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Research Paper

Elemental composition of hair as a marker for forensic human identification

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ABSTRACT

Background: Hair is one of the most common evidence types found in criminal investigations. Analysis of human hair reveals the mineral composition accumulated within it over time spent in a specific area, thereby providing additional information for forensic identification.
Materials and methods: To identify patterns of the elemental composition of hair in territories with different natural and anthropogenic features, hair samples of 1238 residents and 217 corpses of Central Kazakhstan were studied. The determination of 14 chemical elements in hair by inductively coupled plasma atomic emission spectrometry were presented. The data were analysed in terms of place of residence, gender, age and condition.
Results: The results showed that the concentration of trace elements like Cu, Fe, Cd, and As significantly differed among all regions ($p < 0.05$). The composition of hair samples obtained from women significantly differed from those obtained from men for certain major and trace elements ($p < 0.05$). Concentrations of Ca and Mg in men were significantly lower than in women ($p < 0.05$) and were decreasing with age ($p < 0.05$).
Conclusions: The present investigation revealed a relationship between the elemental composition of hair and the place of permanent residence of a person, formed under the influence of regional industrial complexes, and determining gender and age-related differences. These findings enhance the possibilities of forensic human identification.

1. Introduction

Hair samples are essential resources in the forensic analysis of crime scenes, often providing valuable information that can help identify a suspect or victim. Hair analysis has been receiving increased attention for years. Currently it has become the third most fundamental biological matrix used for drug testing in forensic toxicology, after blood and urine.[1] Although molecular genetic methods currently dominate human identification, they are not always effective in identifying unknown cadavers due to a lack of comparison material.[2] Thereby alternative techniques are of interest, including those using hair.[3,4] Human hair is an attractive biological material because of its ease of sampling, transport, storage, processing and higher concentrations of trace elements.[5] The diagnostic value of hair analysis has been confirmed by many authors who have proven a correlation between major element concentrations in hair and the body both in physiological and pathological states.[6,7] Adverse environmental impacts can affect public health and serve as indicators for human forensic identification.[8] Some researchers have analysed hair for hazardous elements such as As, Zn, Cu, Cd, Pb to trace sources of pollution and have found an increase in trace elements in hair through environmental sorption.[9–11]

The trace element determination is a subject of ongoing interest in the biomedical and environmental sciences. Changes in chemical element contents in the environment lead to corresponding changes in human biological substrates.[12] The trace elements accumulate in the body over time. Consequently, they reflect the biomedical and environmental history of the body and long-term metabolic changes throughout a lifetime.[13] Therefore, elemental analysis is widely used in medical and environmental research.[14] According to Evrenoglou et al. element concentrations in human biological samples vary from country to country depending on the geographical variation, dietary habits and environmental factors.[15] Thus, detecting regionally defined traits enhances the possibilities for forensic identification of a person using hair samples.[16]

Central Kazakhstan is one of the leading industrial regions of the country. The development of industrial complexes leads to pollution of the environment with chemical elements and their accumulation in high concentrations in various biological environments.[5,17–19] In 2019, 593

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thousand tonnes of pollutants were emitted into the atmosphere, accounting for 26.1% of the total emissions in Kazakhstan.[20] The population of these regions is exposed to a complex impact of harmful factors associated with geochemical features of the regions and intensive environmental pollution, such as emissions from large city-forming enterprises. Subsequent studies have shown that the deposition of heavy metals in human hair is related to exposure and inhalation of these substances from the environment.[21] A literature review showed that reference values for most chemical elements in hair have not yet been officially established, as they will depend on the region.[7,10,21] The quantitative composition of trace elements of hair of the population of Central Kazakhstan in different regions has not been studied yet. Atomic emission and argon inductively coupled plasma mass spectroscopy (ICP-OES and ICP-MS, respectively) are prevalent techniques that can significantly enhance the diagnostic capabilities of several human biomedical features.[11,22,23] Analysis of human hair reveals the mineral composition accumulated within it over time spent in a specific area, thereby providing additional information for forensic identification. The information obtained allows the refinement of the search area involved in cadaver identification activities.

Thereby, this research's objective was to identify quantitative patterns of the elemental composition of human hair in regions with different natural and anthropogenic features. Determining the most typical indicators might help forensic identification by ranking and determining individuals' permanent residence, gender and age.

2. Materials and Methods

2.1. Sampling regions and methodology

The elemental composition of hair was studied in 1238 residents and 217 corpses of central Kazakhstan (Table 1). According to the location of the principal industrial complexes, four regions exerting a technogenic impact on the environment were selected: the cities of Karaganda, Temirtau, Balkhash, and Jezkazgan. Hair samples were analysed, ranging by gender and age ranges (21–30, 31–40, 41–50, 51–61 years old and 61 years and older).

Full-length hair samples (0.1 g at least) were cut with sterilized stainless steel scissors from the back of the head. The samples were sealed in paper envelopes and stored in a dry place at room temperature.

2.2. Data acquisition and ethical approval

Personal and medical histories of the individuals involved in the study were obtained using a questionnaire based on the recommendations of the World Health Organization.[24] Personal data contained information about the subject's condition (alive or dead), gender, age, region of permanent residence, profession, etc.

The study was approved by the Committee on Research Bioethics of the National Research Ethics Service (Protocol No. 23 dated December 19, 2019) to use human tissue. The material (hair samples) was collected following the rules adopted by the Ethical Commission of the University (Republic of Kazakhstan). In all cases, written informed consent was obtained from donors of hair samples and relatives if samples were taken from corpses.

Table 1
Distribution of the studied hair samples of individuals (by region).

Parameters	Living individuals (n = 1238)		Deceased individuals (n = 217)	
	men	women	men	women
Karaganda region	154	156	28	27
Temirtau region	152	156	27	27
Balkhash region	154	156	27	27
Jezkazgan region	154	156	27	27

2.3. Sample pretreatment procedures

Hair samples were pre-washed with a non-ionic detergent and deionized water. Samples were treated with acetone for 15 min to remove surface contamination and degrease, then washed three times with deionized water. Then, samples were dried at room temperature for 30 min and stored in desiccators. Immediately before analysis, the hair samples were crushed. Sample decomposition was carried out using the microwave digestion technique: a 0.5 g weighted portion of the sample was placed into a PTFE reactor chamber, adding 5 ml of nitric acid (69%). The autoclave containing the sample in the reactor chamber was placed into a microwave oven. According to the following program, the sample was digested: the autoclave was heated to 200 °C, held at this temperature for 5 min and then cooled down to 45 °C. The cooled autoclave was shaken to mix the contents, and the lid was opened slightly to balance the pressure. The dissolved sample was transferred to a 15 ml test tube containing 1 ml of deionized water, shaking continuously. Each washing was transferred into a 10 mL test tube containing deionized water, then closed and mixed. A 1 ml aliquot of the obtained solution was collected using an automatic dispenser with a replaceable tip, adjusted to 10 ml with 0.5% nitric acid, sealed with a laboratory film and stored until analysis. The aliquot and dilution volume data were entered into the spectrometer software together with the sample name and sample weight.

2.4. Research methods

Measurements of 14 elements (Cu, Zn, Co, Fe, Cr, Mn, Cd, As, Pb, Ni, P, CA, K and Mg) in hair were performed on an inductively coupled plasma atomic emission spectrometer Ultima 2 (Horiba Jobin Yvon, Japan, France). The device's characteristics allow to minimize matrix influences and carry out the analysis of ultra-low concentrations of elements at preservation of the possibility of the analysis of average and high concentrations. The guaranteed detection limits achievable on spectrometers of this class were fractions of a milligram per litre. The serial optical emission spectrometer with inductively coupled plasma from HORIBA Jobin Yvon - model "ULTIMA 2" is the last generation instrument. It consists of a monochromator, a 40.68 MHz solid-state generator and a microcomputer, under the control of which the analysis conditions are programmed and carried out. The Czerny-Turner monochromator has the following characteristics: (i) focal length - 1.0 m; (ii) holographic grating - 2400 strokes/mm, first and second order, size 110 × 110 mm; (iii) spectral range - standard 160–800 nm, with an option for UV 120–180 nm; (iv) resolution - 5 p.m. at 120–320 nm and - 10 p.m. at 320–800 nm. The optics were filled with nitrogen and thermostabilised to ±1 °C. The grating was moved by a stepper motor with a step size of 0.001 nm and mechanical repeatability of 0.0004 nm. The trace elements were determined using the following wavelengths (nm): Cu - 324.754; Zn - 213.856; Co - 228.616; Fe - 259.94; Cr - 267.716; Mn - 280; Cd - 228.802; Pb - 220.353; Ni −221.647; P - 213.618; Ca - 1064.

The content of chemical elements in the examined hair samples was compared with reference values of concentrations of chemical elements in hair obtained by ICP-AES (interquartile range (q25–q75),[25]). Biologically acceptable levels of chemical elements in hair were set to be equal to threshold limit values of toxic chemical element contents developed for individuals in contact with toxic metals.[24,26] The analysis results were entered into an electronic database containing the medical and biological characteristics of the studied individuals.

The obtained data were processed in the Microsoft Excel XP table editor (Microsoft Corp., USA) using the Statistica 10.0 (StatSoft Inc., USA) and SPSS 20 statistical software packages. A nonparametric Mann-Whitney test was used when comparing quantitative features in two independent samples when analysing quantitative data by condition and gender. The Kruskal-Wallis test was used to compare three or more independent groups when performing quantitative inter-comparison of regional data and age. Differences in values were considered statistically significant at a probability level of more than 95% ($p < 0.05$).

3. Results

3.1. Analysis of elemental concentrations by region

The concentrations of essential elements (Cu, Zn, Co, Fe, Cr and Mn), toxic and potentially toxic elements (Cd, As and Pb), conditionally essential trace (Ni) and major (P, Ca, K and Mg) elements in the hair samples taken from residents and deceased individuals (corpses), who had previously lived in the same region of central Kazakhstan, are presented in Fig. 1. It should be noted that, for most elements, a wide distribution of concentration values was observed with a tendency towards elevated values concerning the average reference values.

The present investigation has shown that the concentration of trace elements like Cu, Fe, Cd, and As significantly differed among all regions ($p < 0.05$). Comparing the Cu levels for hair samples taken from residents of Karaganda, Balkhash and Jezkazgan regions, each with the reference value significantly differed ($p < 0.05$). In each of these regions, the concentration of Cu in the hair samples was higher than the norm. Moreover, in the Balkhash region, the median value of the Cu concentrations was over ten times higher than the threshold concentration ($p < 0.05$). In the samples of residents of the Temirtau region, the median concentrations of this metal in hair samples were within the reference range.

When comparing the levels of Fe and Cd found in hair samples of residents of the surveyed territory in Kazakhstan with reference values, significant discrepancies were found ($p < 0.05$). Amidst samples, those taken from residents of the Temirtau region showed drastically elevated

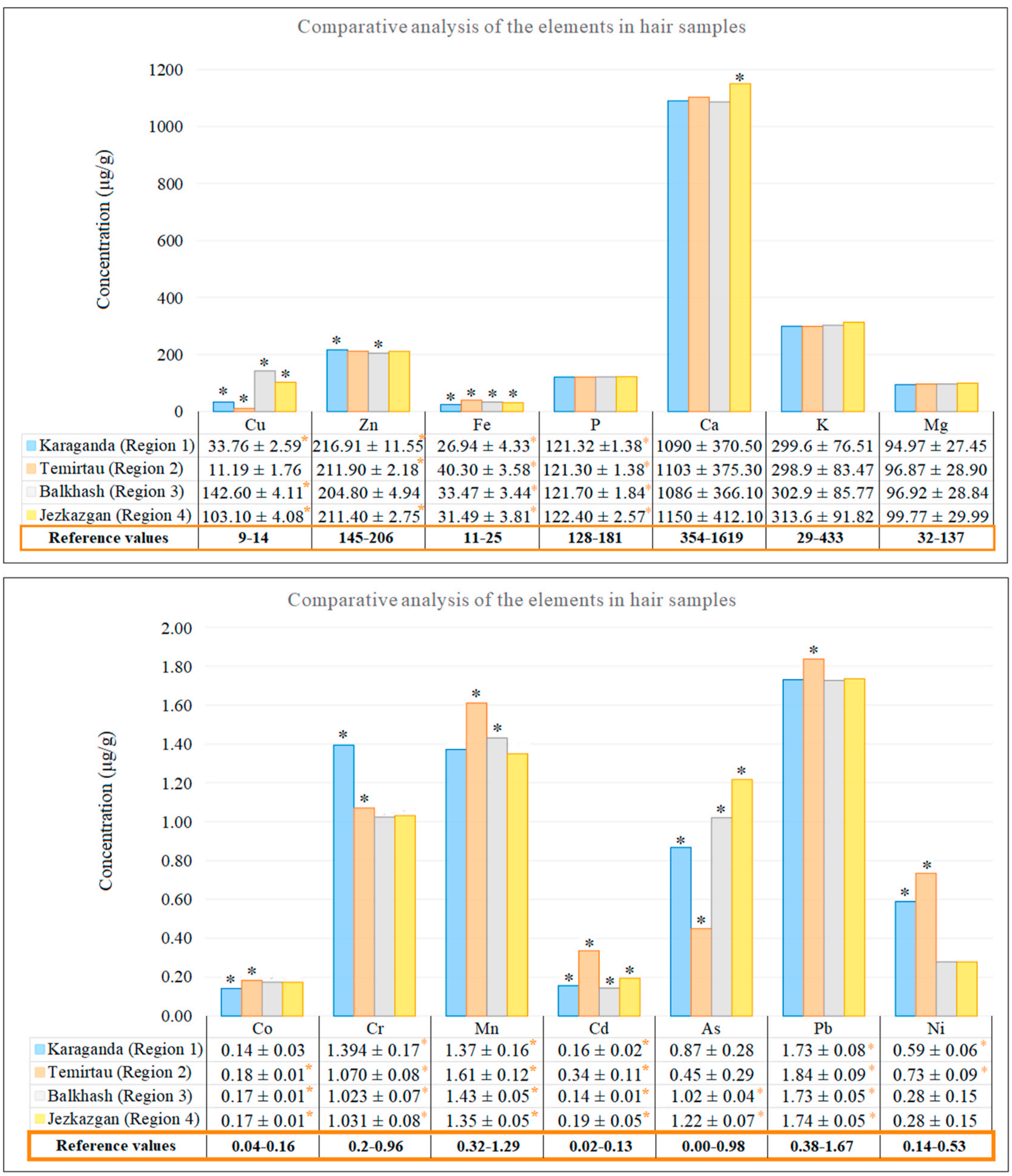

Fig. 1. Comparative analysis of the quantitative levels of different elements from hair samples from all individuals depending on the region presented in the vertical column bar graph and table (below) with mean ± SD (µg/g), reference values of concentrations of chemical elements in hair obtained by ICP-AES (interquartile range (q25–q75),[25]). A significant difference was noted when $p < 0.05$, the difference among all groups displayed above the bars (*), and between reference values in the table (*).

concentrations of Fe and Cd ($p < 0.05$ and $p < 0.05$), compared to other regions and reference values. The highest levels of As, which were above the threshold limit, were found in the hair of residents of the Balkhash ($p < 0.05$) and the Jezkazgan ($p < 0.05$) regions, and the lowest values were observed in the Temirtau region ($p < 0.05$).

Estimating the trace element status of the population based on Cr and Pb elements showed significantly higher concentrations compared to the corresponding reference value ($p < 0.05$ and $p < 0.05$), respectively. The highest Cr levels were found in hair samples taken from residents of the Karaganda region ($p < 0.05$). Compared to other regions, the highest Pb concentration was found in the Temirtau region's hair ($p < 0.05$). Average concentrations of Ni in hair from the Balkhash and Jezkazgan regions fall within the reference range. Significant elevation of the concentration of this element was found in the hair of residents of Temirtau and Karaganda regions ($p < 0.05$ and $p < 0.05$).

It was found that the Zn concentration in the hair samples in all regions, except the Balkhash, was elevated ($p < 0.05$). The amount of Co in the hair of all residents in 2, 3, and 4 regions is marginally higher than the threshold limit ($p < 0.05$), and in the Temirtau (region 2), it was significantly higher ($p < 0.05$), compared to other regions. The content of Mn in all hair samples was elevated compared to reference values. The most pronounced elevation in Mn levels was found for residents of Temirtau and Balkhash regions ($p < 0.05$ and $p < 0.05$), respectively. Compared to the hair samples obtained from the residents of these two regions, those from Karaganda and Jezkazgan regions showed lower concentrations of Mn, yet these remain higher ($p < 0.05$ and $p < 0.05$) than the threshold limit concentration, respectively.

The analysis of the major element content of hair taken from residents of the surveyed territory showed a deficit in P, compared to the reference values ($p < 0.05$). The major elements Ca, K and Mg were within the reference range. No differences between the residents from all regions were found, except Ca, higher in the Jezkazgan region ($p < 0.05$).

3.2. Analysis of elemental concentrations by gender and age

Each region was then investigated individually, and the difference between gender and age was determined. The conducted investigations have shown that the quantitative composition of hair samples obtained from women is significantly different from those obtained from men for certain major and trace elements. Looking at the data obtained for the Karaganda region, in comparison with samples obtained from women, those obtained from men contain significantly higher concentrations of all trace elements (Cu, Zn, Co, Fe, Cr, Mn, Cd, As, Pb, Ni) as well as the major element K (see Fig. 2) ($p < 0.05$). While concentrations of Ca and Mg in men were significantly lower than in women ($p < 0.05$) and ($p < 0.05$), respectively. A similar trend was observed in other regions: Temirtau (see Supplement 1. Fig. 1S.), Balkhash (see Supplement 1. Fig. 2S.) and Jezkazgan (see Supplement 1. Fig. 3S.).

The age-related differences in element concentrations were then analysed separately for men and women. Analysing the age differences in men form the Karaganda region, the group from 21 to 30 years had a significant difference in element concentrations (Fig. 2). Lower concentrations of trace elements (Cu, Zn, Co, Fe, Cr, Mn, Cd, Pb, Ni and K) were found and significantly differed from all other age groups ($p < 0.05$). Mg and Ca concentrations in this group (21–30 years) were significantly higher than in all other groups; except Ca, no significant difference was found between the 31–40 years' group. It was noted that Ca and Mg concentrations decreased significantly with age. The same pattern was observed for most elements in other regions; see Supplement 1 (Figs. 1S, 2S and 3S). Concentrations of trace elements (Cu, Zn, Co, Fe, Cr, Mn, Cd, Pb, Ni and K) were lower in men from 21 to 30 years old ($p < 0.05$), while Ca and Mg were higher than in other studied groups ($p < 0.05$). Also, Ca and Mg concentrations were decreasing with age.

In women from Karaganda aged 21–30, the concentrations of Fe, Cr, Mn, Ni, Ac and Mg were significantly lower than other groups ($p < 0.05$) (Fig. 2). A similar pattern in some element concentrations in young women (21–30 years) was observed in other regions; see Supplement 1. In the Temirtau region, lower concentrations were detected in Mn, Cd, Ni, Pb, As and Mg, compared to other age ranges ($p < 0.05$) (Fig. 1S). In the Balkhash region, lower concentrations were detected in Ni, As, and Mg elements ($p < 0.05$) compared to other groups (Fig. 2S).

No significant differences were observed in the Jezkazgan region (Fig. 3S). A significantly higher concentration of K in women aged 21–30 and 31–40 years, compared to other age ranges, was noted in all studied regions ($p < 0.05$).

3.3. Analysis of elemental concentrations by condition

Then an analysis was carried out to find out if there is a difference between living and deceased individuals (Fig. 3). Only in the Karaganda and Temirtau regions differences between the living and the dead individuals were identified. In the Karaganda region, differences in elements such Cu, Zn, Fe, Co, Cr, Mn, Cd, As, Pb and Ni were found significant ($p < 0.05$). No differences were detected for P, Ca, K and Mg elements. In the Temirtau region, a significant difference was observed only for As, Pb and Ni ($p < 0.05$). As and Pb concentrations in deceased individuals were lower ($p > 0.05$), while Ni concentration was significantly higher ($p < 0.05$). No statistically significant differences in the concentration of trace and major elements between hair samples obtained from living vs deceased individuals in the remaining regions were detected.

4. Discussion

Hair evidence is one of the most common types of evidence encountered in criminal investigations. During the normal hair-growth cycle, hairs are readily lost from individuals and might be transferred during criminal activity.[27] However, the science of macroscopic hair examination might not result in identification; to conclude, a hair came from one individual to excluding all others. Nevertheless, the vast amount of microscopic information available from hair analysis can provide a solid basis for an association and certainly provides solid evidence.[27] The unique physicochemical structure of the hair shaft protects it from any post-biogenic changes for extended periods, making the hair a unique instrument both for archaeological and forensic investigation.[28,29]

The results obtained from the study of the elemental composition of hair taken from residents of the four main regions of central Kazakhstan showed a dependence of the concentrations of trace elements in the hair on the residents' level and amount of exposure of a specific region to those elements. It was established that in hair samples taken from residents of different central Kazakhstan regions, the concentrations of 14 major and trace elements differ both from reference values and each other.[25] The data analysis shreds of evidence that the levels of certain elements in hair samples of residents for central Kazakhstan differ from those reported by other authors in other investigated areas.[26] A characteristic feature of the elemental status of the population of central Kazakhstan is excess levels of Cu, Zn, Mn, Fe, Cr, Cd, As, Ni, and Pb, together with a deficiency in the major elements (such P). According to the data presented by Hu et al. an imbalance in the elements is mainly due to the development of specific industrial production complexes in the region.[30] Observations obtained by other authors on the permanent active presence of a variety of pollutants in the environment are strictly specific to each industry and thus create a characteristic pattern of departures of the composition of hair in a particular region compared to the reference values.[14,31–33] This observation is sufficiently specific and unvarying and makes it possible to identify the region of permanent residence of a person based on the absolute and relative composition quantitative indicators.

To summarize the obtained data, it is worth noting the elevated concentrations of Cd, Cu, Cr, Zn, and Pb in the hair of the residents of the

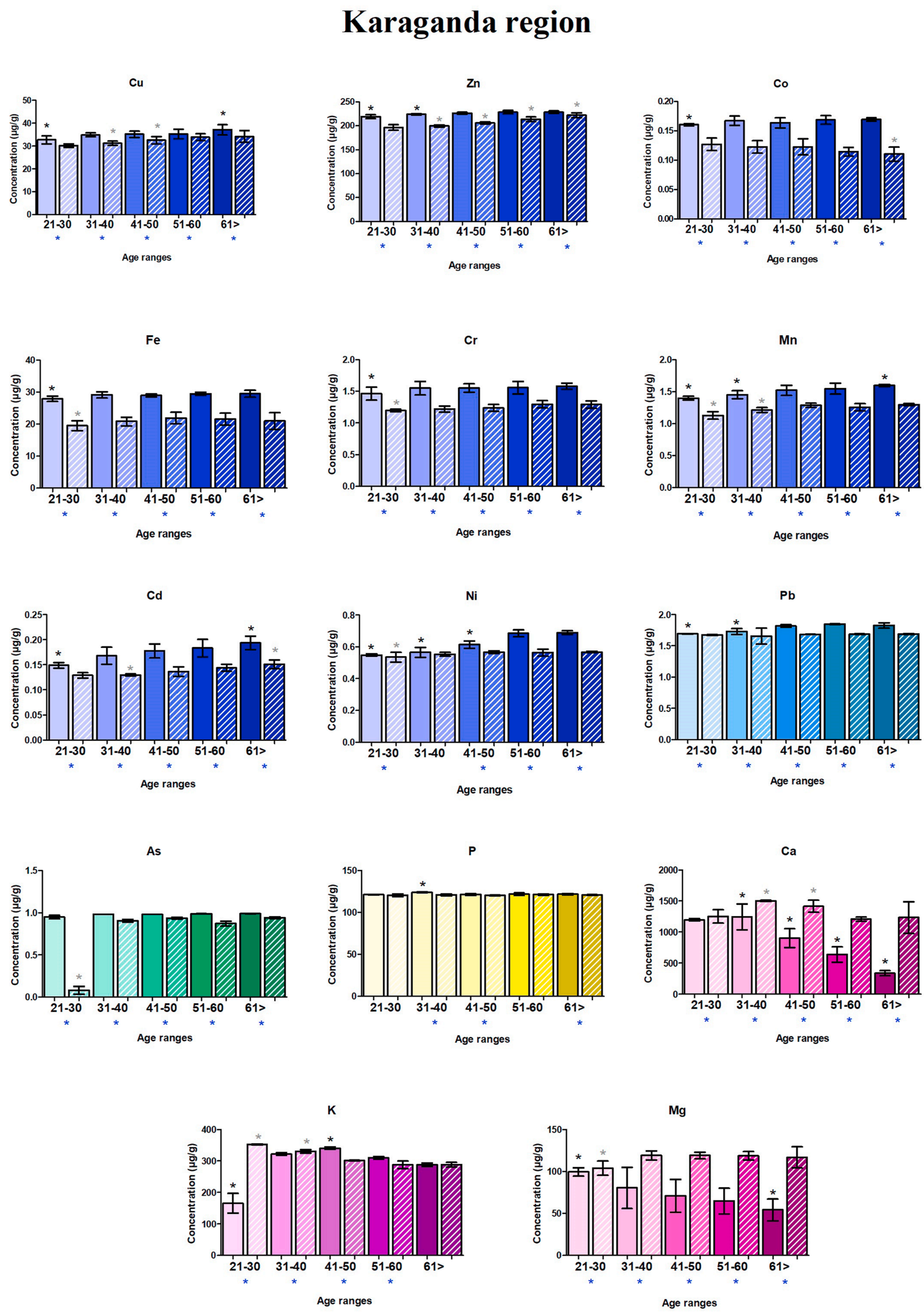

Fig. 2. Vertical column bar graphs (mean with ±SD), representing comparative analysis of the quantitative levels of different elements from the hair samples from men and women in different age groups from the Karaganda region. The colour coding of the elements was chosen according to the element groups of the periodic table; men (solid bars), women (shaded bars). The significant difference noted when $p < 0.05$ (*): gender differences are indicated under the bars (*), differences among age ranges, when differs from all groups, are displayed above the bars (*). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

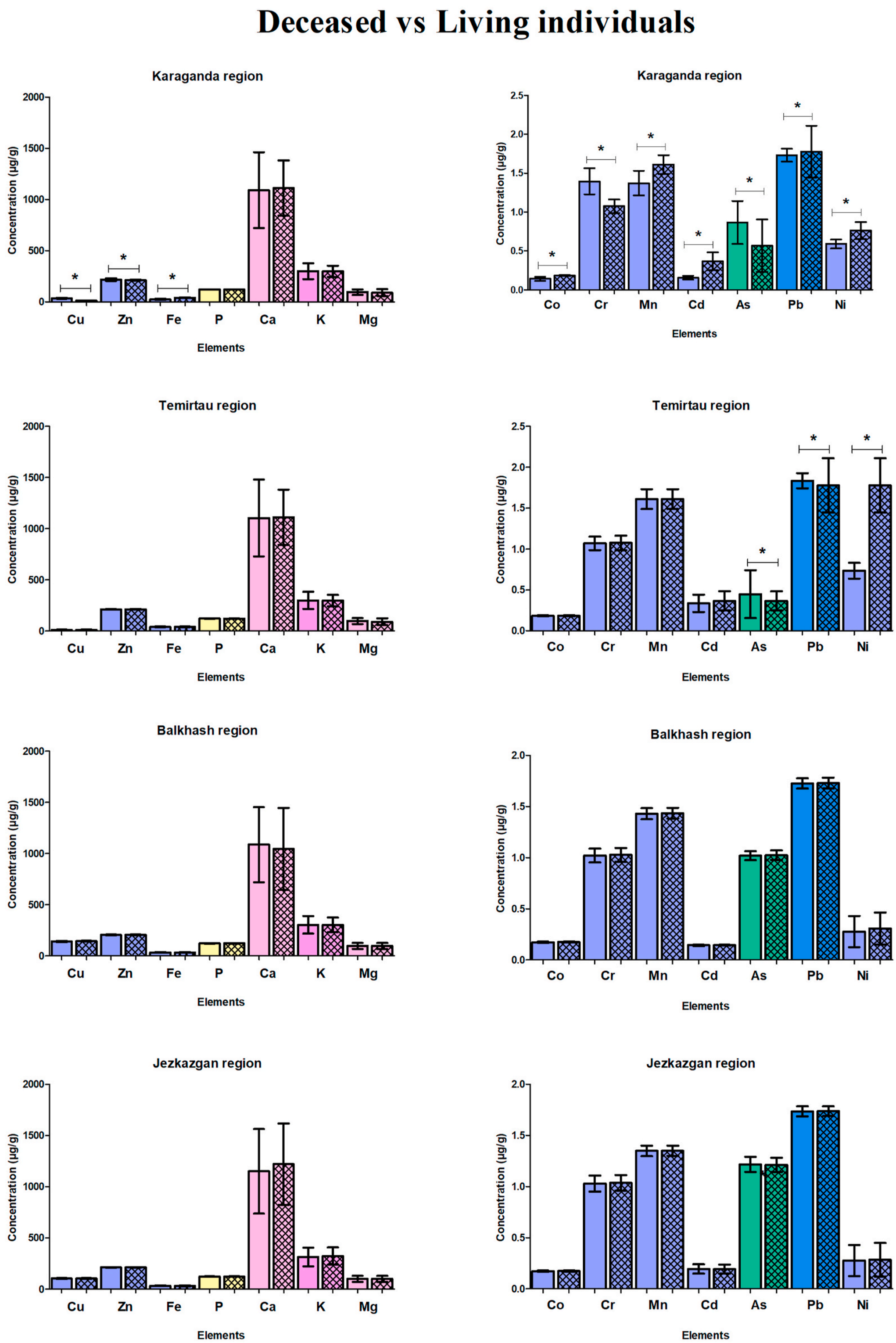

Fig. 3. Vertical column bar graphs (mean with ±SD), representing comparative analysis of the quantitative levels of different elements from the hair samples from living and deceased individuals from each region. The colour coding of the elements was chosen according to the element groups of the periodic table; living individuals (solid bars), deceased (crossed out bars). The significant differences between living and deceased individuals noted when $p < 0.05$ (*). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Karaganda region, which differ significantly from reference values. In this region, the highest level of Zn in all the central Kazakhstan was recorded. It is prominent only in residents of this region, most likely to be caused by the particular influence of the coal mining industry, which is typically accompanied by an increase in pollution of the environment by toxic metals, which are contained in elevated concentrations overburden rocks and coal.[34,35] Pb, Fe, and Ni levels were the highest in the Temirtau region's residents' hair. The concentration of lead in the hair of residents of this city, a large industrial centre, was highest compared to all remaining regions. The excess of lead in the hair is likely caused by the ongoing and multitudinous emissions of lead into the atmosphere by the steel plant located in the area.[14,36] The high level of Fe observed in the studied samples can be attributed to iron oxide vapours in the environment, particularly in territories adjacent to metallurgical plants, contributing to the excess accumulation of iron oxides. Michalak et al. have shown that the inhalation of nickel carbonyl vapours by workers at metallurgical plants or individuals living in the immediate area causes elevated levels of Ni, which is in agreement with the results of the present investigation.[37] The concentration of cadmium in territories in the vicinity of metallurgical plants is 25–50 times higher than in remote areas due to the sedimentation of Cd from the atmosphere.[35] Thus, following these results, the air of large industrial cities has a characteristically high concentration of Cd, which exceeds the threshold limits over 15 times. In the soil, Cd is retained for extended periods after no more external sources are present. The tendency of toxic metals to bioaccumulation and the exposure characteristics result in the long-term stability of these elements' determination in the analyses carried out to identify human remains.

The conducted investigations have shown that the elemental composition of women's hair is different from men's hair, as indicated by some elements. The presence of sexual dimorphism evidences specific features related to the metabolism of these elements that depend on gender.[38,39] Thus, hair samples taken from women in central Kazakhstan show higher concentrations of Ca and Mg than male samples, independent of regional features. In the opinion of Zhou et al. these elements are involved in vital processes of the female organism, such as the formation of the endocrine profile and physiological changes involved in pregnancy and childbirth.[40] A generalization and comparison of the obtained data showed that the concentration of Fe is higher in men's hair, which is also possibly due to the characteristics of the female organism. The levels of toxic metal pollutants are higher in samples of men's hair than in samples of women's hair. These parameters may indicate that men are more frequently in contact with pollutants in a professional setting (in factories) or domestic settings (owing to features of their diet or due to the influence of such exogenous factors as smoking).[17,31] Also, lower physiological levels of antagonistic elements: calcium, magnesium and zinc was noted in men. The identified distinction makes it possible to determine the gender of a hair's donor within the framework of forensic investigations.

A tendency towards increased levels of microelements in hair samples with age was found for all residents of central Kazakhstan, irrespective of sex. This increase in the concentration of chemical elements can be related to ongoing, long-term exposure to environmental factors and deposition processes.[30] It might be noted that the concentrations of most of the investigated elements in hair samples increased substantially across the age groups spanning from 21 to 60 years old. A literature review showed that age-related disbalance is developed to different extents for different trace elements due to insufficient trace elements to the human body with food, and dysfunctions in absorbing, metabolizing, and accumulating compounds.[41,42] Dysfunctions can be traced back to the location of permanent residence and depend on the influence of different environmental factors of region and profession.[43] The established deviations of the hair trace element composition might make it possible to estimate the age of their donor.

The population of central Kazakhstan is subjected to a complex influence of harmful factors, arising from both geochemical features of the region and substantial environmental pollution through emissions and dumping from large industrial complexes. The differences in geochemical peculiarities of territories, social factors, gender and health condition affect the chemical composition of hair, which is the reason for their informativeness as a source of identifying features for addressing many forensic issues.

5. Conclusions

The present study indicates a high level of accumulation of certain trace elements in the hair of residents of central Kazakhstan in specific proportions, making it possible to match a person (or a corpse) to a specific region. The investigation of hair samples of the residents of this region, living in ecologically burdened areas, made it possible to establish background levels for the concentrations of chemical elements, characteristic for each region, to be used for comparison during the forensic identification of individuals. Discovered differences in gender and age make it possible to adjust search boundaries and localize search activities to a specific region. The identified distribution patterns of the chemical elements in hair samples in men and women during their lifetime also make it possible to determine hair's donor within the framework of forensic investigations. Thus, identifying individuals by the elemental content of their hair is a new step in developing modern medicine and forensic science, making it possible to estimate the place of permanent residence of a person, gender, and age.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jflm.2021.102182.

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