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A STUDY ON CHEMICAL ELEMENT DETERMINATIONS IN HUMAN NAILS BY NEUTRON ACTIVATION ANALYSIS

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ABSTRACT

Nail analyses have been the object of study in order to assess the levels of elements accumulated in the human organism and to use this tissue to monitor environmental and occupational exposure, to evaluate the nutritional status, to verify intoxication by toxic metals and to diagnose or to prevent diseases. Nail analyses present advantages due to easy sample collection, storage, transportation and this tissue provides element level accumulation over time. However, there is controversy regarding the application of nail analysis data due to difficulties to establish reliable reference values or element concentration ranges as control values. The objective of this study was to evaluate the factors that can affect nail element concentrations for further sample analyses of a group of individuals by applying neutron activation analysis (NAA). Fingernails and toenails collected from adult individuals of both genders, aged 18 to 71 years, living in the São Paulo Metropolitan Region were cut in small fragments, cleaned and dried for analyses. Samples and element standards were irradiated for 16 h under a thermal neutron flux of about 4.5 x 10^{12} n cm^{-2} s^{-1} at the IEA-R1 nuclear research reactor followed by gamma ray spectrometry. Element concentrations for As, Br, Ca, Co, Cr, Cs, Fe, K, La, Na, Rb, Sb, Sc, Se and Zn were determined. For quality control of the analytical results, certified reference materials were analysed and the results showed good accuracy and precision with relative errors and relative standard deviations lower than 5.1 % and 11.6 %, respectively. Preliminary assays indicated that the contribution due to impurities from plastic involucres used in the irradiation as well as those from nail polishes is very low and could be considered negligible. Results from the nail sample cleaning process using distinct procedures indicated that HNO_3 solution may cause sample dissolution. Sample homogeneity was verified by analysis of a sample in replicate. A comparison of finger and toenail results indicated significant differences (p= 0.05) for the elements Br, Co and Zn. Element concentration comparisons were also made with gender, age and body mass index (BMI) parameters and for the most part showed no significant differences. The differences were found for Zn concentrations when compared between genders in fingernail, for Cs when compared between ages in fingernail. Elements concentrations obtained for both finger and toenail samples presented wide variability and they were of the same order of magnitude or within ranges as those reported in published literature.

1. INTRODUCTION

The determination of elemental composition in human tissues such as fingernails has aroused great interest to assess the nutritional status, in the diagnosis of diseases, especially those systemic and to evaluate occupational exposure to toxic elements. There are 25 elements recognized as essentials for human and animal life, being those 11 of them are present at the trace levels [1]. The human nail tissue is composed of keratin and it acts as a final protection of the fingers and the nail helps to do delicate movements, to hold small objects [2]. Due to the slow growth of nails, they are not affected by transient factors that alter the levels of minerals in blood serum.

Various elements of interest by its essentiality or toxicity are accumulated on the nails, whose ions have an affinity with the atoms N, O, S group of proteins of the nails [3].

The analysis of hair and nails are being conducted to see if there is a correlation between the concentrations of elements found in the samples with nutritional status [4], pathological [5] and for use as an indicator of environmental contamination and occupational [6,7].

Ribeiro et al (1995) reported that knowledge about the nails and their illnesses attracted interest since the beginning of medicine. Before the Christian, Celsius has made reference to diseases such as paronychia, an infection characterized by bacterial or viral infection that can affect the entire distal part of the finger [8]. Studies were conducted on the nail growth by Robert Boyle in 1684. In 1852, he was presented a description of the "complex nail" in Handbook of Human Histology. The first work on nail diseases found in Brazilian medical literature was Clovis Castro 1948, which describes a case of nail pigmentation related to the use of cream containing mercury.

It is worth emphasizing that the analysis of the nails have advantages due to its collection not invasive, and can be easily collected by the donor, with ease of storage and transportation due to its stability. Moreover, unlike the fluid samples such as blood serum and urine concentrations of elements that indicate newly ingested, the nails serve as a biological indicator of accumulated long period elements, as each nail trimming is several weeks of growth and incorporation of elements [4, 5, 9, 10, 11].

Cheng et al, (1994) called attention to the fact that for the nails being used as biomonitor of trace elements should be checked for contamination originating from the ground, nail polish and as the influence of age and gender. Already KUCERA et al, (2004) found it difficult to remove exogenous contamination of nails and these researchers stress on the absence of a standard protocol for cleaning nails.

BANK et al. (1981) report that there are many differences in the elemental composition reported on the nails. Most researchers uses extensive washing procedure to minimize contamination of the samples exogenous nails, however, with washes runs the risk of drawing elements connected to the nail matrix.
However, the nails are exposed to soaps, nail polishes and other substances that may remain attached to its outer surface [15]. For nails to be useful as a trace element monitor factors, such as contamination by soil, nails polish, and the influence of age, sex, and longitudinal variation must be understood [12].

Therefore establishing a protocol for cleaning nails, before the analysis is required to remove only the exogenous contaminant and for the comparison results between the studies.

The purpose of this study was determination of chemical elements present in nails of a group of individuals residing in the Metropolitan Region of São Paulo - Brazil and compare with the values found in the literature.

2. EXPERIMENTAL

2.1. Procedure for Neutron Activation Analysis

2.1.1. Preparation of synthetic standards of elements

Certified standard solutions of elements acquired from Spex CertiPrep USA were used to prepare synthetic element standards. From the stock standard solutions, diluted solutions containing one or more elements were prepared. Aliquots of 50 µL of these solutions were pippetted on small sheets of n°. 40 Whatman filter paper with the sizes 1.5 cm x 3.5 cm. These sheets were placed in a desiccator for drying the aliquots at room temperature. The micropipette used was previously checked in relation its calibration. The sheets were folded and placed in plastic bags. The plastic (polyethylene) foils used to prepare these bags were previously cleaned using dilute nitric acid solution and purified water.

2.1.2. Irradiation, measurements and calculations

The cleaned nails were dried at room temperature before the weighing. Each nail sample or certified reference material was weighed (140 to 200 mg) in polyethylene bags. Each bag containing the sample or standard was wrapped with aluminum foil. This set of samples and standards were placed in an aluminum irradiation device called "rabbit." Irradiation was performed in the IEA-R1 nuclear research reactor for 16 hours under a thermal neutron flux from 4.0×10^{12} n cm^2 s^{-1} to 5.0×10^{12} n cm^2 s^{-1}.

After adequate decay time of 4 days, samples and standards were placed individually in stainless steel planchets for gamma ray measurements. The induced gamma ray activities measurements were carried out using a hyperpure germanium detector from EG & ORTEC connected to a gamma ray spectrometer and associated electronic system. The system used had a resolution (FWHM) of 0.81 keV for 122.06 keV peak of ^{57}Co and of 1.97 keV for 1332.50 keV peak of ^{60}Co. The analysis of gamma spectra was performed using a computer program VISPECT2 in TURBO BASIC language. The samples and standards were measured in three different decay times of about 5, 12 and 19 days in order to eliminate the problem of interferences and to increase the number of elements to be determined. In the first measurement, the As, Br, Ca, K, La, and Na were determined, in the second the elements Cr, Fe, Rb, Sb and Sc, and in third Co, Cs, Se and Zn. The radioisotopes measured were identified according to their half-lives and gamma-ray energies. The calculations for obtaining the element concentrations were performed by the comparative method.

2.2. Analysis of Certified Reference Materials

For quality control in relation to the precision and accuracy of the results reference materials NIST SRM 1577b *Bovine Liver* and NIST SRM 1566b *Oyster Tissue* provided by the National Institute of Standards & Technology, USA were analyzed.

Percentages of humidity of these certified reference materials were determined in order to obtain the results on a dry basis. For this determination approximately 250 mg of each reference material were basis weighed drying in an Universal oven at a temperature of 85 °C, for 24 hours. The percentages of humidity obtained in this drying process were 4.71 % for the certified reference material NIST 1577b *Bovine Liver*, 3.2 % for NIST 1566b *Oyster Tissue*. These percentage values were used to calculate the element concentrations on a dry basis.

2.3. Analysis of the Polyethylene Material used for Sample Irradiation

This test was performed since the samples are irradiated in the plastic bags and they are not removed for counting. Elemental analysis of plastic samples pre-cleaned with dilute nitric acid solution and purified MILLIQ water was performed by neutron activation analysis. For this assay a polyethylene foil of 10 cm x 10 cm and weighing 0.33305 g was irradiated for 16 hours.

2.4. Qualitative Analysis of Nail Polish Samples

As some clipping nails presented with nail polishes before their cleaning, nail polishes were analyzed to verify the possible presence of their chemical elements absorbed into the nail samples. Nail polishes were analyzed qualitatively by NAA.

Three samples of nail polishes with different colors and following characteristics were analyzed: 1) with anti-allergy component, sparkling pearl nail polish, 2) red nail polish of known brand in the market and a relatively high commercial value and 3) the orange nail polish with a little commercial value brand. For these tests, samples of nail polish were placed in plastic bags, where they kept for 24 hours at room temperature for drying. After drying the samples these plastic bags were heat sealed and irradiated at the nuclear research reactor.

2.5. Collection of Clipping Nail Samples for Cleaning Assays

Clipping nail samples were obtained from adult individuals of both genders of a population considered "healthy" residing in the metropolitan region of São Paulo, Brazil. The nail clippings of the hands and feet were collected using a clipper or a pair of scissors and they were placed in plastic bags. The present study was previously approved by the internal ethical committee of Faculty of Public Health, University of São Paulo.

2.5.1. Treatment of nail samples for the analyses

Initially the clipping nail samples were kept immersed in a solution of 70 % ethanol without stirring for a period of 10 minutes to reduce risks to the microbiological contaminants such as fungi and bacteria during the handling of samples by the analyst. Then the nails were cut into small pieces (fragments) using a pair of scissors with titanium coating to prevent sample contamination.

The nail treatment procedure [16]: three consecutive washings with acetone p.a, with mechanical shaking for 1 minute, 3 consecutive treatments with 2 % Triton X100, with mechanical shaking for 1 minute, followed by two washings with MILLIQ purified water, with mechanical shaking for 20 seconds. A shaker with agitation frequency of 3 rpm was used for shaking. The separation of the nail fragments from the washing solution was performing by the process of filtration using n°. 41 Whatman filter paper. The filtrate was also washed twice using MILLIQ water, and finally two washes with acetone p.a. The filter paper was placed with the nails in Petri dishes. The cleaned samples were kept at room temperature for a period from 24 to 48 hours for drying.

2.5.2. Analysis in triplicate of clipping nails

The homogeneity of nail samples prepared was verified by the analysis of a sample in triplicate by neutron activation analysis.

3. RESULTS AND DISCUSSION

3.1. Results of Certified Reference Materials

Tables 1 and 2 show the results obtained in the analysis of certified reference materials NIST 1577b *Bovine Liver* and NIST 1566b *Oyster Tissue* respectively. Results obtained in these reference materials are in good agreement with their respective certified values, showing that the activation analysis procedure used was adequate for the determination of several elements. The percentages of relative errors (RE) of the results obtained for the material NIST 1577b *Bovine Liver* were lower than 4.5 % and the relative standard deviations (RSD) were lower than 5.3 %. The percentages obtained for the RE for NIST 1566b *Oyster Tissue* were lower than 5.1 %, and RSD were lower than 11.6 %.

A comparison of the results obtained with the values of the certificates indicated good agreement showing that the procedure for activation analysis was used to determine the appropriate number of elements [17].

To evaluate the accuracy of the results obtained in the analysis of certified reference materials were calculated from standardized difference or Z-score. A result is considered acceptable when the value of Z-score is between -3 and 3. The Z-score values obtained were below 2 indicate that the results obtained are within the range of certified values at a level of significance of 5% [18]. The value of Z-score for the concentration of an element can be calculated using the relationship 1.0 [18]:

$$\text{Z-score} = (C_i - C_{ref,i}) / (\sigma_i^2 + \sigma_{ref,i}^2)^{1/2} \qquad (1.0)$$

where:

Ci is the value of the concentration of the element **i** obtained from the analysis; **Cref**, **i** is the concentration of the certificate value for the element **i, σi** is the uncertainty of

the concentration obtained for element **i**. In this study we used the standard deviation value for **σi**;
σref, i is the uncertainty of the certified value for the element **i**.

The Z-score values obtained are also shown in Tables 3.1 and 3.2 for NIST NIST 1577b and 1566b Bovine Liver Tissue Oyster respectively.

Table 3.1. Concentrations of elements in certified reference material NIST 1577b *Bovine Liver*. Results in mg kg^{-1}

Element	M±DP(n)	DPR (%)	ER (%)	Z-score	Certificate values [19]
Br	10.10±0.33 (7)	3.3	-	-	(9.7)*
Co	0.243±0.012(7)	5.0	-	-	(0.25)*
Fe	189±10(6)	5.3	2.9	0.3	184±15
Na	2473±63(7)	2.5	2.2	0.1	2420±60
Rb	13.07±0.41(6)	3.1	4.5	-0.5	13.7±1.1
Se	0.749±0.027(6)	3.6	2.7	0.3	0.73±0.06
Zn	123.9±3.0(6)	2.4	2.4	-0.2	127±16

M = arithmetic mean, SD = standard deviation, n = number of determinations, RSD = relative standard deviation, RE = relative error.
* Number in parentheses indicates informational value

Table 3.2. Concentrations of elements in reference material NIST 1566b Oyster Tissue. Results in mg kg^{-1} unless indicated.

Elemento	M±DP(n)	DPR (%)	ER (%)	Z-score	Valor do certificado [20]
As (µg kg^{-1})	7349±338(6)	4.6	3.9	-0.9	7650±650
Br	52.8±1.7 (7)	3.2	-	-	-
Ca	875±102(5)	11.6	4.4	0.3	838±20
Co (µg kg^{-1})	356±11(7)	3.3	3.9	1.2	371±9
Fe	196.6±7.6(5)	3.9	4.4	-0.9	205.8±6.8
K	6181±499(4)	8.0	5.1	-0.6	6520±90
Na	3362±76(6)	2.2	1.9	0.8	3297±53
Rb	3.20±0.13(6)	4.1	1.8	-0.4	3.26±0.14
Se	2.068±0.056(6)	2.7	0.4	0.08	2.06±0.15
Zn	1378±61(7)	4.4	3.2	-0.7	1424±46

M = arithmetic mean, SD = standard deviation, n = number of determinations, RSD = relative standard deviation, RE = relative error.
* Number in parentheses indicates informational value

3.2. Results Obtained in the Preliminary Assays

3.2.1. Analysis of plastic material and nail polishes

Table 3.3 shows the concentrations of elements found in the plastic material used for sample irradiation as well as those obtained in nail samples. From these data the amounts of elements present in plastic bags and in nail samples were calculated. The comparison between the masses of the elements present in the nail and those in a plastic bag indicated that the quantities of elements of a plastic bag are very low and can be considered negligible. In the third column of Table 3.3 the masses of the elements in a plastic bag of 22.24 mg used for irradiation are shown. In the fourth column are the masses of the elements present in about 200 mg of nail.

The nail polish samples were irradiated for 16 hours and the qualitative analysis indicated the presence of radioisotopes of various elements, but at very low counting rates, similar to the counts obtained in plastic bags. The sample which presented peaks more intense in the gamma spectrum was the orange nail polish. The radioisotopes ^{131}Ba, ^{47}Ca and ^{46}Sc due to these low counting rates obtained in the analysis of nail polish samples were detected the contribution of absorption of the element of nail polishes after washing was considered negligible.

Table 3.3. Concentrations of elements in a sample of treated plastic.

Elements	Concentration in the plastic	Mass of element in 22.24 mg of plastic bags	Concentration in nail	Mass of element in 200 mg of nail
Br (mg kg^{-1})	0.142±0.001[a]	3.1x10^{-6} mg	1.748±0.008[a]	3.5x10^{-4} mg
Ca (mg kg^{-1})	26.02±2.90	5.7x10^{-4} mg	957.63±36.65	1.9x10^{-1} mg
Co (µg kg^{-1})	21.1±0.6	4.6x10^{-7} mg	31.6±1.6	6.3x10^{-3} mg
Cr (µg kg^{-1})	51.6±4.3	1.13x10^{-6} mg	251.4±14.7	5.0x10^{-2} mg
Cs (mg kg^{-1})	10.2±0.6	2.2x10^{-4} mg	7.7±2.1	1.5x10^{-3} mg
Fe (mg kg^{-1})	2.8±0.3	6.1x10^{-5} mg	34.6±1.0	7.0x10^{-3} mg
K (mg kg^{-1})	21.2±0.8	4.6x10^{-4} mg	114.9±4.3	2.3x10^{-2} mg
La (µg kg^{-1})	3.35±0.13	7.4x10^{-8} mg	134.66±1.28	2.6x10^{-2} mg
Na (mg kg^{-1})	12.96±0.04	2.8x10^{-4} mg	309.89±0.44	6.2x10^{-2} mg
Sb (µg kg^{-1})	8.1±0.3	1.8x10^{-7} mg	24.1±3.7	4.8x10^{-3} mg
Sc (µg kg^{-1})	0.34±0.05	7.5x10^{-9} mg	2.64±0.15	5.2x10^{-4} mg
Zn (mg kg^{-1})	11.00±0.06	2.4x10^{-4} mg	117.09±0.63	2.3x10^{-2} mg

[a] Results of one determination. The uncertainties of the results were calculated using statistical counting errors of the sample and standart.

3.2.2. Results of nail analysis in triplicate

Table 3.4 shows the results obtained for analysis of the nail samples in triplicate. For most of elements, the results presented relative standard deviations lower than 15 % indicating the homogeneity of the prepared sample. For the elements As, Cs, La an Rb results showed relative standard deviations higher than 17.7 % due to low concentrations of these elements in the sample and to poor statistical counting obtained in the gamma ray measurements.

Brockman *et al*, 2009 observed large variation in duplicate analysis of the nails. The average relative standard deviation on the samples were 17 % for Se and 4 % for Zn, they used 10% HNO_3 in washing procedure. Supposed this variation by contamination of the nails before collection or removal of these elements of the nail matrix during cleaning procedure.

In the present study, the analysis in triplicate using a solution with Triton X100 + acetone + water in washing procedure was obtained the percentages of RSD of 1.6% for Se and 1.8 % for Zn.

Table 3.4. Results of a nail analysis in triplicate.

Elements	M[a]±SD[b]	RSD[c] (%)
As (µg kg^{-1})	28.3±6.2	22.0
Br (mg kg^{-1})	1.85±0.03	1.6
Ca (mg kg^{-1})	862.3±48.2	5.6
Co (µg kg^{-1})	14.8±1.1	7.6
Cr (µg kg^{-1})	214±14	6.7
Cs (mg kg^{-1})	11.4±4.0	35.0
Fe (mg kg^{-1})	18,42±2,55	13.8
K (mg kg^{-1})	402.2±56.3	14.0
La (µg kg^{-1})	12.7±2.6	20.2
Na (mg kg^{-1})	324.9±12.4	3.8
Rb (µg kg^{-1})	1.2±0.2	17.7
Sb (µg kg^{-1})	12.5±1.9	14.8
Sc (µg kg^{-1})	2.5±0.3	12.7
Se (µg kg^{-1})	372.8±5.9	1.6
Zn (mg kg^{-1})	79.6±1.5	1.8

[a] arithmetic mean, [b] standard deviation, [c] relative standard deviation.

3.3. Results of analysis of nails

3.4. Analysis of the fingernails and toenails

Applying the procedure proposed to NAA analysis fingernails was possible to determine the elements As, Br, Ca, Co, Cr, Cs, Fe, K, La, Na, Sb, Sc, Se and Zn. The element Rb can not always be detected under the experimental conditions so that was not considered for this study. We calculated the statistical parameters of the arithmetic and geometric means with their respective standard deviations, medians and ranges of concentrations (maximum and minimum) to the data obtained for the population studied.

Tables 3.5 and 3.6 are the results of the fingernails and toenails respectively. These results show that both the fingernails and toenails in the elements Ca, Fe, K, Na and Zn were obtained at higher levels of the order of mg kg-1 and the other elements As, Br, Co, Cr, cs, La, Sb, Sc and Se in the order of mg kg^{-1}. As indicated in these Tables 3.5 and 3.6, certain elements could not be determined due to the low concentration in the sample or due to the practical problems of detecting or measuring the radioisotope decay time established.

The results in Tables 3.5 and 3.6 also show that analyzes allow the determination of the nails of many elements that play an important metabolic role in the human body and they can indicate nutritional individuals. Among these are the essential elements Ca, Fe, K and Zn. With respect to Ca, the nails were obtained in the order of levels mg kg^{-1}. Ca is an important element in bone metabolism and its deficiency has been linked to disease osteoporosis. However, VECHT-Hart et al (1995) found that the concentrations of Ca present in toenails do not exhibit a strong correlation bone density so that analyzes of this fabric can not be a reference for evaluating the development of this disease.

Most of Fe lies within the cells of the body. The nails are derived from epithelial cells of the deepest layer which gives rise to the nail matrix, in which cells that differentiate into keratin will form the nail bed where they are firmly established. Sobolewski, et al, (1978) found a positive relationship of iron concentration in nails obtained with the treatment of iron deficiency anemia. As for the Zn, CARMEIRO et al (2011) demonstrated that there is an association between the concentration of the element present in the nails with the development of childhood asthma.

Also found in analyzes of nails If the element, but at lower levels ranging from 0.362 to 1.297 mg kg^{-1} in fingernails and from 0.322 to 1.315 mg kg^{-1} in the toenails. Among the toxic elements found in fingernails and toenails is o, but this element was obtained in very low concentrations, less than 0.084 mg kg-1 in fingernails and less than 0.073 mg kg^{-1} in the toenails. Regarding the determination of As in nails, Slotnick & Nriagu, (2006) who conducted a review on the validity of the analysis of human nails as a biomarker of As and if they draw attention to the need for future studies in view of the various routes to accumulation of these elements in nails.

The Cr considered toxic element, depending on its chemical form was detected in the samples of nails. The Cr in the form Cr^{6+} is considered toxic and is associated with lung cancer, since the Cr^{3+} is an essential nutrient but can be toxic if ingested in large

quantidade4. Ayodele & Ajala (2009) determined the concentration of Cr and Cu in a population group in Nigeria, but failed to complete a contamination with search results because of possible contamination. Rajpathak et al (2004) found lower levels of Cr among men with diabetes and cardiovascular disease compared to the control group.

Table 3.5. Elements concentrations in mg kg^{-1} in fingernails.

Elements	n	M ± DP	Maximum	Minimum	MG x÷ DPMG	Median
As	16	0.0442 ± 0.0147	0.0842	0.0213	0.0421 x÷ 1.4	0.0420
Br	18	2.2 ± 1.4	6.5	0.91	1.9 x÷1.7	1.8
Ca	18	784 ± 360	1994	327	724.1 x÷ 1.5	728
Co	18	0.052 ± 0.058	0.248	0.017	0.037 x÷ 1.7	0.029
Cr	17	0.322 ± 0.212	0.840	0.112	0.273 x÷ 1.8	0.267
Cs	16	0.0135 ± 0.0086	0.0377	0.0043	0.0115 x÷ 1.8	0.0114
Fe	18	35.5 ± 26.4	112.0	10.1	28.9 x÷ 1.9	27.7
K	13	207 ± 186	723	42.1	147.1 x÷ 2.6	193
La	16	0.0230 ± 0.0172	0.0607	0.0002	0.0118 x÷ 5.8	0.0224
Na	17	220 ± 180	838	66.1	177.1 x÷ 1.9	160
Sb	17	0.050 ± 0.053	0.249	0.019	0.040 x÷ 1.8	0.033
Sc	17	0.0050 ± 0.0049	0.0189	0.0010	0.0036 x÷ 2.2	0.0030
Se	18	0.668 ± 0.266	1.297	0.362	0.623 x÷ 1.3	0.667
Zn	18	121.1 ± 22.9	178.1	92.0	119.2 x÷1.2	119.4

n = number of samples, M ± SD = arithmetic mean and standard deviation; DPMG MG x ÷ M= geometric mean and geometric standard deviation

Table 3.6. Elements concentrations in mg kg^{-1} in toenails.

Elements	n	M ± DP	Maximum	Minimum	MG x÷ DPMG	Median
As	16	0.0500 ± 0.0159	0.0734	0.0247	0.0475 x÷ 1.4	0.0488
Br	16	1.3 ± 0.6	2.8	0.5	1.2 x÷ 1.5	1.3
Ca	15	818 ± 250	1363	421	781.8 x÷ 1.4	797
Co	16	0.018 ± 0.007	0.033	0.010	0.017 x÷ 1.4	0.016
Cr	16	0.221 ± 0.221	0.962	0.062	0.165 x÷ 2.1	0.152
Cs	15	0.0104 ± 0.0045	0.0205	0.0036	0.0094 x÷ 1.6	0.0095
Fe	15	22.3 ±18.6	82.0	9.15	18.29 x÷ 1.8	16.48
K	16	344 ± 226	752	78.2	275.3 x÷ 2.0	261
La	14	0.023 ± 0.022	0.090	0.003	0.016 x÷ 2.4	0.016
Na	16	326 ± 172	750	122	287.8 x÷ 1.7	300
Sb	15	0.032 ± 0.024	0.103	0.009	0.026 x÷ 1.9	0.028
Sc	15	0.0035 ± 0.0035	0.0153	0.0007	0.0026 x÷ 2.1	0.0024
Se	16	0.503 ± 0.237	1.315	0.322	0.470 x÷ 1.4	0.433
Zn	16	96.2 ± 30.3	183.0	70.2	92.8 x÷ 1.3	87.8

n = number of samples, M ± SD = arithmetic mean and standard deviation; DPMG MG x ÷ = geometric mean and geometric standard deviation

3.5. Comparison between concentrations of elements of the fingernails and toenails

Although the small number of samples analyzed nails not ideal for statistical analysis, samples of 18 to 16 hands and toenails, it was decided to make a comparative study between the data element concentrations obtained for the fingernails and feet. To verify that the element concentrations obtained significant differences compared groups, we applied the Student t test. The statistical test was applied to the significance level of 5% or p = 0.05 [29].

The results of the comparison between the concentrations obtained in fingernails and toenails are in Table 3.7. The results of the t test performed at a significance level of 5% are shown in this Table 3.7 for the elements Br, Co and Zn with concentrations of the fingernails and toenails showed significant differences by t-test (p = .05). Therefore these results are consistent with the statements made by BARBOSA, et al, (2005), according to these researchers; the toenails are less affected by exogenous contamination of the hands. Consequently toenails have the advantage of low exogenous contamination and reflect the content of an element accumulated over time, in view of the rate of growth of the toenails is smaller than the hands.

Table 3.7. Comparison of the concentrations (mg kg^{-1}) obtained in fingernails and toenails.

Elements	Fingernails		Toenails	
	n	M ± DP	n	M ± DP
As	16	0.0442 ± 0.0147 **a**	16	0.0500 ± 0.0159 **a**
Br	18	2.2 ± 1.4 **a**	16	1.3 ± 0.6 **b**
Ca	18	784± 360 **a**	15	818 ± 248 **a**
Co	18	0.052 ± 0.058 **a**	16	0.018 ± 0.007 **b**
Cr	17	0.322 ± 0.212 **a**	16	0.221 ± 0.221 **a**
Cs	16	0.0135 ± 0.0086 **a**	15	0.0104 ± 0.0045 **a**
Fe	18	36 ± 26 **a**	15	22 ±19 **a**
K	13	207 ± 186 **a**	16	344 ± 226 **a**
La	16	0.023 ± 0.017 **a**	14	0.023 ± 0.022 **a**
Na	17	220 ± 180 **a**	16	326 ± 172 **a**
Sb	17	0.050 ± 0.053 **a**	15	0.032 ± 0.024 **a**
Sc	17	0.0050 ± 0.0049 **a**	15	0.0035 ± 0.0035
Se	18	0.668 ± 0.266 **a**	16	0.503 ± 0.237 **a**
Zn	18	121.1 ± 22.9 **a**	16	96.2 ± 30.3 **b**

In the lines, the concentrations of an element followed by the same lowercase letter indicate no significant difference by t-test at a significance level of 5%.

3.6. Comparison between concentrations of elements of the fingernails and toenails in relation to the gender of the individuals.

In Table 3.8 are the results of the comparison between the concentrations of elements obtained in fingernails of males and females applying the t-test (p = 0.05) only the element Zn showed a significant difference.

The results of the comparison between the concentrations obtained in the toenails of the male and female group in Table 3.9 showed no significant difference for any of the elements determined.

In the analysis of the fingernails and toes in the results indicated no significant difference for the elements determined in this work with the exception of the element Zn in fingernails. However for the fingernails VANCE et al, (1998) had high concentrations of Ag, Au, Se and Zn and low K and Na in individuals of the female group than masculine. For Ca, Co, Cr, Hg and Sc concentrations were not affected by gender. Already researchers HOSSEINIMAKAREM & Tavassoli (2011) showed high concentrations of Al, Fe, H, K, Mg, N, Na, and if the male group and high concentrations of Ca and Ti in the female group. These researchers HOSSEINIMAKAREM & Tavassoli, (2011) using the technique of discriminant function analysis to their analytical data obtained distribution of subjects into two groups. The results obtained for the fingernails to the elements Co, Cr, Se and Zn are consistent with the VANCE et al, (1998). Żukowska et al, (2009) had high concentrations of Se nail group females than males in a population residing in Poland.

Table 3.8. Comparison of element concentrations (mg kg^{-1}) in fingernails of the male and female group.

Elements	Male group		female group	
	n	M ± DP	n	M ± DP
As	6	0.044 ± 0.009 **a**	10	0.045 ± 0.011 **a**
Br	7	1.65 ± 0.55 **a**	11	2.50 ± 1.33 **a**
Ca	7	714 ± 232 **a**	11	828 ± 135 **a**
Co	7	0.034 ± 0.027 **a**	11	0.063 ± 0.083 **a**
Cr	6	0.224 ± 0.086 **a**	11	0.376 ± 0.330 **a**
Cs	6	0.0104 ± 0.0028 **a**	10	0.0154 ± 0.0059 **a**
Fe	7	30.5 ± 22.1 **a**	11	38.7 ± 18.4 **a**
K	6	232 ± 251 **a**	7	186 ± 102 **a**
La	7	0.023 ± 0.017 **a**	9	0.023 ± 0.020 **a**
Na	7	284 ± 261 **a**	10	175 ± 69 **a**
Sb	6	0.032 ± 0.010 **a**	11	0.060 ± 0.088 **a**
Sc	6	0.0054 ± 0.0067 **a**	11	0.0048 ±0.0026 **a**
Se	7	0.652 ± 0.337 **a**	11	0.677 ±0.265 **a**
Zn	7	104.9 ± 11.2 **a**	11	131.4 ± 27.3 **b**

In the lines, the concentrations of an element followed by the same lowercase letter indicate no significant difference by t test at a significance level of 5%.

Table 3.9. Comparison of element concentrations (mg kg^{-1}) in the toenails of the male and female group.

Elements	Male group		female group	
	n	M ± DP	n	M ± DP
As	7	0.0580 ± 0.0158 **a**	9	0.0438 ± 0.0137 **a**
Br	7	1.190 ± 0.446 **a**	9	1.394 ± 0.648 **a**
Ca	6	742 ± 243 **a**	9	868 ± 252 **a**
Co	7	0.0166 ± 0.0063 **a**	9	0.0197 ± 0.0076 **a**
Cr	7	0.1224 ± 0.0439 **a**	9	0.2977 ± 0.2741 **a**
Cs	7	0.0120 ± 0.0056 **a**	8	0.0089 ± 0.0030 **a**
Fe	6	18.3 ± 12.3 **a**	9	24.9 ± 22.1 **a**
K	7	361 ± 186 **a**	9	330 ± 262 **a**
La	6	0.0114 ± 0.0083 **a**	8	0.0320 ± 0.0252 **a**
Na	7	347 ± 232 **a**	9	310 ± 118 **a**
Sb	7	0.0322 ± 0.0207 **a**	9	0.0326 ± 0.0277 **a**
Sc	6	0.0025 ± 0.0015 **a**	9	0.0041 ± 0.0044 **a**
Se	7	0.564 ± 0.345 **a**	9	0.455 ±0.099 **a**
Zn	7	85.2 ± 9.8 **a**	9	104.8 ±38.3 **a**

In the lines, the concentrations of an element followed by the same lowercase letter indicate no significant difference by t test at a significance level of 5%.

3.7. Comparison between concentrations of elements of the fingernails and toenails in relation to the age of individuals.

The results of this comparison between the concentrations of elements of the fingernails in relation to age are given in Tables 3.10 and 3.11 for the nails of the hands and feet respectively. This comparison was made between the age groups 19-40 years and 41-71 years. Applying the t test to the data of Tables 3.10 and 3.11 for p = 0.05 was found that only the element Cs of the fingernails significant difference among age groups considered.

VANCE et al (1998) who analyzed fingernails found that Ca, Co, Cr, Hg and Sc are not affected by age and HOSSEINIMAKAREM & Tavassoli, (2011) using the technique of discriminant function analysis to data analysis could nail separate individuals into five groups of different age groups as follows: less than 10 years, 11-18 years old, 19-35 years old, 36-50 years and above 50 years.

Żukowska et al, (2009) showed a statistically significant difference between the ages of the subjects. Participants older than 50 years had a lower concentration of Se in toenails.

Table 3.10. Comparison of element concentrations (mg kg^{-1}) of the fingernails for different age groups.

Elements	Age ranged from 19 to 40 years old		Age ranged from 41 to 71 years old	
	n	M ± DP	n	M ± DP
As	9	0,0418 ± 0,0073 **a**	7	0,0474 ± 0,0212 **a**
Br	10	1,90 ± 0,86 **a**	8	2,51 ± 1,88 **a**
Ca	10	707 ± 202 **a**	8	880 ± 494 **a**
Co	10	0,0488 ± 0,0704 **a**	8	0,0550 ± 0,0418 **a**
Cr	9	0,279 ± 0,214 **a**	8	0,370 ± 0,213 **a**
Cs	9	0,0096 ± 0,0038 **a**	7	0,0186 ± 0,0106 **b**
Fe	10	28,4 ± 19,3 **a**	8	44,4 ± 32,5 **a**
K	8	247 ± 221 **a**	5	144 ± 101 **a**
La	10	0,0193 ± 0,0161 **a**	6	0,0291 ± 0,0188 **a**
Na	10	255 ± 223 **a**	7	169 ± 81 **a**
Sb	9	0,0608 ± 0,0727 **a**	8	0,0387 ± 0,0112 **a**
Sc	9	0,0042 ± 0,0056 **a**	8	0,0059 ± 0,0041 **a**
Se	10	0,624 ± 0,299 **a**	8	0,717 ± 0,232 **a**
Zn	10	112,9 ±13,6 **a**	8	131,4 ± 28,6 **a**

In the lines, the concentrations of an element followed by the same lowercase letter indicate no significant difference by t test at a significance level of 5%.

Table 3.11. Comparison of element concentrations (mg kg^{-1}) toenails for different age groups.

Elements	Age ranged from 19 to 40 years old		Age ranged from 41 to 71 years old	
	n	M ± DP	n	M ± DP
As	11	0,0547 ± 0,0154 **a**	5	0,0398 ± 0,0129 **a**
Br	11	1,12 ± 0,41 **a**	5	1,71 ± 0,68 **a**
Ca	10	827 ± 303 **a**	5	800 ± 92 **a**
Co	11	0,0185 ± 0,0064 **a**	5	0,0178 ± 0,0090 **a**
Cr	11	0,194 ± 0,258 **a**	5	0,281 ± 0,101 **a**
Cs	11	0,0106 ± 0,0050 **a**	4	0,0097 ± 0,0034 **a**
Fe	10	15,9 ± 5,8 **a**	5	35,1 ± 28,7 **a**
K	11	408 ± 229 **a**	5	202 ± 155 **a**
La	10	0,0181 ± 0,0126 **a**	4	0,0359 ± 0,0361 **a**
Na	11	376 ± 182 **a**	5	217 ± 82 **a**
Sb	10	0,0391 ± 0,0272 **a**	5	0,0192 ± 0,0089 **a**
Sc	10	0,0026 ± 0,0015 **a**	5	0,0053 ± 0,0057 **a**
Se	11	0,506 ± 0,283 **a**	5	0,494 ± 0,100 **a**
Zn	11	99,9 ± 35,5 **a**	5	88,1 ± 13,3 **a**

In the lines, the concentrations of an element followed by the same lowercase letter indicate no significant difference by t test at a significance level of 5%.

3.8. Comparison between concentrations of elements of the fingernails and toenails in relation to the range of body mass index (BMI) of individuals.

The results of this comparison are presented in Tables 3.12 and 3.13 for the nails of the hands and feet respectively. The body mass index (BMI) was calculated as the ratio of the donor's weight (in kg) and the square of height (in m). According to the World Health Organization [34] individuals are ranked according to this index as: underweight (BMI <18.50), normal (BMI between 18.50 and 24.99), overweight (BMI for > 25.00) and obese (BMI> 30.00). For this study, the BMI data were divided into two ranges, namely 21 to 24.3 kg m^{-2} and range from 26.2 to 30.5 kg m^{-2}. The results of the statistical test showed that t is no difference between the concentrations of the different elements BMI ranges.

Püchau et al, (2010) determined that Cu, Se and Zn in fingernails of healthy young adults of the Caucasus with an average age of 20.9 ± 2.7 years found high concentrations of Zn and Cu for individuals with lower BMI.

Żukowska et al, (2009) determined If the nails of a population of Poland and obtained concentrations of Se significantly lower for participants with a BMI greater than 25 kg m^{-2}. These researchers also observed that individuals with BMI below 18.5 kg m^{-2} achieved

significantly higher results If, however, the number of people with that condition was very small.

Table 3.12. Comparison of element concentrations (mg kg^{-1}) of the fingernails for different body mass index (BMI).

Elements	BMI range of 21 to 24.3 kg m^{-2}		BMI range of 26.2 a 30.5 kg m^{-2}	
	n	M ± DP	n	M ± DP
As	7	0.04073 ± 0.0084 **a**	9	0.0469±0.0183 **a**
Br	9	2.26 ± 1.84 **a**	9	2.08 ± 0.84 **a**
Ca	9	822 ± 452 **a**	9	746 ± 259 **a**
Co	9	0.0471 ± 0.0421 **a**	9	0.0560 ± 0.0730 **a**
Cr	9	0.245 ± 0.092 **a**	8	0.409 ± 0.276 **a**
Cs	9	0.0132 ± 0.0099 **a**	7	0.0139 ± 0.0074 **a**
Fe	9	38.1 ± 34.4 **a**	9	32.9 ± 16.9 **a**
K	8	198 ± 111 **a**	5	221 ± 287 **a**
La	9	0.0372 ± 0.0614 **a**	7	0.0301 ± 0.0212
Na	9	213 ± 72 **a**	8	230 ± 261 **a**
Sb	9	0.0377 ± 0.0192 **a**	8	0.0648 ± 0.0748 **a**
Sc	9	0.0055 ± 0.0065 **a**	8	0.0044 ± 0.0024 **a**
Se	9	0.625 ± 0.288 **a**	9	0.717 ± 0.249 **a**
Zn	9	116.8 ± 15.8 **a**	9	125.5 ± 28.7 **a**

In the lines, the concentrations of an element followed by the same lowercase letter indicate no significant difference by t test at a significance level of 5%.

Table 3.13. Comparison of element concentrations (mg kg^{-1}) toenails for different body mass index (BMI).

Elements	BMI range of 21 to 24.3 kg m^{-2}		BMI range of 26,2 a 30,5 kg m^{-2}	
	n	M ± DP	n	M ± DP
As	10	0,0507 ± 0,0169 **a**	6	0,0489 ± 0,0154 **a**
Br	10	1,30 ± 0,62 **a**	6	1,31 ± 0,50 **a**
Ca	10	812 ± 297 **a**	5	827 ± 127 **a**
Co	10	0,0171 ± 0,0059 **a**	6	0,0203 ± 0,0087 **a**
Cr	10	0,229 ± 0,276 **a**	6	0,206 ± 0,093 **a**
Cs	9	0,0104 ± 0,00475 **a**	6	0,01027 ± 4,60 **a**
Fe	9	18,3 ± 10,5 **a**	6	28,3 ± 26,7 **a**
K	10	352 ± 234 **a**	6	329 ± 231 **a**
La	8	0,0215 ± 0,0130 **a**	6	0,0254 ± 0,0316 **a**
Na	10	296 ± 119 **a**	6	375 ± 241 **a**
Sb	9	0,0341 ± 0,0297 **a**	6	0,0301 ± 0,0152 **a**

Sc	9	0,0025 ± 0,0016 **a**	6	0,0049 ± 0,0051 **a**
Se	10	0,505 ± 0,292 **a**	6	0,498 ± 0,117 **a**
Zn	10	101,1 ± 36,9 **a**	6	88,2 ± 13,5 **a**

In the lines, the concentrations of an element followed by the same lowercase letter indicate no significant difference by t test at a significance level of 5%.

3.9. Comparison between concentrations of elements of the fingernails and toenails with literature values.

Tables 3.14 and 3.15 show the results obtained in fingernails and toenails with the literature data, respectively, for most elements, the comparison indicates that the results are of the same order of magnitude are within the limits or values presented in literature. Therefore the data from these tables 3.14 and 3.15 provide us with an estimate of the baseline element concentrations in the general population of the Metropolitan Region of São Paulo. A rigorous comparison between the data obtained and literature values is difficult mainly due to the different procedures used in cleaning the nails for analyzes. This shows that the use of such nails analysis in the diagnosis of the health of individuals becomes very difficult without the establishment of the cleaning of the nails and the reference values or control.

Table 3.14. Comparison between concentrations of elements obtained (mg kg^{-1}) in fingernails with literature values.

Elements	This Work		Literature Values			
			Ref. 1	Ref. 2	Ref. 3	Ref. 4
	M ± DP	MG x÷ DPMG	M ± DP	M	M / MG	MG x÷ DPMG
As	0.0442 ± 0.0147	0.0421 x÷ 1.4	0.269±0.190	0.0545	0.43 / 0.35	0.1095 x÷ 2.7
Br	2.2 ± 1.4	1.9 x÷ 1.7	23±14	2.11	1.36 / 1.28	3.6 x÷ 1.6
Ca	784 ± 360	724.1 x÷ 1.5	670±240	665	257.9 / 194.0	1054.8 x÷ 1.6
Co	0.052 ± 0.058	0.037 x÷ 1.7	0.035±0.031	0.0421	0.05 / 0.05	0.1155 x÷ 2.3
Cr	0.322 ± 0.212	0.273 x÷ 1.8	1.16±1.05	0.898	1.90 / 1.62	1.2799 x÷ 2.7
Cs	0.0135 ± 0.0086	0.0115 x÷ 1.8	0.0041±0.0043	0.0054	-	-
Fe	35.5 ± 26.4	28.9 x÷ 1.9	42±30	19.5	122.7 / 110.2	133.0 x÷ 1.7
K	207 ± 186	147.1 x÷ 2.6	210±260	94.6	45.80 / 38.89	223.1 x÷ 3.9
La	0.0230 ± 0.0172	0.0118 x÷ 5.8	0.120±0.180	-	0.03 / 0.01	-
Na	220 ± 180	177.1 x÷ 1.9	240±240	157	31.67 / 28.11	170.6 x÷ 2.0
Sb	0.050 ± 0.053	0.040 x÷ 1.8	0.053±0.054	0.0366	0.23 / 0.17	-
Sc	0.0050 ± 0.0049	0.0036 x÷ 2.2	0.0084±0.012	0.0027	0.02 / 0.02	-
Se	0.668 ± 0.266	0.623 x÷ 1.3	0.940±0.210	1.003	0.68 / 0.68	0.5140 x÷ 1.3
Zn	121.1 ± 22.9	119.2 x÷1.2	120±29	147	36.42 / 31.24	161.4 x÷ 1.7

Ref. 1. Rodushkin & Axelsson (2000); Ref. 2. Chaudhary *et al* (1995); Ref. 3. Xiao *et al* (1995); Ref. 4. Aguiar & Saiki (2000)
M = Arithmetic mean, SD = standard deviation of the arithmetic mean, GM = geometric mean; DPMG = standard deviation of the geometric mean.

Table 3.15. Comparison between concentrations of elements obtained (mg kg^{-1}) in toenails with literature values.

Elements	This Work		Literature Values			
			Ref. 1	Ref. 2	Ref. 3	Ref. 4
	M ± DP	MG x÷ DPMG	M ± DP	M ± DP	M ± DP	Faixa
As	0.0500 ± 0.0159	0.0475 x÷ 1.4	0.12 ± 0.13	0.10 ± 0.22	0.12 ± 0.27	-
Br	1.3 ± 0.6	1.2 x÷ 1.5	0.96 ± 3.56	-	2.41 ± 1.22	-
Ca	818 ± 250	781.8 x÷/ 1.4	714 ± 303	-	968 ± 436	-
Co	0.018 ± 0.007	0.017 x÷ 1.4	0.037 ± 0.032	0.17 ± 0.86	0.042 ± 0.023	0.2 – 0.3
Cr	0.221 ± 0.221	0.165 x÷ 2.1	0.97 ± 0.91	1.91 ± 1.66	2.39 ± 2.91	1.6 – 4
Cs	0.0104 ± 0.0045	0.0094 x÷ 1.6	-	-	-	-
Fe	22.3 ±18.6	18.29 x÷ 1.8	25.2 ± 20.4	-	42.5 ± 26.3	78 – 323
K	344 ± 226	275.3 x÷ 2.0	-	-	-	-
La	0.023 ± 0.022	0.016 x÷ 2.4	-	-	-	-
Na	326 ± 172	287.8 x÷ 1.7	25.2 ± 40.2	-	-	-
Sb	0.032 ± 0.024	0.026 x÷ 1.9	-	-	-	-
Sc	0.0035 ± 0.0035	0.0026 x÷ 2.1	0.0026 ± 0.0024	-	0.0053 ± 0.0062	0.004 – 0.1
Se	0.503 ± 0.237	0.470 x÷ 1.4	0.83 ± 0.23	0.82 ± 0.46	0.92 ± 0.15	-
Zn	96.2 ± 30.3	92.8 x÷ 1.3	111 ± 29.9	-	110 ± 28.9	57 - 290

Ref. 1. Cheng *et al* (1994); Ref. 2. Slotnick *et al* (2005) ; Ref. 3. Garland *et al* (1993); Ref. 4. Menezes *et al* (2004)
M = Arithmetic mean, SD = standard deviation of the arithmetic mean, GM = geometric mean; DPMG = standard deviation of the geometric mean.

4. CONCLUSIONS

In analytical control, the results of analysis of reference materials obtained were consistent with the values of the certificates, demonstrating accuracy of the data obtained . Results were also obtained with a good precision, which could be evaluated by the values of relative standard deviations obtained. The values of | Z -score | obtained were less than 2 indicating that the data obtained are within the certified values at a significance level of 5 %.

With respect to plastic material used in the manufacture of plastic enclosures for the irradiation of the samples in the reactor was possible to verify that it resists the activation conditions, suffering no change that may rupture and cause leakage of the sample. On the other hand , the results of this analysis indicated that polyethylene sheath elements Br, Ca , Co , Cr , Cs , Fe , K, La , Na, Sb , Sc and Zn are present in this material at very low levels may be considered negligible when compared with the present nail.

The analysis results of a sample in triplicate nail indicated homogeneity analyzed nail can be concluded that the preparation of the nails were cut into small pieces suitable for determining the elements of this work.

Regarding the wash procedure nail verified the importance of establishing an appropriate protocol for cleaning. The use of acetone solution + Triton X100 + purified water were adequate for the purpose of this work. From the results of the analyzes nail tested with dilute nitric acid can be concluded that depending on the hardness of the nails may be dissolved or demineralization also may occur elements of endogenous origin.

The results obtained in the analysis of fingernails proved the feasibility of applying the procedure to the NAA analysis fingernails due mainly to multielement analysis capability, simplicity and quality of results. Applying the experimental conditions were determined Nail elements As, Br , Ca , Co , Cr , Cs , Fe , K , La , Na, Sb , Sc , Se and Zn.

Applying the t test to the set of results obtained in this study it was concluded that the fingernails have higher levels of Br , Co and Zn that the feet due to low contamination of toenails by exogenous elements . These results indicate that the toenails are more appropriate for the elemental analysis of the hands. However toenails present growth rate slower than the hands.

The comparison between the element concentrations between groups of different genders , age groups and BMI indicated that for various elements is no significant difference and these findings were not always consistent with the literature.

A comparison of the results with literature data indicates that both analysis of the fingernails as the legs are of the same order of magnitude or within values are reported in the literature. However, the results obtained in this work as well as the values reported in the literature for various elements present a wide variability in their concentrations.

Given these results, it can be concluded that for the establishment of reference values or ranges of concentrations is necessary to conduct further studies to establish mainly adopt an appropriate protocol for cleaning the nails and selection criteria for the control group. There are several papers on analysis of nails for the most varied applications, but these still indicate

insufficient to understand the different effects that affect the comparison of elementary nails. It is concluded that to obtain narrower intervals for the values of reference is the need to standardize the various parameters in the sample thoroughly as in the select group of individuals.

Regarding the number of samples analyzed in this study was that 18 samples of fingernails and 16 of toenails, is of great interest to extend this analysis to a larger number of samples for statistical examination.

The results obtained in this work and the literature shows that most studies may contribute to the applications of data analysis of fingernails.

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