

Determination of Toxic Concentrations of Heavy Metals in Selected Samples of Tobacco Products

Hani Yahya Alfaifi, Majed Marji Alenezy, Talal Telhi Alenezy, Faiz Nasser Alenazy, Ahmed Mohammed Aleneze

Department of Biochemistry, Faculty of Science, University of Jeddah, Jeddah, Kingdom of Saudi Arabia

Received: 01.07.2022 • Accepted: 26.07.2022 • Published: 06.08.2022 • Final Version: 06.08.2022

Abstract: The foremost threats to human health from heavy metals are related with use tobacco when inserted in the human body by smoking (shisha and cigarette) or chewable like shammah which is harm of health. The tobacco species have been collected from different places, natural or included in the installation of some of the species sold in the markets. The samples were analyzed by wet digestion method and standardized. International protocols were followed for the preparation of material and analysis of heavy metals contents and analyzed by Inductively coupled plasma spectrometer Model-Varian720 ES in center Laboratory in Meteorology, Environment and Arid Land Agriculture, and Center of Excellence in Environmental Studies, King Abdulaziz University (KAU). Results obtained using ICP-OES technique for determination of heavy metals in Tobacco products. Through this study the results were shown the heavy metals which found in tobacco samples is clearly. the objective of the study showing the concentrative the heavy metals in tobacco and compared with levels of heavy metals allowing in the World Health Organization (WHO). What the results indicate is the seriousness of the presence of heavy metals, which cause serious diseases such as cancer and failure in most organs. The use of tobacco should be abandoned.

Keywords: Tobacco, Human Health, Heavy Metals, cadmium, arsenic, copper, nickel, zinc.

1. Introduction

Any metallic chemical element with a relatively high density which is at low concentrations toxic or poisonous is defined as heavy metal. Some examples of heavy metals which differ by their various properties as atomic number, atomic weight, toxicity and density include mercury (Hg), cadmium (Cd), arsenic (As), chromium (Cr), thallium (Tl), and lead (Pb).

Heavy metals as a natural component cannot be degraded or destroyed are found in the Earth's crust. They enter our bodies to a small extent via air, drinking water and food. Many heavy metals (e.g. copper, selenium, zinc) play crucial role as trace elements to maintain the metabolism of the human body. However, poisoning occurs post exposing to higher concentrations.

Bioaccumulation is defined as an increase over time in the chemical concentration in a biological organism, compared to the chemical's concentration in the environment (1). The main metals and/or metalloids from environmental view point are classified according to their toxicity and/or biological importance are the following: chromium (Cr), lead (Pb), cadmium (Cd), arsenic (As), copper (Cu), nickel (Ni) and zinc (Zn) (2) . The International Agency for Research on Cancer (IARC) has classified heavy metals into three classification groups depending on their metal's carcinogenicity on human (3): Group 1 metals and/or metalloids which are carcinogens to human. Group 2A compounds may be carcinogens and group 2B compounds are rarely carcinogens. Group 3 compounds

are non-carcinogens to humans. Based on above, heavy metals are distributed in groups according to their carcinogenic nature: As and its elements, Cd and its elements, hexavalent Cr and metallic, Ni and its element come in group 1, which are carcinogens for humans through the inhalation and ingestion ways of exposure, therefore they are mostly accompanied with lung, liver, nose and kidney cancers (4). Inorganic Pb compounds categorized in group 2A (5). Metallic Cr, trivalent Cr (6), metallic Hg (7) and organic Pb elements are listed in group 3 elements.

1.1. Arsenic

We can find in rock, soil, water and air. Inorganic arsenic is present in groundwater used for drinking in several countries. whereas organic arsenic compounds (e.g. arsenobetaine) are primarily found in fish.(8)

In the production of energy from fossil fuel are the major class industrial processes that lead to arsenic contamination of air, water and soil by the process smelting. (9).

1.2. Cadmium and Zinc

Cadmium mainly found naturally in ores together with zinc, lead and copper. Cadmium complex are used as stabilizers in PVC products, colour pigment, several alloys and in re-chargeable nickel-cadmium batteries. Metallic cadmium is used as an anticorrosion agent (cadmiation). Cadmium is also been found as a pollutant in phosphate fertilizers (10). Although zinc is an essential requirement for good health, excess zinc can be harmful. Excessive absorption of zinc suppresses copper and iron absorption (11).

1.3. Lead

The studies demonstrate that above 50% of inhaled inorganic lead may be absorbed in the lungs. Adults take above 10–15% of lead in food, in the other hand, children may absorb up to 50% via the gastrointestinal tract. Lead in blood is bound to erythrocytes, and excretion is slow and mainly via urine. Lead is precipitated in the skeleton and is only slowly released from this body compartment. Half-life of lead in blood is around 1 month and in the skeleton 20–30 years (12).

1.4. Copper

Copper is the third most used metal in the world [13]. Copper is an important micronutrient that is needed in the growth of both plants and animals. In humans, it facilitates in the production of blood haemoglobin. In plants, Cu is mainly important in seed production, disease resistance, and regulation of water. Copper is an important element, but in high doses it can cause anaemia, liver and kidney damage, and stomach and intestinal irritation (14).

1.5. Nickel

Nickel is an important element that found in the environment only at very low levels and is beneficial in small doses, but it can be hazardous when the maximum high amounts are exceeded. This can cause different types of cancer on different sites of body organs of animals. Ni is used in application of steel and other metal products. The general sources of nickel contamination in the soil are metal plating industries, combustion of fossil fuels, and nickel mining.[15]

1.6. Chromium

It is not found naturally in elemental form, but only in compounds. Chromium is a primary raw product in the form of the mineral chromite. Major sources of Cr contamination include releases from electroplating produces and the disposal of Cr containing excess. (16)

2. Aims

The aim of these study is to know the toxic concentrations of heavy metals in some random samples in the markets of local and global tobacco products such as cigarettes, shammah, and moasel , as well as compare the global rates in terms of toxicity as well as the comparison of natural products developed locally in southern Saudi Arabia and know where to grow with those imported industrial products. And the measurement of heavy metals in it through various modern technologies and writing scientific recommendations to educate people addicted to the danger to health and knowledge of the chronic organic effect of its used.

3. Material and methods

3.1. Inductively Coupled Plasma ICP-OES

The principle used in the inductively coupled Plasma Optical Emission Spectroscopy (ICP-EOS) is when the samples are exposed to plasma energy from outside and the atoms were stimulated. the stimulated atoms return to low energy position, emission rays are spared and the emission rays that matched to the photon wavelength are measured. The element kind is determined basically on the position of the photon rays, and the content of each element is determined basically on the rays intensity. To activate plasma, first, argon gas is added to torch coil, and high frequency electric current is given to the work coil at the tip of the torch tube. An electromagnetic field produced in the torch tube by the high frequency current, argon gas is ionized, and plasma is activated. The temperature of the plasma (10000k) and has high electron density and this energy is applied in the excitation-emission of the sample. The final solution samples are providing into the plasma in an atomized state through the narrow tube in the center of the torch tube (17).

3.2. Characteristics (ICP)

The main analytical advantages of the ICP reproducible vaporization, atomization, excitation, and ionization for a wide range of elements in defferent sample matrices. This is mainly due to the high temperature from 6000 to 7000 K, in the observation zones of the ICP. The temperature of the ICP is high makes it capable of stimulating refractory elements and makes it less prone to matrix interferences. There is another electrical-discharge-based source, for example, direct current and alternating current arcs and sparks ICP is typically less noisy and better able to handle liquid samples and this is a good advantage. Also, there is no contamination from the impurities present in an electrode material due to that ICP is an electrode source less.

ICP source characteristics:

- ❖ The temperature is very high (up to 8000 K).
- ❖ The electron density high (up to 10^{16}cm^3).
- ❖ The degree of ionization for most elements activate multi element capability.
- ❖ Stability is high, resulting to excellent accuracy and precision
- ❖ Detection limits is accurate for a lot of elements (0.1 –100 ng mL⁻¹)
- ❖ Dynamic range is a wide linear.
- ❖ Effective in analyses test (18).

3.3. Samples Collection:

The tobacco species have been collected from different places, natural or included in the installation of some of the species sold in the markets. We started collecting natural leaves from tobacco trees (fig A), which are located in a few places according to the nature of the environment and suitable place for growth in southern Saudi Arabia, Second is shamma(fig B), which is mixed with other compounds and is sold undercover. In the local markets, the third regular cigarettes brand (Davidoff and one Germany) are also available in the markets (fig C), forth is Shisha (Moasel) (fig D) collected from the tobacco product markets which provide different types of different flavors and one type was purchased with apple flavor and strawberry.



figA: natural leaves from tobacco trees



figB: is shammah



figC: cigarettes brand (Davidoff and one Germany)



figD: is Shisha (Moasel)

3.4. Samples preparation

1.000 g of oven dried (120 °C, 24 h) powdered sample was taken in Pyrex flask. The sample is treated with 10 ml of aqua-regia (HNO₃/HCl) and heated slowly at 100 °C till it dissolves the residue. This process was repeated thrice with the addition of 10 ml aqua-regia to get the complete digestion. The solution was filtered through Whatman filter paper No. 42 and transferred to 100 ml volumetric flask and diluted with Milli Q water. The blank was prepared by the same procedure excluding the sample. The digestion and quantification of metal content were performed in triplicate by (ICP-OES) technique. The metal contents were analyzed by using the Inductively coupled plasma spectrometer Model-Varian720ES. Before analyzing the samples, the instrument was calibrated with a standard blank and the multi-element calibration standard. The dilution of the standard heavy metal form

(AppliChem Panreac 766333.1208) was used to determine concentrations of most heavy metals. There was also a dilution of the standard solution (INORGANIC VENTURES. CGAS10-1) to determine the concentration of the As element. In order to obtain concentrations of 4.0, 2.0, 1.0, 0.5, in order to make a standard curve for the device. Where we used the following equation ($M1XV1=M2XV2$) to dilute the previous solutions and then injected into the device.

3.5. Statistical methods:

The results for the identification of the heavy metal in the tobacco samples were obtained after the results were measured and a graph was drawn to determine these concentrations by the Microsoft® Office Proofing Excel.

4. Results:

Results were determined as mean \pm SE of dry weight from three replicates in each test. The samples were analyzed by wet digestion method and standardized. International protocols were followed for the preparation of material and analysis of heavy metals contents and analyzed by Inductively coupled plasma spectrometer Model-Varian720 ES in center Laboratory in Meteorology, Environment and Arid Land Agriculture, and Center of Excellence in Environmental Studies, King Abdulaziz University (KAU). Results obtained using ICP-OES technique for determination of heavy metals in Tobacco products:

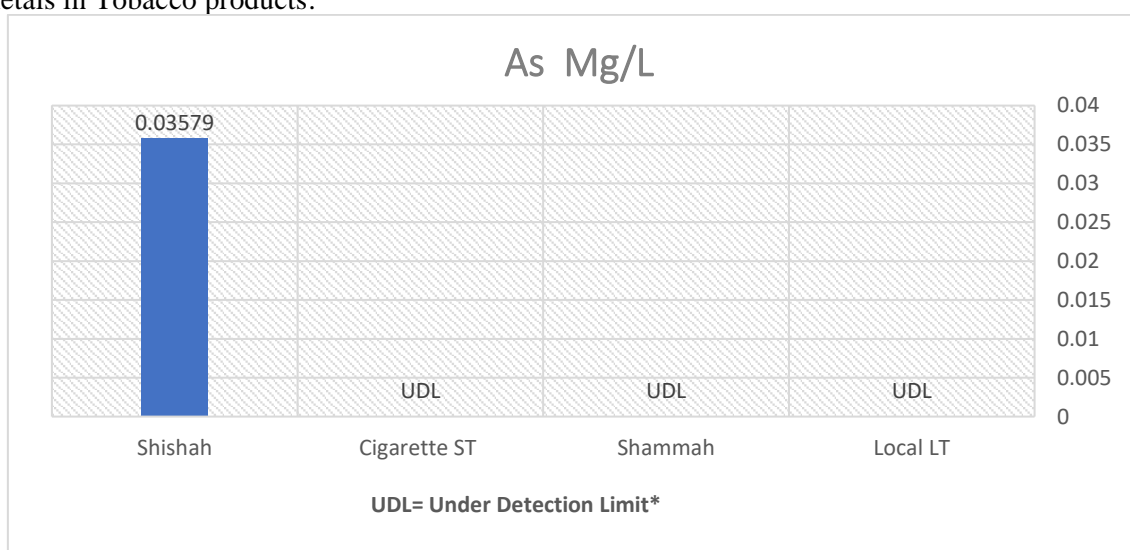


Figure 1. The mean content of Arsenic (Mg/L ± SE) in the studied Shisha, Cigarette ST, shammah, and Local LT.

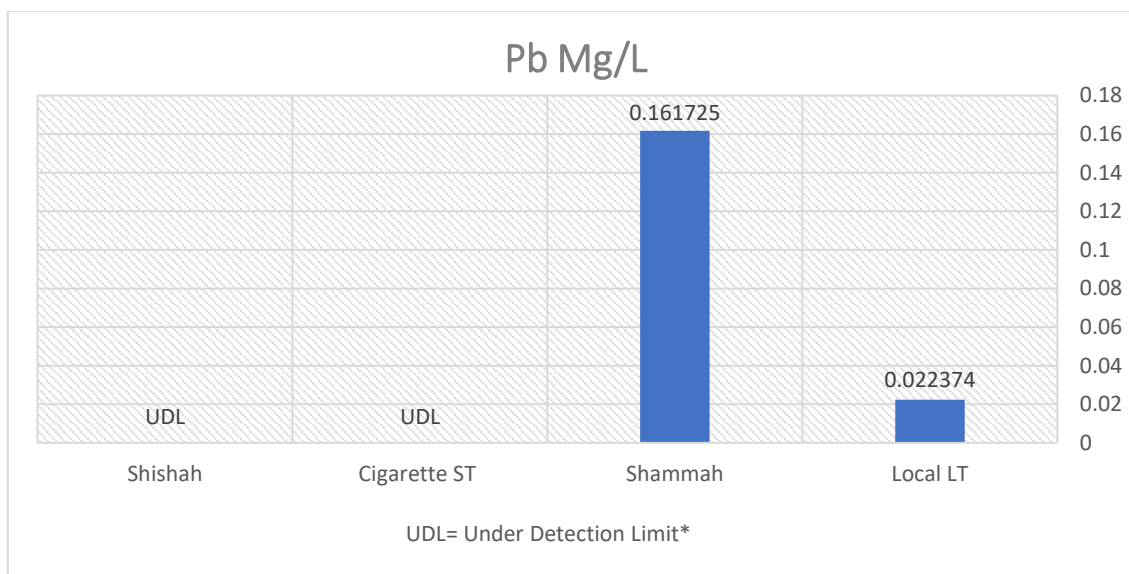


Figure 2. The mean content of Lead (Mg/L ± SE) in the studied Shisha, Cigarette st, shammah, and Local LT

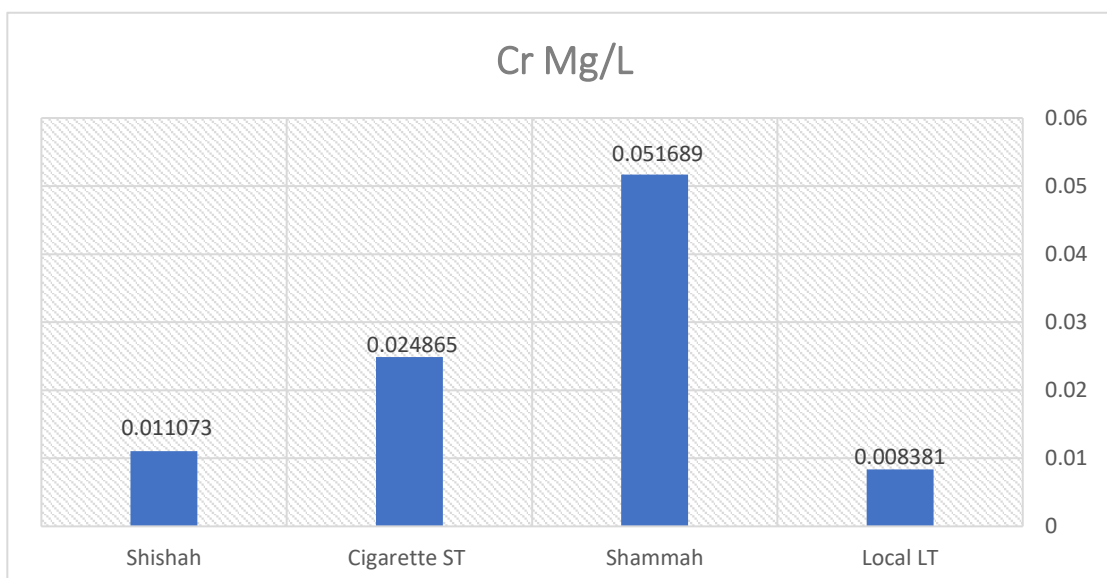


Figure 3. The mean content of chromium (Mg/L ± SE) in the studied Shisha, Cigarette st, shammah, and Local L T.

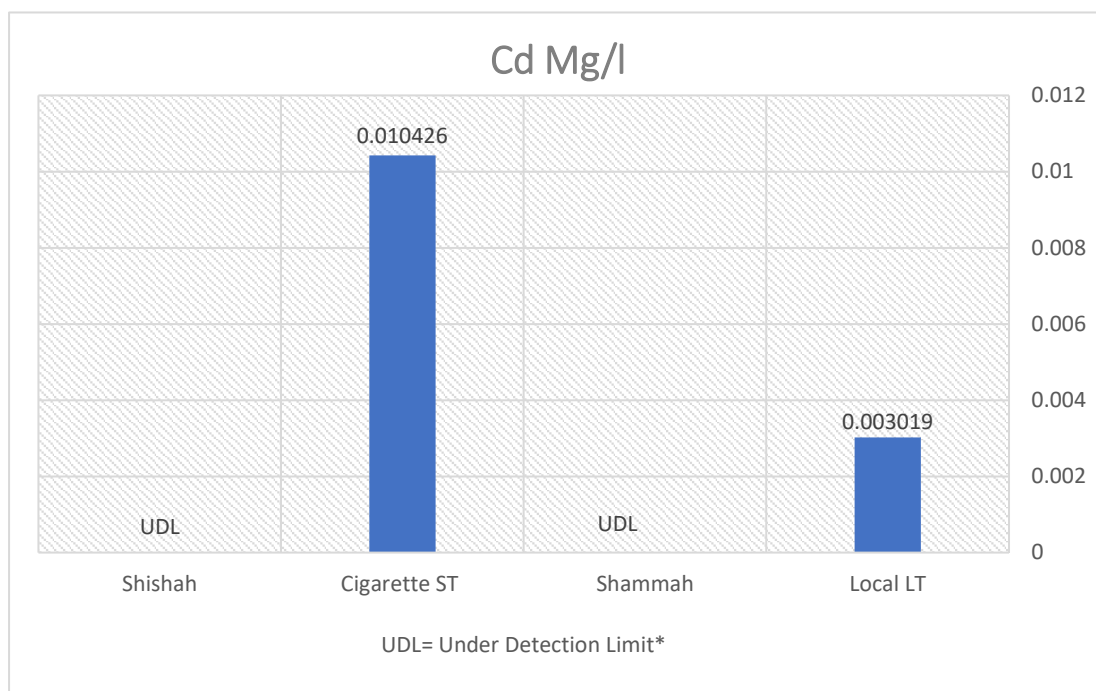


Figure 4. The mean content of Cadmium (Mg/L \pm SE) in the studied Shisha, Cigarette ST, shammah, and Local LT

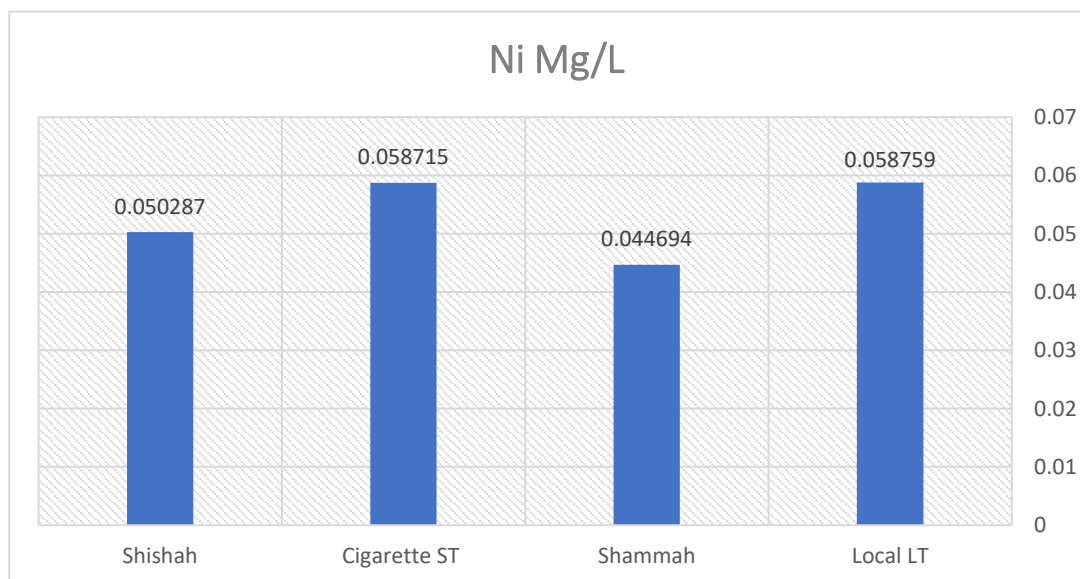


Figure 5. The mean content of Nickel (Mg/L \pm SE) in the studied Shisha, Cigarette ST, shammah, and Local LT

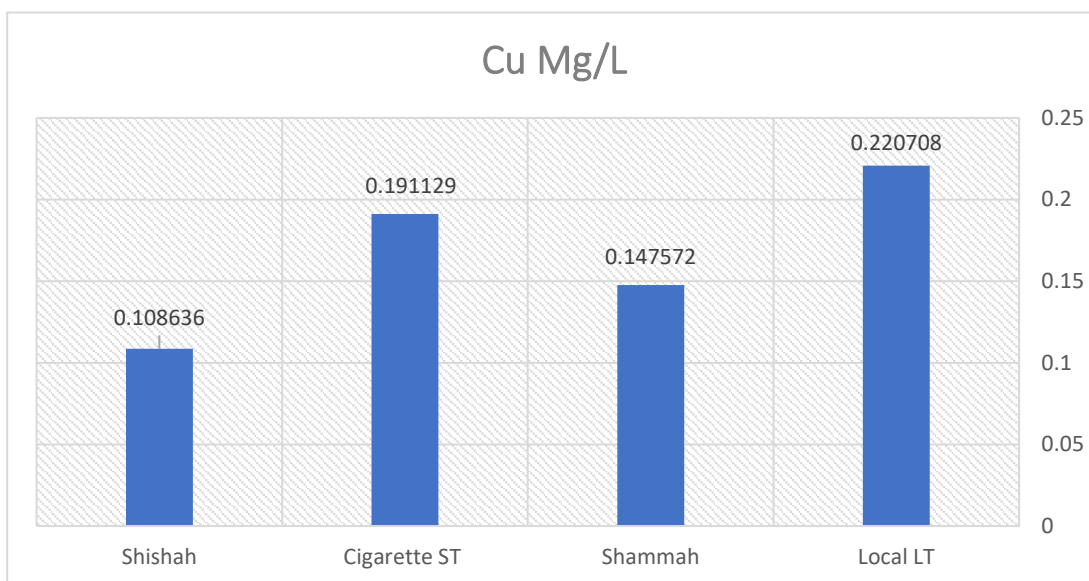


Figure 6. The mean content of Cupper (Mg/L \pm SE) in the studied Shisha, Cigarette ST, shammah, and Local LT

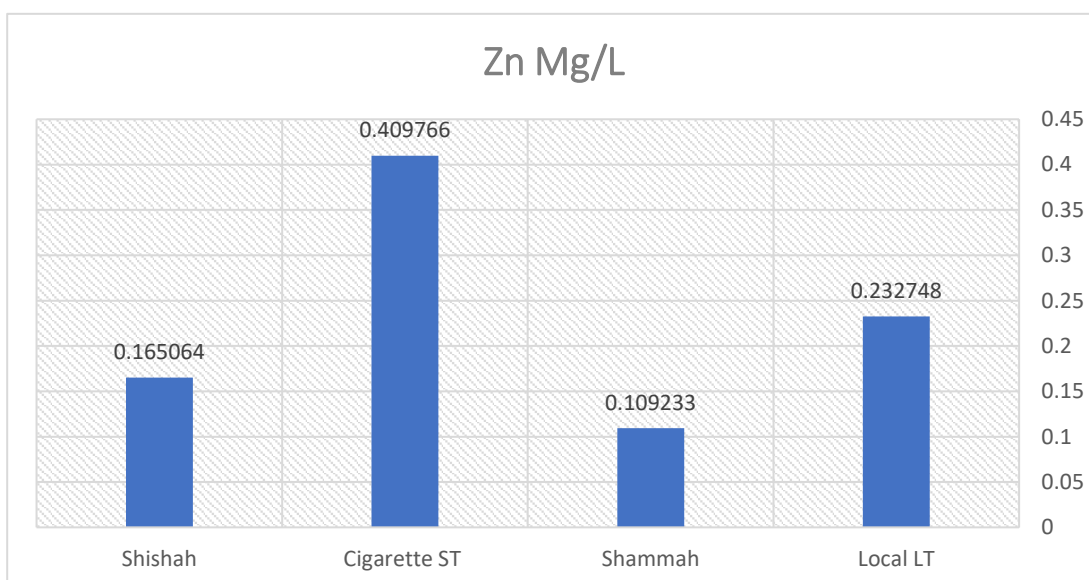


Figure 7. The mean content of Zinc (Mg/L \pm SE) in the studied Shisha, Cigarette ST shammah, and Local LT

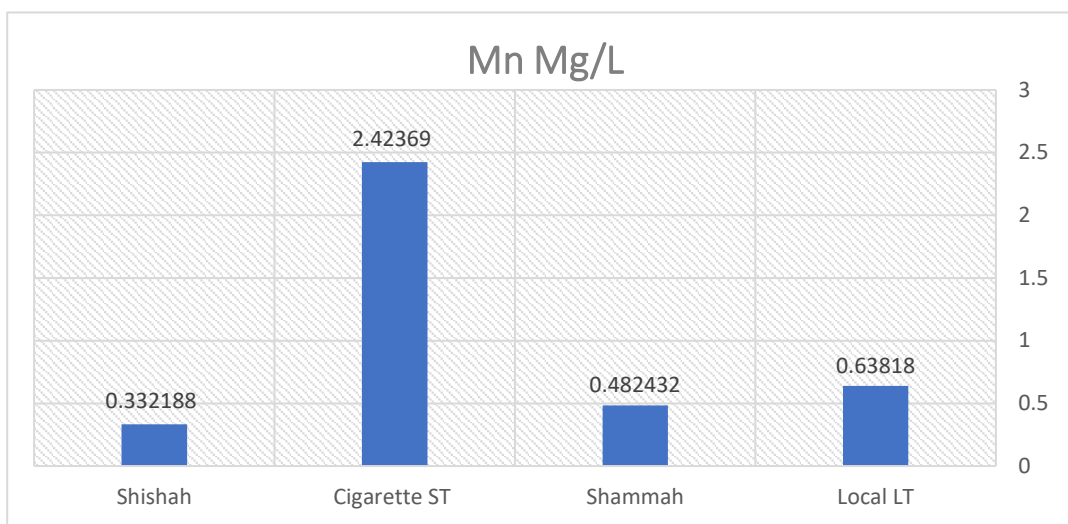


Figure 8. The mean content of manganese (Mg/L \pm SE) in the studied Shisha, Cigarette ST, shammah, and Local LT

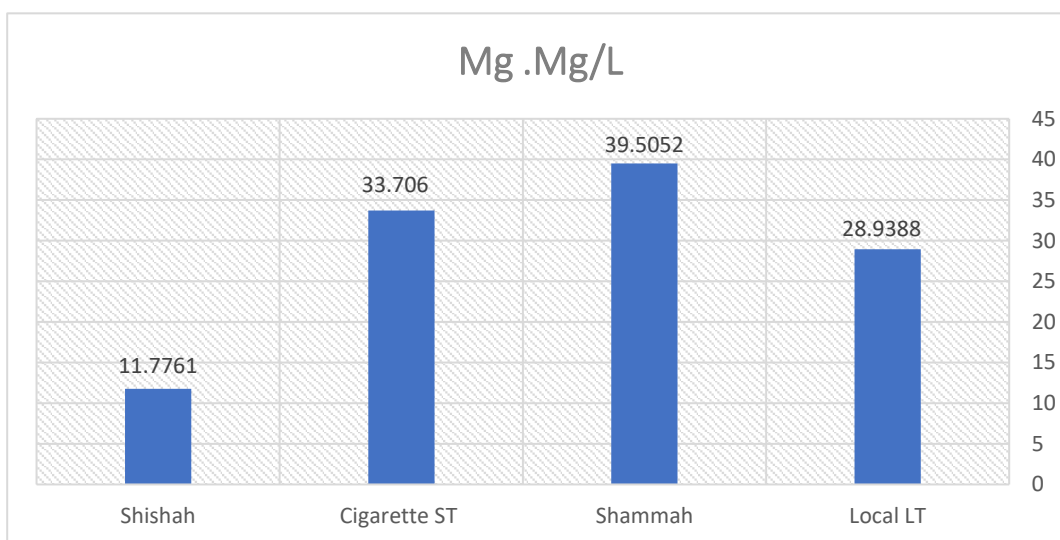


Figure 9. The mean content of Magnesium (Mg/L \pm SE) in the studied Shisha, Cigarette ST, shammah, and Local LT

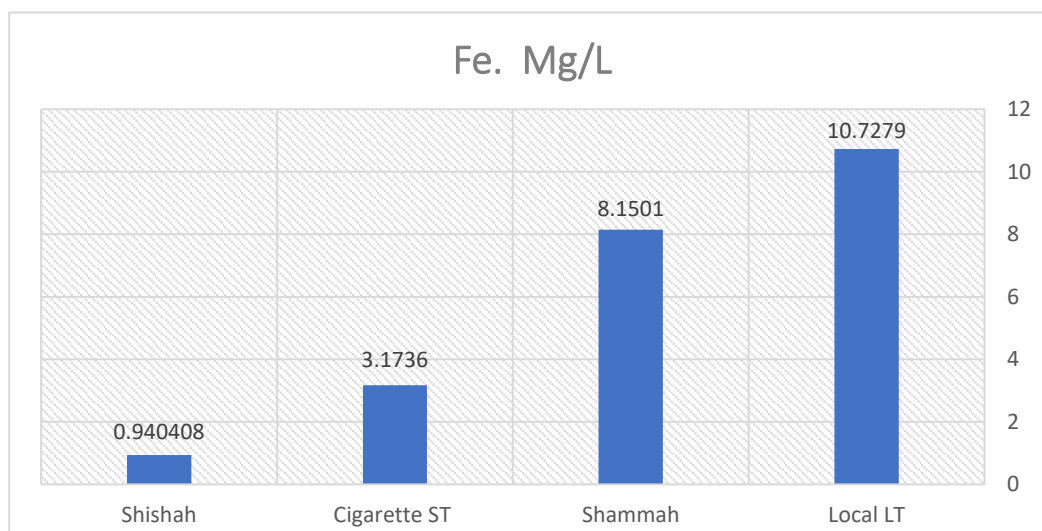


Figure 10. The mean content of ferrum (Mg/L \pm SE) in the studied Shisha, Cigarette ST, shammah, and Local LT

5. Discussion

This work focuses on some toxic heavy metals such as (Cr, Cd, Ar, Cu, Zn, Mg, Fe, Mn, Ni, and Pb), in four samples that were collected (*i.e.* The tobacco species were collected from different places, natural leaves were collected from the tobacco trees, which are found in a few in south Saudi Arabia, in Jazan city, Shammah, ordinary cigarettes (Davidoff one-Germany) and Shisha (moasel) collected from the widely available tobacco products markets. The concentrations of heavy metals were measured and determined by Inductively Coupled Plasma (ICP-OES). The results show that heavy metal levels were higher in tobacco leaves and some selected cigarettes form. Figure 1 shows that the mean level of some heavy metals analysis of the samples using ICP technique. As in shishah, high level of As (Arsenic) at 0.03579 Mg/L which lead to its effect as toxic concentration compared with the World Health Organization (WHO) which is 0.01 Mg/L. (19).

However in the other samples (shammah, local LT, cigarette st) were demonstrated (UDL) under detection limit. Figure 2 shows the results of the present study of the lead Pb was observed in Shammah at 0.161725 mg/L which is very harmful and toxic because it is ten times in comparison to World Health Organization / Food and agriculture organization (WHO/FAO) which is at 0.015 Mg/L and tobacco leaves at 0.022374 mg/L which effect some of the body part of the human (20). As we see in figure 4 there is a high level of cadmium Cd in the sample of Cigarette ST 0.010426 Mg/L more than Local LT while (WHO) level at 0.005 Mg/L (21).

The results in Figure 3 of the study showed that chromium was clear in the table of all samples, and Shammah is the highest concentration as shown in the table 0.051689 Mg/L compared to other samples. As we can see there are comparison between Cigarette ST between shisha and Local LT. Meanwhile, comparing to united stat Food and drug association (USFDA) level at 0.1 Mg/L (22).

Figure 5 The results of the study showed that nickel Ni was clear in the table of all samples and their level is evident. In shisha 0.050287 Mg/L, Cigarette ST 0.058715 Mg/L and Local LT 0.058789 Mg/L the levels are very close rather than Shammah 0.044694 Mg/L according to World Health Organization (WHO) which is at level of 0.07 Mg/L so the results are slightly near and there is an alert to be advised (23).

Here in Figure 6 every two samples are almost the same level concentration of Copper Cu shisha 0.108636 Mg/L and Shammah 0.147572 Mg/L. In addition that we can see the levels of Cigarette ST 0.191129 Mg/L and Local LT 0.2207807 Mg/L and it is below World Health Organization / Food and agriculture organization (WHO/ FAO) level which is at 2.0 MG/L(24).

The recorded result in Figure 7 as shown a high level of Zinc Zn in cigarette ST 0.0409766 Mg/L , while the other three samples Shisha 0.165064 Mg/L , Shammah 0.109233 Mg/L and Local LT 0.232748 Mg/L and all the samples are below the limits of World Health Organization (WHO) at 5 Mg/L (25). Here in Figure 8 the results of manganese Mn show a high level in Cigarette ST at 2.42369 Mg/L which is above the WHO in five times, while the remaining samples levels are the same level of WHO level (26). In the Figure 9 the Shammah result shown are the higher level of Magnesium Mg which is 39.5052 Mg/L comparing to the other collected sample Cigarette ST 33.706 Mg/L, Local LT 28.9388 Mg/L and Shisha 11.7761 Mg/L, where the normal range 0.14 Mg/L to 529 Mg/L (27). We observe in figure 10, the ferrous Fe in Local LT is the highest amount at 10.7279 Mg/L which is a way above at 0.3 Mg/L according to the scale of (WHO) in comparing to Shammah at 8.1501 Mg/L, Cigarette ST 3.1736 Mg/L and Shisha 0.940408 Mg/L(28).

In general, there was some statistical difference in concentrations for the heavy metals between the four sampling that was collected for whole the tobacco species were collected from different places, natural leaves were collected from the tobacco trees, Shammah, ordinary cigarettes (Davidoff one-Germany) and Shisha (moasel) and tobacco products market. In general, we can see that the concentrations level for Arsenic in shisha which is harmful and toxic, there is no identification for the other heavy metals or under detection limits (UDL) in figure 1. In Shammah, the level of lead (Pb) is very high than the other samples as seen in figure 2 and level of Chromium in figure 4. Cadmium level in Cigarette is high in figure 3. Nickel (Ni) the three samples are the same and few changes in the other one figure 5. Copper (Cu) Shammah and Shisha are the same level nearly in other hand, the two Cigarette and Local LT at the same also. In figure 9 results of Magnesium (Mg) in Shammah is the highest level comparing to the remaining three samples figure 10.

6. Conclusion:

The present study shows that shisha represent hazard exposure to the heavy metals and the only sample that contain arsenic compared to the other samples. So, it is the highest toxic because it is above level of arsenic compared to WHO. On the other hand, the shammah finding indicate high level of lead that is more than the limit consumption of lead to ten times based on the WHO. In addition, the shammah highest concentration of chromium compared to another sample and USFDA. Chromium is the major cause to the spread of cancer. It has been shown in our study that the cigarette contains high level of cadmium compared to WHO.

7. Recommendation

The purpose of the study is to detect the concentration of heavy metals in tobacco products because the recent prevalence of more than one percent of people to consume to lead the spread of many diseases.

The purpose of the study founding concentration of the heavy metals in shisha, shammah, cigarette and tobacco leaves because the spread recent times increase percent person to intake it to lead spread of many disease.

We recommend a person who takes a shisha that shows no symptoms, any pigmentation on the skin and pain in the stomach should be achieved from arsenic in the blood as well as the person who takes the cigarette test is working continuously on kidneys, liver and lungs. If you have any ulcers or a tooth loss, consult your doctor for the necessary tests to make sure there are no serious illnesses.

We recommend that the Ministry of Health and the SFDA repeat the examination of the level of measurement of cigarettes, shisha, shammah and tobacco in heavy metals and write the concentration of all heavy metals in the cover to see people before use.

We also propose the intensification of smoking-cessation clinics in all health centers. And also facilitate the cooperation of research centers with researchers and the provision of specialized centers for the measurement of heavy metals.

Acknowledgment:

First, many thanks to Allah how gave me comfort at the difficult times during this study.

Secondly, we would like to express my sincere gratitude to my supervisors Dr. Mohanad and Dr. Mohammad.

Thirdly, we would like to extend our thanks to the central laboratory in Meteorology, Environment and Arid Land Agriculture, and Center of Excellence in Environmental Studies, King Abdulaziz University (KAU).

Finally, special thanks to our families for their encouragement, support and patience.

References

- [1] Suvarapu LN, Seo YK and Baek SO (2014) Heavy metals in the Indian atmosphere: a review. *Research Journal of Chemistry and Environment* 18(8): 99–111.
- [2] Hogan MC (2010) Heavy metal. *Encyclopedia of Earth*. National Council for Science and the Environment. Available at: <http://www.eoearth.org/view/article/153463> (accessed 28 December 2015).
- [3] IARC (International Agency for Research on Cancer) (1987) IARC monographs on the evaluation of carcinogenic risks to humans. Some metals and metallic compounds. Volume 23. Available at: <http://monographs.iarc.fr/ENG/Monographs/vol23/volume23.pdf> (accessed 15 December 2015).
- [4] IARC (2012) IARC monographs on the evaluation of carcinogenic risks to humans. Arseni, metals, fibres, and dusts. A review of human carcinogens. Volume 100C. Available at: <http://monographs.iarc.fr/ENG/Monographs/vol100C/index.php> (accessed 12 December 2015).
- [5] IARC (2006) IARC monographs on the evaluation of carcinogenic risks to humans. Inorganic and organic lead compounds. Volume 87. Available at: <http://monographs.iarc.fr/ENG/Monographs/vol87/index.php> (accessed 12 December 2015).
- [6] IARC (1990) IARC monographs on the evaluation of carcinogenic risks to humans. Chromium, Nickel and Welding. Volume 49. Available at: <http://monographs.iarc.fr/ENG/Monographs/vol49/index.php> (accessed 20 December 2015).
- [7] IARC (1990) IARC monographs on the evaluation of carcinogenic risks to humans. Chromium, Nickel and Welding. Volume 49. Available at: <http://monographs.iarc.fr/ENG/Monographs/vol49/index.php> (accessed 20 December 2015).
- [8] WHO. Arsenic and Arsenic Compounds. *Environmental Health Criteria*, vol. 224
- [9] Chilvers DC, Peterson PJ. Global cycling of arsenic. In: Hutchinson TC, Meema KM (eds) *Lead, Mercury, Cadmium and Arsenic in the Environment*. Chichester: John Wiley & Sons, 1987; 279-303
- [10] Jarup L, Berglund M, Elinder CG, Nordberg G, Vahter M. Health effects of cadmium exposure—a review of the literature and a risk estimate. *Scand J Work Environ Health* 1998, 24(suppl 1):1-51
- [11] Fosmire, Gary J. "Zinc toxicity." *The American journal of clinical nutrition* 51.2 (1990): 225-227.
- [12] WHO, Lead, *Environmental Health Criteria*, vol. 165. Geneva: World Health Organization, 1995

- [13] VCI, Copper history/Future, Van Commodities Inc., 2011, <http://trademetal futures.com/copperhistory.html>.
- [14] C. E. Mart'inez and H. L. Motto, "Solubility of lead, zinc and copper added to mineral soils," *Environmental Pollution*, vol. 107, no. 1, pp. 153–158, 2000.
- [15] [59] A. P. Khodadoust, K. R. Reddy, and K. Maturi, "Removal of nickel and phenanthrene from kaolin soil using different extractants," *Environmental Engineering Science*, vol. 21, no. 6, pp. 691–704, 2004.
- [16] L. A. Smith, J. L. Means, A. Chen et al., *Remedial Options for Metals-Contaminated Sites*, Lewis Publishers, Boca Raton, Fla, USA., 1995.
- [17] Inductively Coupled Plasma –Optical Emission Spectroscopy: A Review. Somsubhra Ghosh^{1*}, V. Laxmi Prasanna¹, B. Sowjanya¹, P. Srivani¹, M. Alagaraja¹, Dr. David Banji¹ Nalanda College of Pharmacy, Nalgonda, Andhra Pradesh – 508001, India *Corresponding Author E-mail: somsubhraghosh@gmail.com
- [18] Rodolfo Fernández-Martínez,quel Caballero et al., Application of ICP-OES to the determination of CuIn_{1-x}GaxSe₂ thin films used as absorber materials in solar cell devices *Analytical and Bio analytical Chemistry*, 2005, Volume 382, Number 2, Pages 466-470
- [19] Kamaruzzaman, B.Y., Rina, Z., Akbar John, B. and Jalal, K.C.A. (2011). Heavy metal accumulation in commercially important fishes of South west Malaysian coast, *Research journal of environmental sciences*, 5: 595-602.
- [20] Akintujoye, J.F., Anumudu, C.I. and Awobode, H.O. (2013). Assessment of heavy metal residues in water, fish tissue and human blood from Ubeji, Warri, Delta State, Nigeria, *Journal of Applied Science and Environmental Management*, 17 (2) 291-297.
- [21] El-Moselhy, Kh.M., Othman, A.I., Abd El-Azem, H. and El-Metwally, M.E.A. (2014). Bioaccumulation of heavy metals in some tissues of fish in the Red Sea, Egypt, *Egyptian Journal of Basic and Applied Sciences*, 1(2): 97–105, doi: 10.1016/j.ejbas.2014.06.001
- [22] Javed, M. and Usmani, N. (2014). Assessment of heavy metals (Cu, Ni, Fe, Co, Mn, Cr, Zn) in rivulet water, their accumulations and alterations in hematology of fish *Channa punctatus*, *African Journal of Biotechnology*, 13(3): 492-501, doi: 10.5897/AJB2013.13131.
- [23] *International Journal of Applied Science and Technology* Vol. 1 No.4; July 2011 90 Measurement of Heavy Metals (Zn, Cu, Fe, Ni, Cd, Co) in Chadormalu Underground Water Resources and Presentation of an Environmental Management Plan
- [24] El-Moselhy, Kh.M., Othman, A.I., Abd El-Azem, H. and El-Metwally, M.E.A. (2014). Bioaccumulation of heavy metals in some tissues of fish in the Red Sea, Egypt, *Egyptian Journal of Basic and Applied Sciences*, 1(2): 97–105, doi: 10.1016/j.ejbas.2014.06.001
- [25] El-Moselhy, Kh.M., Othman, A.I., Abd El-Azem, H. and El-Metwally, M.E.A. (2014). Bioaccumulation of heavy metals in some tissues of fish in the Red Sea, Egypt, *Egyptian Journal of Basic and Applied Sciences*, 1(2): 97–105, doi: 10.1016/j.ejbas.2014.06.001
- [26] El-Moselhy, Kh.M., Othman, A.I., Abd El-Azem, H. and El-Metwally, M.E.A. (2014). Bioaccumulation of heavy metals in some tissues of fish in the Red Sea, Egypt, *Egyptian Journal of Basic and Applied Sciences*, 1(2): 97–105, doi: 10.1016/j.ejbas.2014.06.001
- [27] Sources of variability in livestock water quality over 5 years in the Northern Great Plains. M. K. Petersen,² J. M. Muscha,* J. T. Mulliniks,† R. C. Waterman,* A. J. Roberts,* and M. J. Rinella*. *Fort Keogh Livestock and Range Research Laboratory, USDA-ARS, Miles City, MT 59301; and †Department of Animal Science, University of Tennessee, Crossville 38571
- [28] El-Moselhy, Kh.M., Othman, A.I., Abd El-Azem, H. and El-Metwally, M.E.A. (2014). Bioaccumulation of heavy metals in some tissues of fish in the Red Sea, Egypt, *Egyptian Journal of Basic and Applied Sciences*, 1(2): 97–105, doi: 10.1016/j.ejbas.2014.06.001