IOSR Journal of Applied Chemistry (IOSR-JAC)
e-ISSN: 2278-5736.Volume 15, Issue 7 Ser. II (July 2022), PP 46-52
www.iosrjournals.org

Trace element levels in healthy livers of live human beings from Northwestern Argentina

Carlos Ariel Marrades[1], Adriana María Sales[1], María del Pilar Balverdi[1], Carla María Rubio[1], Lino Edmundo Campero[2], Ricardo Figueroa[3], Héctor Jorge Lazarte[3], MaríaAngética Véliz[1]

[1]*Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán, Tucumán, Argentina*
[2]*Hospital de ClínicaPresidente Dr. Nicolás Avellaneda, Tucumán, Argentina*
[3]*Hospital Centro de SaludZenónSantillán, Tucumán, Argentina*

Abstract:
Trace elements play an important role in human metabolism. The aim of this work was to determine iron (Fe), copper (Cu), cadmium (Cd), lead (Pb) and arsenic (As) concentrations in healthy livers by the atomic absorption spectrometry technique and set normal reference ranges for the region. Analytical methods were duly validated by evaluating figures of merit. This topic is important because there are a few research works about trace element contents in the liver of live human beings, so no data were found in the literature in similar papers. Analyses of 25 liver tissue samples were carried out (12 male and 13 female subjects, between 18 and 85 years old). Sample number and size were important critical issues, since the possibility of obtaining samples from healthy living subjects was limited. The obtained concentration ranges, in µg.g-1 dry weight, were the following: Fe (527 - 1095), Cu (10.12 - 27.53), Cd (0.56 - 1.59) and Pb (0.76 - 7.86). Arsenic levels were below 0.02 µg.g-1. Correlations were not found between individual ages, sex and metal levels. The obtained results were compared with data reported in other publications made for diseased and healthy livers from autopsies.
Key Word*:human liver; biopsies; trace elements; atomic spectrometry.*

Date of Submission: 14-07-2022 Date of Acceptance: 29-07-2022

I. Introduction

Trace elements play an important role in human metabolism, however a deficiency or excess of them can cause risky diseases; for this reason, they have been the subject of research for a long time (Filteau and Tomkins 1994). The incorporation of most trace elements into the body occurs through the digestive tract with forced transit through the liver, which has a high capacity to store them. Metabolism alteration of certain metals has a direct impact on this organ and can cause various liver diseases, thus leading, in some cases, to early death (Muñoz et al. 2011). Some elements, such as iron (Fe) and copper (Cu) were related to different liver diseases such as chronic hepatitis, cirrhosis, nonalcoholic fatty liver and autoimmune diseases (Himoto and Masaki 2020). Some authors associated fatty changes in the liver with decreased lead (Pb) concentrations, as well as with Wilson's disease with elevated Cu levels in the liver (Griesmann et al. 2009). Cu accumulation has also been associated with chronic hepatitis C (Hatano et al. 2000). Other investigators found high Fe levels in the liver of patients with hemochromatosis (Adams et al. 2006). Arsenic (As) and Pb have been shown to represent a major source of morbidity and mortality for children and adults (Quansah et al. 2015). It has been established that metals such as As, cadmium (Cd) and Pb have negative health impacts (Rehman et al. 2018). Metal amount in liver tissue is a direct measure of each element deposition in liver. Iron quantification in liver by using electrothermal atomic absorption spectrometry in adults with chronic liver diseases was carried out (Adams et al. 2006). Metal concentration data in a healthy liver of postmortem individual´s samples have been reported (Katoh et al. 2002; Zhang et al. 2004; Rahil-Khazen et al. 2002) and in liver tissue from biopsies of individuals with a diseased liver (Varga et al. 2005; Şahin et al. 2018), as well. In scientific publications no reference values for trace metals in liver of live individuals were found under normal physiological conditions that could be used as "reference ranges". In Argentina, trace metal levels have not been reported in healthy livers from live human beings.

The aim of this work was to determine levels of Fe, Cu, Cd, Pb and As elements in liver tissue of individuals from northwestern Argentina by atomic absorption spectrometry. These determinations were carried out in the healthy liver of live humans and they are a valuable contribution to establish the range of normal reference values for the region.

II. Material And Methods

2.1. Reagents and standard solutions

All reagents were analytical grade. Ultrapure water (18 MΩ cm resistivity) from NANOpure (Barnstedt, IA, USA) were used. For sample digestion, 30% (w/w) hydrogen peroxide (Cicarelli, Argentina) and 65% (w/w) HNO_3 redistilled in a sub-boiling (Merck, Darmsatdt, Germany) were used. The laboratory material was decontaminated by immersion in 30% (v/v) HNO_3 for 24 h and rinsed with ultrapure water. Five intermediate dilutions from 1000 mg L^{-1} stock standard solutions of Fe, Cu, As, Cd y Pb (Merck) were prepared with 5% (v/v) nitric acid at the following concentrations: 100 mg L^{-1} for Fe, 1 mg L^{-1} for Cu, 150 mg L^{-1} for As, 1mg L^{-1} for Cd and 10 mg L^{-1} for Pb.

2.2. Instrumentation

Samples were weighed on a Mettler-Toledo AG 245 analytical scale (sensitivity 0.01 mg) and digested through a microwave system (Anton Paar, Multiwave PRO, Germany).

Analyses were performed with a Perkin Elmer (Norwalk, CT, USA) AAnalyst 100 atomic absorption spectrometer equipped with a deuterium lamp background corrector, a HG800 pyrolytic graphite furnace integrated to the platform (part N° B3000407, Perkin Elmer) and an AS-72 autosampler. As purge gas, high purity argon (99,9%) at 250 mL.min^{-1} was used. Individual hollow cathode lamps were used as a radiation source. A slit width of 0.7 nm for Cu, As, Cd and Pb, and 0.2 nm for Fe was used. The following wavelengths (in nm): 248.3 for Fe, 324.8 for Cu, 193.7 for As, 228.8 for Cd and 283.3 for Pb were employed.

2.3. Sample collection and preparation

Twenty-five individuals, between 18 and 85 years old, who were admitted to the Surgery Service of Centro de Salud "ZenónSantillán" hospital, between 2016 and 2018, were studied. Liver samples were taken by biopsies according to the bioethical rules of the aforementioned hospital and with the subjects' signed consent involved in this study with anonymity guarantee and confidentiality.

Adults without liver disease and without a history of toxic habits (drugs and alcohol) were considered to be as inclusion criteria. Neonates, children and adolescents under 18 years of age, pregnant women and individuals treated with dietary supplements were excluded from this study.

Liver tissue samples were placed in individual decontaminated polypropylene tubes and stored in a freezer until treatment. They were dried at 95°C for 5 hours at constant weight. Accurate amounts of samples, between 50 and 150 mg of dry weight were weighed for digestion and placed directly into digester vessels, 0.5 mL of 65% HNO_3, 1 mL of 30% H_2O_2 and 1.5 mL of ultrapure H_2O being added. The digestion program used was as follows: ramping for 10 min to reach 450 W, then holding for 20 min at 450 W and finally cooling to 70°C. At the same time, a reagent blank was made and subjected to the same treatment. In all cases the final volume was 10 mL.

2.4. Determinations of trace elements

Fe determination was carried out by the atomic absorption spectrometry technique with flame atomization (FAAS) and Cu, As, Cd and Pb quantification by electrothermal vaporization (ETAAS). For the ET AAS technique, temperature programs suggested by the equipment manufacturer was used after verifying that the conditions were optimal for the work. The drying temperature (120°C) and the cleaning temperature (2600°C) were the same in all cases. Pyrolysis and atomization temperatures were 1400 and 2400°C for Fe, 1000 and 2300°C for Cu, 1300 and 2300°C for As, 700 and 1600°C for Cd, 700 and 1800°C for Pb. As a matrix modifier for arsenic, 0.015 mg of Pd and 0.01 mg of Mg $(NO_3)_2$ were used, while for cadmium and lead 0.2 mg of potassium dihydrogen phosphate were employed. Calibration curves were performed for each element by using five standard solutions in the concentration range of 0.5-5.0 mg L^{-1} for Fe, 20-100 µg L^{-1} for Cu, 30-120 µg L^{-1} for As, 0.5-5.0 µg L^{-1} for Cd and 10-100 µg L^{-1} for Pb. Liver tissue sample readings were done in triplicate. Concentrations were expressed in µg g^{-1} referred to dry weight of liver tissue.

2.5. Statistical Analysis

A descriptive statistical study was carried out to characterize the samples by calculating mean, median, and standard deviation (SD), maximum and minimum values and their corresponding quartiles. Concentration data distribution was evaluated by using Box Plots. Correlation between pairs of metals and between metal and age was analyzed by using Pearson's correlation coefficient, while the relationship between metal and sex was performed by Tukey's test. In all cases statistical software (Minitab) and a significance level of 0.05 were used.

III. Result and Discussion

Analytical method validation

Figures of merit for each elemental determination method were evaluated. LOD (limit of detection), three times the standard deviation of the blank signal, was obtained while the LOQ (limit of quantification) being ten times the SD of the blank signal, was calculated.

Calibration curves of absorbance (in FAAS) or integrated absorbance (in ET AAS) versus standard solution concentrations of each analyte were made. The linear regression analysis to obtain the equations of the corresponding lines by evaluating the linearity by Pearson's correlation coefficient (r) was applied. LOL (limit of linearity) was also obtained. Accuracy through recovery tests by adding known amounts of standard solutions before sample treatment was evaluated. Data obtained are shown in Table 1.

Table 1*LOD, LOQ, LOL, Pearson´s coefficient and recovery*

Element	LOD ($\mu g\ L^{-1}$)	LOQ ($\mu g\ L^{-1}$)	LOL ($\mu g\ L^{-1}$)	Pearson's coefficient	Recovery (%)
Fe	40.0	120	5000	0.9999	92
Cu	2.3	7.7	100	0.9998	94
As	8.0	27.0	120	0.9987	90
Cd	0.2	0.5	5.0	0.9970	89
Pb	2.6	8.6	100	0.9997	89

Precision was evaluated for each method in terms of repeatability and reproducibility by calculation of relative standard deviation (RSD%). In all cases, RSD% values calculated under repeatability conditions were less than 5% and under reproducibility conditions were less than 6%. Results indicated adequate precision of the method and they are shown in Table 2.

Table 2*Precision parameters of each method*

Element	Precision	
	Repeatability RSD%	Reproducibility RSD%
Fe	2.8	3.3
Cu	2.4	2.9
As	4.9	5.4
Cd	0.40	0.48
Pb	2.9	3.6

Matrix effect in both techniques (FAAS and ETAAS) was assessed by using the standard addition method. This method was applied to cow livers due to the small amount of human liver samples available for analysis. A statistical comparison, using test t, between the slopes of calibration lines carried out with aqueous standards and with the addition of standards to a sample portion was performed. No significant difference was obtained in both techniques between the slopes for a significance level of 0.05, therefore matrix interference in metal quantification was discarded.

Trace element levels

Fe, Cu, As, Cd, and Pb concentrations were expressed in $\mu g\ g^{-1}$ of dry tissue. Data obtained from the descriptive statistical analysis performed on concentrations are shown in Table 3.

Table 3*Statistics of human liver samples studied. Values are expressed in µg g^{-1} (dry weight) with three significant figures (n=25)*

Parameter	Fe	Cu	Cd	Pb
Mean	779	18.5	1.41	4.94
Median	726	14.9	1.13	2.06
Standard deviation	313	9.10	1.33	6.11
Minimum	322	6.66	0.221	0.340
Maximum	1.349	34.8	6.18	23.8
Q_1	527	10.1	0.561	0.760
Q_3	1.095	27.5	1.59	7.86
n ≤ LD	0	1	1	3

n ≤ LD: number of samples below the limit of detection.

From the statistical data expressed in Table 3, concentration ranges of the studied analytes can be established. For iron, a median value of 726 (between Q_1= 527 and Q_3=1095) has been found, for copper 14.9 (between 10.1 and 27.5), for cadmium 1.13 (between 0.56 and 1.59) and for lead 2.06 (between 0.76 and 7.86), all expressed in µg g^{-1} for dry weight with three significant figures. Arsenic levels were below the LOD of 0.02 µg.g^{-1} for most of the samples. Only one sample yielded an As value of 10.5 µg g^{-1} for dry weight, below the LOQ. Standard deviations obtained indicated a high data dispersion, giving a wide range of values for all analytes.

Elemental concentrations in human liver have been reported by different authors. Table 4 shows results that correspond to liver tissue samples obtained by autopsy from individuals from countries outside South America. Results with wide dispersion ranges can be seen in the aforementioned table.

Table 4 *Element concentrations in human liver reported by different authors, expressed in µg g^{-1} of dry tissue, as means ± SD. The range of values is indicated in brackets*

Author	Country	Fe	Cu	As	Cd	Pb
Bona *et al*, 1992	Spain	-	-	-	-	4.43 ± 3.12 (0.42- 9.25)
Bush *et al*, 1995	USA	885 ± 573 (158-2434)	20 ± 11 (4-47)	-	5.20 ± 4.54 (0.7-21.8)	-
Griesmann, 2009	USA	1342.2±140 (220.9-4251.2)	20.56±1.69 (5.53–48.44)	-	8.79 ± 1.17 (0.77-24.66)	3.09 ± 0.46 (0.48-3.91)
Hudnik y Gomiscek, 1984	Yugoslavia	-	30.8 ± 17.1 (8.2-71.0)	-	6.4 ± 4.4 (1.2-18.2)	-
Julshamn *et al*, 1998	Norway	281 (75-890)	-	-	1.7 ± 1.5 (0.39-5.5)	
Katoh*et al*, 2002	Japan	837 ± 522 (79-2660)	32.2 ± 20.0 (2.6-116)	-	16.7 ± 19.1 (0.7-117)	-
Trebbe*et al*, 1998	Canada	-	14.2 ± 7.0 (1.7-32.4)	-	-	-
Yinsong *et al*, 1991	China	-	34.11 ± 13.65	0.16 ± 0.11	8.60 ± 5.32	-
Zhang *et al*, 2004	China	705±321 (132-1530)	-	0.060±0.009 (0.04-0.069)	5.45 ± 1.56 (3.92-8.16)	-

In this work, the minimum value of iron concentration found in healthy liver was 322 µg g^{-1} and the maximum value was 1349 µg g^{-1}. This range goesfrom the lowest value (132 µg g^{-1}) reported by Zhang (2004) to the highest value (4251.2 µg g^{-1}) reported by Griesmann (2009). In the same way, the obtained values agreed with those reported by other authors (Katoh et al. 2002; Bush et al. 1995; Julshamn et al. 1989).

A similar situation can be observed for copper, the minimum value obtained in our studywas 6.66 µg g^{-1} and that published by Trebbe (1998) was 1.7 µg g^{-1}. The highest concentration published by Katoh (2002) was 116 µg g^{-1} and far exceeded the result of 34.8 µg g^{-1}.

Very low levels for arsenic were obtained in the samples studied, values being undetectable or unquantifiable. Some authors reported contents of 0.060 μg g^{-1} (Zhang et al. 2004) and 0.16 μg g^{-1} (Yinsong et al. 1991).

Cadmium concentrations reported were between a value of 0.39 μg g^{-1} (Julshamn et al. 1989) and a value of 21.8 μg g^{-1} (Bush et al. 1995) in agreement with the values between 0.221 and 6.18 μg g^{-1} in this work.

Lead content in human liver was the least studied metal, and values between 0.340 and 23.8 μg g^{-1} were obtained. The widest range published corresponds to Bona et al. (1992) with concentrations between 0.42 and 9.25 μg g^{-1}.

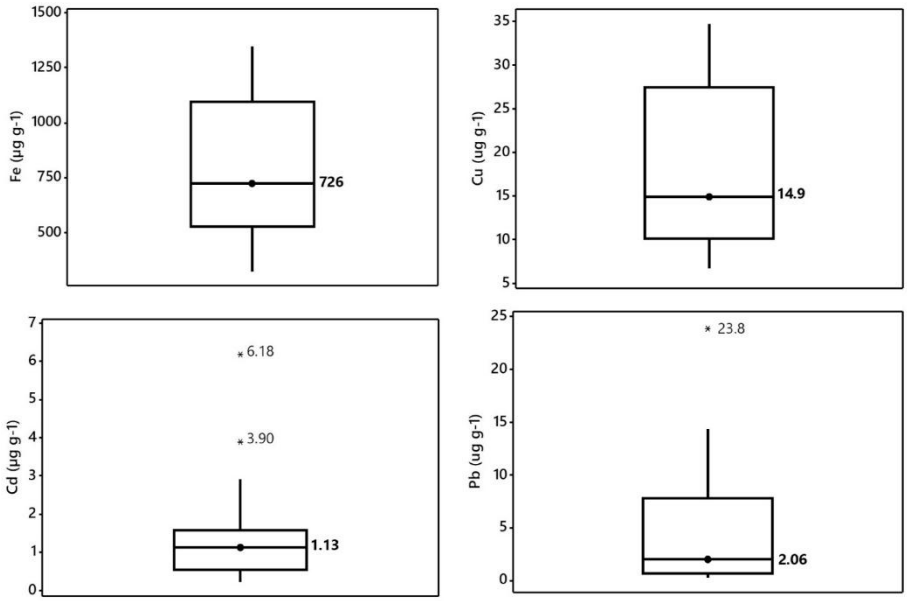
Data recorded in the literature mostly refer to liver tissue samples obtained by autopsies from individuals with a healthy liver and only, in some cases, refer to those obtained by biopsies from individuals with some liver disease. There are no reported values of metal concentrations in the liver of individuals without liver disease that can be used as estimates of "normal values".

The Box Plot was made for each element as is shown in Figure 1. For iron, a slightly skewed distribution of liver concentrations can be seen. Fifty percent of the concentrations found for this metal was between 527 and 1095 μg g^{-1} of dry tissue, but there were individuals with concentrations as low as 322 μg g^{-1} and others with concentrations as high as 1349 μg g^{-1}.

Plot made for copper showed a median equal to 14.9 μg g^{-1} that divides the box asymmetrically. Most individuals studied present a hepatic copper concentration between 10.1 and 27.5 μg g^{-1}, although some gave values as low as 6.66 μg g^{-1} or as high as 34.8 μg g^{-1}.

Two very high concentrations have been found for cadmium (3.90 and 6.18 μg g^{-1} of dry tissue) which are shown with an asterisk (*) in the corresponding box plot. Fifty percent of the concentrations found were between 0.561 and 1.59 μg g^{-1}, although there were individuals with concentrations as low as 0.221 μg g^{-1} or as high as 6.18 μg g^{-1}. The box plot for lead showed one very high concentration (23.84 μg g^{-1}) in relation to the rest of the results. Data distribution was markedly skewed. Most individuals studied presented a lead concentration in liver tissue ranging from 0.760 to 7.86 μg g^{-1}. Concentrations as low as 0.340 μg g^{-1} or as high as 23.8 μg g^{-1} could be seen.

Figure 1 *Box Plot for iron, copper, cadmium and lead concentrations*



In Table 5, a comparison of the mean concentrations for each element was made according to the sex of the person, for which Tukey's method with 95% confidence was used. Significant differences were not found.

Table 5*Mean concentrations related to the sex of individuals. The values are expressed in μg g^{-1} (dry weight)*

Element	Sex	Mean	Group*
Fe	M	752.8	A
	F	735.5	A
Cu	M	20.37	B
	F	16.66	B

Cd	M	1.510	C
	F	1.322	C
Pb	M	5.01	D
	F	4.88	D

*The same letter corresponds to means that do not have a significant difference.

Correlation between age of the individuals and concentration of each metal was also studied by calculating Pearson's correlation coefficient. Obtained results are shown in Table 6. Correlations yielded values of r negative for most cases except for the Pb-age pair which gave a low positive correlation. Absolute value of r was less than 0.256 in all cases, indicating the existence of a low correlation between the variables analyzed, but statistically non-significant with values of $p > 0.05$.

Table 6*Correlation between metal concentrations and ages of the individuals*

Pair of variables	Coefficient of Correlation (r)	p-value
Fe – age	-0.196	0.347
Cu – age	-0.256	0.226
Cd – age	-0.101	0.638
Pb – age	0.071	0.755

Correlation between the concentrations of the different metals was also studied. Relationships between Fe-Cu, Cu-Cd, Cd-Pb, Fe-Cd, Fe-Pb and Cu-Pb were obtained and the results of Pearson's correlation coefficient for each pair studied with its corresponding p value are shown in Table 7.

Table 7*Correlation between different pairs of metals*

Correlated concentrations	Coefficient of Correlation (r)	p-value
Fe – Cu	-0.181	0.398
Cu – Cd	0.386	0.069
Cd – Pb	0.722	0.000
Fe – Cd	-0.251	0.237
Fe – Pb	-0.229	0.306
Cu – Pb	0.401	0.072

A strong positive correlation ($| r | > 0.7$) between cadmium and lead concentration in liver samples was observed, being statistically significant with a value of $p < 0.05$. A moderate correlation between copper and lead ($0.4 < | r | < 0.7$) was obtained, while for copper and cadmium a slight correlation ($0.3 < | r | < 0.4$) was obtained. In both cases, they were not statistically significant. Other pairs (Fe with Cu, Fe with Cd and Fe with Pb) yielded a low negative correlation.

Correlation between the contents of elements in human tissue was very little studied, but the existence of a connection with some synergistic or antagonistic effect in organisms was considered (Zhang et al. 2004). Knowledge of the causes and effects of these relations requires a lot of experimental evidence. Taking into account that there is little information in this subject, correlations of metal contents in human liver found in this work could be useful for future research on the interrelationships of trace metals in other human tissues.

IV. Conclusion

A total of 25 liver tissue samples from female and male individuals, between 18 and 85 years old, was analyzed. Sample number and size were important critical issues, since the possibility of obtaining samples from healthy living subjects was limited. Only between 50 and 150 mg of solid material were possible to be obtained from biopsies, so its use has been restricted to certain analyses. Analytical methods were optimized to quantify Fe, Cu, As, Cd and Pb in the samples of live individuals with healthy liver. Values found in this work were in agreement with those reported by other authors, although the latter refer to samples from autopsies. Results of the elements studied show that there is no statistically significant relationship between their concentration and

their sex or age. A strong correlation was found between Pb-Cd concentrations. Considering that there is little information on this subject, correlations of metal contents in human liver found in this work could be useful for future research on the interrelations of trace metals in other human tissues.

Results obtained from physiologically normal liver tissue made it possible to establish ranges of elemental concentrations that can be taken as reference regional levels.

References

[1]. Adams PC, Passmore L, Chakrabarti S, Reboussin DM, et al (2006) Liver Diseases in the Hemochromatosis and Iron Overload Screening Study. ClinGastroenterolHepatol 4(7):918-923. https://doi.org/10.1016/j.cgh.2006.04.013

[2]. Bona MA, Castellano M, Plaza L, et al (1992) Determination of Heavy Metals in Human Liver. Hum ExpToxicol 11:311–313. https://doi.org/10.1177/096032719201100502

[3]. Bush VJ, Moyer TP, Batts KP, et al (1995) Essential and toxic element concentrations in fresh and formalin-fixed human autopsy tissues. ClinChem 41: 284–294. https://doi.org/10.1093/clinchem/41.2.284

[4]. Filteau, SM, Tomkins, AM (1994) Micronutrients and tropical infections. Trans R Soc Trop Med Hyg 88(1):1-26. https://doi.org/10.1016/0035-9203(94)90480-4

[5]. Griesmann GE, Hartmann AC, Farris FF (2009) Concentrations and correlations for eight metals in human liver. Int J of Environ Health Res 231-238. https://doi.org/10.1080/09603120802641546

[6]. Hatano R, Ebara M, Fukuda H, et al (2000) Accumulation of copper in the liver and hepatic injury in chronic hepatitis C. J GastroenterolHepatol 15: 786–791. https://doi.org/10.1046/j.1440-1746.2000.02199.x

[7]. Himoto T, Masaki T (2020) Current Trends of Essential Trace Elements in Patients with Chronic Liver Diseases. Nutrients 12(7):2084. https://doi.org/10.3390/nu12072084

[8]. Hudnik V, Marolt-Gomišček M, Gomišček S (1984) The determination of trace metals in human fluids and tissues: Part 1. Estimation of "Normal Values" for Copper, Zinc, Cadmium and Manganese in Blood Serum and Liver Tissue. Anal ChimActa 157:143-150. https://doi.org/10.1016/S0003-2670(00)83614-4

[9]. Julshamn K, Andersen K, Svendsen E, et al (1989) Trace elements intake in the Faroe Islands III. Element concentrations in human organs in populations from Bergen (Norway) and the Faroe Islands. Sci Total Environ 84:25-33. https://doi.org/10.1016/0048-9697(89)90367-7

[10]. Katoh T, Sato T, Yamamoto Y (2002) Determination of multielement concentrations in normal human organs from the Japanese. Biol Trace Elem Res 90:57–70. https://doi.org/10.1385/BTER:90:1-3:57

[11]. Muñoz M, García-Erce JA, Remacha ÁF (2011) Disorders of iron metabolism. Part II: iron deficiency and iron overload. J ClinPathol 64:287-296. http://dx.doi.org/10.1136/jcp.2010.086991

[12]. Quansah R, Armah FA, Essumang DK, et al (2015) Association of arsenic with adverse pregnancy outcomes/infant mortality: a systematic review and meta-analysis. Environ Health Perspect 123(5):412-21. https://doi.org/10.1289/ehp.1307894

[13]. Rahil-Khazen R, Botann BJ, Myking A, et al (2002) Multi-element analysis of trace element levels in human autopsy tissues by using inductively coupled atomic emission spectrometry technique (ICP-AES). J Trace Elem Med Biol 16:15-25. https://doi.org/10.1016/S0946-672X(02)80004-9

[14]. Rehman K, Fatima F, Waheed I, Akash MSH (2018) Prevalence of exposure of heavy metals and their impact on health consequences. J Cell Biochem. 119:157-184. https://doi.org/10.1002/jcb.26234

[15]. Şahin M, Karayakarb F, Erdoganc KE, et al (2018) Liver tissue trace element levels in HepB patients and the relationship of these elements with histological injury in the liver and with clinical parameters. J Trace Elem Med Biol 45:70–77. https://doi.org/10.1016/j.jtemb.2017.09.014

[16]. Treble RG, Thompson TS, Lynch HR (1998) Determination of copper, manganese and zinc in human liver. Biometals 11:49–53. https://doi.org/10.1023/A:1009209408034

[17]. Varga I, Szebeni Á, Szoboszlai N, et al (2005) Determination of trace elements in human liver biopsy samples by ICP–MS and TXRF: hepatic steatosis and nickel accumulation. Anal BioanalChem 383:476–482. https://doi.org/10.1007/s00216-005-0010-0

[18]. Yinsong W, Guisun Z, Mingguang T, et al (1991) Distribution of some elements in human hair and internal organs, determined by neutron activation analysis. J RadioanalNuclChem 151:257-8. https://doi.org/10.1007/bf02035489

[19]. Zhang P, Chen C, Horvat M, R. et al (2004) Element content and element correlations in Chinese human liver. Anal BioanalChem 380:773–781. https://doi.org/10.1007/s00216-004-2834-4

Carlos Ariel Marrades, et. al. "Trace element levels in healthy livers of live human beings from Northwestern Argentina." *IOSR Journal of Applied Chemistry (IOSR-JAC)*, 15(07), (2022): pp 46-52.