppm. Two (2) products representing 12.5% contained

acceptable limit of copper out of the sixteen (16) products that were observed to be contaminated with the heavy metal. Though Copper is a heavy metal, but in minute and acceptable limit, supports the human body in developing new blood vessels and promote healthy immune system functioning (34). It was reported that copper toxicity shows series of sicknesses which is an indication of oxidative stress. Excess of copper is cytotoxic and generates highly damaging free radicals(34, 36).

Conclusion

There is insufficient research on herbal medicine products despite increasing use. Heavy metals are known contaminants or adulterants of many traditional remedies. Collectively, this study shows that between 70 -80% of the herbal products were contaminated with at least one of the heavy metals. It was also determined that only few of the products have heavy metal contamination within the acceptable limits as globally recommended. It is recommended that appropriate monitoring Agencies of the Government put in place policies to stem this dangerous trend.

Consent

It's not applicable.

ETHICAL APPROVAL

It's not applicable.

References

1. Ashvini Y. Parbat , Gunjan P. Malode , Aayasha R. Shaikh , Wrushali A. Panchale , Jagdish V. Manwar and Ravindra L. Baka (2021). Ethnopharmacological review of traditional medicinal plants as immunomodulator, *World Journal of Biology Pharmacy and Health Sciences*, 2021, 06(02), 043–055. <https://doi.org/10.30574/wjbphs.2021.6.2.0048>.
2. World Health Organization, “Inorganic mercury: environmental health criteria 118,” in *International Programme on Chemical Safety*, World Health Organization, Geneva, Switzerland, 1991.
3. Tiwari R, Latheef SK, Ahmed I, Iqbal HMN, Bule MH, Dhama K, Samad HA, Karthik K, Alagawany M, El-Hack MEA, Yatoo MI, Farag MR (2018). Herbal Immunomodulators - A Remedial Panacea for Designing and Developing Effective

- Drugs and Medicines: Current Scenario and Future Prospects. *Curr Drug Metab.* 19(3):264-301.
4. Zhu F, Wang X, Fan W, Qu L, Qiao M, Yao S (2013) Assessment of potential health risk for arsenic and heavy metals in some herbal flowers and their infusions consumed in China. *Environ Monit Assess* 185:3909–3916. <https://doi.org/10.1007/s10661-012-2839-y>
 5. Singh D, Chaudhuri PK.(2017) Chemistry and Pharmacology of *Tinospora cordifolia*. *Nat Prod Commun.* 12(2):299-308.
 6. Eghbal N, Nasrabadi T, Karbassi AR, Taghavi L (2019) Evaluating the potential of plants (leaves) in removal of toxic metals from urban soils (case study of a District in Tehran City). *Pollution* 5:387–394. <https://doi.org/10.22059/POLL.2019.272090.555>
 7. Gudalwar BR (2021). *Allium sativum*, a potential phytopharmacological source of natural medicine for better health. *GSC Advanced Research and Reviews.*; 06(03):220–232.
 8. Rubio C, Lucas JRD, Gutiérrez AJ, Glez-Weller D, Pérez Marrero B, Caballero JM, Revert C, Hardisson A (2012) Evaluation of metal concentrations in mentha herbal teas (*Mentha piperita*, *Mentha pulegium* and *Mentha* species) by inductively coupled plasma spectrometry. *J Pharm Biomed Anal* 71:11–17. <https://doi.org/10.1016/j.jpba.2012.07.015>
 9. Badukale NA (2021) Phytochemistry, pharmacology and botanical aspects of *Madhuca indica*: A review. *Journal of Pharmacognosy and Phytochemistry*; 10(2): 1280-1286.
 10. Tungmunnithum D, Pinthong D, Hano C (2018). Flavonoids from *Nelumbo nucifera* Gaertn., a Medicinal Plant: Uses in Traditional Medicine, *Phytochemistry and Pharmacological Activities*. *Medicines* (Basel). 23;5(4):127
 11. Liu YM, Luo J, Bennett C. (2010) Adaptive immunity: Based on the dual recognition responses of $\alpha\beta$ T cells. *Self Nonself*;1(1):62-66.
 12. Cetin M, Sevik H, Cobanoglu O (2020) Ca, Cu, and Li in washed and unwashed specimens of needles, bark, and branches of the blue spruce (*Picea pungens*) in the city of Ankara. *Environ Sci Pollut Res* 27:21816–21825. <https://doi.org/10.1007/s11356-020-08687-3>
 13. Nourmohammadi E, Hosseinkhani S, Nedaeinia R, et al. (2020) Construction of a sensitive and specific lead biosensor using a genetically engineered bacterial system with a luciferase gene reporter. <https://doi.org/10.21203/rs.3.rs-18087/v1>

14. Alaqouri HAA, Genc CO, Aricak B, Kuzmina N, Menshikov S, Cetin M (2020) The possibility of using Scots pine needles as biomonitor in determination of heavy metal accumulation. *Environ Sci Pollut Res* 27:20273–20280. <https://doi.org/10.1007/s11356-020-08449-1>
15. Sevik H, Cetin M, Ozel HB, Ozel S, Zeren Cetin I (2020b) Changes in heavy metal accumulation in some edible landscape plants depending on traffic density. *Environ Monit Assess* 192:192. <https://doi.org/10.1007/s10661-019-8041-8>
16. Ghosh, K. and Indra, N. 2018. Cadmium treatment induces echinocytosis, DNA damage, inflammation, and apoptosis in cardiac tissue of albino Wistar rats. *Environ. Toxicol. Pharmacol.* 59, 43–52. doi:10.1016/j.etap.2018.02.009.
17. The Ministry of Environment (2005). Review on the occurrence of cadmium and lead in the environment of the czech republic
18. Martelli A, Rousselet E, Dycke C, Bouron A, Moulis J.M, (2006). Cadmium toxicity in animal cells by interference with essential metals. *Biochimie*.88; 1807-1814.
19. Nawrot T, Plusquin M, Hogervorst J, Roels HA, Celis H, Thijs L,(2006). Environmental exposure to cadmium and risk of cancer: a prospective population-based study. *Lancet Oncol* 7(2):119-126.
20. Curtis D., Klaassen, J.L., and Choudhuri S., (1999). METALLOTHIONEIN: An Intracellular Protein to Protect Against Cadmium Toxicity. *Annu. Rev. Pharmacol. Toxicol.* . 39:267–9.
21. Lars Järup, Agneta Åkesson(2009).Current status of cadmium as an environmental health problem.*Toxicology and Applied Pharmacology* 238 201-208.
22. Mohammad Faisal Siddiqui (2010). Cadmium induced renal toxicity in male rats, *Rattus rattus*. *Eastern Journal of Medicine* 15: 93-9.
23. Takiguchi M, Achanzar WE, Qu W, Li G, Waalkes MP (2003) Effects of cadmium on DNA-(cytosine-5) methyltransferase activity and DNA methylation status during cadmium-induced cellular transformation. *Exp Cell Res* 286:355–365. [https://doi.org/10.1016/s0014-4827\(03\)00062-4](https://doi.org/10.1016/s0014-4827(03)00062-4).
24. Oyedeji F.O , M.O Alagbala and A.B Fawehinmi(2021)Determination Of Heavy Metal Levels In Lipsticks Samples Sold In Akilapa Market, Ibadan, Nigeria. *International Journal of Scientific & Engineering Research* Volume 12, Issue 7, 1411 ISSN 2229-5518 IJSER © 2021 <http://www.ijser.org>
25. US CDC Advisory Committee on Childhood Lead Poisoning Prevention. CDC updates blood lead reference value to 3.5µg/dL. Atlanta: US Centres for Disease Control and Prevention; 2021 (<https://www.cdc.gov/nceh/lead/news/cdc-updates-blood-lead-reference-value.html>).
26. Angrand (2012). Relation of blood lead levels and lead in gasoline: an updated systematic review. *Environmental Health* 21:138 <https://doi.org/10.1186/s12940-022-00936-x>.
27. Arsenic primer: Guidance on the investigation and mitigation of arsenic contamination. New York: United Nations Children’s Fund and the World Health Organization; 2018.
28. Quansah R, Armah FA, Essumang DK, Luginaah I, Clarke E, Marfoh K, et al. *Environ Health Perspect.* (2015);123(5):412-21.Association of arsenic with adverse pregnancy outcomes/infant mortality: a systematic review and meta-analysis.

29. Farzan SF, Karagas MR, Chen Y. (2013). In utero and early life arsenic exposure in relation to long-term health and disease. *Toxicol Appl Pharmacol.*;272(2):384-90.
30. Kadar E, Costa V, Santos R. S (2005) Distribution of micro-essential (Fe, Cu, Zn) and toxic (Hg) metals in tissues of two nutritionally distinct hydrothermal shrimps. *Sci Total Environ.* 65 - 70.
31. Burger J, Jeitner C, and Gochfeld M, (2011) "Locational differences in mercury and selenium levels in 19 species of saltwater fish from New Jersey," *Journal of Toxicology and Environmental Health*, vol. 74, no. 13, pp. 863–874.
32. Berlin M, Zalups R.K, and Fowler B.A, (2007) "Mercury," in *Handbook on the Toxicology of Metals*, G. F. Nordberg, B. A. Fowler, M. Nordberg, and L. T. Friberg, Eds., chapter 33, Elsevier, New York, NY, USA, 3rd edition.
33. Mutter J, Curth A, Naumann J, Deth R, and Walach H, (2010) "Does inorganic mercury play a role in Alzheimer's disease? A systematic review and an integrated molecular mechanism," *Journal of Alzheimer's Disease*, vol. 22, no. 2, pp. 357–374.
34. Hordyjewska A, Popiołek Ł, Kocot J.(2014) The many "faces" of copper in medicine and treatment. *Biometals.* ;27(4):611-21. [PMC free article] [PubMed]
35. Sinkovic A, Strdin A, Svensek F (2008). Severe acute copper sulphate poisoning: a case report. *Arh Hig Rada Toksikol.* ;59(1):31-5. [PubMed]
36. Amos O. Abolaji, Kehinde D. Fasae , Chizim E. Iwezor , Michael Aschner , Ebenezer O. Farombi(2020). Curcumin attenuates copper-induced oxidative stress and neurotoxicity in *Drosophila melanogaster*. *Toxicological report*. Pages 261 -268.
37. Kohzadi S, Shahmoradi B, Ghaderi E, Loqmani H, Maleki A (2019) Concentration, source, and potential human health risk of heavy metals in the commonly consumed medicinal plants. *Biol Trace Elem Res* 187:41–50. <https://doi.org/10.1007/s12011-018-1357-3>
38. Sevik H, Ozel HB, Cetin M, Özel HU, Erdem T (2019) Determination of changes in heavy metal accumulation depending on plant species, plant organism, and traffic density in some landscape plants. *Air Qual Atmos Health* 12:189–195. <https://doi.org/10.1007/s11869-018-0641-x>
39. Mohammadi M, Riyahi Bakhtiari A, Khodabandeh S (2014) Concentration of Cd, Pb, Hg, and Se in different parts of human breast cancer tissues. *J Toxicol*:1–5. <https://doi.org/10.1155/2014/413870>