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Morphological and elemental analysis of alpaca hair using scanning electron microscopy with energy-dispersive X-ray spectroscopy (SEM-EDX)

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Summary

The aim of the study was to evaluate the microstructure and elemental compositions of alpaca (Vicugna pacos) hair. The hair from eight alpacas that came from different parts of Poland were analyzed by scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM-EDX). A quantitative analysis was performed for nutritionally important elements (calcium and phosphorous); elements which are part of building blocks of hair structure (silicon and sulfur) and trace elements (copper, zinc, selenium). Moreover, elemental mapping was carried out. The SEM images showed that microstructure of hair is irregular and rough. The average diameter of hair is 32.27 ± 4.06 µm. The obtained results showed content of elements in hair and also their distribution in the structure of hair. These findings can be a good benchmark for further studies.

Keywords: alpaca, hair analysis, scanning electron microscopy, energy-dispersive X-ray spectroscopy

Alpacas (*Vicugna pacos*) are domesticated representatives of South American camelids, whose origin and importance in science or industry are the subject of numerous academic publications (2, 8, 18, 27). Currently, the main reason for breeding alpacas in the high Andes is their meat and fibers. The largest breeding centers of alpacas are located in Peru, Bolivia and Chile. Nevertheless, since the 1980s, the breeding of these mammals is spreading to other continents (6). Initially, alpacas were brought to Australia and New Zealand, primarily for fiber industry development. Thereafter, their breeding has started in the United States and in European countries, such as Great Britain, Spain, France, Austria and Germany. Well-developed and prosperous alpaca breeders in Germany have become the object of interest of Polish entrepreneurs. In 2012, the Polish Alpaca Breeders Association was established, the activities of which consisted in disseminating information about alpaca raising, improving breeding quality and supporting Polish breeders (http://pzha.pl/). Alpaca breeding is led by both private breeders and universities. At present, these animals can be found in universities, among others, in Rzeszów and Wrocław. Alpacotheraphy – one of the branches of animal-assisted therapy based on a cooperation of a therapist, patient and animal seems to be an interesting development (22). Such therapeutic actions improve human well-being in the social, emotional and physical spheres. This type of collaboration with alpacas is possible due to their unprecedented characteristics. They are initially over-modest, however, relatively quickly they establish contact with man, thanks to their inquisitive nature, extraordinary intelligence and eye-friendly appearance (19). Additionally, alpacas are well-adapted to harsh environmental conditions, their nourishment consists mainly of hay and pasture grass, which allows breeding in different parts of the world (10). There is an increasing number of alpacotheraphy farms in Poland; nevertheless, the main reason for alpaca breeding is their unique hair. Alpaca fibers, due to their remarkable properties, are the most produced camelid filaments in South America (14).

Wool of this fiber is light, soft and delicate, yet at the same time it has high mechanical strength as well as good isolation properties. It is considered a luxurious hair fiber (4). Alpaca wool is successfully used in the textile industry; moreover, it also has potential in various industrial applications as a natural fiber material (7). It is necessary to analyze alpaca hair in order to select good quality fibers and wool. Scanning electron microscopy with energy-dispersive X-ray spectroscopy (SEM-EDX) is one of the instruments used to evaluate hair microstructure and elemental composition (11, 20). The effectiveness of this method has been demonstrated in earlier publications that examined equine hair (15, 17). It is worth stressing that hair analysis makes it possible to determine the content of even trace elements in the organism, because their concentrations are higher in hair than in other organs and fluids. Additionally, sampling is simple and not invasive (3). Trace elements that can be determined during the experiment include, inter alia, copper (Cu), zinc (Zn) and selenium (Se) (12, 13). These elements are part of functional groups that form several crucial enzymes; they stimulate the synthesis of proteins and nucleic acids as well as eliminate free radicals. There are suppositions that selenium may protect against atherosclerosis, while zinc deficiency may lead to glucose intolerance and diabetes (9, 13). Calcium and phosphorous are major minerals that affect metabolic homeostasis (15). Sulfur and silicon are the elements that form compounds of the hair. Cystine, methionine, cysteine and cysteic acid are basic amino acids of hair that contain sulfur, while silicon forms keratin – the key fibrous structural protein (23).

The main purpose of the study was to observe alpaca hair microstructure, evaluate the elemental hair composition and their distribution in the cuticle, cortex and medulla.

Material and methods

Eight alpacas that originated from different parts of Poland were selected for the experiment. All individuals were fed in the same manner, without specially adapted nutrition. The basic elements of diet were pasture and hay without additional supplements. The hair was collected from each individual, and then the samples were washed in a mixture of distilled water and detergent. Subsequently, the cleaned hair was dried at room temperature and cut with a scalpel. The prepared samples were glued to the tables by carbon ribbon. Before the examination, the samples were sputtered with a thin layer of gold (ScanCoat Six, Edwards).

The microstructure of the selected sample was observed using a scanning electron microscope (EVO LS 15, Zeiss). Diameters of ten hair of each alpaca were measured using the SmartSEM Software Manual. The elemental analysis and mapping were performed by an additional energy-dispersive X-ray system (Quantax Esprit 1.8.2, Bruker). The acceleration voltage during imaging was 10 kV, and the voltage was increased to 20 kV for the elemental analysis. The working distance was the same for each analysis and

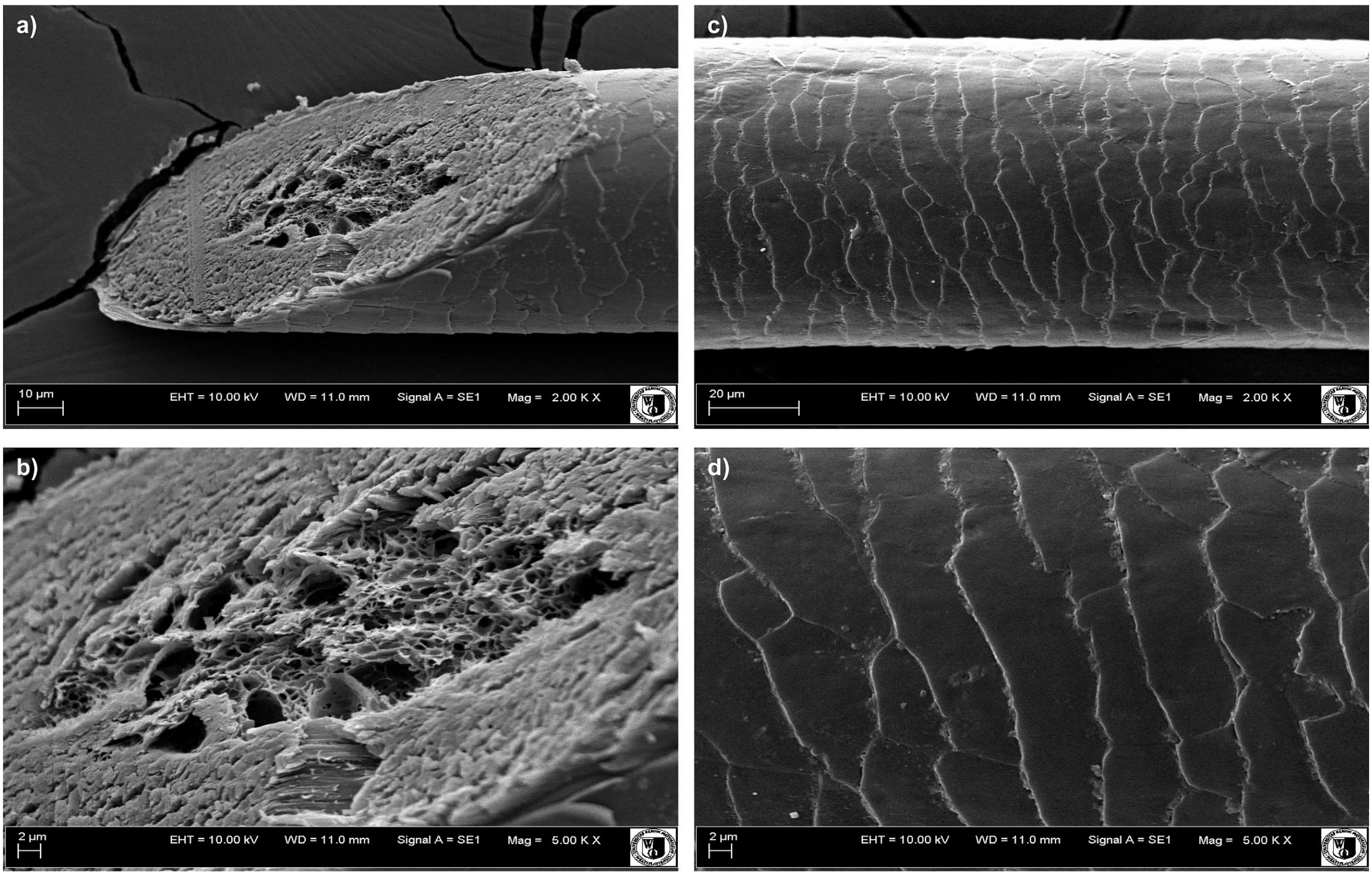

Fig. 1. Microstructure of alpaca hair: (a, b) cross-section (2000 ×, 5000 ×), (c, d) surface (2000 ×, 5000 ×)

was equal to 11 mm. SEM microphotographs were taken using 2000 × and 5000 × magnifications, whereas energy dispersive spectroscopy was performed under 2000 × magnification. Furthermore, the elemental mapping was carried out on the surface and cross-section of the hair. The research was conducted under high vacuum conditions. The elemental analysis was performed for ten hairs of each alpaca and the results were statistically analyzed.

Results and discussion

Morphological and elemental analyses are useful methods for evaluating hair properties. The methods used in this experiment are relatively quick, accessible and non-invasive. They enable characterizing the finished product, such as wool; additionally, they provide the opportunity to analyze, control and enhance

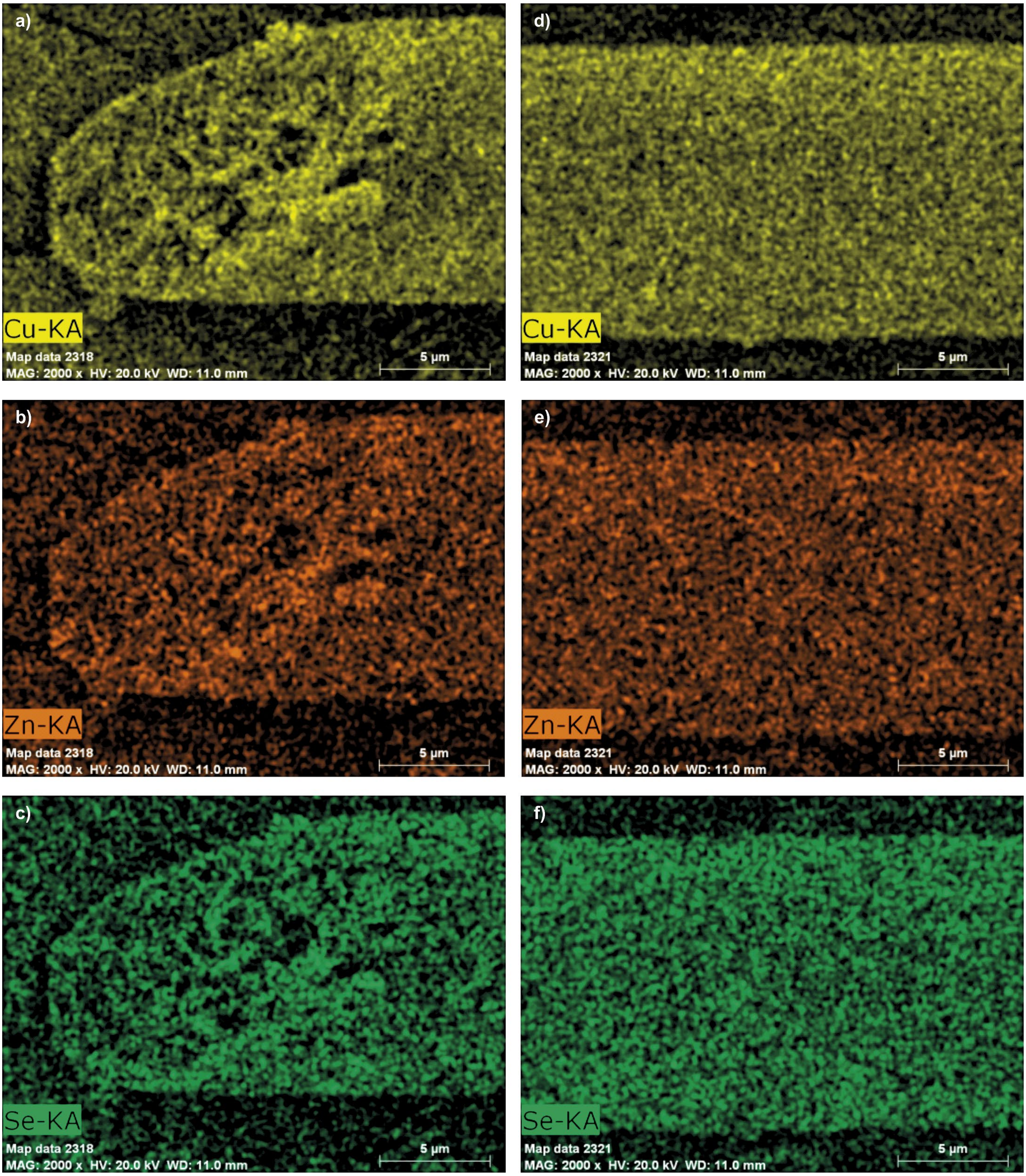

Fig. 2. Mapping of elements on cross-section (a-c) and surface (d-f): Cu – yellow, Zn – orange, Se – dark green

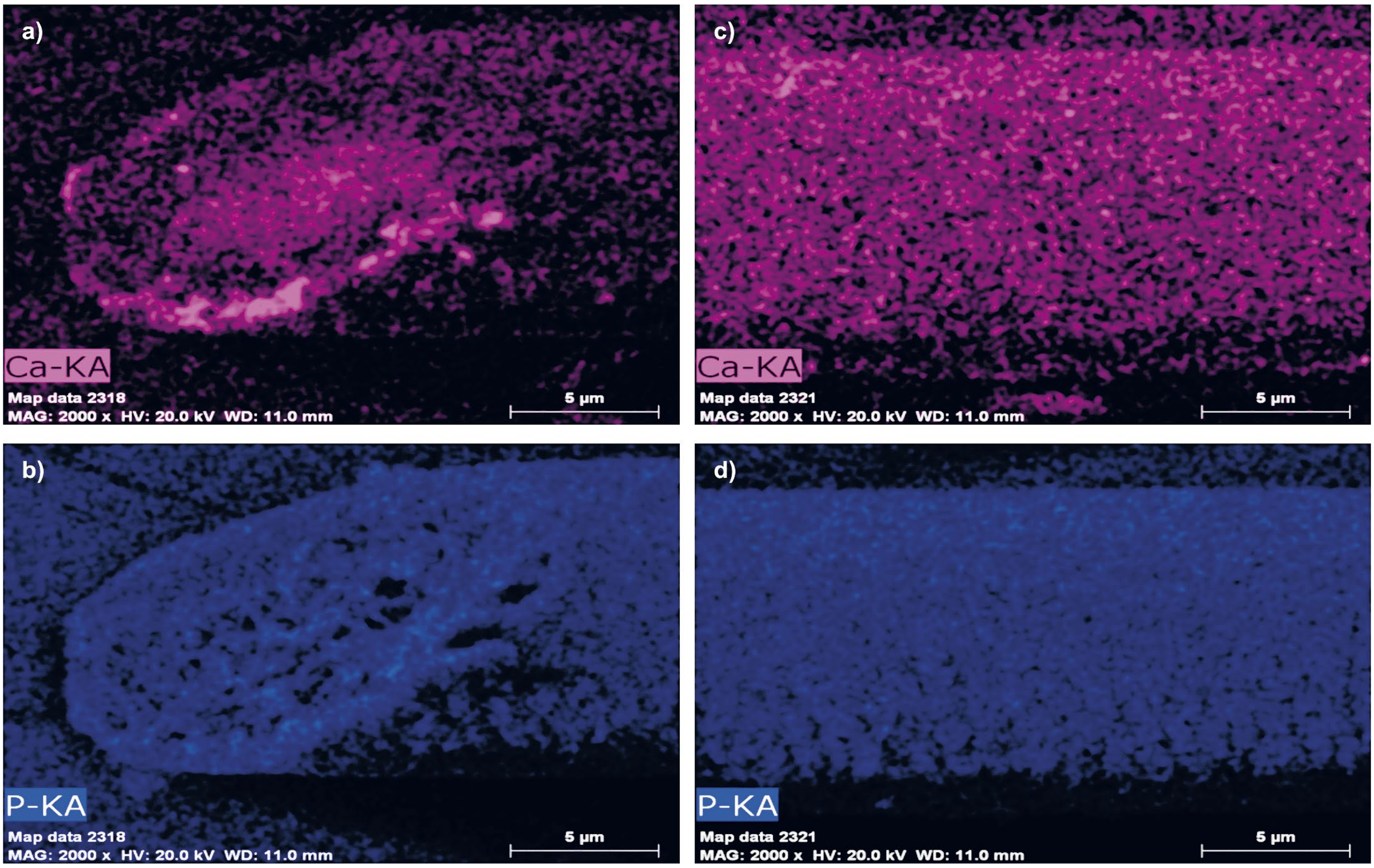

Fig. 3. Mapping of elements on cross-section (a, b) and surface (c, d): Ca – pink, P – dark blue

tailored diets for individual animals. Marycz et al. (16) have shown that SEM-EDX analysis makes it possible to evaluate hair condition and determine the elements present in the hair. Based on these results, one can choose the appropriate diet that improves the health of an animal as well as the quality of the products derived from it. Although alpacas theoretically have no particular nutritional requirements, their diet and supplementation are becoming the subject of an increasing number of scientific studies (24-26).

The microstructure of healthy hair does not depend on the mammalian species. The hair shaft contains three types of layers: cuticle, cortex and medulla. The first is a thin single layer of cells. The cortex, composed of numerous concentric layers of hair cells, is the thickest part of the hair. The third – medulla – consists of several layers of cubic cells. Cuticle cells are arranged around the cortex like roof tiles. Healthy hair scale edges are even and smooth (5). Hair microphotography (Fig. 1c, 1d) shows that the microstructure of alpaca hair is irregular, scale edges are rough; empty spaces appear between cuticle cells. Moreover, they are noticeably chipped and the lifting of individual cuticle cells is visible. However, it is worth noting that no perpendicular cracks are visible. The cross-section makes it possible to observe the internal parts of the hair. SEM images depict cortex layers that overlap the medulla. The porous structure of the medulla is visible. It is possible to observe different pore sizes (Fig. 1a, 1b). Their diameter ranges from a few micrometers to even nanometers (Fig. 5a). In addition, the average fiber diameter was measured (Fig. 5b) and calculated. These measurements are summarized in the form of a histogram (Fig. 6). The results showed that most of the fibers had a diameter of 30-40 µm. The average diameter size, calculated from 80 measurements, was 32.27 ± 4.06 µm. According to the data provided by Alfonso et al. (1), the best quality fibers had a diameter lower or equal to 23 µm. However, it should be noted that the analyzed hair was natural and had not undergone any chemical, mechanical or thermal treatment that would alter the fiber diameter.

The elemental analysis was performed using an energy-dispersive X-ray spectroscope. The elements were divided into 3 groups. Copper, zinc and selenium were classified into trace element groups. Calcium and phosphorus were included in nutritionally important elements, while silicon and sulfur represented the group of elements that were parts of the building blocks of hair structure. A similar division into groups was also applied by Prashanth et al. (21). The obtained

Tab. 1. Atomic concentration (%) of elements in alpaca hair

Elements	Atomic concentration (%)
Cu	0.25 ± 0.03
Zn	1.59 ± 0.28
Se	0.75 ± 0.17
Ca	0.14 ± 0.02
P	2.85 ± 0.34
Si	0.20 ± 0.03
S	3.21 ± 0.18

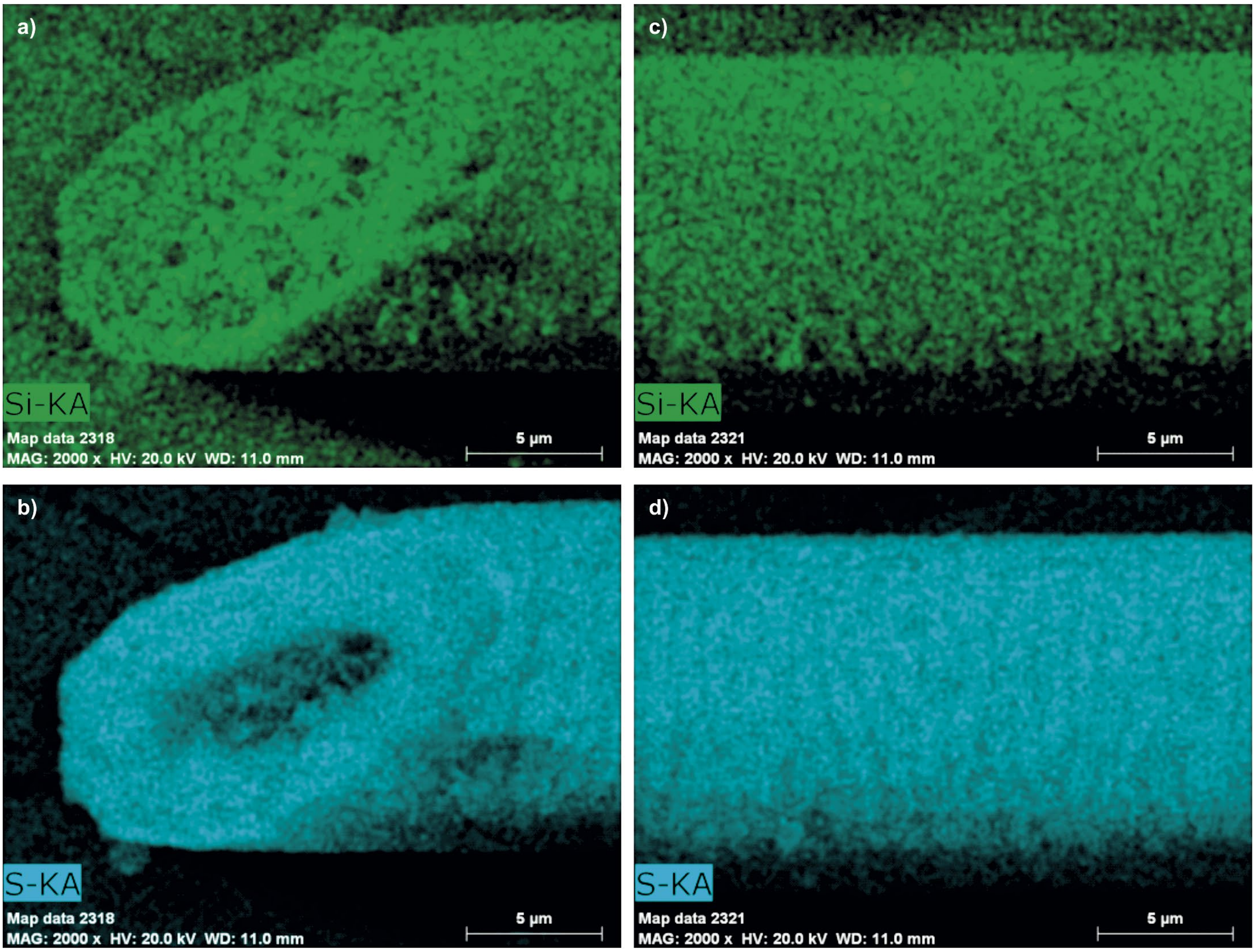

Fig. 4. Mapping of elements on cross-section (a, b) and surface (c, d): Si – green, S – light blue

results are summarized in Tab. 1. After quantitative analysis, the elemental mapping was performed. Yellow (Cu), orange (Zn) and dark green (Se) colors were homogenously distributed across all hair layers (Fig. 2). Quantitative analysis showed that Zinc had the highest atomic concentration: approximately 1.59 ± 0.28 at.%. An ANOVA statistical test was performed for this group of elements. For all analyses, p values < 0.05 were considered statistically significant. Pink (Ca) color was spread throughout the hair and particularly concentrated in the hair medulla (Fig. 3a, 3c). However, it should be noted that Ca clusters were present in the cuticle (Fig. 3a). Dark blue color (P) was noticeable in all hair parts (Fig. 3b, 3d). The content of

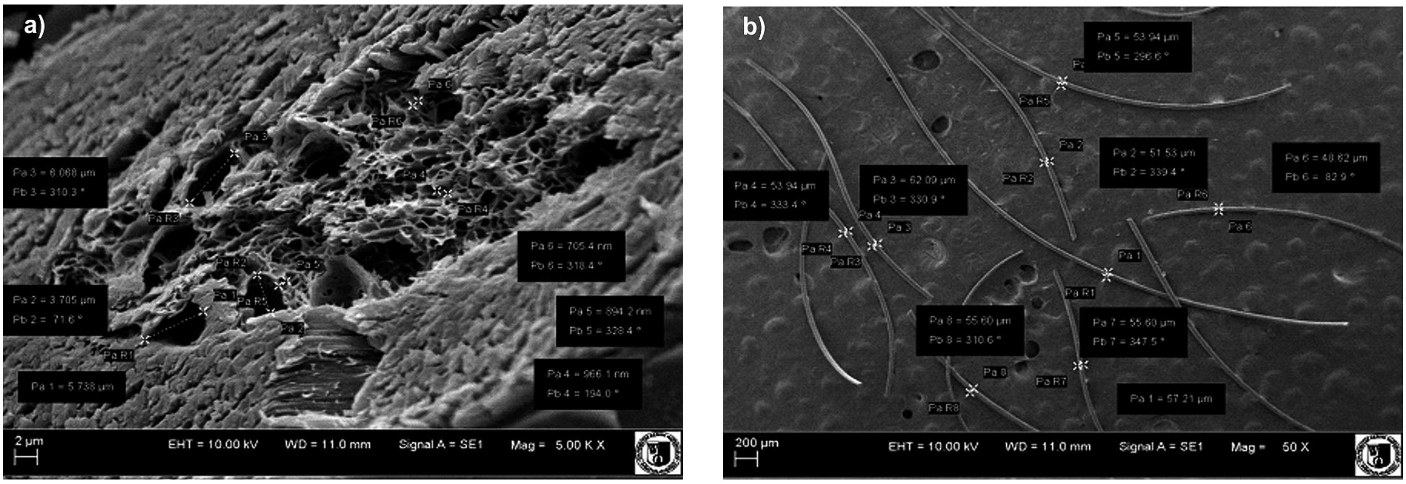

Fig. 5. Morphometric parameters of alpaca hair measured during SEM analysis

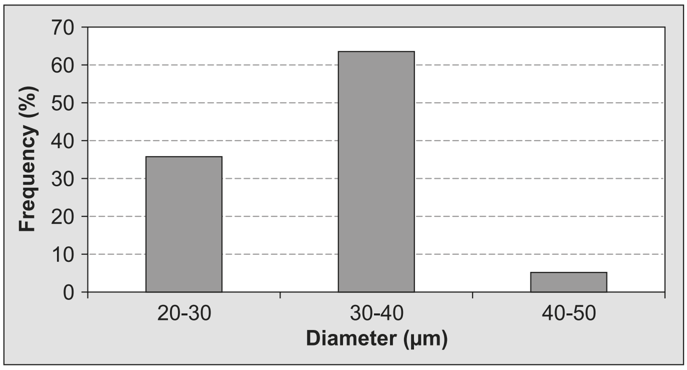

Fig. 6. Histogram of alpaca hair diameter

calcium was definitely less than the average concentration of phosphorous. Differences in the concentration and in element mapping were also recorded for silicon and sulfur. Green (Si) color, similarly to Cu, Zn and Se, was proportionally spread in the medulla, cortex and cuticle (Fig. 4a, 4c). In contrast to silicon, sulfur was present primarily in the cortex and cuticle (Fig. 4b, 4d). The microphotograph with light blue (S) color clearly showed an empty medulla (Fig. 4b). The percentage proportion of these elements was significantly different.

Alpaca breeding is a growing branch of agriculture and veterinary in Poland. Despite many studies and experiments performed in Europe and in the world, there are many issues that are still not fully understood. One of them is the microstructure and elemental composition of alpaca hair. The purpose of our research was to evaluate the concentration of essential elements that affect the microstructure of hair and the correct functioning of organisms. The obtained microphotographs showed that the microstructure of examined hair was not completely normal. This suggests that nutritional modification and additional supplementation will result in positive changes in the health and well-being of alpacas. Quantitative and qualitative analyses included in this publication can be a very good benchmark for further research.

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