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Abstract. Laser-induced breakdown spectroscopy (LIBS) is applied to analyze human fingernails using nanosecond laser pulses. Measurements on 45 nail samples are carried out and 14 key species are identified. The elements detected with the present system are: Al, C, Ca, Fe, H, K, Mg, N, Na, O, Si, Sr, Ti as well as CN molecule. Sixty three emission lines have been identified in the spectrum that are dominated by calcium lines. A discriminant function analysis is used to discriminate among different genders and age groups. This analysis demonstrates efficient discrimination among these groups. The mean concentration of each element is compared between different groups. Correlation between concentrations of elements in fingernails is calculated. A strong correlation is found between sodium and potassium while calcium and magnesium levels are inversely correlated. A case report on high levels of sodium and potassium in patients with hyperthyroidism is presented. It is shown that LIBS could be a promising technique for the analysis of nails and therefore identification of health problems.
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Keywords: Laser-induced breakdown spectroscopy; nail analysis; sex and age discrimination.

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1 Introduction

Laser-induced breakdown spectroscopy (LIBS) is a laser-based spectroscopic analysis method based on optical emission following pulsed-laser ablation of a sample and it is applied to the detection and analysis of some elements in the samples. The technique, which has been utilized in a wide variety of applications, has numerous advantages over other competing spectrochemical analysis techniques. Screening of surfaces by scanning laser pulses along a surface is feasible so that spatial information is maintained. Analysis could be done rapidly and can be computerized, removing the requirement of the expertise. It does not require any special sample preparation. A highly focused laser beam is directed on the surface of the sample so that a high spatial resolution on the target can be obtained. Finally, the analysis detects most elements without bias.[1,2] However, like any other technique, LIBS also has several drawbacks. The spectroscopic signals are instable due to laser intensity fluctuations. Changes in atmosphere and gas pressure above the sample affect plasma properties. Also, spectral line intensities depend on chemical and physical matrix effect.[2]

The application of LIBS for elemental analysis of biological samples has previously been reported. Several authors have proposed the use of laser-induced breakdown spectroscopy for analysis of teeth, hair, and nails,[3–5] Nail is a biosample that contains biological information from the subject. Nails keep information on the nutrition, habitat, and other environmental conditions. Elements normally found in the body (e.g., Ca, Mg, Fe, etc.) can also be found in the nail but with inconsistent levels. Nail analysis is also useful for detecting those elements that are not normally found in the body. Trace elements in nails indicate exposure to hazardous material during the previous 2 to 18 months.[6] On the other hand, nail as a biomarker has some merits for the elemental analysis. Sample collection is noninvasive and simple, special storage conditions are not required, and since a large number of samples can be obtained and stored, population studies can be performed. Moreover, the level of elements in the nail remains isolated from other metabolic activities in the body with no fluctuation, thus the composition of the nail remains unchanged after collection. Therefore, elemental analysis of nail can be used to detect element imbalance or toxicity in the human body.[7]

There has been increasing interest in the study of human fingernails and hair in connection with the trace elements contained in these materials and possible applications for medical or forensic purposes.[8] The forensic analysis of trace elements in nails and hair can provide valuable crime scene evidence. By analyzing and matching the trace elements in hair and nail samples found on the scene of the crime with those of the criminals, their identities can be proven.[9]

Atomic absorption spectrometry[10–12] (AAS), inductively coupled plasma emission spectrometry[13] (ICP-OES), neutron activation analysis,[14] and inductively coupled plasma mass spectrometry (ICP-MS) are other analytical techniques used for trace element analysis in nail samples.[15–17] However, in the present work, human fingernails are analyzed by LIBS. A number of samples are randomly gathered. The data obtained from their spectra is processed to see if there is a discriminating factor between LIBS spectra of nails and sex and age of the subjects. We tried to see if the group membership of an unknown subject can be identified by analyzing the LIBS spectrum of its fingernail. Also, some of our subjects suffer from Hyperthyroidism. The gathered data from these subjects are investigated to see if the LIBS spectra of their nails differ from those of other subjects.

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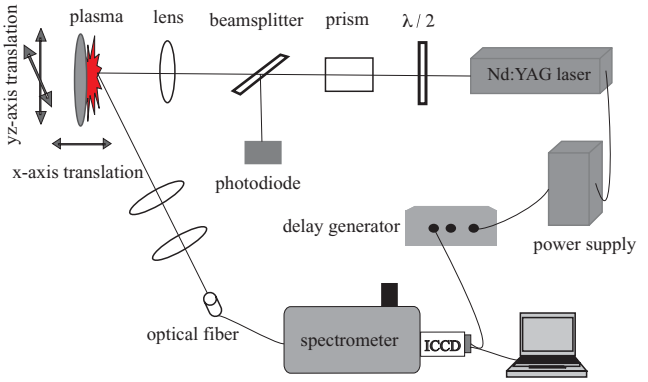

Fig. 1 Experimental setup.

Table 1 Distribution of nail samples among different age groups and genders.

	Group	Number
Sex	Women	25
	Men	20
Age	Under10	1
	10 to18	2
	19 to 35	34
	36 to 50	6

2 Experimental

The experimental setup used to perform LIBS on the nail samples is schematically shown in Fig. 1. Laser pulses from a Q-swiched Nd:YAG laser operating at its fundamental wavelength, 1064 nm with pulse duration of 6 ns and repetition rate of 0.3 Hz are used to ablate the nails. The laser beam is guided through a λ/2 rotator and Glan-Taylor prism to a lens focusing the laser beam on the nail samples. The measurements are performed in ambient air. The λ/2 rotator and Glan-Taylor prism are used to control the laser energy while keeping the operational conditions of the laser source constant. An energy meter is employed to monitor the pulse energy. In the present work, the laser pulse energy is 45 mJ/pulse. The laser beam is focused on the nail with a 20-cm focal length lens. The plasma emission is collected from the whole plasma by a quartz lens and focused into a fused-silica optical fiber, then guided to an intensified charge-coupled device (ICCD) through an Echelle spectrometer. The fused silica optical fiber is mounted on a micro-*xyz*-stage. According to the spectrograph datasheet, the resolving power is 1700. The ICCD is triggered by an output signal from the laser system. All spectra are collected at 1 μs delay and 20 μs gate width.

For improving the reproducibility of the results, laser pulses are focused slightly below the samples. The spectra are taken from the exposed part of the nails. Samples are mounted on a cylindrical holder maintaining the natural curvature of nails as they are moved using a micro-*xyz*-stage. The samples are moved in a way that the laser pulses are focused on the central part of nails maintaining the same distance from the focusing lens.

3 Results and Discussion

3.1 *Human Fingernail Spectrum*

Samples were randomly collected from people of Tehran. Subjects were categorized into two sex groups and five age groups. Table 1 represents the distribution of nail samples among different age and sex groups. Aspects of gender, age, nutrition, and disease were assessed by questionnaires. All samples were cleaned with alcohol, acetone, and distilled water to remove the external contaminants and then dried before the experiment. We could identify 13 key elemental species and a molecule by measuring the samples. A representative LIBS spectrum of nail is shown in Fig. 2. This spectrum is an average of three spectra. Each of these spectra is formed by accumulation of 10 pulses ablating different locations of the sample. The sample is moved perpendicular to the incident laser beam in the vertical direction so that every laser shot hits a new target position. The emission lines are atomic and ionic lines from organic and inorganic elements, which have been labeled on the graph. It is known that the dominant elements in nail composition are Ca, Mg, P, Na, K, Fe, Zn, and A1 while other elements like Cu, Cr, Se, Ni,

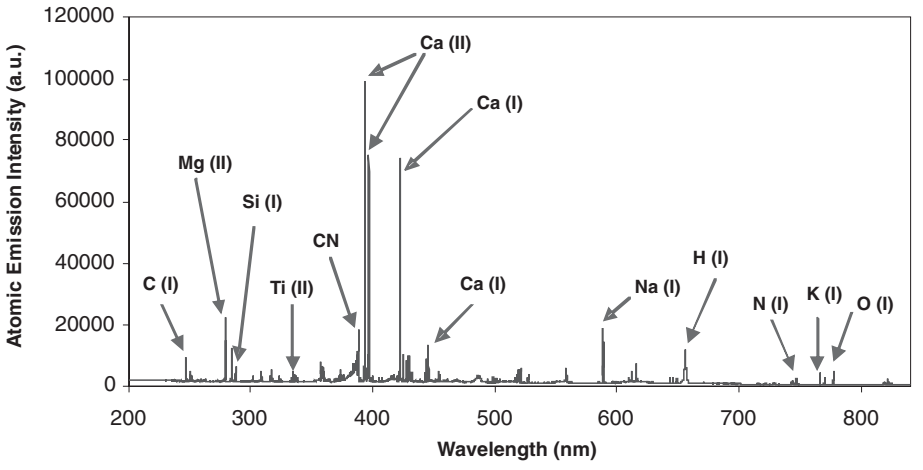

Fig. 2 Typical LIBS spectrum of the human nail.

Table 2 Identification of 63 atomic and ionic emission lines in human fingernail spectrum along with the Wilks' lambda for sex and age group discriminations.

Wavelength (nm)	Species	Wilks' lambda Sex group	Wilks' lambda Age group
247.97	C I	0.986	0.976
460.73	Sr I	0.985	0.961
358.16	Fe I	0.852	0.885
373.48	Fe I	0.952	0.885
373.72	Fe I	0.898	0.939
421.54	Fe I	0.923	0.912
279.56	Mg II	0.949	0.934
280.30	Mg II	0.975	0.966
285.22	Mg I	0.951	0.975
308.22	Al I	0.915	0.961
309.29	Al I	0.911	0.965
394.4	Al I	0.913	0.955
396.16	Al I	0.891	0.954
288.16	Si I	0.969	0.962
315.90	Ca II	0.979	0.760
317.96	Ca II	0.999	0.802
393.36	Ca II	0.978	0.788
396.84	Ca II	0.982	0.826
422.68	Ca I	0.905	0.890
430.26	Ca I	0.968	0.975
430.78	Ca I	0.951	0.938
443.56	Ca I	0.930	0.950
445.48	Ca I	0.879	0.925
526.56	Ca I	0.917	0.908
527.11	Ca I	0.957	0.953
558.20	Ca I	0.943	0.959
558.88	Ca I	0.937	0.934
559.44	Ca I	0.946	0.938
559.84	Ca I	0.922	0.916
610.28	Ca I	0.912	0.951
612.22	Ca I	0.869	0.896
616.22	Ca I	0.922	0.937
643.88	Ca I	0.959	0.940
646.26	Ca I	0.946	0.957
588.98	Na I	0.913	0.948
589.58	Na I	0.928	0.933
766.46	K I	0.921	0.923
769.88	K I	0.937	0.913
656.30	H I	0.896	0.875
777.18	O I	0.843	0.816
334.94	Ti II	0.926	0.986
335.48	Ti I	0.949	0.983
336.14	Ti II	0.923	0.989
337.28	Ti II	0.931	0.985
338.38	Ti II	0.952	0.969
363.54	Ti I	0.931	0.985
364.30	Ti I	0.911	0.982
365.34	Ti I	0.930	0.988
368.52	Ti II	0.936	0.984
394.86	Ti I	0.936	0.981
359.82	Ti I	0.928	0.991
398.16	Ti I	0.919	0.985
399.98	Ti I	0.935	0.988
399.86	Ti I	0.915	0.984
498.16	Ti I	0.940	0.986
499.08	Ti I	0.936	0.987
500.70	Ti I	0.949	0.992
386.16	CN 2-2	0.864	0.819
387.08	CN 1-1	0.929	0.893
388.34	CN 0-0	0.903	0.866
746.84	N I	0.855	0.802
744.22	N I	0.924	0.879
746.72	N I	0.868	0.813

Pb, V, Co, Cd, Mn, As, Sb, Sn, and Hg can be found in trace or subtrace levels.[18] In 1971, Harrison et al.[8] detected up to 30 elements in human fingernail samples by spark source mass spectrometry including Na, Mg, Al, Si, P, K, Ca, Cr, Mn, Fe, Ni, Cu, Zn, Ge, As, Se, Rb, Sr, Zr, Mo, Ag, Cd, Sn, Sb, Cs, Ba, La, Ce, Pb, and Bi. However, the elements detected with the present system are: aluminum (Al), carbon (C), calcium (Ca), iron (Fe), hydrogen (H), potassium (K), magnesium (Mg), nitrogen (N), sodium (Na), oxygen (O), silicon (Si), strontium (Sr), and titanium (Ti) as well as CN. Table 2 shows 63 emission lines related to these 14 species. The line signals are obtained by subtracting background emission from the peak area under the spectral lines. Here, an imaginary line between two either sides of each spectral line is considered and the area under this line is subtracted as the background. Also, the corresponding Wilks' lambda for each emission line is presented in Table 2. The Wilks' lambda will be discussed in Sec. 3.2 Although the calcium content is only 0.1% in human nail,[19] the LIBS spectrum of nail is dominated by calcium, about 65% of the total spectral power. The total spectral power can be calculated by summing the intensities of all spectral lines in each spectrum.

Here, we discuss concentration levels of elements in two sex groups. In order to do so, all emission lines of the same species are added up and then normalized to the total spectral power. This value shows the fractional spectral power of each element. Table 3 represents the average of the fractional spectral power of each species among 45 nail samples. The comparison between concentrations of elements of two sex groups is shown in Fig. 3. The x axis represents different sex groups and the z axis represents the average fractional spectral power of each element in every group. The average concentrations of Al, CN, Fe, H, K, Mg, N, Na, O, and Si are more elevated in men while concentrations of Ca and Ti are higher in the female group. The elevated concentration of Na and K in males in comparison with females was also reported by Vance et al.[20] They analyzed 117 subjects by instrumental neutron activation analysis. However, they reported that Ca concentration had no effect on either age or sex, which is in contradiction with our analysis. Concentrations of C and Sr are equal within the experimental error in both groups. Dittmar et al.[21] reported that carbon content in male and female subjects was not different, which is in accordance with our analysis. In 1988, Takagi et al. detected 18 elements in human nails by argon inductively coupled plasma emission spectroscopy.[18] They reported that Ca, Mg, Na, K, and Fe generally exist at higher levels in males compared to females, which is in accordance with our results except for the Ca concentration. The average concentrations of each species in different age groups are displayed in Fig. 4. As the age increases, the overall carbon level in the fingernails increases, although there are slight fluctuations. This was also observed by Dittmar et al.[21] They performed analysis on 225 individuals (93 males and 132 females). Takagi et al. reported that Al concentration decreased with age, which is not exactly in accordance with our results.[18]

Table 3 Fractional spectral powers for each species in human fingernails (average from 45 samples).

Species	Average fraction of total spectral power
Ca	0.649
H	0.068
Na	0.058
CN	0.053
Ti	0.035
Al	0.025
Mg	0.025
N	0.022
K	0.019
Fe	0.016
O	0.011
C	0.006
Si	0.002
Sr	0.002

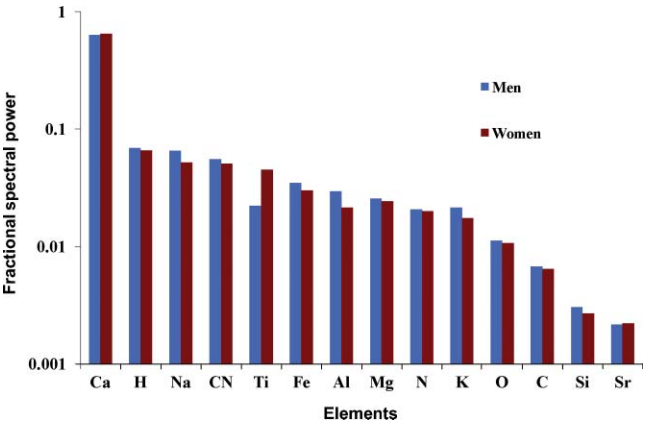

Fig. 3 Fractional spectral power of elements in nails of men and women. *Y*-axis has a logarithmic scale.

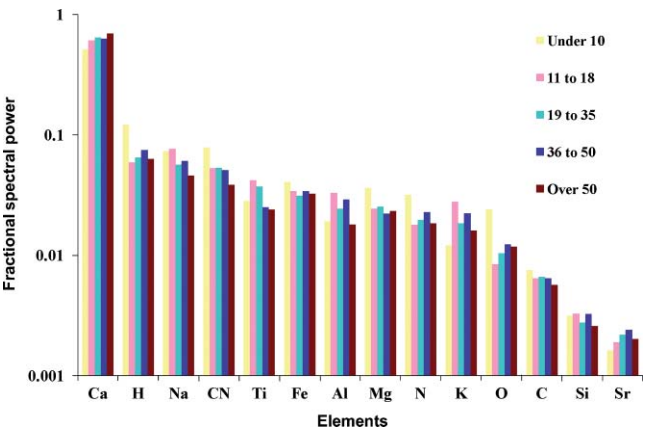

Fig. 4 Fractional spectral power of elements in nails in different age groups. *Y*-axis has a logarithmic scale.

However, they performed testing on 512 samples (278 men and 134 women) from different countries of Japan, India, U.S.A, Canada, and Poland.

It should be mentioned that a number of samples in the present work and their distribution is not enough for making a statistically meaningful analysis. Also, the number of samples in the present work differs from the number of samples in other authors' research[18,20,21] The limited number of samples tested in the present work (45 samples) and also the number of samples in the original works, ranging from 117 to 512, do not allow one to draw definite conclusions on systematic difference in elements' concentrations between different groups.

3.2 *Discriminant Function Analysis*

Discriminant function analysis (DFA) is a data reduction analysis technique that is used to determine which variables discriminate between different groups. DFA uses a set of independent variables to separate cases based on groups one defines; the grouping variables are the dependent variables that are categorized as the variables of sex and age group in our analysis. Given a set of independent variables, DFA attempts to find linear combinations of those variables that best separate the groups of cases. These combinations are called discriminant functions. A canonical correlation analysis produces the discriminant functions and their canonical roots that are the eigenvectors and eigenvalues of the data. N-1 discriminant functions are constructed for discrimination among N groups. Canonical discriminant analysis constructs discriminant functions that separate the categories as much as possible. The first function separates the groups the most. The second function provides the second most separation. The procedure continues adding functions in this way until the maximum number of functions is reached. All of these constructed functions are uncorrelated.[22] In this work, subjects are categorized in two sex groups and five age groups.

The null hypothesis states that the groups or categories under consideration are statistically the same or exhibit the same behavior without any significant difference. To reject the null hypothesis, a significance test is carried out by checking the differences in the mean values of the groups. The groups are significantly different if the difference of their mean values is greater than the variance of all samples constituting one normal group. Therefore, if the test is significant, the groups are discriminated according to the corresponding canonical discriminant function scores. By constructing discriminant functions, DFA can find small similarities in the variances of dependant variables.[22] By giving each dependant variable a score, it can classify them. When the scores are plotted, we can observe the validity of our predictions.

Here, two approaches are used. In the first approach, the sum of all of the emission lines belonging to a species is used for discrimination.[23] In the second, all emission lines are individually inserted into DFA software (SPSS v16). The results of the first approach do not show a significant discrimination between groups, i.e., 73.3% for sex discrimination and 80% for age group discrimination. Therefore, the second approach is utilized in which 63 individual emission lines of 13 elements and one molecule are input into the software. Figure 5 shows a graph of the discriminant function scores for each subject. In Fig. 5, the first function accounts for the most discrimination (79.1% of the variance) and the second one provides the second most (20.9% of the variance). For the age groups, four discriminant functions are constructed, which account for 59.2, 21.6, 13.7, and 5.5% of the variance, respectively. The graphical output of the DFA analysis depicts only the scores from the first two functions.

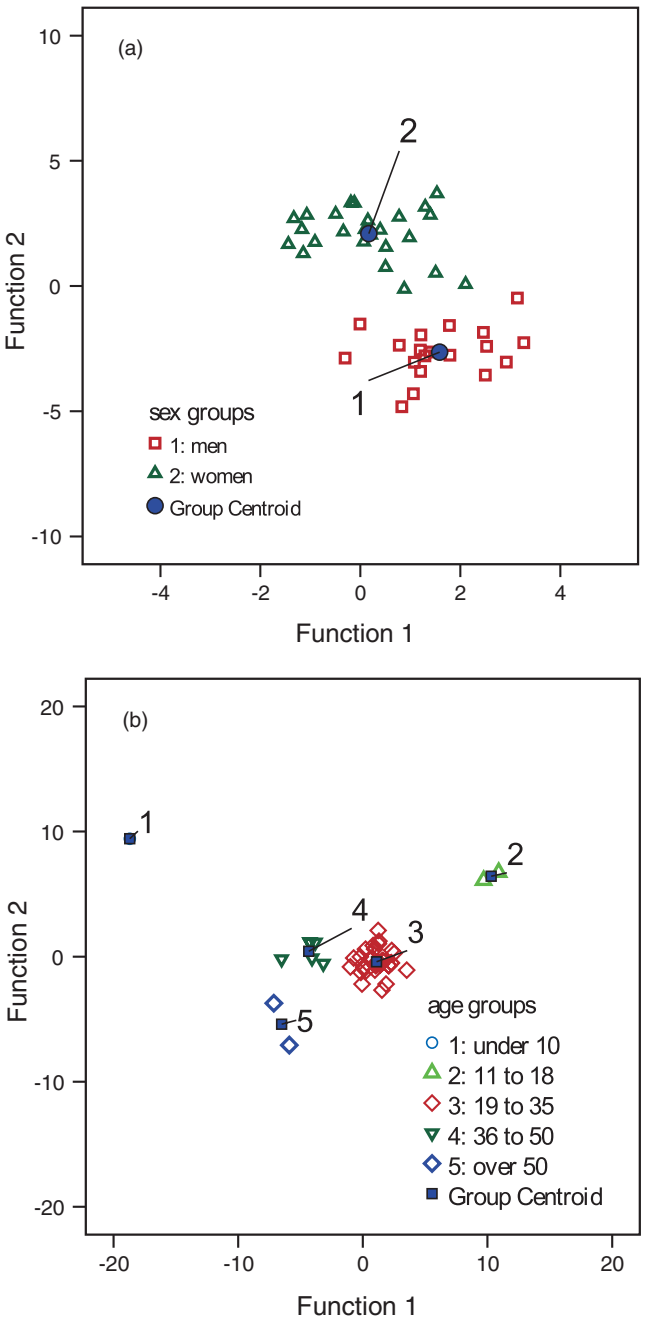

Fig. 5 DFA plot showing the first two function scores that resulted from LIBS spectra obtained from nail samples. (a) Two groups of men and women. (b) Five age groups.

3.2.1 *Sex discrimination*

Figure 5(a) shows the DFA plot for discrimination of sex groups. Each colored object in Fig. 5 is related to the nail spectrum of a subject that is depicting the subject's function 1 score against his or her function 2 score. In Fig. 5, the "group centroid" is

Table 4 Pearson correlation between concentrations of elements in 45 nail samples.

	Al	C	Ca	CN	Fe	H	K	Mg	N	Na	O	Si	Sr	Ti
Al							0.40					0.61		
C			−0.41	0.84	0.36	0.84			0.74		0.73		−0.59	
Ca		−0.41		−0.43	−0.27	−0.56	−0.30	−0.49	−0.55	−0.32	−0.53	−0.38	0.51	−0.43
CN		0.84	−0.43		0.57	0.65			0.67		0.54		−0.52	
Fe		0.36	−0.27	0.57										
H		0.84	−0.56	0.65					0.92		0.97		−0.54	
K	0.40		−0.30							0.88		0.33		
Mg			−0.49									0.65		0.42
N		0.74	−0.55	0.67		0.92					0.91		−0.42	
Na			−0.32				0.88							
O		0.73	−0.53	0.54		0.97			0.91				−0.48	
Si	0.61		−0.38				0.33	0.65						
Sr		−0.59	0.51	−0.52		−0.54			−0.42		−0.48			
Ti			−0.43					0.42						

also presented, which is the "center of mass" of each group. As it is observed, 100% of the original grouped cases are correctly classified. However, it should be noted that the small number of subjects here does not allow one to definitely generalize the idea to a larger set. The number of samples in each group is not enough to validate the results with untested samples. Although no validation with untested samples is provided, results of DFA show that discrimination between age and sex groups is possible.

Wilks' lambda is a test statistics used to determine the role of each independent variable in discrimination. The smaller the Wilks' lambda for an independent variable, the more that variable contributes to the discrimination. Lambda varies from 0 to 1. As can be seen from Table 2, the smallest Wilks' lambda respectively belongs to the emission lines of O I at 777.18, Fe I at 358.16, N I at 746.84, CN 2–2 at 386.16, N I at 746.72, Ca I at 445.48, Al I at 396.16, and H I at 656.30 nm. These results show that the concentrations of organic elements such as O, N, H, and CN and elements like iron and calcium are a determining factor in sex discrimination.

3.2.2 *Age group discrimination*

Individuals were categorized into five age groups. The distribution of subjects in different age groups can be found in Table 1. The results of age group discrimination according to LIBS analysis of nails is represented in Fig. 5(b). In this analysis, 100% of the original grouped cases are correctly classified. This classification should be carefully considered since the number of members in each group is not equal. Additionally, in three of five groups, the numbers of samples are too small for the results to be applied to a larger set. In this analysis, 80.8% of the variance between all groups is described by the first two canonical discriminant functions where 59.2% is attributed to F1 and 21.6% to F2. Here, the smallest Wilks' lambdas correspond to Ca II at 315.90, Ca II at 393.36, N I at 746.84, N I at 746.72, CN 2–2 at 386.16, O I at 777.18, Ca II at 393.84, CN 0–0 at 388.34, H I at 656.30, and N I at 744.22 nm. So, the concentrations of Ca, N, O, H, and CN in fingernails can be indicative of age group differences. It can also be concluded that LIBS analysis of human nails coupled with DFA is not suitable for determining the exact age of the person. However, it is particularly useful for identifying the person's age group.

3.3 *Correlation Between Concentrations of Elements*

Pearson correlations between concentrations of elements are shown in detail in Table 4. Only the significant correlations are displayed in Table 4. The Pearson correlation is obtained by dividing the covariance of the two variables by the product of their standard deviation. Concentrations of sodium and potassium are positively correlated in 45 nail samples at the level of 0.88. Nowak also observed a significant positive correlation between sodium and potassium in fingernails.[24] Calcium and magnesium concentrations are negatively correlated at the level of 0.49. This may be due to the fact that Ca and Mg compete for absorption on the gut surface.[25] Authors in Ref. 26 refer to this fact that magnesium inhibits the calcification. This fact may explain the negative correlation between Ca and Mg. Magnesium correlates with silicon at the level of 0.65. Berlyne et al. showed that the urinary silicon was highly correlated with the urine magnesium concentration.[27] Silicon is bound with magnesium cations when

excreted from the body mainly in the form of urine.[28] Nails can be considered as another depletion mechanism of elements in the body.[29] So, Si and Mg are simultaneously excreted by the nails. There is no significant correlation between pairs of elements like sodium-iron and sodium-silicon. In addition to that, aluminum, magnesium, and titanium do not significantly correlate.

3.4 *Hyperthyroidism*

In this section a case report of hyperthyroidism based on the LIBS spectra of fingernails is presented. According to the questionnaires, case numbers 2, 3, 5, and 16 suffer from hyperthyroidism and case number 43 suffers from high blood pressure. Hyperthyroidism, an illness of the thyroid, is associated with an overproduction of the thyroid hormone. The symptoms of hyperthyroidism are palpitations, heat intolerance, nervousness, insomnia, breathlessness, increased bowel movements, light or absent menstrual periods, fatigue and fast heart rate, which are caused by the effects of too much thyroid hormone on tissues of the body.[30] By looking at the box plot depicting sodium (Na) concentration in Fig. 6(a), it is shown that the Na concentrations of case numbers 3, 5, and 16, who are siblings, are outliers. An outlier is an observation that is numerically far from the rest of the data.[31] If the groups of males and females are separately considered [Fig. 6(a)], case number 16 who is a hyperthyroidism patient is located in the normal distribution, while the sodium concentration of case number 43 in the female group is an outlier. By observing the box plot for potassium level in Fig. 6(b), we can see that case numbers 2, 3, 5, 15, 16, and 43 have outlying levels of potassium in their fingernails. As it is mentioned, the cases 2, 3, 5, and 16 suffer from hyperthyroidism while case number 43 is a 47 year old woman who has high blood pressure. However, case number 15 does not suffer from any disease. If the sex groups are separately observed [Fig. 6(b)], all male subjects suffering from hyperthyroidism (2, 5, and 16) are diagnosed as healthy, which is a false negative diagnosis. Generally, it is observed that there is an agreement between the elevated levels of potassium and sodium in the fingernails and blood test results indicating hyperthyroidism and high blood pressure. Potassium deficiency is one of the symptoms of hyperthyroidism.[32] On the other hand, the high level of an element in hair and nail may indicate depletion of that element in the body.[29] Thus, the elevated level of potassium in nails of hyperthyroidism patients can be due to the potassium depletion. In general, it should be mentioned that only individual cases were investigated for hyperthyroidism, and a more statistical approach is demanded to ascertain whether our results are of a more general nature.

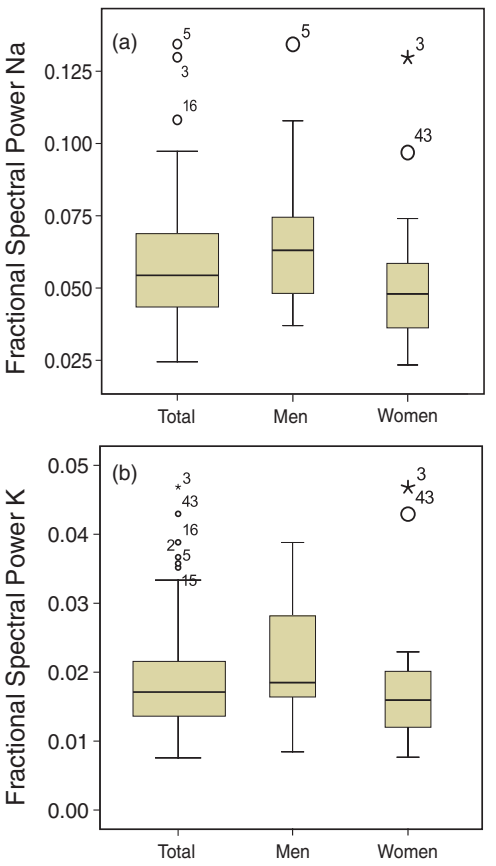

Fig. 6 Box plot of concentration of (a) sodium and (b) potassium, and representation of elevated levels in cases of hyperthyroidism.

4 Conclusion

Elemental analysis of fingernails is performed on 45 samples using laser-induced breakdown spectroscopy in ambient air. Laser pulses are focused slightly below the exposed part of nails. The plasma emission is collected from the whole plasma at 1 μs delay and 20 μs gate width. The spectra of Al, C, Ca, Fe, H, K, Mg, N, Na, O, Si, Sr, Ti, as well as CN molecule are detected. The line signals are obtained by subtracting background emission from the peak area under the spectral lines. The fractional spectral power of each species is calculated and compared between different groups. Although calcium concentration in nail tissue is about 0.1%, its fractional spectral power is as high as 65%. Comparison between the levels of elements in different genders shows that men have higher levels of Al, CN, Fe, H, K, Mg, Na, O, and Si while women have higher concentrations of Ca and Ti. The Pearson correlations between elements show that there is a negative correlation between calcium and magnesium, which can be related to their competition in absorption at gut surface. The samples are categorized in two sex and five age groups. Discriminant function analysis is applied to discriminate among different groups. The DFA method significantly discriminated among the sex and age groups and 100% of cases are correctly classified. This analysis shows that applying DFA on LIBS spectrum of fingernails offers a simple and well suited approach to sex and age group discrimination. The results should always be carefully considered since the number of samples in each group is too small. Study of a few cases shows that the levels of essential elements change in health disorders such as hyperthyroidism and high blood pressure and such changes in concentration of elements are an indicator that can be utilized in the monitoring of human health status. However, this method, like any other, has weaknesses that can be strengthened through further research.

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