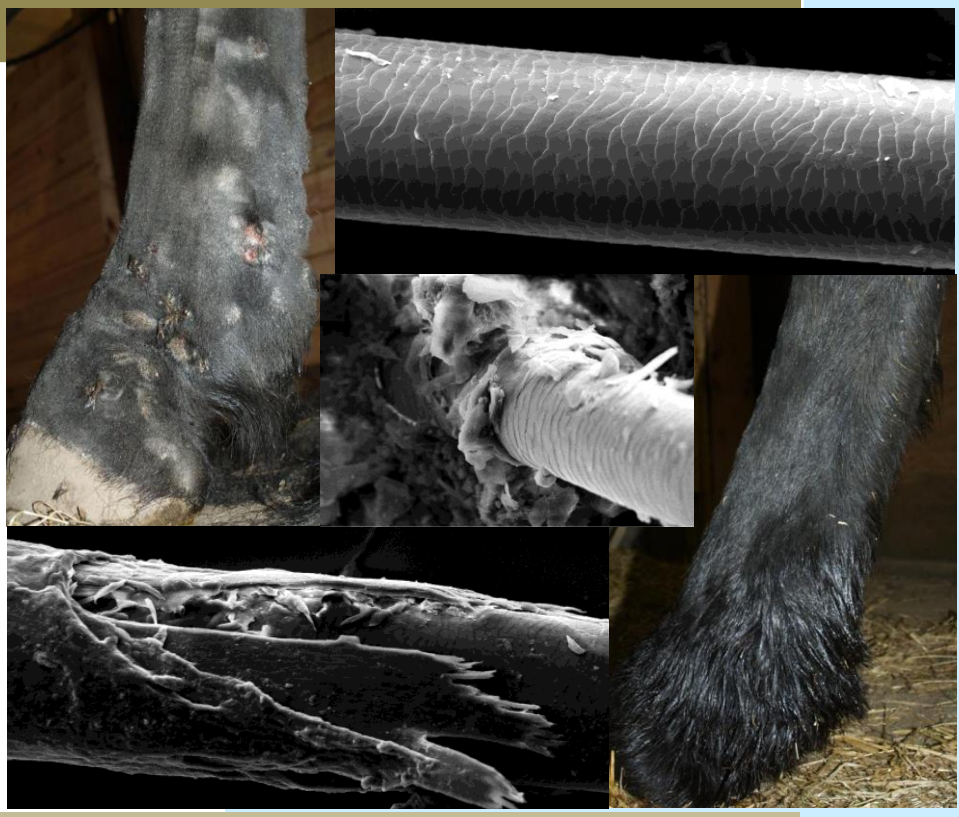
2010

An Evaluation of the Clinical Effect of Oral Zinc Supplementation to Horses with Distal Limb Skin Disease and an Assessment of Different Paraclinical Techniques to Estimate Equine Zinc Status



For a period of two month, seven horses with distal limb skin disease were supplemented orally with 525-735 mg of organic zinc/day/horse. Before and after zinc supplementation, clinical observations were made, samples were obtained from blood, skin biopsies from the diseased area, and hair from the mane and hair from the diseased area. The samples were analyzed for the concentration of zinc in serum and in hair and skin using scanning electron microscopy. Morphology of hair and skin was studied using gold staining and scanning trichoscopy. Results showed that all the horses improved clinically. There was a better morphological structure of hair and skin in the diseased area and in mane hair individually.
There was an increase in zinc concentration in the majority of the horses' mane hair, and blood serum, but in hair and skin from the diseased area there were no unambiguous increase. The most reliable parameters to estimate the equine zinc status, is evaluated to be zinc concentration in mane hair, hair from diseased area and serum zinc. The conclusion is that whether the zinc status is above or below the normal values, the oral supplementation of organic zinc is beneficial to equines with distal limb skin diseases.
There still remains to be established normal values for mane hair and hair from diseased area from a larger number of individuals. International standardized analysis need to be established to unanimously interpretation of the results, when trying to estimate zinc status in the horse.

Keywords: Zinc, serum, hair, skin, equine, atomic absorption spectrophotometer, scanning trichoscopy, scanning electron microscope

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Introduction

Zinc is a trace element of essential biological importance. Zinc serves as structural ions in transcription factors and is stored and transferred in metallothionein, it is found in nearly 200 metalloenzymatic systems or zinc containing proteins. Three basic functions of relevance of this study have been demonstrated: catalytic, structural and as regulator for keratinocytes proliferation and differentiation, Ott et al. (1995, 2001). Marycz et al (2009) found morphologic improvement of hair after feeding with zinc enriched food. Alcohol dehydrogenase possess antioxidant properties and also helps speed up wound healing, and zinc deficiency has been shown to play a role in increased susceptibility to infections and delayed wound healing Wound healing of the skin and the cell metabolism both depend on zinc as a catalyst enzyme in DNA synthesis, and in metallothionein mRNA. Zinc indirectly activates part of a cellular differentiation process: the keratinization that transforms live epithelial cells into corneous cells that are structurally stable and with no metabolic activity, Iwata (1999). Pories et al. (1967) found that zinc sulphate significantly accelerates wound healing. In proteins, Brandt et al. (2009) studied the structural site of horse liver alcohol dehydrogenase and found that Zinc ions often were coordinated to the amino acid side chains of aspartic acid, glutamic acid, cysteine and histidine. Zinc ions are effective antimicrobial agents even at low concentrations, McCarthy et al (1992).

Distal limb skin disease is a common debilitating problem in horses, mostly caused by widespread fungal infection or bacterial infection in the pastern area also known as mud fever or greasy heel. The pain from the inflamed skin can cause lameness and the disease is often associated with heavy workload for the owner. If pastures and paddocks are muddy, it may be hard to provide a dry place, and in the constantly damp environment of the pastern bacteria can thrive and grow. The mechanical protection from the coat and the skin is crucial and weakness of the hair and epithelial barrier can potentially promote bacterial and fungal infections.

It is of importance for the practitioner to know which paraclinical technique gives the best estimate of equine zinc status, as interpretation of concentration levels within low normal references values is challenging.

Studies of zinc deficiency, including hair zinc levels have been reported in man and normal zinc status trough hair analysis seems potentially useful in experimental medicine but its use in clinical medicine will remain limited until validation by the standard methods of clinical investigation is achieved, Klevay et al. (1987). Clinical zinc deficiency has not been unequivocally described so far in the horse Kienzle and Zorn, (2006), but several studies have been conducted in order to quantify the normal mineral status in horses. Zinc concentration in hair (Table 1) and serum (Table 2) are so far the most well studied paraclinical parameters to equine zinc status.

Table 1 Zinc studies on equine hair.

Year	Author	No.	Breed	Finding
1990	Wells	391	Thoroughbred	80-120ppm zinc in hair. Significant correlation between feed mineral intake and hair mineral content.
2002	Wichert	106	Various	126±38 mg zinc/kg dry matter hair
2003	Dunnett	29	Various	Morphologic study on permanent equine hairs. Mane has slowest growth rate near withers and highest near poll. Tail hair show constant growth. No effect of age or gender on growth rate in tail or mane.
2005	Biricik	1	Warmblood	39, 48-220,44mg zinc/kg dry matter hair (winter/summer), no correlation between zinc hair concentration and intake. Sampled from 8 parts of the body in both January and July.
2009	Marycz	12	Thoroughbred	Morphologic improvement of hair after feeding with zinc enriched feed.

Table 2 Studies on zinc level in serum. Right most column is an attempt to compare the values between the different studies with the conversion factor (µmol/l x 65,4 = µg/l).The zinc serum concentration in clinically healthy horses in these studies varied between 360 µg/l and 2000 µg/l .

Year	Author	No.	Breed	Concentration	Converted values
1983	Stubley	300	Thoroughbred	170 ± 54µg/dl, stabled 111 ± 45 µg/dl, pasture	1700± 544µg/l 1111±450µg/l
1985	Gromadzka	8	Shetland pony	1.07 ± 0.04 µg/ml , highest in January	1070±- 400µg/l
1986	Cymbaluk	215	Various	10, 9-19, 8 µmol/L Standardbred and thoroughbred foal 30-80% higher. than adultsValues are higher at birth, stabile as yearling	712-1294µg/l
1988	Auer	83	Thoroughbred	0,47 ± 0.09µg/ml, stabled 0, 47 ± 0,11 µg/ml, pasture	470±90µg/l 470±110µg/l
1990	Bridges	10 10	Mixed breed Foal	0,58-2 µg/ml (supplement high concentration, of zinc in feed induces high serum levels and Osteochondrosis dissecans) 0,36-0,85µg/ml(control)	580-2000µg/l 360-850µg/l
1995	Ott	33	Various	0,43-0,68 mg/l supplemented in feed	430-680µg/l
1995	Okumura	10 Mare 10	Foal	73.2±13.1±µg/dl(1week old) 38.3±5.9µg/dl(17month old) Values higher at birth, stabile as yearling. 56,9±4,8µg/dl (1 week post partum) 51.7±10.7µg/dl (17 month post partum)	732±131µg/l 380±59µg/l 569±48µg/l 517±107µg/l
2001	Stark	104	Islandic horses	9.4 ±1.5 µmol/l horses with Culicoides hypersensitivity 10.0 ± 1.5 µmol/l controls	614±98µg/l 654±98µg/l
2005	Biricik	23	Warmblood	0,46-0,59µg/ml, highest in summer	460-590µg/l
2005	Kolm	104	Icelandic horses	9,0-10,1µmol/L	588-660µg/l
2006	Maia	120	Various	0,42-0,87µg/ml.	420-870µg/l

Many studies in various species have been made regarding the influence zinc has on skin, in acceleration of wound healing Poriers et al (1967), Senepati et al (1990), and a part of a cellular differentiation process: the keratinization that transforms live epithelial cells into corneous cells that are structurally stable and protective Iwata et al (1999). Chester (1999) showed that zinc has influence on both cell replication and differentiation. Others have shown effect of treatment on skin problems such as alopecia, superficiel flaking of dried epidermis, poor healing of abrasion and recurrent infections with edema, parakeratosis, seborrhea sicca and crusting dermathosis Prasad (1969), Harrington (1973), Van Den Broeck (1986), Sousa (1988). When choosing the zinc source to equine feed, absorption must be considered. The absorption of zinc occurs primarily in the small intestine Weigand et al. (1976), Hambidge et al. (1998). Once absorbed in plasma, zinc is bound to and transported by albumin and transferrin, Chester (1981) and Duchateau (1981). Since transferrin also transports iron, excessive iron reduces zinc absorption, and vice-versa. The intake of excessive amounts of iron induces a decrease of plasma zinc levels in ponies. The intake of iron must be above 800 to 2000 mg/kg feed per day per horse before effects are detectable, Lawrence et al. (1987). A similar reaction occurs with copper, as metallothionein absorbs both zinc and copper. In intestinal cells metallothionein is capable of adjusting absorption of zinc by 15–40%. However, inadequate or excessive zinc intake can be harmful Fosmire (1990). Zinc supplementation with 161 mg zinc/kg of dry matter (DM) feed along with excessive amount of calcium 1,25% DM feed showed a statistically significant fall of zinc concentration in blood being 1,03 mg/l before and 0,86 mg/l after, Danek et al.(1999). Crozier et al. (1997) found that hay containing the amount of zinc recommended by American National Research Council (1989) (NRC) still may require supplementation with phosphor, sulfur, cupper, and crude protein to optimize the zinc absorption.

Oral zinc preparations contain zinc in different types of salts and chelates which in turn affects the bioavailability of zinc. Bioavailability can be defined as a measurement of the rate and extent of a nutrient that reaches the systemic circulation and is available at target tissue level, Kienzle and Zorn (2006). Krayenberg (2003) and Wichert et al. (2002) showed that the oral supplementation with zinc as zinc sulphate and zinc sulphate chelates to horses resulted in the highest increase in plasma zinc concentration compared to zinc oxide and zinc lactate. The same tendency has been shown in chicks Edwards et al. (1999) and Wedekind et al. (1992). Although an increase in serum zinc levels after long term oral supplementation of zinc oxide to ponies has been shown Schryver (1980), the tendency has later been shown to be of lower significance Wichers (2002). Lowe et al (1994) showed that the rate of growth of hair and the amount of zinc deposited in the hair was significantly higher in dogs fed diets containing zinc as the amino acid chelate, than in dogs fed zinc as zinc oxide or as a zinc polysaccharide complex.

The 1989 NRC recommend that all classes of horses require 40 mg zinc/kg feed of dry matter per day. Jackson, (1997) suggests the intake to be 400mg Zn/day for horses at light work and 500mg/day for a horse at moderate or heavy work, due to the zinc loss in sweat in working horses that Meyer found in 1986 (20-21 mg zinc/l of sweat). Meyer et al. (2002) found that zinc requirement seems to be higher if the diet contains high levels of phytate, calcium or copper. When the diet is supplemented with as high as 1000 or 2000 mg zinc per kg feed the copper metabolism is affected adversely in foals, Bridges and Muffit (1990). The ratio of zinc to copper should not exceed 4:1 to 5:1 Cymbaluk et al. (1993).

Distal limb skin disease is a common debilitating problem in horses, and to know whether the zinc status is below or above the normal range is essential in planning of treatment.

This pilot study was conducted in order to observe the clinical effect of 2 month of oral organic zinc compound supplementation to seven horses with distal limb skin disease. Furthermore to observe the distribution of zinc in serum, hair and skin before and after oral zinc supplementation to horses with distal limb skin disease and hence to evaluate the paraclinical methods used to estimate equine zinc status.

Materials and methods

Experimental design

All observations were made between October 2009 and February 2010. The cases in this study are comprised of 7 horses between 2 and 16 years of age, weighing 500-700 kg, and performing at different levels. Inclusion criteria were distal limb skin disease of various etiologies that the patient had not received treatment for during last month. In addition their general health should be sound as evaluated by standard blood chemistry panel. Exclusion criteria were deep profound dermatitis with need of antibiotic treatment, fever and pregnancy in the last trimester if the horse needed to be sedated. During the study, the horses were stabled at home in 7 different stables without any changes in pasture habits or other daily routines.

Except for the oral supplement of zinc the horses were not subject to any dietary changes during the study. Every horse was observed twice with a two-month interval. Prior to the study and immediately after the zinc supplementation they were all photographed and clinically evaluated visually for edema, alopeci, erythem, crustae, ulceration, lichenification, scaling and pustule registered and scored from — (no reaction), +(minimal reaction) to ++++(severe reaction), (Table 4). Their initial diets were evaluated according to adequacy in zinc. If needed the horse was sedated and the following samples were taken from each horse: Blood serum,

mane hair, hair from the diseased skin area and biopsies from the diseased area.

All samples were stored at room temperatures until analysis. All horses were then treated for a two-month period with an oral supplement of 525 – 735 mg zinc daily per horse. The source of this supplement was HestaPlus Zink, St. Hippolyt, a pelleted feed supplement also containing dosages of 2,6 mg manganese, 3,8 mg ion and 1,4 mg cupper daily per horse. The zinc source was zinc sulfate monohydrate chelated to amino acid. After the two month treatment period blood serum, mane hair, hair from the diseased skin area and biopsies from the diseased areas were again obtained from each horse.

Zinc concentration was measured in serum, using a Atomic Absorption Spectrophotometer. Hair and skin were analyzed for elemental content using Scanning Electron Microscope - Energy Dispersive Spectroscopy (SEM-EDS) analysis and photos were made of the hair and skin after covering with gold Scancoat 6 (Edwards). The material was observed in Scanning Electron Microscope LEO ZEISS 435 Vp (Oberkohen). Histological preparations were made of the skin.

Sampling

Hair from the sample area was cut off with a pair of scissors without prior surgical preparation. The hair was gently removed 2 mm above the skin surface. Care was taken not to destroy the surface of the epidermis before and during collection of the biopsies. From each horse two biopsies were collected by biopsy punch (0,7 cm ø) or scalpel blade (0,5 x 0,5 cm) prior to and after treatment. One skin biopsy was placed in 10% buffered neutral formalin. The volume of formalin was 10 times the volume of the skin sample. The second skin biopsy was placed in 3, 5 % glutaraldehyde in a phosphate buffer (ph 7, 2-7, 49). Approximately 50 hair from the mane (preferably hair growing in the center of the mane to avoid direct environmental contamination) with hair bulbs were collected and taped to a piece of paper and placed in a plastic bag. In addition to these approximate 50 hairs from the affected area on the limbs were collected. In case of alopecia in affected area the hair were collected as close as possible with hair bulbs. All skin and hair samples were sent to the laboratory at Wroclaw University of Environmental and life sciences (Uniwersytet Przyrodniczy we Wroclawiu) in Poland. Approximately 10 ml of peripheral blood were obtained from each horse by venepuncture into a plastic EDTA tube and a coagulation tube.

The EDTA blood was analyzed by Nørlund Blood Laboratory for Horses, Denmark. The blood from the coagulations tubes were left at room temperature for 2 hours and the serum subsequently separated, frozen and sent to the laboratory at Wroclaw University of Environmental and life sciences (Uniwersytet Przyrodniczy we Wroclawiu) in Poland. These serum samples were later analyzed for trace minerals.

Analysis

SEM-EDS analyses: The hairs were cleaned from solid matter, washed and degreased in

demineralised water with detergent, then rinsed three times and dried. The hair samples were divided in two. One sample were analyzed regarding content of elements: Carbon, oxygen, Sulfur, Selenium, Manganese, Magnesium, Zinc, Cobalt, Siliceum and Calcium, using the microroentgenographic detector Roentec, an X-ray microanalyzer combined with SEM – EDS. The second hair samples were covered with gold Scancoat 6 and used for ultra structural analysis. The material was observed in Scanning Electron Microscope LEO ZEISS 435 Vp and photos were made for later evaluation (appendix).

Histological examination: The skin specimens fixed in 10 % buffered formalin were embedded in paraffin and 5 µm sections cut by Zeiss Microm HM 340E. The sections were stained with haematoxylin and eosin (Shandon) and analyzed by means of light microscope (Axio Imager A1). The second part of the skin samples were used for ultrastructural analysis and covered with gold in Scancoat 6.

The blood serum: The concentration of zinc in serum was determined by flame atomic absorption spectrophotometer using a polarized Zeemann atomic absorption spectrophotometer (Z1800; Hitachi).

Results

The clinical appearance

In all the horses there were improvements in clinical hair coat appearance and skin condition after 2 months of oral zinc supplementation. All owners noticed an improvement compared to previous treatments efforts such as wash or topical treatment. Before treatment there were different degrees of dermatitis (Table 4). The general condition of the coat was dull, lack of luster in varying degrees and most of the horses were irritated, painful and rubbing the coat or kicking the ground. After treatment all the horses improved with healthier shining coats, the dermatitis healed and the horses were no longer irritated or rubbing the legs. The morphology of mane hair, hair from diseased area and skin evaluated by SEM-EDS all showed improvements. The evaluation of the histological sektions, showed a decrease in inflammatory cells and recovery of epithelial abnormalities.

The overall improvement in clinical appearance and paraclinical values are compared (table 5). The clinical improvement is documented by photo taken before and after the zinc supplementation (Table 6).

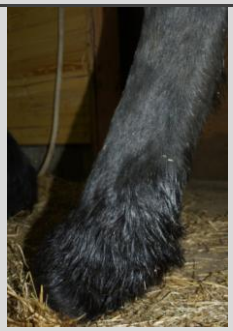
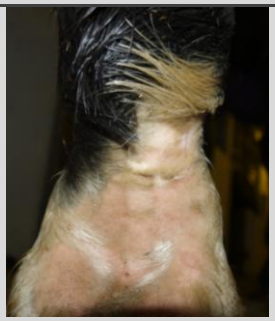
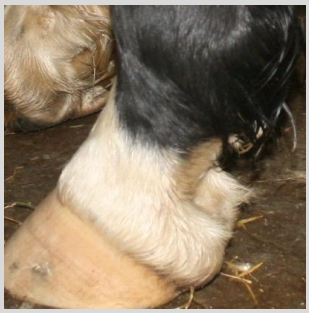
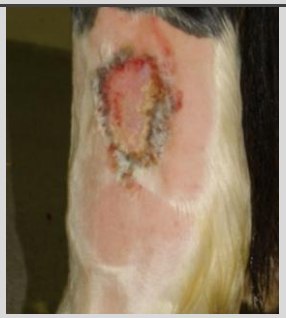
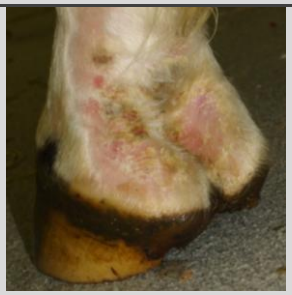
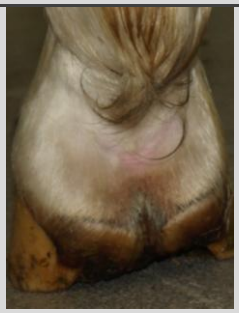
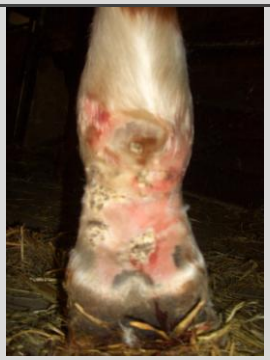
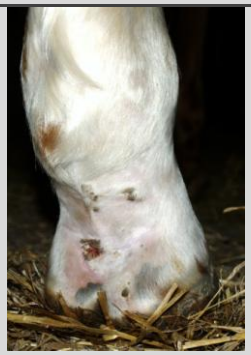
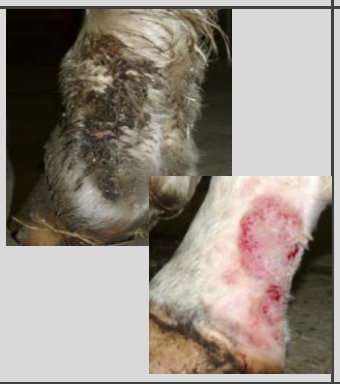
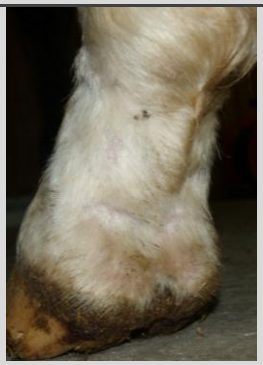
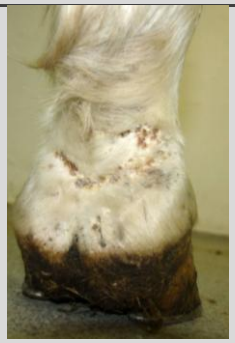
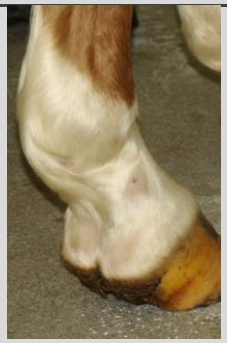
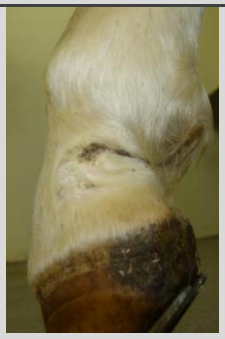
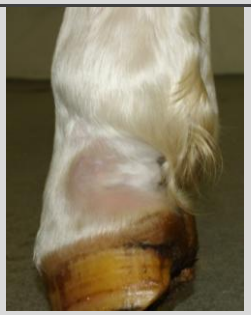
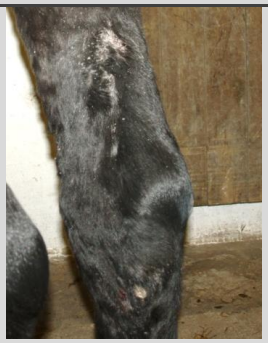
Table 4 Clinical observations scored from — (no reaction), + (minimal reaction) to ++++ (severe reaction).

Horse no: Before/after	Total Zinc in mg/day/horse /recommendati	Pain/Rubbing/ kicking	Edema	Alopeci	Crustae	Ulceration	Erythem	Lichenification	Scales	Pustule
1 before	878/540	++++	++++	+++	+++	++	++	++	+++	++++
1 after		—	—	—	—	—	—	—	+	—
2 before	794/450	++	+	—	+	—	—	++	++	—
2 after		—	—	—	—	—	—	—	—	—
3 before	1308/480	++	++	+++	++++	++++	+++	—	—	—
3 after		—	—	—	++	—	+	—	—	—
4 before	713/540	++	++++	++++	++++	+++	++++	++	++	—
4 after		+	+	+	++	+	+	—	—	—
5 before	638/360	+++	++	+	++++	++++	++++	—	+++	—
5 after		—	—	—	—	—	—	—	+	—
6 before	1490/450	++	+	++	++	+	+	—	+	—
6 after		—	—	—	—	—	—	—	—	—
7 before	897/540	+++	++	++++	++	++	+	++	++++	+
7 after		+	—	+	—	—	—	—	+	—

Table 5 Clinical and paraclinical improvements scored from ▼ (exacerbation), (▼) (minimal exacerbation), — (no improvement), (✔) (minor improvement) and ✔improvement.

Horse no/Improvement	1	2	3	4	5	6	7
Clinically	✔	✔	✔	✔	✔	✔	✔
Morphology of mane hair (SEM-EDS)	✔	✔	✔	✔	✔	✔	✔
Morphology of hair - diseased area(SEM-EDS)	✔	✔	✔	✔	✔	✔	✔
Morphology of skin (SEM-EDS)	(✔)	✔	✔	(✔)	(✔)	✔	✔
Histopatological examination	✔	✔	✔	✔	✔	(✔)	(✔)
Serum zinc	(✔)	(✔)	▼	▼	✔	✔	✔
Mane Hair zinc	✔	✔	▼	✔	✔	✔	(✔)
Extremity hair zinc	✔	✔	(▼)	(✔)	(▼)	▼	✔
Skin zinc	✔	(▼)	—	(▼)	▼	✔	✔

Table 6 Clinical observations in photo

	Before	After		Before	After
1			2		
3			3		
4			5		
6			6		
7			7		

SEM-EDS analysis of hair

The ultrastructure of hair shaft, cuticle cells, hair medulla and medulla/hair shaft diameter ratio were observed and revealed improvement in all of the horses (Table 5 and figure 1-2). Before treatment there were defects concerning the hair shaft, hair cuticle and the structure of the medulla. There were flattening, twisting and splitting of the hair shaft and ragged endings of the hair (figure 1). After treatment the central medulla of the mane hair, that before treatment contained loosely packed rectangular cells and empty vacuoles now had closer packed cells and filled vacuoles (figure 3). Before treatment the outer keratin cuticle consisted of irregular overlapping cells with ruptures and loose scales. After treatment the outer cuticle shoved regularity and smoothness.

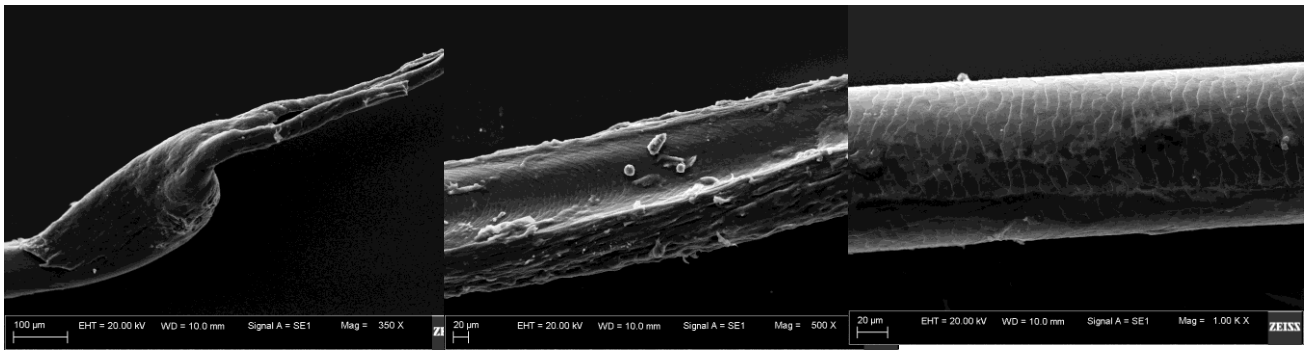


Figure 1 Before treatment SEM-EDS analysis of the mane revealed changes of hair shafts shape namely flattening, twisting and splitting. Some of hair shaft had longitudinal surface ruptures.

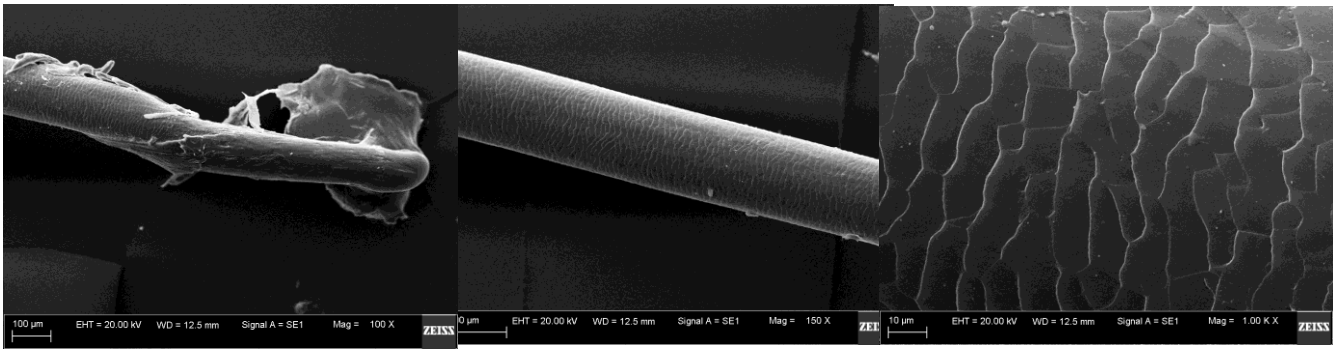


Figure 2 After treatment SEM-EDS analysis of the mane hairs revealed no major changes of hair shafts shape but a reasonable amount of hair bulbs were in telogen phase.

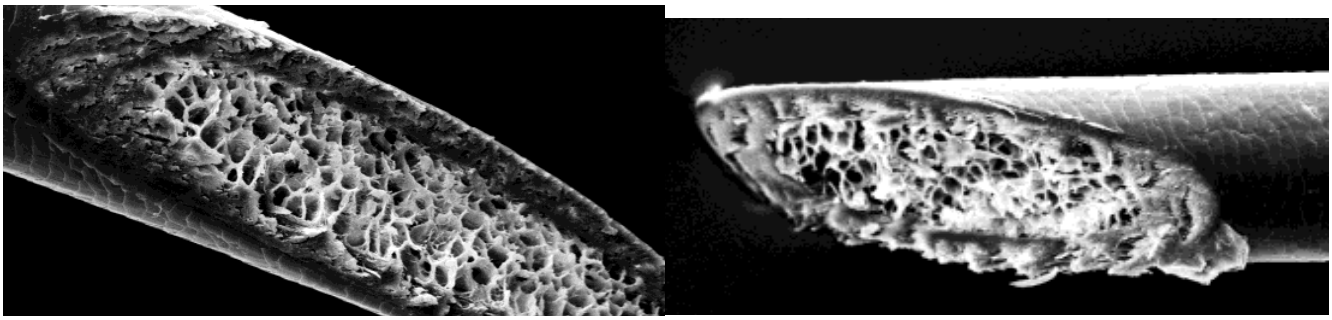


Figure 3: Before treatment - loosely packed rectangular cells and empty vacuoles. After treatment- have closer packed cells and filled vacuoles.

SEM-EDS analysis of skin

In all the skin samples taken before treatment (figure 4) there were yeast organisms mainly on the surface of the hair shafts and in close proximity of the hair follicles. Also a lot of desquamated keratin scales lining the surface of the skin and the hair shaft. A few blood cells were observed. After treatment (figure 5) there was marked reduction in yeast organisms close to the hair bulb and fewer keratin scales on the surface.

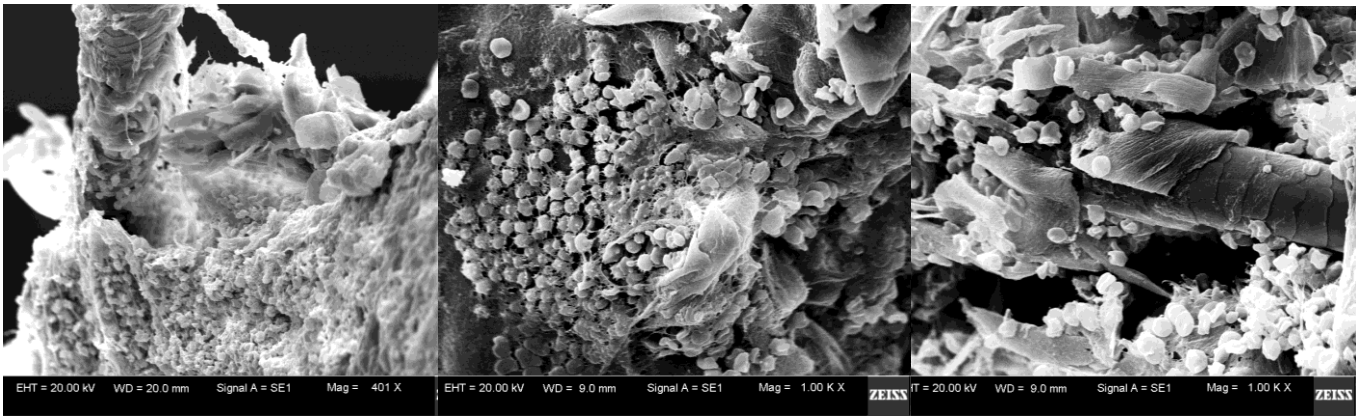


Figure 4 Before treatment SEM examination of the lesional skin surface revealed a lot of desquamated keratin scales lining the surface of the skin and the hair shaft. Among keratin masses there were significant quantities of yeast organisms observed, mainly in the close proximity to hair shafts areas. A few blood cells were observed.

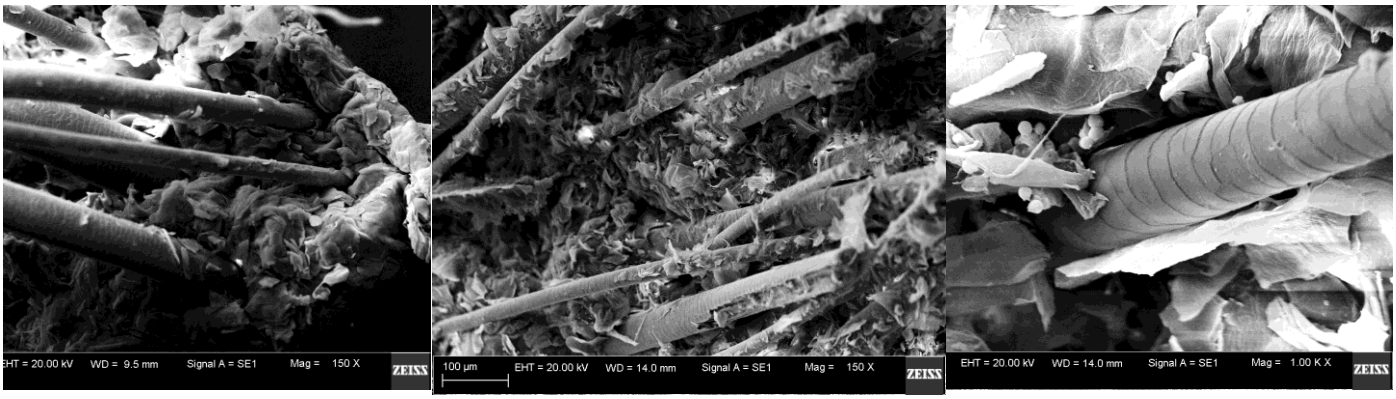


Figure 5 After treatment SEM pictures of the surface of the skin coming from affected areas revealed mainly relatively big keratin scales covering the surface of the epidermis and some parts of the hair shafts. There were moderate to small quantities of yeasts organisms located mainly some distance from the hair shafts within desquamated epithelium.

Histopathological examination

Before treatment (figure 6) most of the skin samples coming from affected areas reviled marked hyperkeratosis and parakeratosis. There was also some dermal edema noticed. In the dermis area cell infiltrate consisted mainly of activated fibroblasts, plasma cells and some mast cells. They were predominately periadnexal in their location. Some focal fibrosis and ortokeratotic areas were observed in subepidermal compartment. Sebaceous glands observed reviled subtle features of hyperplasia. After treatment (figure 7) there were mild features of hyperkeratosis of the epidermis. Within dermis compartment inflammatory infiltrates were minimal and consisted mainly of fibroblasts and some mononuclear cells. Individual sites were observed with hyperpigmentation. The periadnexal compartment showed no significant abnormalities.

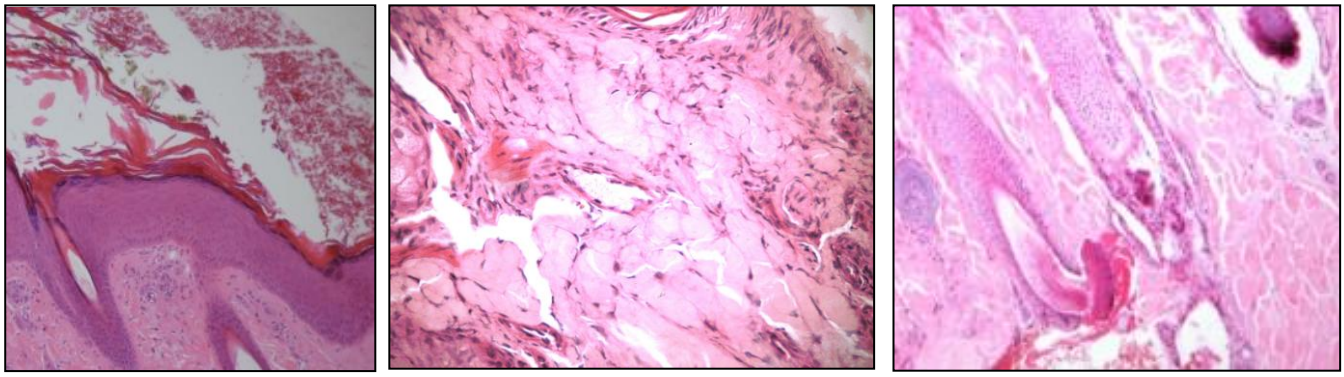


Figure 6 Examples from histological preparations before treatment (for detailed description see the appendix).

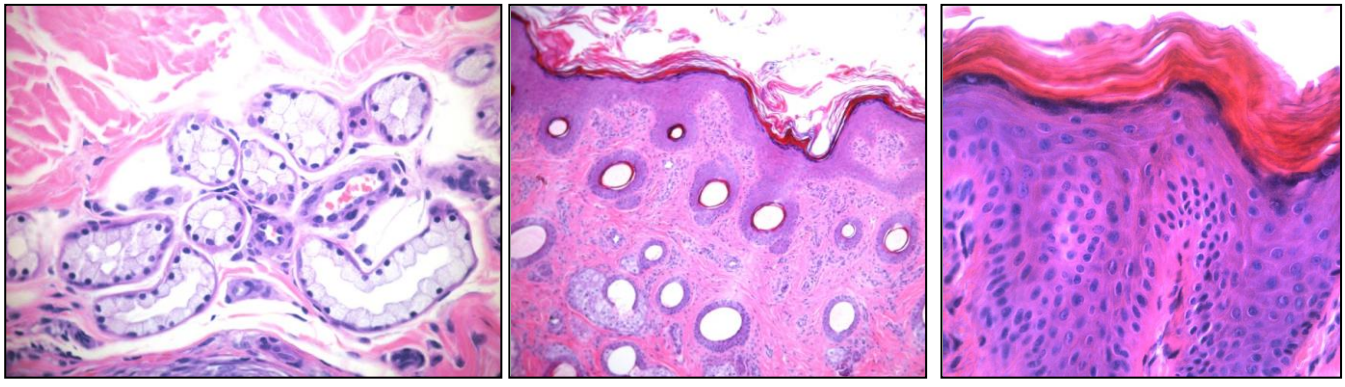


Figure 7 Examples from histological preparations after treatment (for detailed description see the appendix).

Zinc concentration in serum

No overall effect was detected in serum zinc concentration before and after dietary treatment with zinc (figure 8). All the horses were in the lower range of the reference values both before and after the treatment. Horse number 3 and 4 had a decrease in the concentration.

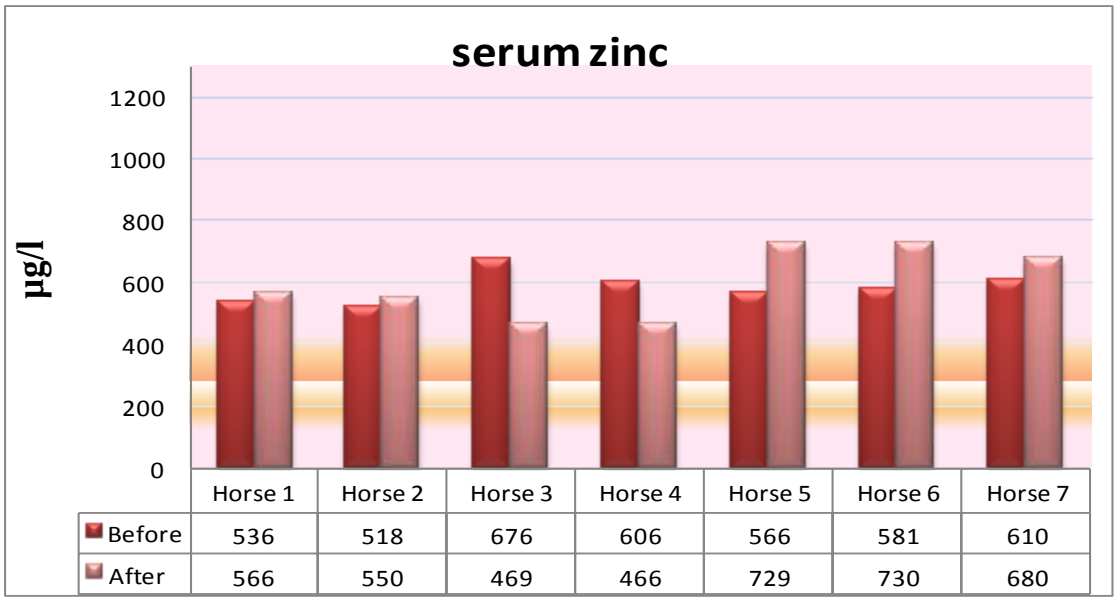


Figure 8
Zinc levels in serum µg/l before and after the 2 month period of zinc supplementation. References for zinc concentration are 500-1300 µg/L.

Elemental mane hair analysis

There was an increase in mane hair zinc concentration (figure 9) in 6 of the 7 samples. The effect of dietary zinc treatment on mane hair zinc concentration varies from 3, 5 % (horse 7) to 155 %(horse 1) increase.

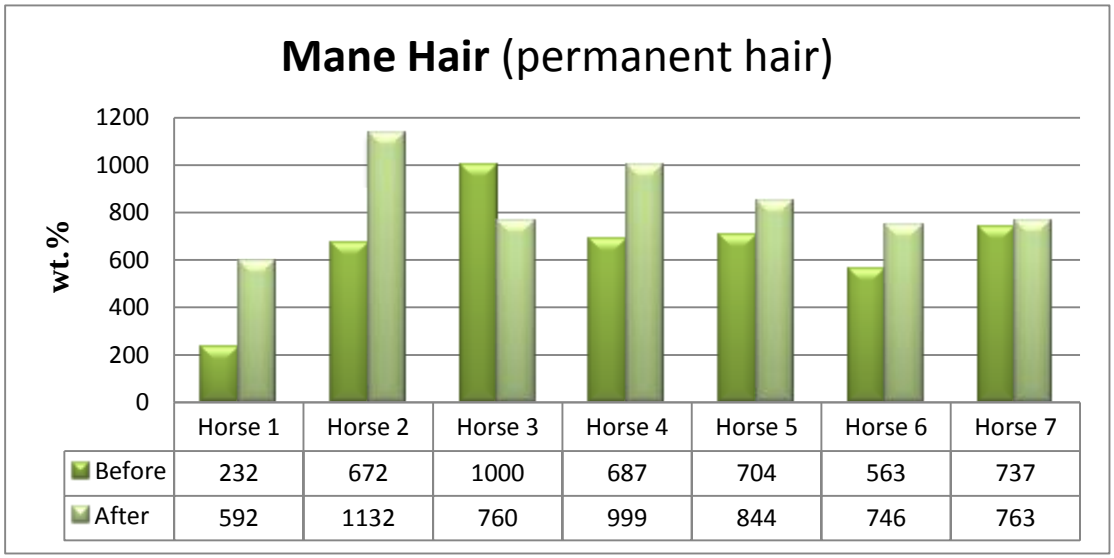
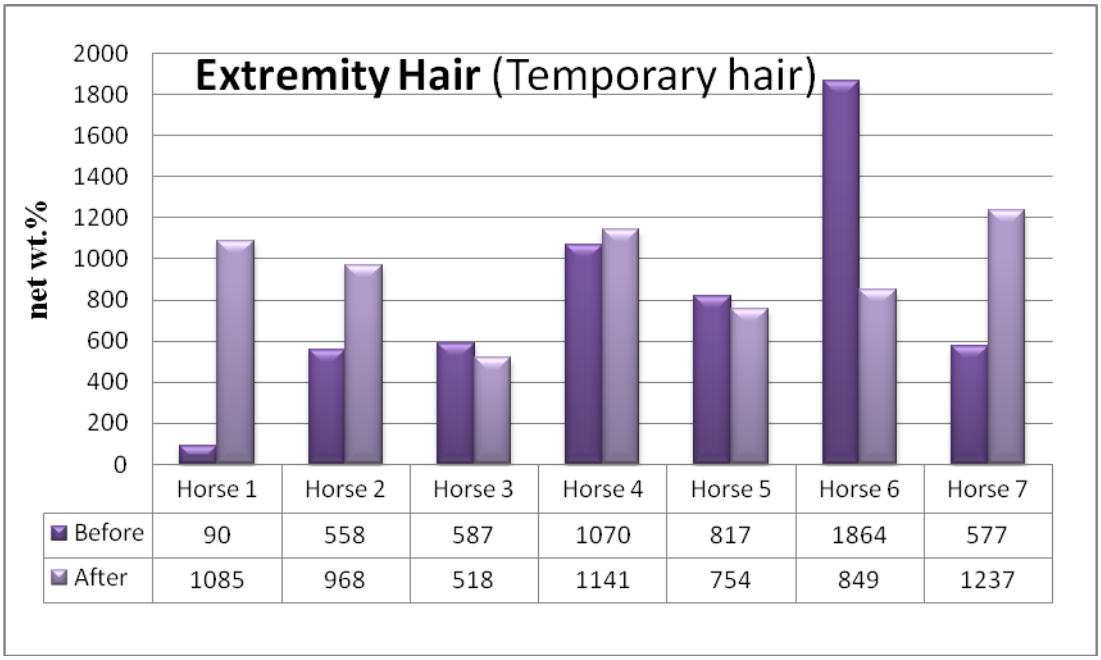


Figure 9 Contents of Zinc in main hair x-ray SEM% before and after the 2 month period of zinc supplementation. Mass fraction [wt. %] is the fraction of one substance to the total mixture mass.

Elemental hair analysis-Extremity hair

Hair zinc concentration before and after dietary treatment with zinc showed both increase and decrease.

There were an increase for horse 1, 2, 4 and 7. Horse number 6 had an extreme decreased in zinc concentration.



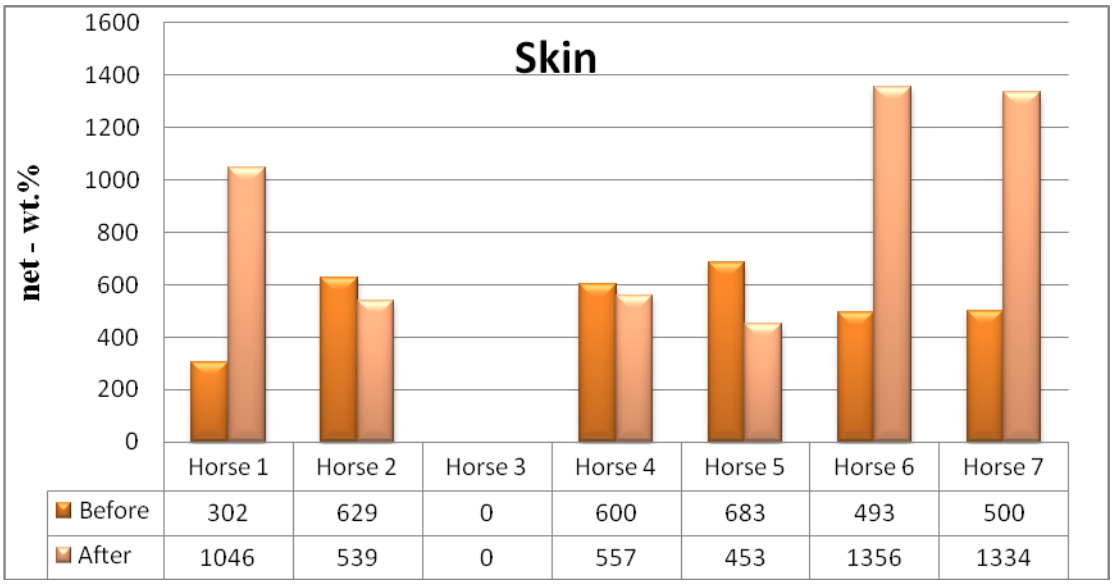
	Horse 1	Horse 2	Horse 3	Horse 4	Horse 5	Horse 6	Horse 7
Before	90	558	587	1070	817	1864	577
After	1085	968	518	1141	754	849	1237

Fig. 4: Contents of Zinc in body hair x-ray SEM% before and after the 2 month period of zinc supplementation. Mass fraction `[wt. %]` is the fraction of one substance to the total mixture mass.

Elemental skin analysis

Regarding to skin zinc analysis it shoved no pattern whether the concentration of zinc in skin can increase by supplementation.

Three of the horses had an increase between 166-246%, the other three a minor decrease



	Horse 1	Horse 2	Horse 3	Horse 4	Horse 5	Horse 6	Horse 7
Before	302	629	0	600	683	493	500
After	1046	539	0	557	453	1356	1334

Fig. 5: Contents of Zn in body hair x-ray SEM% before and after the 2 month period of zinc supplementation. Mass fraction [wt. %] is the fraction of one substance to the total mixture mass. Horse 3 were excluded from the figure due to an extremely high value 4096[wt. %] before treatment and an absence of measurable value after treatment.

Discussion

Evaluation of the **clinical effect** is initially straightforward. There is sufficient improvement in all the horses, to validate the results. However a control groups would have provided stronger evidence. It would exclude the possibility that the improvement could be due to weather, seasons, breed, age or gender. Assessment of the clinical results would quite evidently be stronger if the data material was constituted by a larger number. It would be beneficial to design more specific inclusion criteria about etiologies of the disease and more standardized feeding before and during the study. Regarding **SEM-EDS analysis of hair** it's easy to collect mane hair samples and it is without major inconvenience to the horse. When it comes to hair from the affected area, many of the horses were fairly annoyed at the collection and precautions to avoid being kicked had to be taken. Since there also had to be taken biopsies, there was made perineural nerve block. The photo of magnifications of hair shows clearly the improvements. Regarding **SEM-EDS analysis of skin** and especially looking at the **histopatological examination** it requires more in depth knowledge and experience to interpret. A good laboratory with experience in the field is recommended. The histopatological findings in this study match closely with the results of Sanecki et al. (1985) in puppies with skin problems related to zinc deficiency. Collection of the skin biopsy is more laborious and yields are not entirely worth the effort. The results of this study showed however as previous studies, Poriers et al. (1967), Prasad (1969), Harrington (1973), Van Den Broeck (1986), Sousa et al. (1988), Senepati (1990), Iwata (1999), Chester (1999) and Rostan et al. (2002) that zinc therapy promotes healing and improves coat health. Looking at table 5 the positive effect is easy to spot. Assessment of the different paraclinical techniques to estimate the nutritional zinc status in the horses of this study has been limited by the lack of patients. It makes no sense to perform statistical calculations; they would at best be able to point to trends more than any significant evidence. However the current results are of interest for further study. Regarding z**inc serum concentration** it's easy to collect blood samples and it is without major inconvenience to the horse. The analysis of serum zinc concentrations of serum can be performed in a local laboratory, and is not very time consuming. This paraclinical parameter is the most investigated and normal values are recognized to be applicable, NRC (1989) and Scientific Committee for Animal Nutrition (2003). The results must however be evaluated with caution since many variables come into play when estimating zinc concentration. The fact that all the horses were in the lower range of the reference values both before and after the treatment could be due to the fact that they were chronic infected or visa versa, the low zinc concentration leading to chronic infection. Others had showed correlation

between infection and serum zinc concentration. Dede et al. (2008) shoved a decrease in plasma zinc in horses with piroplasmose and a correlation between a decrease in plasma zinc levels and increased activity of carbonic anhydrates. Carbonic anhydrates is the zinc containing enzyme that regulates the homeostasis of erythrocytes Mafra et al. (2004). Located in the lower end of the reference values or below, the diseased horse can certainly benefit from organic zinc supplements. Attempts to achieve a more accurate reflection of the tissue zinc and hence a precise estimate of horse zinc status using blood samples is done by Milne et al. (1985). They described a method for cell separation and analysis of separated platelets, mononucleated cell, polymorphnucleated cell and erythrocytes and measured individual zinc levels in each cell. Magneson et al. (1986) described the enzyme phosphoglucomutase as an indicator to measure free zinc in equine plasma. However both these to methods are not accessible at laboratories.

Regarding elemental hair analysis of mane hair and extremity hair it's easy to collect hair samples from the mane and as mentioned before more troublesome from affected area. The methods has been used for many years and within the last years of increasing use there has been made efforts on spectroscopic methods to facilitate multi-element analysis. Current analytical techniques provide reliable and rapid methods. The validity of the analytical method for measuring zinc concentration is supported by previous studies, although Seidel et al. (2001) and Hintz (2001) reported a huge variation in results from different laboratories on various minerals. However the reports of zinc contents in hair analysis were reasonable consistent.

The most obvious improvement was seen in the mane hair, probably due to fact that the hair of the mane and tail is permanent and grows continually throughout the year. The hair of the limbs is temporary hair that is metabolically relatively inert and once formed, does not undergo further biogenic turnover. Hence no increase in zinc concentration is possible, except in the hair newly formed in areas of alopecia. The hair on the limbs is made up of mainly cuticle and cortex cells, where the mane hairs contain a greater number of medulla cells. Intermediate cortex containing the longitudinally, spindle shaped keratinocytes that cross-link and provide the hair mechanical strength and melanin which provide pigmentation and resistance against enzyme and bacterial attack Combs et al. (1982) and Dunnett et al. (2003). In conjunction with the knowledge that the cortex contains the most melanin which is very zinc dependant (eumelanin providing black/brown pigmentation and pheomelanin red/yellow pigmentation) and that melanin is even more resistant than keratin to enzyme and microbial attack Dunnet et al. (2003) further study would be of interests to compare the zinc concentration of the white hair versus the colored hair of the limbs. Another factor to be considered in future studies is the gender and age influence on hair zinc concentration of the horse. In 2003 Dunnett made a morphologic study on permanent equine hairs. He found mane hair to have the slowest growth rate near the withers and highest near the poll. The hair of the tail however growth constantly. He found no

effect on age or gender on growth rate in tail or mane, but he didn't measure the zinc concentrations levels. Cape et al. (1982) found correlations between various factors including age and mineral concentration of equine hair. Two of the mares in this study were pregnant, but were not exclude as Kavazis et al. (2002) neither found influence on foal growth or development, nor on the cupper, zinc and iron concentrations of the mare milk, mare serum, nor foal serum. Zakrgynska et al. (1997) however found higher zinc levels in women's hair and Schumacher et al (1993) detected higher zinc levels in the hair of young girls. The same tendencies were found by Cymbaluk in 1986 and Okumura in 1995 were young foal had higher levels than their dames.

A longer period of oral zinc supplementation could possibly lead to more significant results in terms of especially hair zinc concentration, having the turnover of hair growth in mind. Regarding **elemental skin analysis** harvesting of the skin biopsy is more troublesome and time consuming than collecting the hair samples. A weakness of this method is that the measuring unit is not directly comparable to units in other studies, since Mass fraction [wt. %] can not be converted to comparable values. [wt. %] is the fraction of one substance to the total mixture mass, and is mostly comparable with previous measurements of same horse. Horse 3 were excluded from the figure due to an extremely high value (4096[wt. %]) before treatment and an absence of measurable value after treatment. The results shoved no pattern whether concentration of zinc in skin can increase by supplementation. Three of the horses had an increase between 166-246%, the other three a minor decrease. It might seem that a larger data material would strengthen the presumption that zinc concentration would increase after supplementation and skin biopsy to a large extent be used as a parameter to estimate equine zinc status. Zinc deficiency is usually perceived as being caused by insufficient dietary intake, but literature show the importance of zinc source and its bioavailability Wedekind et al. (1992), Lowe et al. (1994), Edwards et al. (1999), Wichert et al. (2002), Wichers (2002), Krayenberg (2003) and Kienzle al.(2006). This is supported by the clinical improvement of all the horses in this study, although ingesting enough zinc in the diet before the experiment, they all had low serum zinc levels and showed signs of having benefited from the supplementation. It can be interpreted that bioavailability in this supplementation are better than in the ingesting roughage and grain. A more controlled study is needed where the horses are held under the same conditions, with a similar diet and similar training, standardized groups of age, breed, gender and control groups will help to better understand the complex metabolism of zinc.

Conclusion

This pilot study was conducted in order to observe the clinical effect of 2 month of oral organic zinc compound supplementation to seven horses with distal limb skin disease. Results showed that all the horses improved clinically. There was a better morphological structure of hair and skin in the diseased area and in mane hair individually.

There still remain to be established normal values of elemental concentration in hair from a large number of individuals to improve interpretation of the results.

The correlations between zinc concentration before and after treatment in extremity hairs, skin and serum was not proven, due to the limited amount of patients and the lack of control patients, but the moreover positive impact on hair coat quality, and the wound healing that were observed, reveal an interest in further study in zinc requirements and normal zinc values in equines.

Diagnosing deficit in the horse requires more precise information about mineral relationship and mineral metabolism in the horses and more detailed study with control group of normal horses. Biases have to be ruled out in a later study such as

The conclusion is that whether the zinc status is below or in low normal range, oral supplementation of organic zinc seems to be beneficial to equines with distal limb skin disease.

The most reliable paraclinical parameters to estimate the equine zinc status, is assessment of zinc concentration in mane hair, hair from diseased area and serum zinc.

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Appendix – all results

Pracownia Mikroskopii Elektronowej
Uniwersytetu Przyrodniczego we Wrocławiu

50-375 Wrocław
ul. Kożuchowska 5b

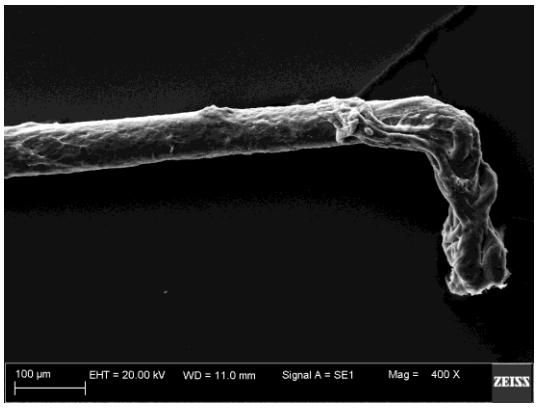
http://microscopy.ar.wroc.pl/

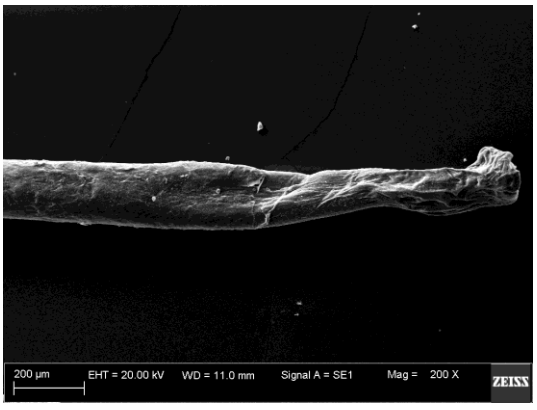
Patient: 1.1. horse, mare. Before treatment

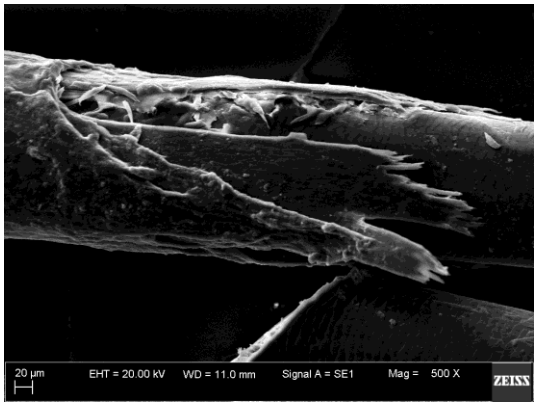
Responsible: dr Lene

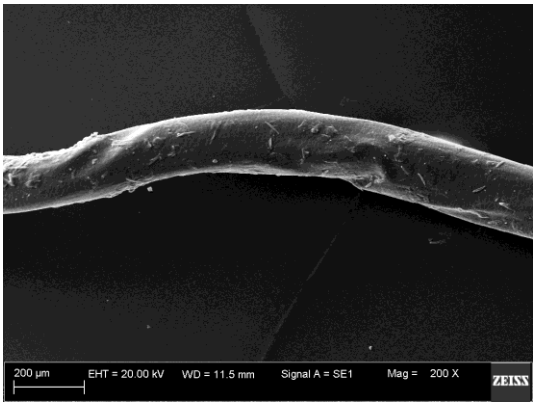
Scanning elektron trichoscopy

SEM analysis of hairs reviled specially with regards to hairs coming from the extremity site misshaped hair shafts, delaminating cuticle and ruptures of the cuticle and cortex of the hairs.









Appendix – all results

Pracownia Mikroskopii Elektronowej
Uniwersytetu Przyrodniczego we Wrocławiu
50-375 Wrocław
ul. Kożuchowska 5b
http://microscopy.ar.wroc.pl/

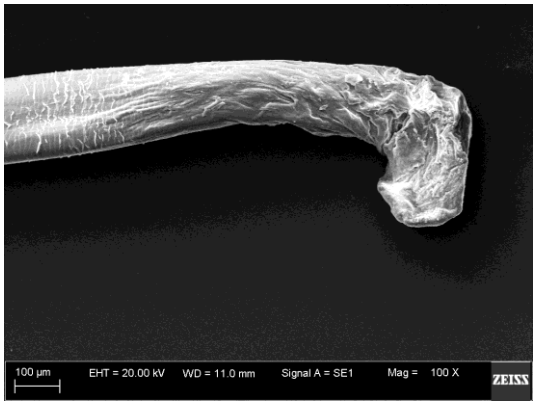
Patient 1.2 : horse, female, (II)After treatment

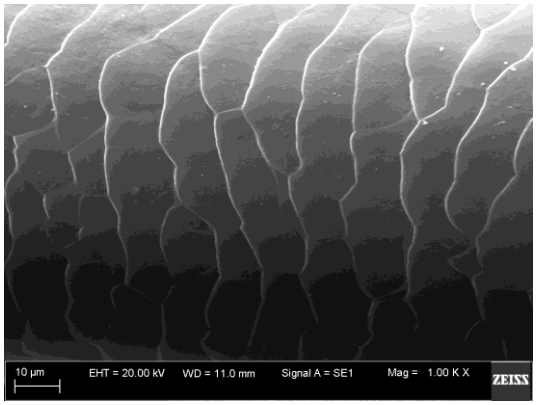
Responsible: dr Lene

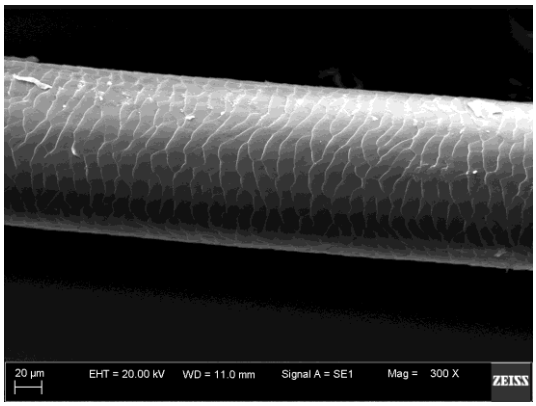
Scanning elektron trichoscopy

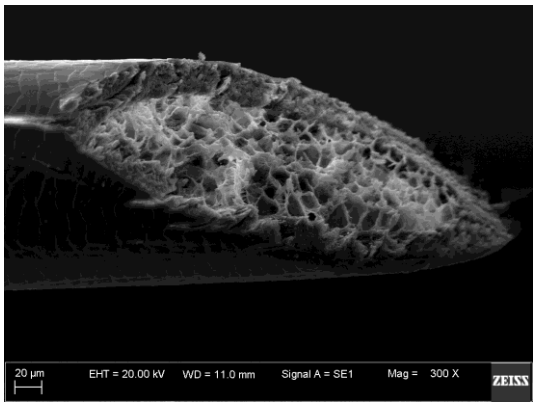
SEM analysis of hairs coming from horse mane reviled no gross abnormalities beside slightly misshaped hair shafts in their peribulbar regions.

Mane hairs





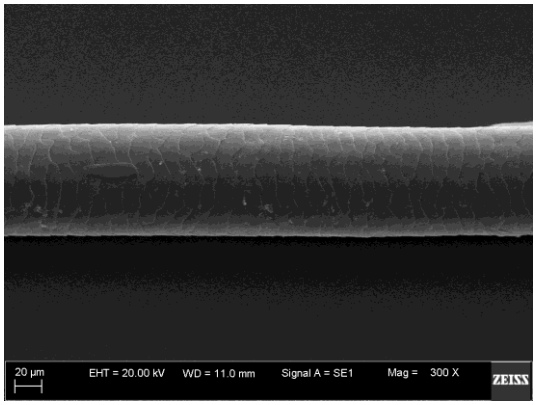


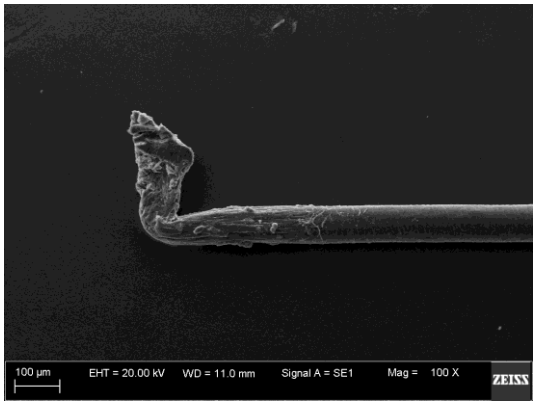


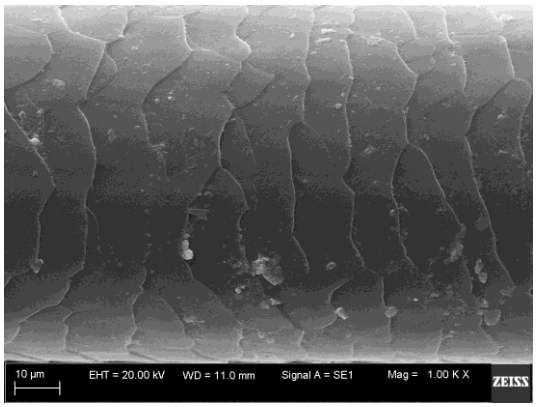
Appendix – all results

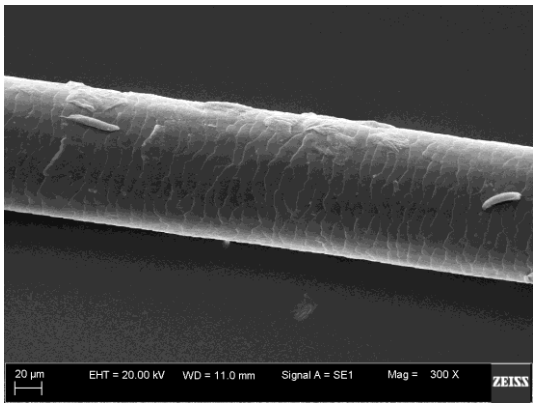
Extremity hairs

With regards to body hairs there was no significant morphological abnormalities observed.









Appendix – all results

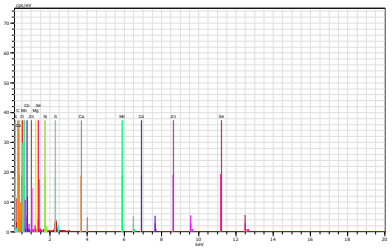
Elemental hair and skin analysis

Analysis of elemental composition of the mane hairs reviled no marked abnormalities except Si deficits. In case of hairs coming from close proximity of the skin lesions there was low level of S indicated.

Mane

Spectrum: Acquisition

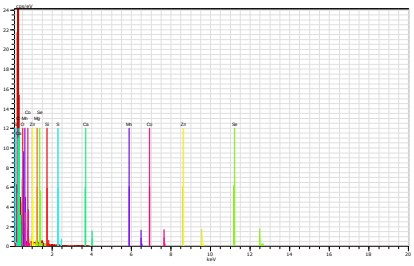
El	AN	Series	Net	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error [%]
C	6	K-series	139430	47.96	47.96	57.37	15.1
O	8	K-series	33245	44.54	44.54	40.00	14.3
S	16	K-series	36043	4.16	4.16	1.86	0.2
Se	34	K-series	187	2.21	2.21	0.40	0.2
Zn	30	K-series	232	0.34	0.34	0.07	0.1
Co	27	K-series	336	0.27	0.27	0.07	0.0
Mg	12	K-series	1196	0.18	0.18	0.11	0.1
Mn	25	K-series	317	0.16	0.16	0.04	0.0
Ca	20	K-series	506	0.12	0.12	0.04	0.0
Si	14	K-series	543	0.05	0.05	0.03	0.0
		Total:		100.00	100.00	100.00	



Extremity hairs

Spectrum: Acquisition

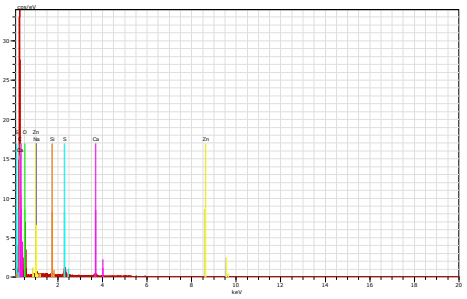
El	AN	Series	Net	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error [%]
C	6	K-series	263704	49.09	49.09	59.39	16.0
O	8	K-series	54104	41.01	41.01	37.25	13.8
Se	34	K-series	64	3.00	3.00	0.55	0.4
Mg	12	K-series	3061	1.79	1.79	1.07	0.2
Zn	30	K-series	90	1.30	1.30	0.29	0.2
Si	14	K-series	2994	1.12	1.12	0.58	0.1
Mn	25	K-series	86	0.83	0.83	0.22	0.1
Co	27	K-series	76	0.70	0.70	0.17	0.1
S	16	K-series	1401	0.59	0.59	0.27	0.1
Ca	20	K-series	269	0.57	0.57	0.21	0.1
		Total:		100.00	100.00	100.00	



Skin surface

Spectrum: Acquisition

El	AN	Series	Net	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error [%]
C	6	K-series	183042	50.34	50.34	57.99	15.5
O	8	K-series	41294	47.62	47.62	41.18	14.9
S	16	K-series	9004	0.96	0.96	0.42	0.1
Ca	20	K-series	3113	0.56	0.56	0.19	0.0
Na	11	K-series	1806	0.26	0.26	0.15	0.1
Zn	30	K-series	302	0.22	0.22	0.05	0.0
Si	14	K-series	669	0.05	0.05	0.02	0.0
		Total:		100.00	100.00	100.00	



Appendix – all results

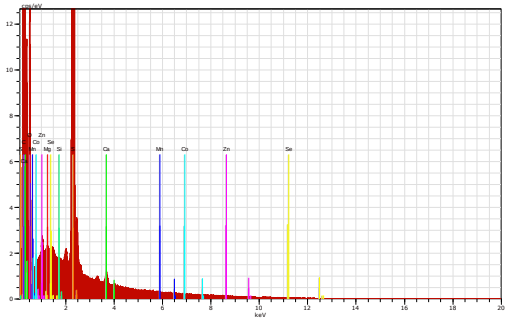
Elemental hair analysis and skin analysis

Analysis of elemental composition of the hairs reviled in case of mane and extremities hairs low level of Mn. With respect to skin surface both Zn and Ca level was relatively low, particularly comparing to original levels . On the other hand S and Si skin concentration was much higher then at the beginning of the research.

mane

Spectrum: Acquisition

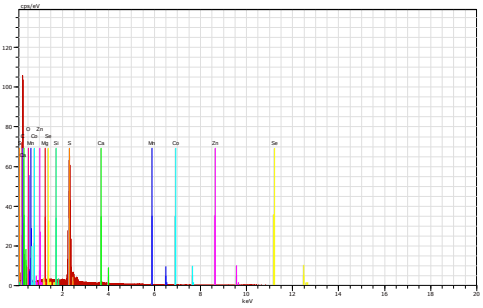
El	AN	Series	Net	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error [%]
C	6	K-series	524253	54.10	54.10	63.71	16.8
O	8	K-series	76005	36.53	36.53	32.29	11.6
S	16	K-series	339734	7.83	7.83	3.45	0.3
Se	34	K-series	526	0.43	0.43	0.08	0.0
Mg	12	K-series	10223	0.33	0.33	0.19	0.1
Ca	20	K-series	6818	0.28	0.28	0.10	0.0
Si	14	K-series	10804	0.21	0.21	0.11	0.0
Zn	30	K-series	592	0.12	0.12	0.03	0.0
Co	27	K-series	850	0.10	0.10	0.03	0.0
Mn	25	K-series	834	0.07	0.07	0.02	0.0
		Total:		100.00	100.00	100.00	



Extremity hairs

Spectrum: Acquisition

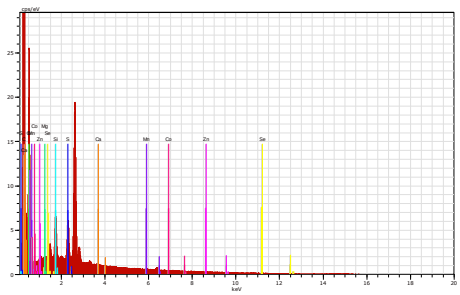
El	AN	Series	Net	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error [%]
C	6	K-series	553110	58.65	58.65	68.28	18.2
O	8	K-series	55994	31.46	31.46	27.50	10.1
S	16	K-series	663712	8.93	8.93	3.89	0.3
Se	34	K-series	537	0.21	0.21	0.04	0.0
Ca	20	K-series	8552	0.19	0.19	0.06	0.0
Si	14	K-series	14124	0.16	0.16	0.08	0.0
Mg	12	K-series	8181	0.15	0.15	0.09	0.0
Zn	30	K-series	1085	0.12	0.12	0.03	0.0
Co	27	K-series	1261	0.08	0.08	0.02	0.0
Mn	25	K-series	1233	0.05	0.05	0.01	0.0
		Total:		100.00	100.00	100.00	



skin surface

Spectrum: Acquisition

El	AN	Series	Net	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error [%]
O	8	K-series	155452	49.20	49.20	43.25	15.2
C	6	K-series	564580	47.18	47.18	55.25	14.5
S	16	K-series	157843	2.52	2.52	1.10	0.1
Si	14	K-series	37161	0.52	0.52	0.26	0.0
Mn	25	K-series	4751	0.19	0.19	0.05	0.0
Se	34	K-series	702	0.16	0.16	0.03	0.0
Zn	30	K-series	1146	0.10	0.10	0.02	0.0
Co	27	K-series	1380	0.08	0.08	0.02	0.0
Ca	20	K-series	1575	0.04	0.04	0.01	0.0
Mg	12	K-series	1125	0.03	0.03	0.02	0.0
		Total:		100.00	100.00	100.00	

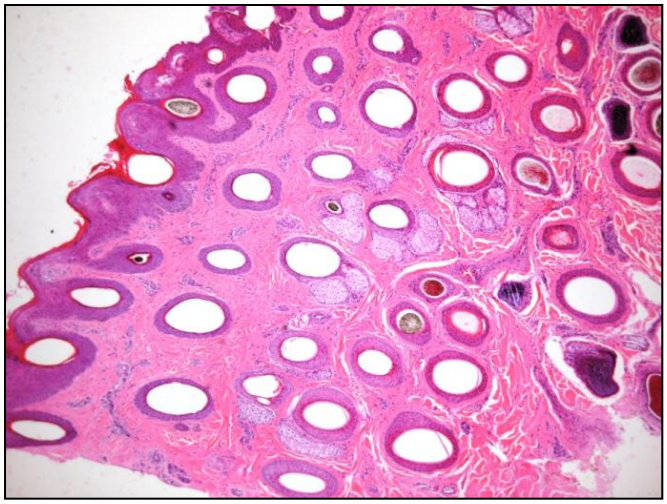


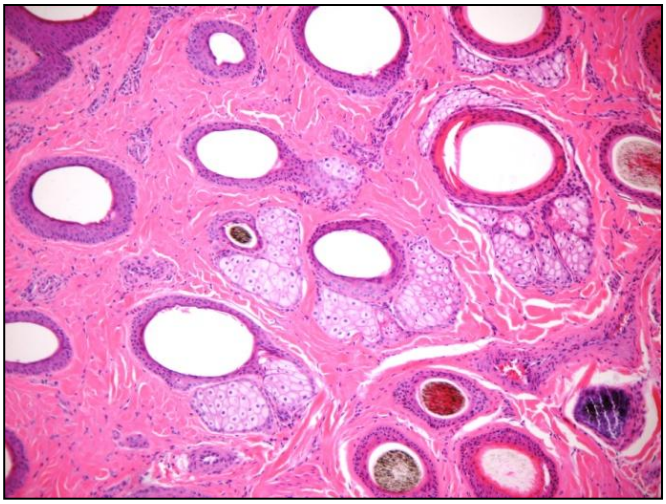
Appendix – all results

Dermatopathological skin examination

Before treatment

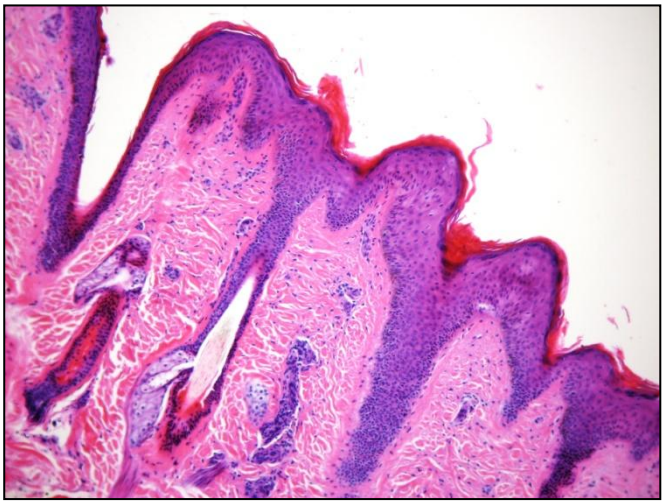
Histopatological examination of the skin samples coming from affected areas reviled marked hyperkeratosis and parakeratosis. In the dermis area cell infiltrate consisted mainly of activated fibroblasts and some mast cells. They were predominately periadnexal in their location. Sebaceous glands observed reviled subtle features of hyperplasia.

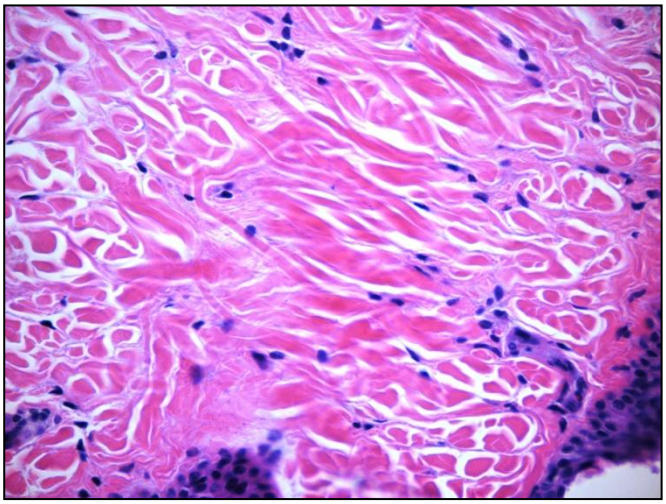




After treatment

Histopatological analysis of the skin samples coming from examined areas reviled mild features of hyperkeratosis of the epidermis. Within dermis compartment inflammatory infiltrates were minimal and consisted mainly of fibroblasts and some mononuclear cells.

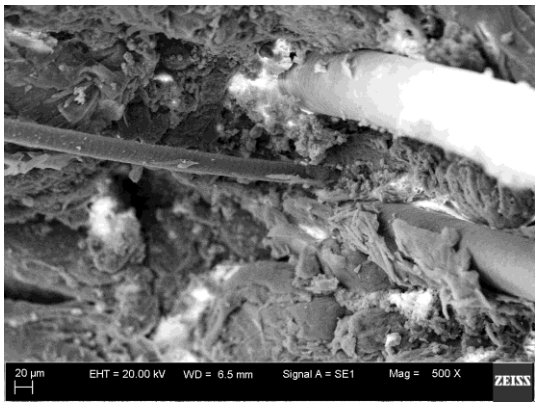


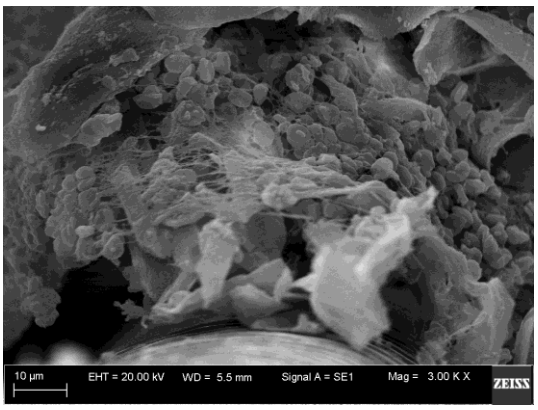


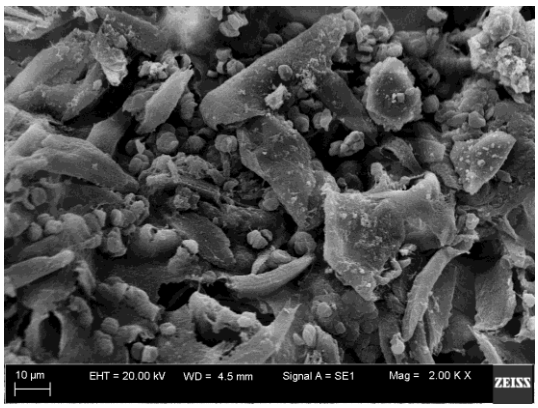
Scanning microscopy examination of the skin

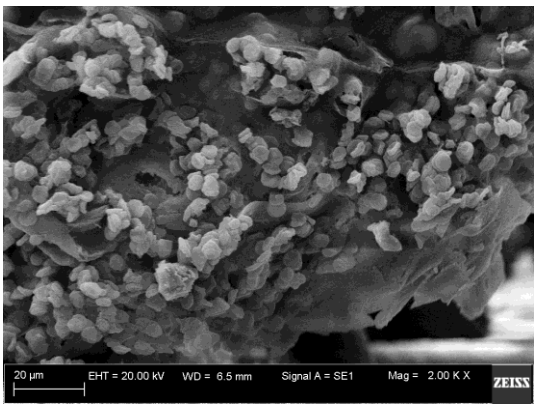
Before treatment 1.1.

SEM pictures of the skin surface. Note some amount of yeasts harboring mainly area of the close proximity of the hair shafts. There are also red blood cells visible.



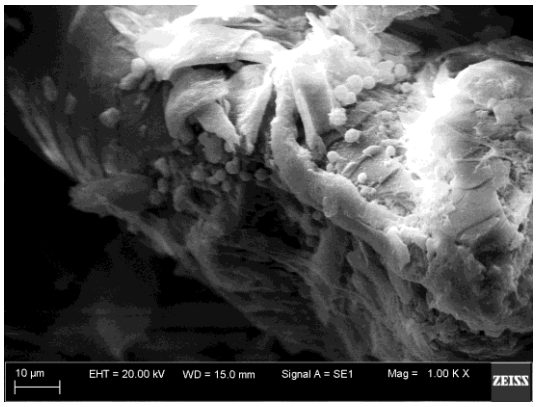


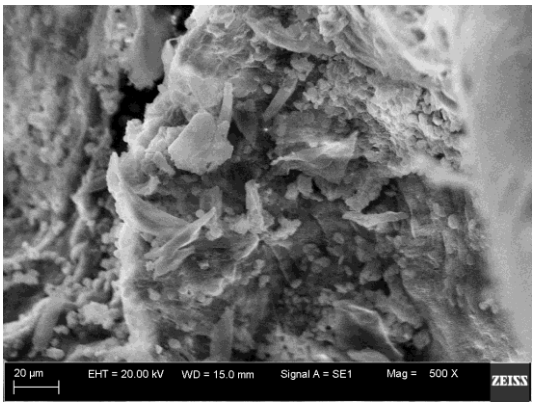


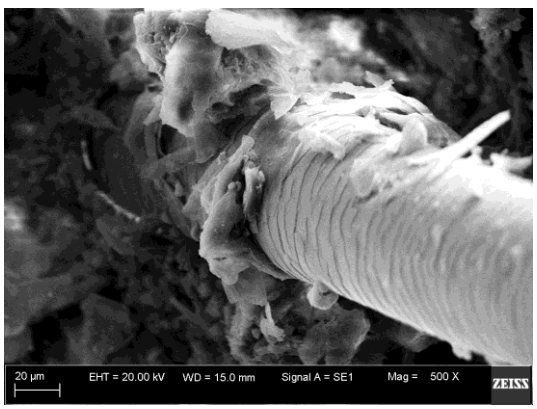


After treatment 1.2.

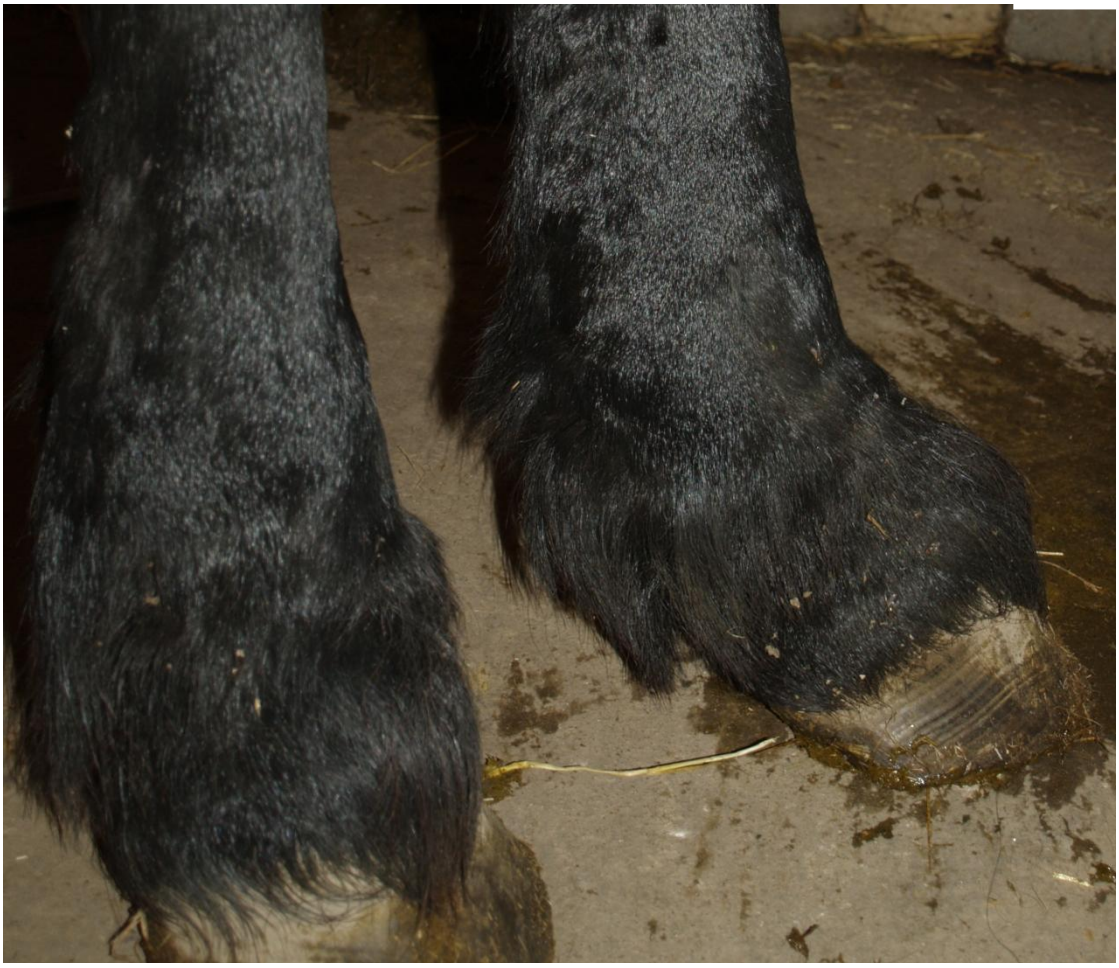
SEM pictures of the skin surface reviled small amount of yeast organisms scattered between hair shafts and some of them on the hair surfaces. There were also some blood cells visible.

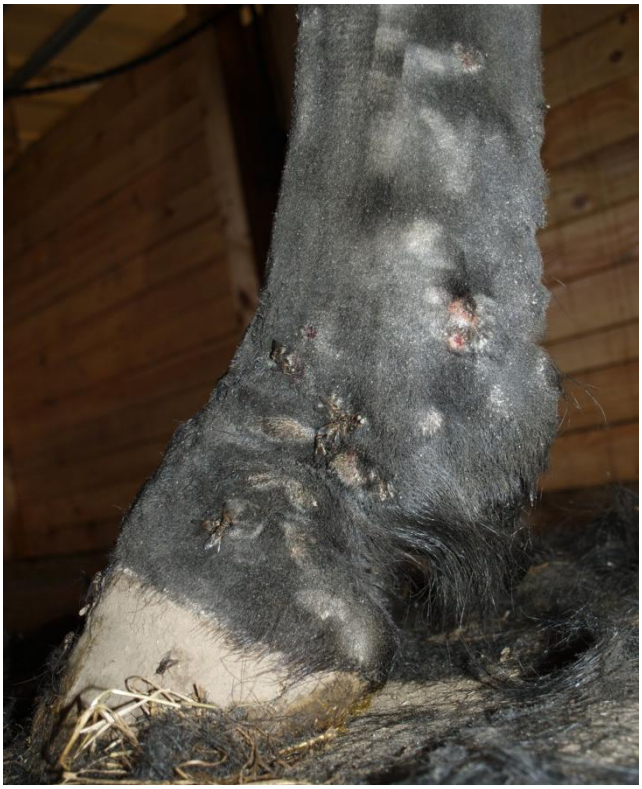


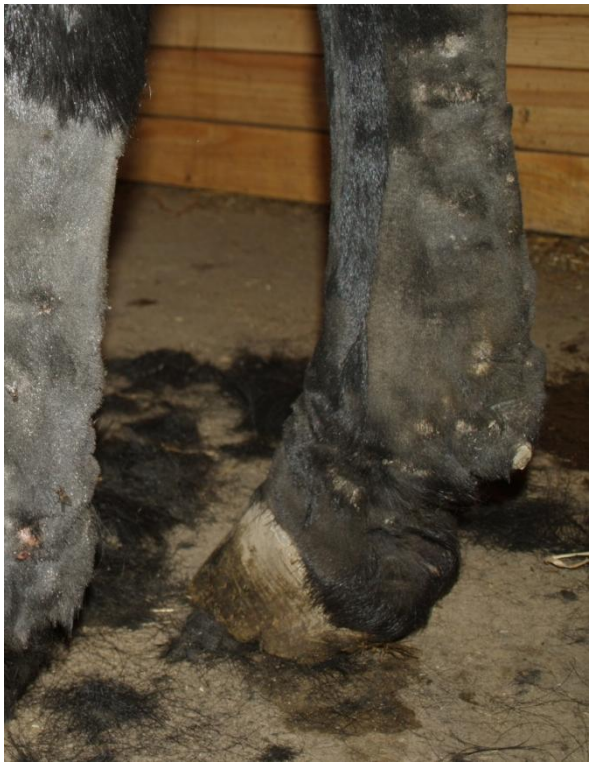




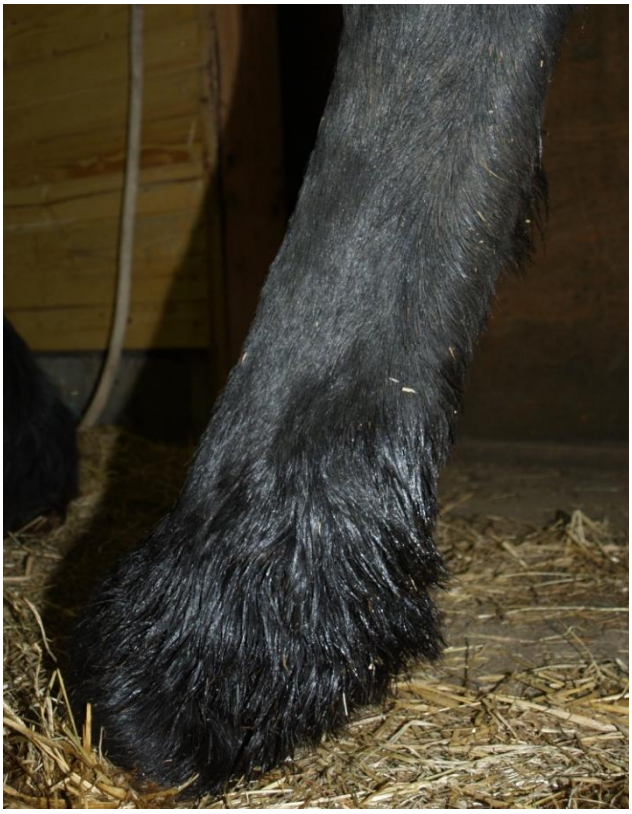
Appendix – all results

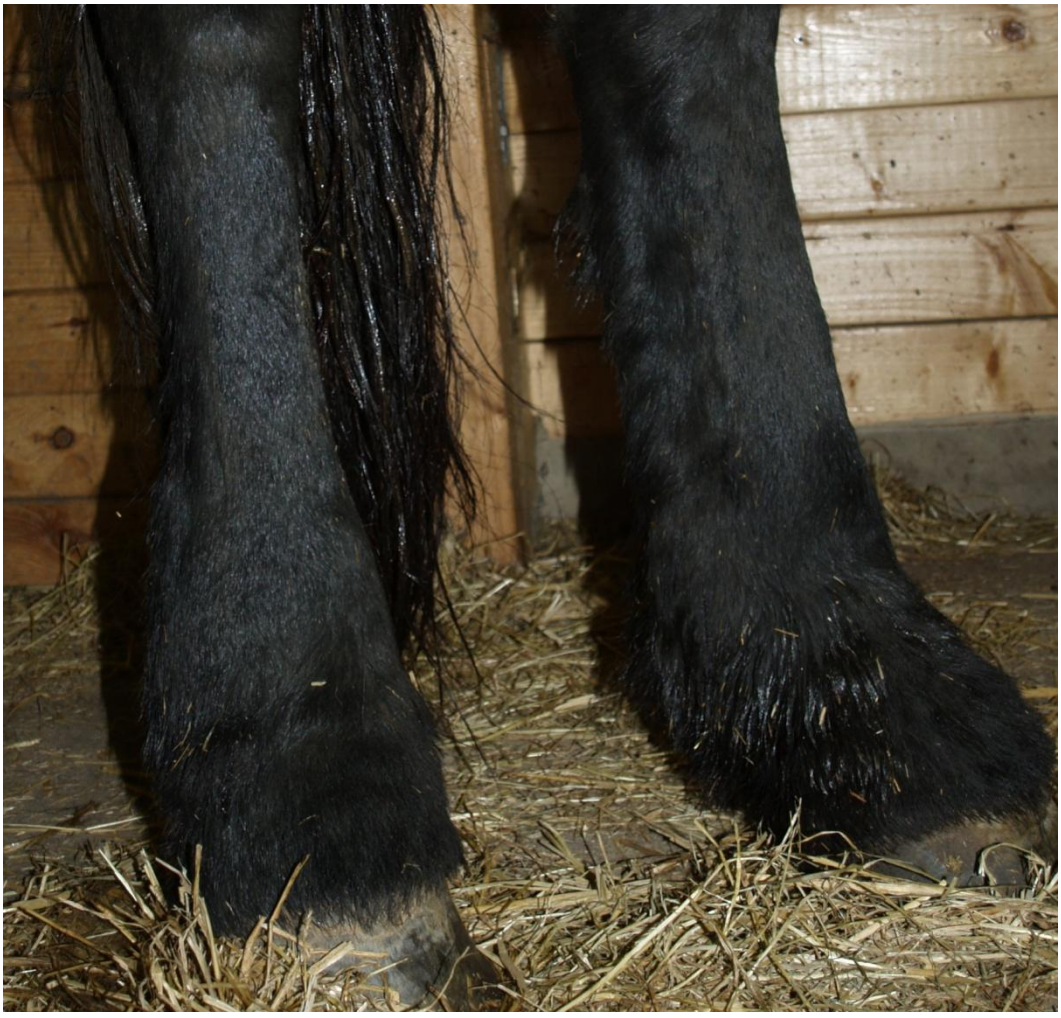






1.1. Clinical appearance before treatment. The limps were swollen with multiple areas of profound pyodermi and suppuration. There were pronounced hyperkeratosis and debris.





1.2. Clinical appearance after treatment. The swelling were totally diminished and only a few small areas with hyperkeratosis were visible.

Appendix – all results

Pracownia Mikroskopii Elektronowej
Uniwersytetu Przyrodniczego we Wrocławiu

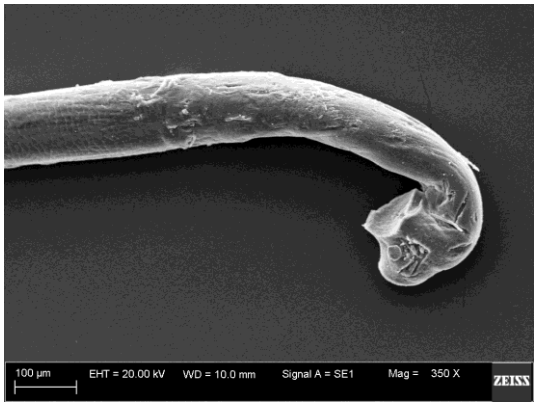
50-375 Wrocław
ul. Kożuchowska 5b

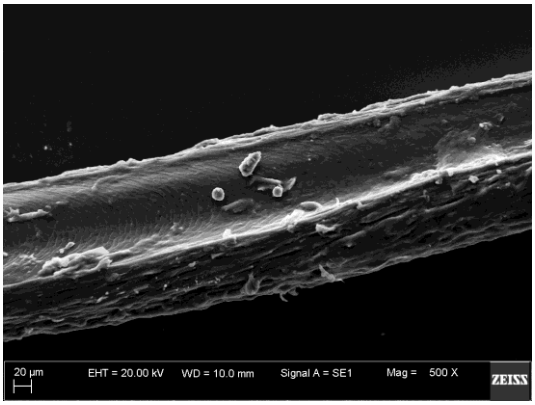
http://microscopy.ar.wroc.pl/

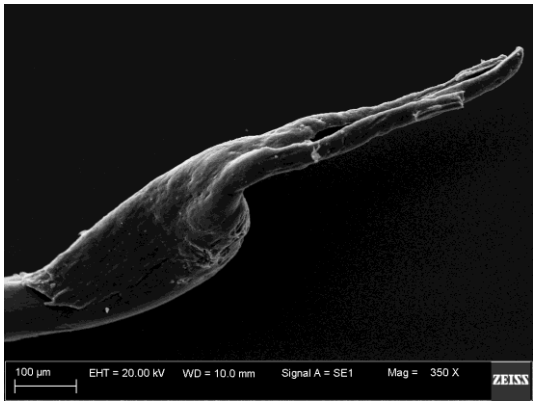
Patient : 2.1. horse, Mare, Before treatment
Responsible: dr Lene

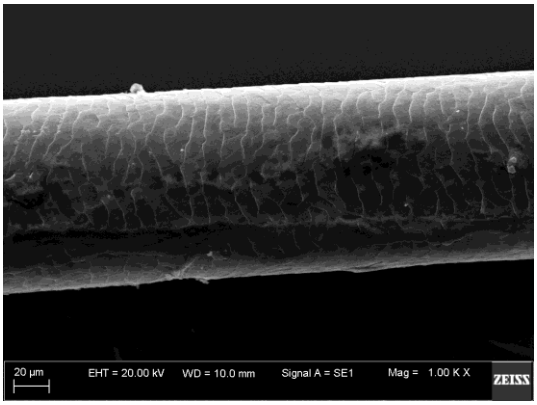
Scanning trichoscopy

SEM analysis of the hairs coming from mane reviled changes of hair shafts shape namely flattening, twisting and splitting. Some of hair shaft had surface longitudinal ruptures.









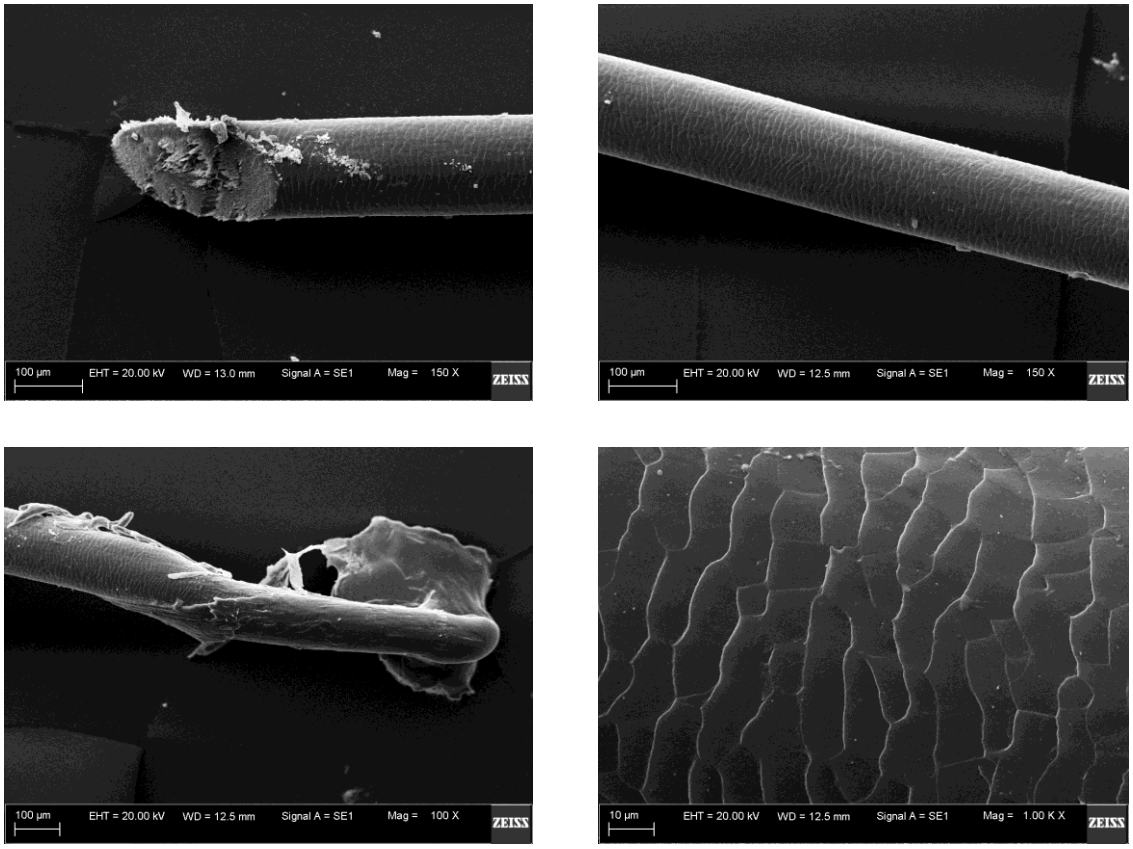
Mane hairs

Patient : 2.2. Horse, mare, After treatment

responsible: dr Lene

Scanning trichoscopy

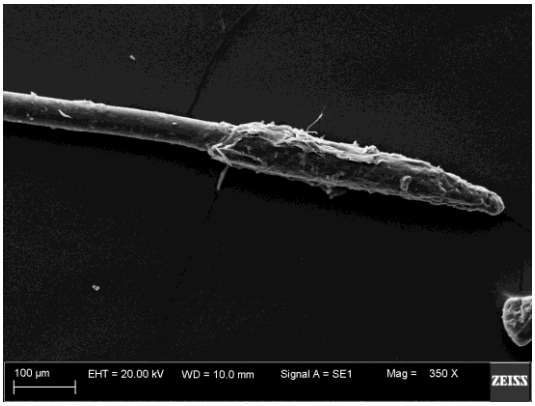
SEM analysis of the hairs coming from mane reviled no serious changes of hair shafts shape but a reasonable amount of hair bulbs was in telogen phase.

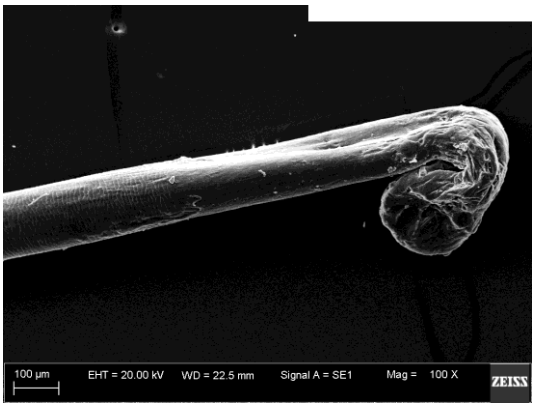


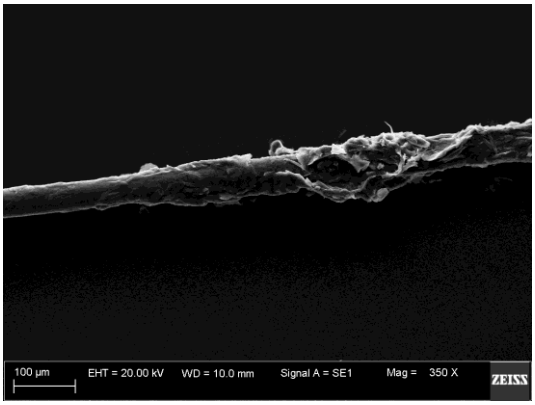
Mane hairs - After treatment

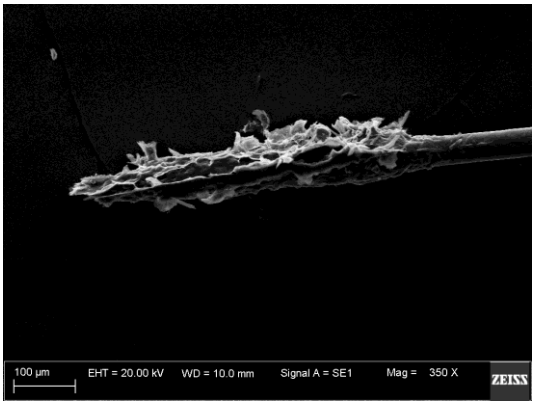
Appendix – all results

2.1. Before treatment

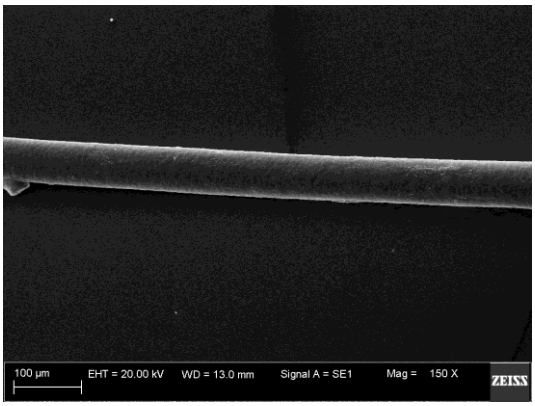


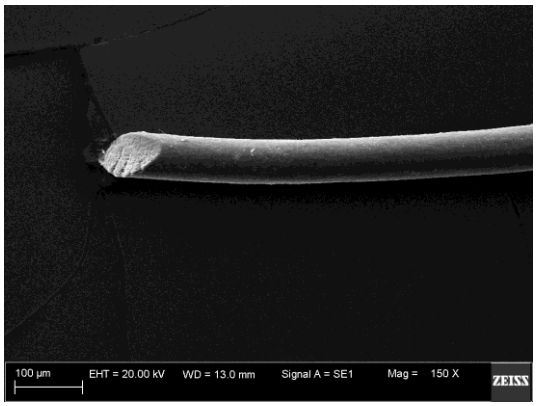


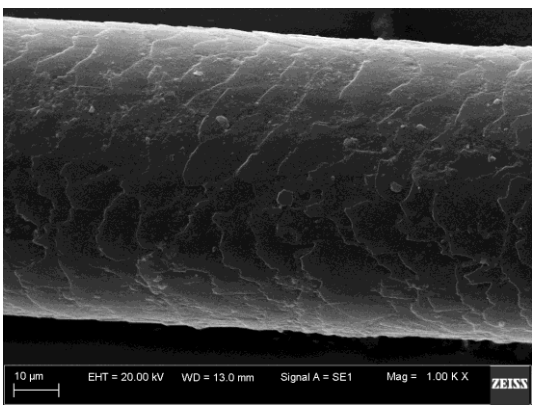


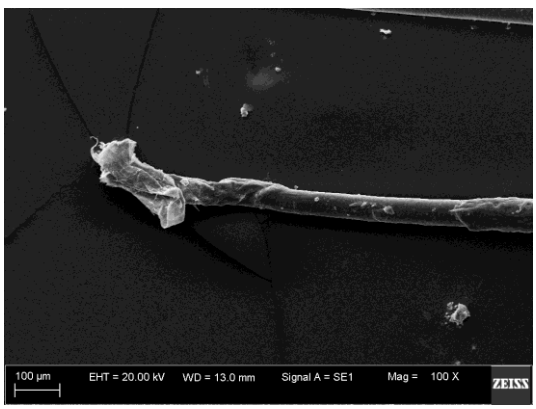


2.1. Extremity hairs - before treatment.There were a lot of keratin debris noticed sticking to hair bulbs and shafts. Moreover some of the hair bulbs were flattened and misshaped.









2.2. Extremity hairs - after treatment. As far as body hairs are concerned there was irregular pattern and misshaped scales building the hair shaft cuticle noticed.

Appendix – all results

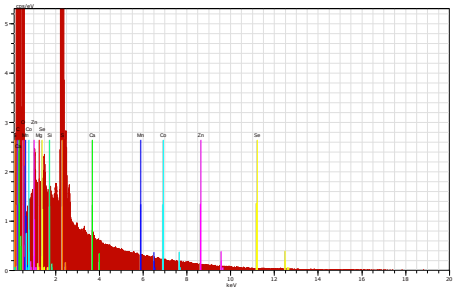
Elemental hair and skin analysis

Elemental analysis of the hairs was performed in peribulbal region of the given hair and on t
As far as mane hairs are concerned there were low levels of Ca and Mn observed. In case of hairs coming from lesional skin low level of Mn was indicated.

Mane hairs

Spectrum: Acquisition

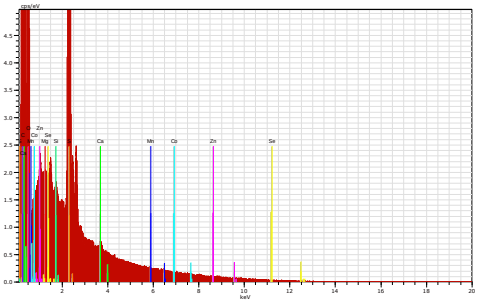
El	AN	Series	Net	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error [%]
C	6	K-series	735475	54.33	54.33	63.43	16.8
O	8	K-series	110558	38.11	38.11	33.40	12.0
S	16	K-series	254828	6.40	6.40	2.80	0.3
Se	34	K-series	380	0.39	0.39	0.07	0.0
Mg	12	K-series	6174	0.22	0.22	0.13	0.0
Si	14	K-series	8142	0.18	0.18	0.09	0.0
Zn	30	K-series	672	0.14	0.14	0.03	0.0
Co	27	K-series	825	0.10	0.10	0.02	0.0
Mn	25	K-series	764	0.06	0.06	0.02	0.0
		Total:		100.00	100.00	100.00	



Extremity hairs

Spectrum: Acquisition

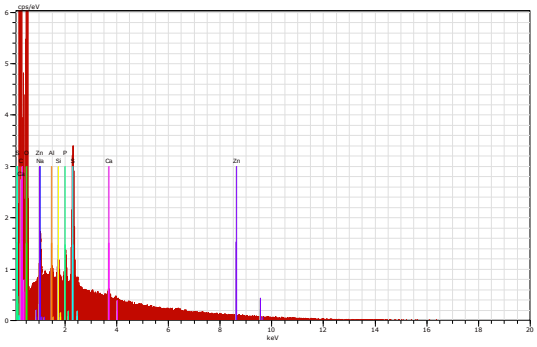
El	AN	Series	Net	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error [%]
C	6	K-series	859794	59.84	59.84	68.72	18.4
O	8	K-series	86585	32.98	32.98	28.44	10.5
S	16	K-series	188406	5.16	5.16	2.22	0.2
Se	34	K-series	579	0.95	0.95	0.17	0.1
Mg	12	K-series	9158	0.34	0.34	0.19	0.1
Si	14	K-series	13129	0.31	0.31	0.15	0.0
Zn	30	K-series	558	0.15	0.15	0.03	0.0
Ca	20	K-series	2298	0.10	0.10	0.03	0.0
Co	27	K-series	694	0.10	0.10	0.02	0.0
Mn	25	K-series	740	0.07	0.07	0.02	0.0
		Total:		100.00	100.00	100.00	



Skin surface

Spectrum: Acquisition

El	AN	Series	Net	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error [%]
C	6	K-series	437934	51.32	51.32	58.77	15.7
O	8	K-series	94450	47.27	47.27	40.64	14.6
S	16	K-series	25112	0.89	0.89	0.38	0.1
Na	11	K-series	3527	0.18	0.18	0.11	0.1
Zn	30	K-series	629	0.14	0.14	0.03	0.0
Ca	20	K-series	2060	0.11	0.11	0.04	0.0
P	15	K-series	2279	0.07	0.07	0.03	0.0
Si	14	K-series	272	0.01	0.01	0.00	0.0
Al	13	K-series	0	0.00	0.00	0.00	0.0
		Total:		100.00	100.00	100.00	



Appendix – all results

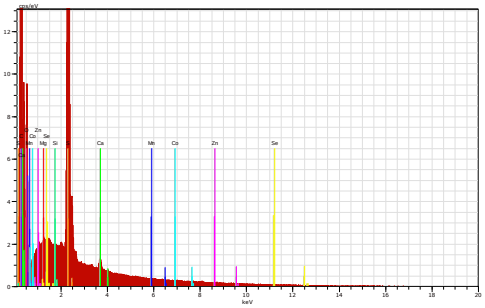
Elemental hair and skin analysis

Elemental analysis of the hairs was performed in peribulbal region of the given hair and on the skin surface. As far as mane hairs are concerned there were observed low levels of Mn .In case of hairs coming from the extremities of the horse low concentration of S and Mn was detected. With respect to skin surface, comparing to the beginning of the research low levels of S, Si and Ca was indicated.

mane hairs

Spectrum: Acquisition

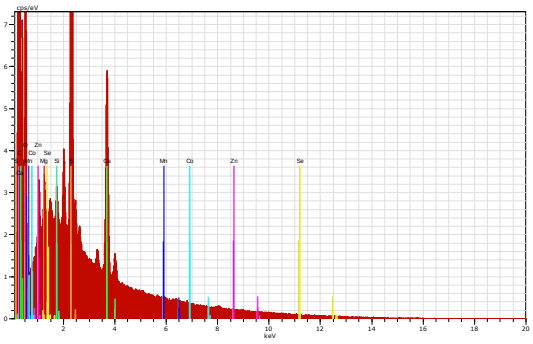
El	AN	Series	Net	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error [%]
C	6	K-series	487816	57.06	57.06	66.63	17.7
O	8	K-series	56521	33.48	33.48	29.35	10.7
S	16	K-series	397818	8.20	8.21	3.59	0.3
Se	34	K-series	595	0.28	0.28	0.05	0.0
Mg	12	K-series	7712	0.22	0.22	0.13	0.0
Ca	20	K-series	6276	0.21	0.21	0.07	0.0
Si	14	K-series	10618	0.19	0.19	0.09	0.0
Zn	30	K-series	1132	0.18	0.18	0.04	0.0
Co	27	K-series	1183	0.11	0.11	0.03	0.0
Mn	25	K-series	997	0.07	0.07	0.02	0.0
		Total:		100.00	100.00	100.00	



Extremity hairs

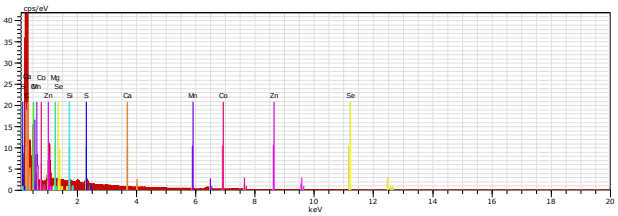
Spectrum: Acquisition

El	AN	Series	Net	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error [%]
C	6	K-series	854181	62.45	62.45	70.31	19.1
O	8	K-series	74633	33.15	33.15	28.02	10.4
S	16	K-series	137457	2.03	2.03	0.86	0.1
Ca	20	K-series	62824	1.51	1.51	0.51	0.1
Mg	12	K-series	10955	0.23	0.23	0.13	0.0
Si	14	K-series	15749	0.20	0.20	0.10	0.0
Se	34	K-series	584	0.20	0.20	0.03	0.0
Zn	30	K-series	968	0.10	0.10	0.02	0.0
Co	27	K-series	1064	0.07	0.07	0.02	0.0
Mn	25	K-series	1241	0.06	0.06	0.01	0.0
		Total:		100.00	100.00	100.00	



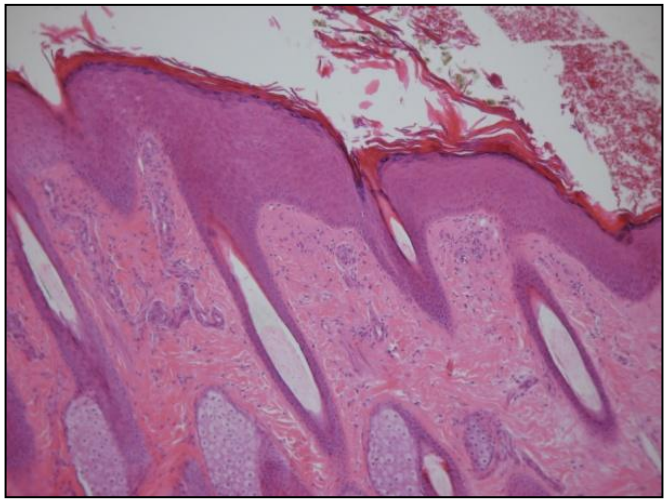
Skin surface

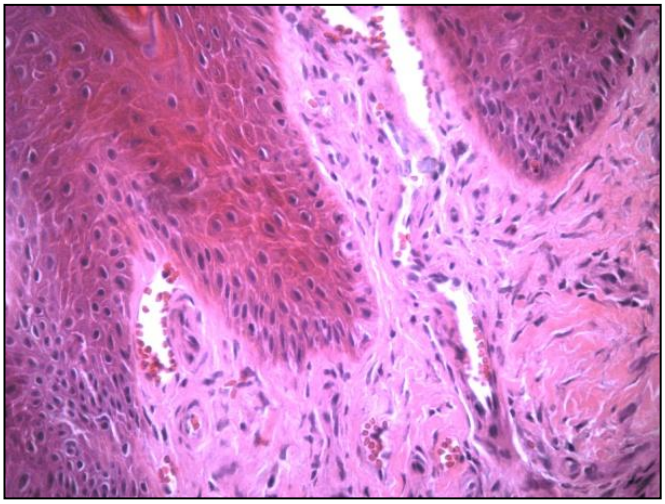
El	AN	Series	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error [%]
C	6	K-series	67.21	67.21	73.73	20.4
O	8	K-series	31.31	31.31	25.78	9.7
Mn	25	K-series	0.34	0.34	0.08	0.0
Mg	12	K-series	0.26	0.26	0.14	0.0
Se	34	K-series	0.22	0.22	0.04	0.0
S	16	K-series	0.21	0.21	0.08	0.0
Si	14	K-series	0.18	0.18	0.08	0.0
Zn	30	K-series	0.12	0.12	0.02	0.0
Co	27	K-series	0.11	0.11	0.02	0.0
Ca	20	K-series	0.04	0.04	0.01	0.0
		Total:	100.00	100.00	100.00	



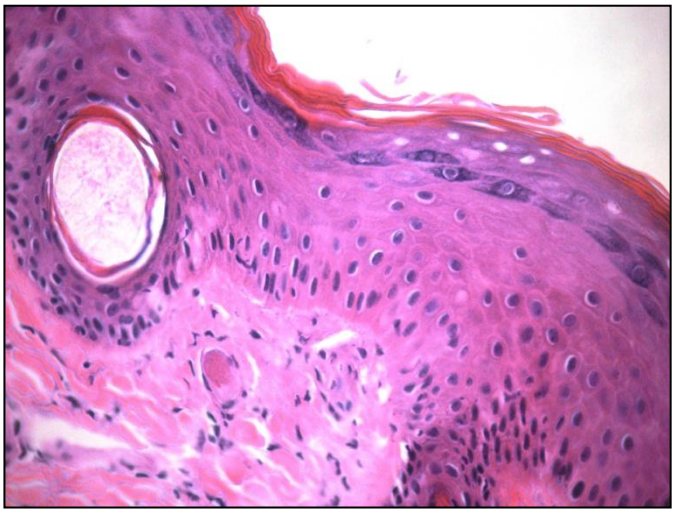
Dermatopathological skin examination

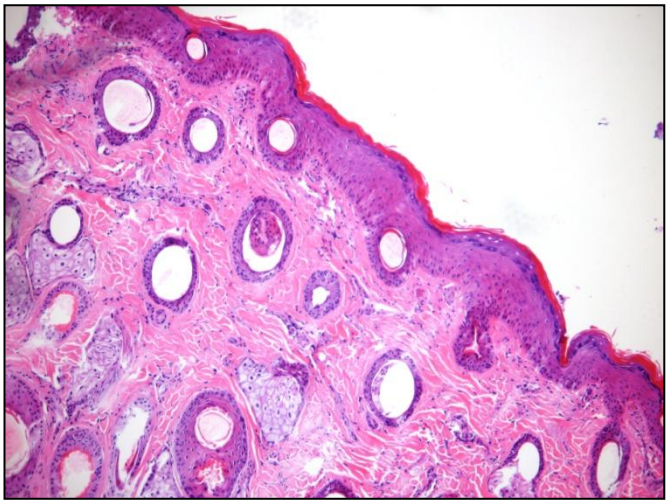
2.1. Before treatment: Dermatopathological examination of the skin reviled hyperkeratosis with parakeratosis of the epidermis. There are some ortokeratotic areas observed as well. In the dermis inflammatory cell infiltrate was most abundant in subepidermal area of the skin (superficial plexus compartment). Mentioned infiltrate consisted mainly of fibroblasts and plasma cells.





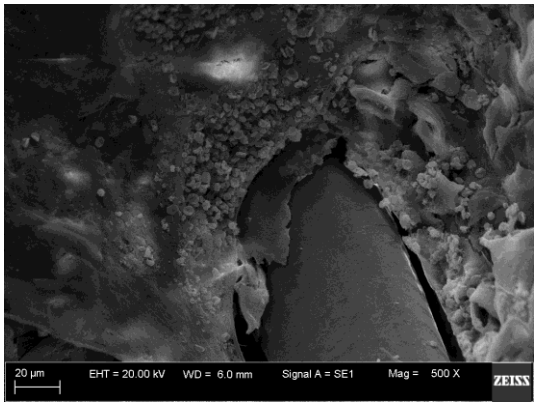
2.2. After treatment: Dermatopathological examination of the skin reviled mild hyperkeratosis with parakeratosis of the epidermis. There was also features of hyperpigmentation observed . In the dermis inflammatory cell infiltrate was localized manly in subepidermal location and consisted predominately of mast cells, fibroblast and some lymphocytes'.

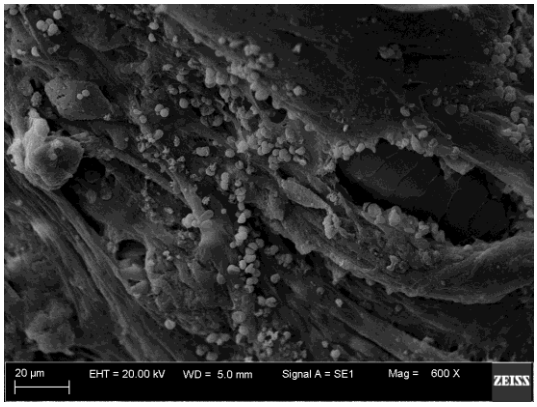


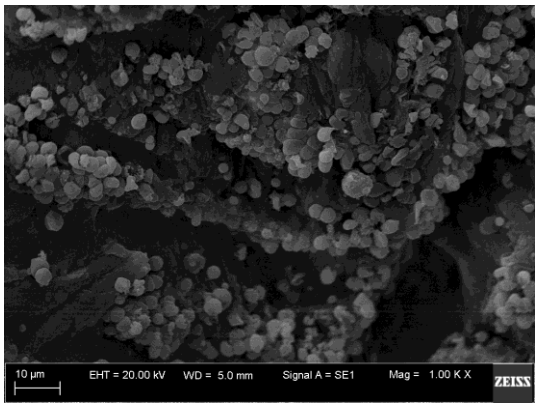


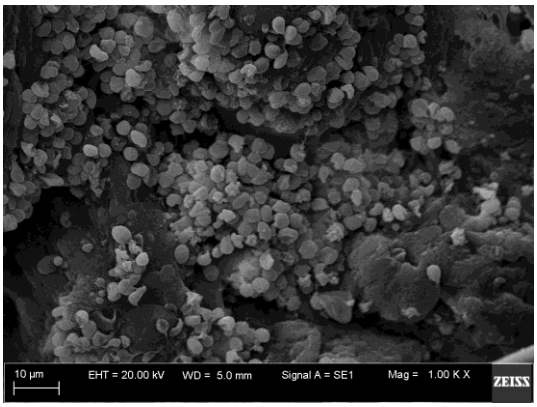
Scanning microscopy examination of the skin

2.1. Before treatment: SEM pictures of the surface of the skin coming from affected areas reviled plenty of yeasts organisms located mainly in some distance of the hair shafts. There are also some blood cells observed.

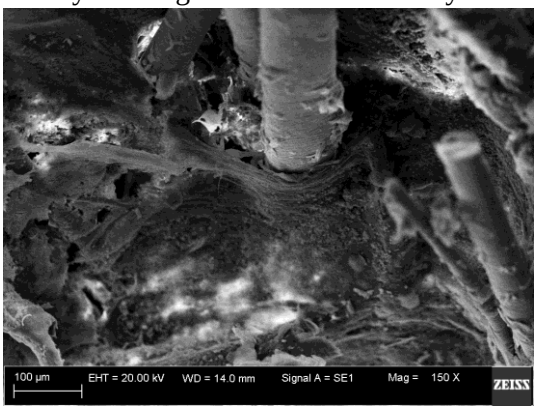


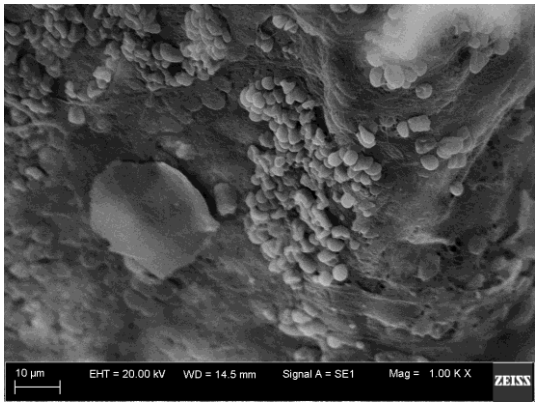


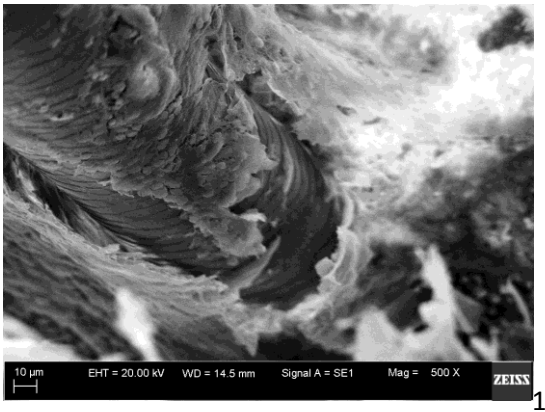


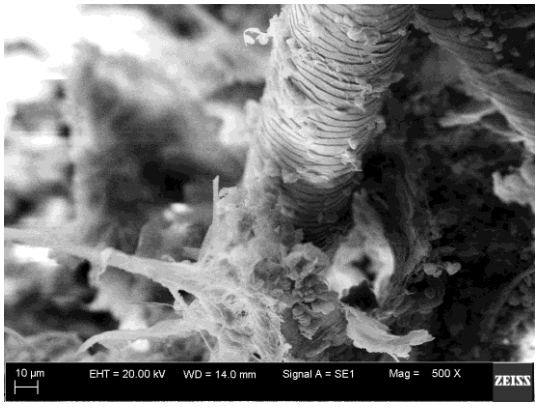


2.2. After treatment: SEM pictures of the surface of the skin coming from affected areas reviled moderate quantities of yeasts organisms located mainly in some distance from the hair shafts.

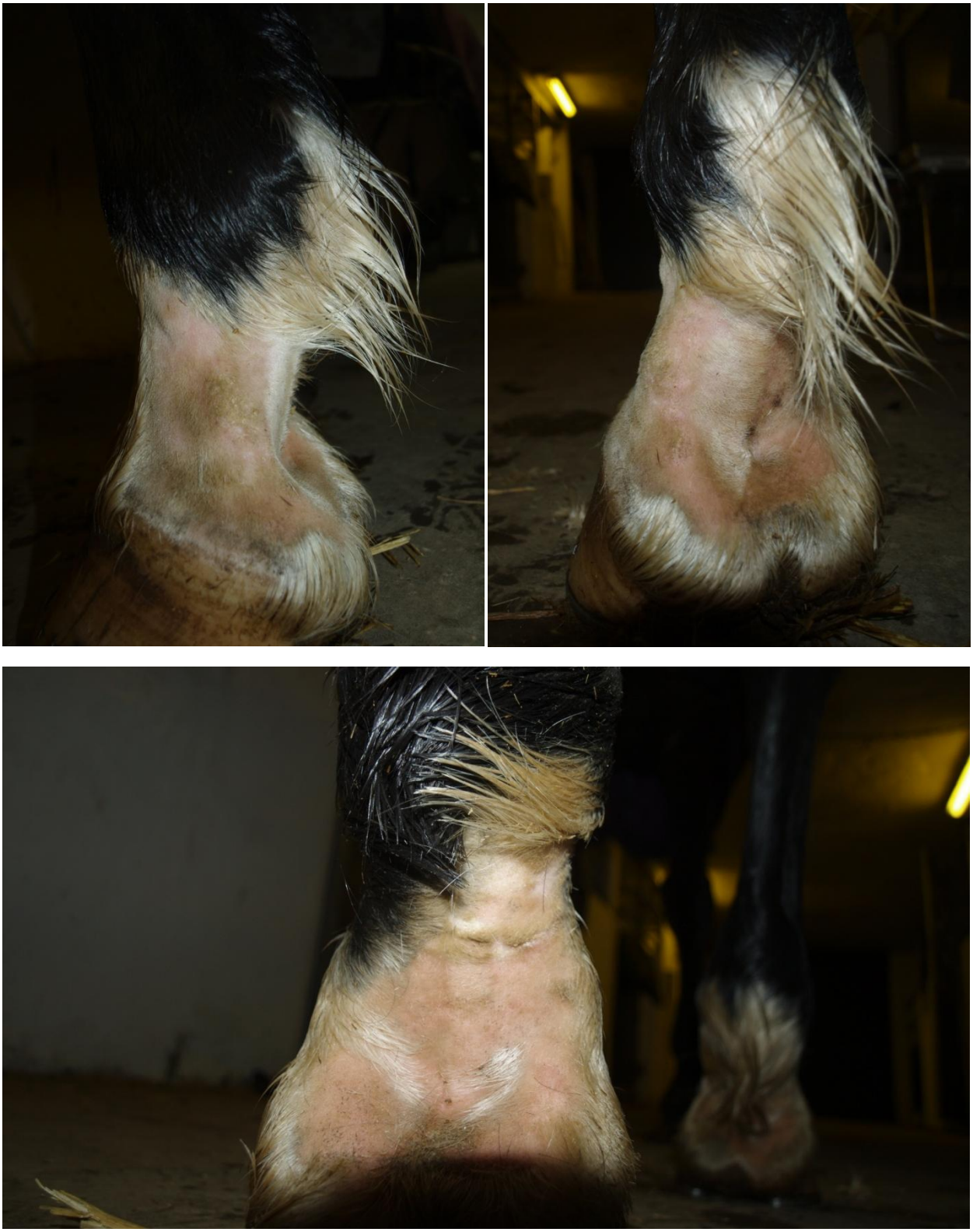








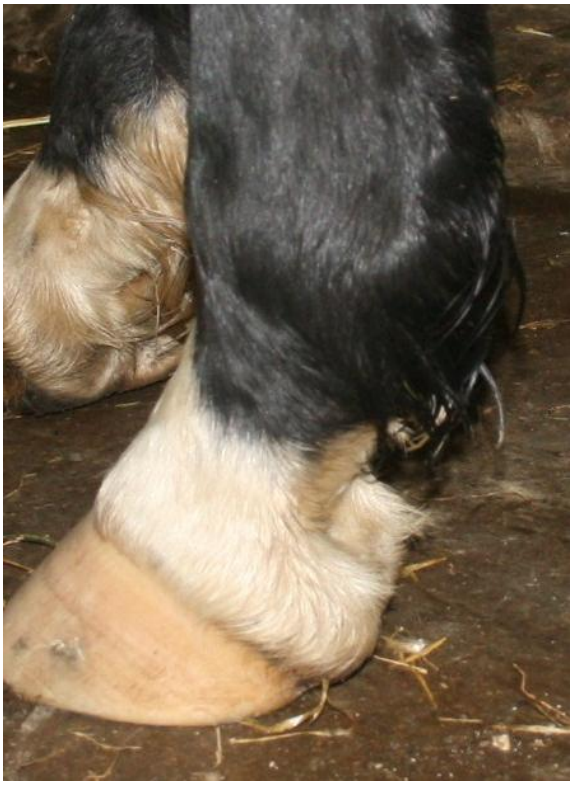
Appendix – all results



2.1.Clinical appearance before treatment. There is some swelling locally in areas were hyperkeratosis were visible.

Appendix – all results

2.2. After treatment



2.2. Clinical appearance after treatment. There is only minimal area with hyperkeratosis left.

Appendix – all results

Pracownia Mikroskopii Elektronowej
Uniwersytetu Przyrodniczego we Wrocławiu

50-375 Wrocław
ul. Kożuchowska 5b

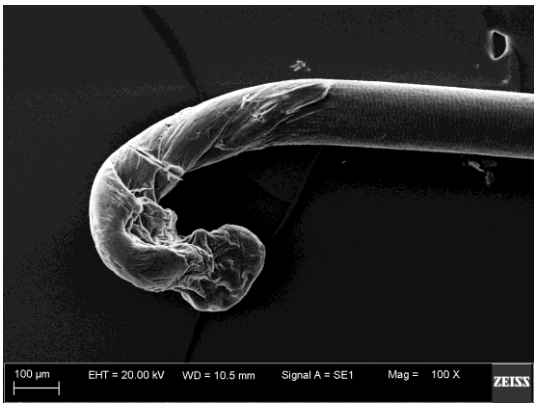
http://microscopy.ar.wroc.pl/

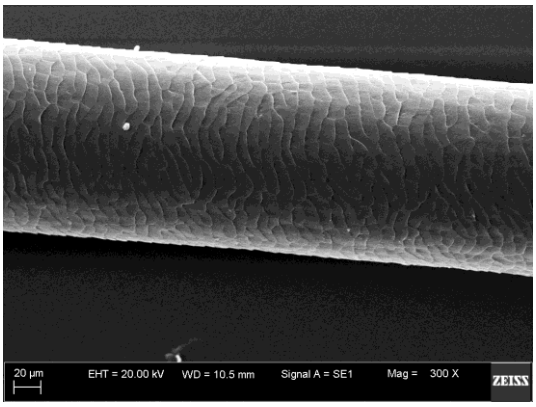
Patient: 3.1. Horse, mare,

Responsible: dr Lene

Scanning trichoscopy

3.1. Before treatment: SEM analysis of the hairs coming from mane reviled no changes of shape of the hair shafts or hairs bulbs.

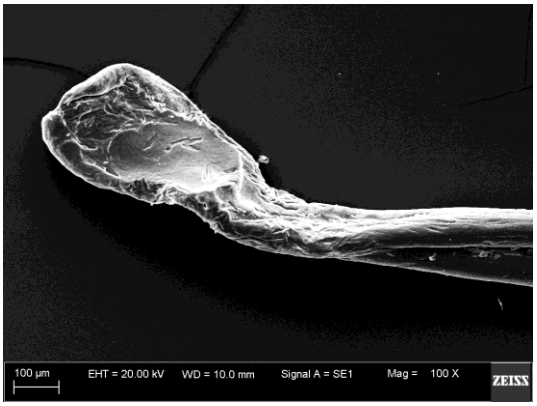


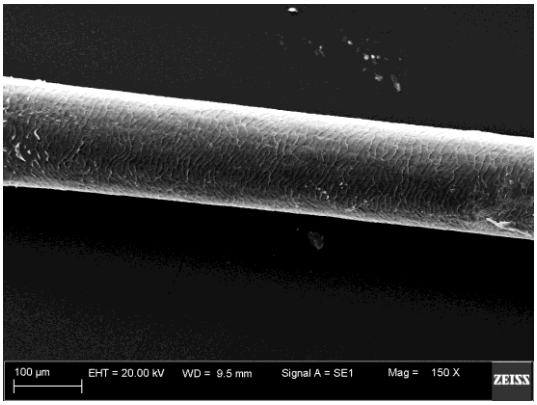


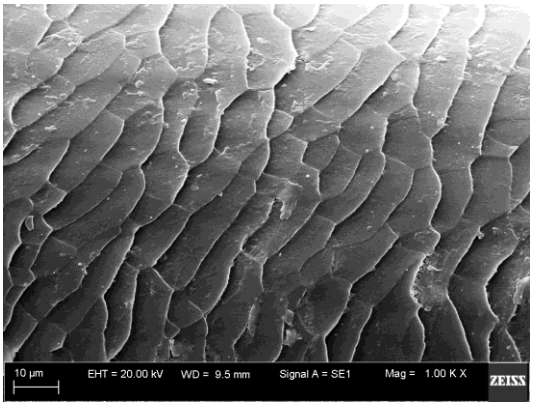
Mane hairs

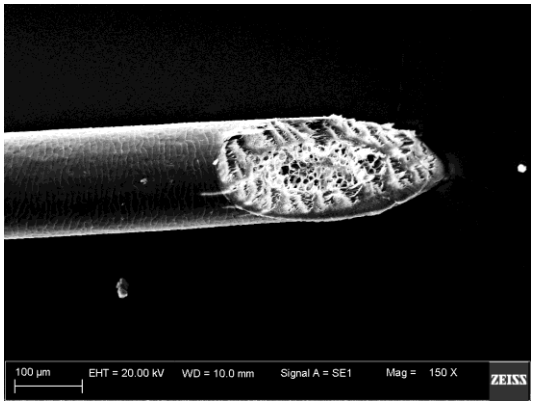
Appendix – all results

3.2. After treatment: SEM analysis of the hairs coming from mane reviled no significance morphological abnormalities of the shape of the hair shafts and hairs bulbs.





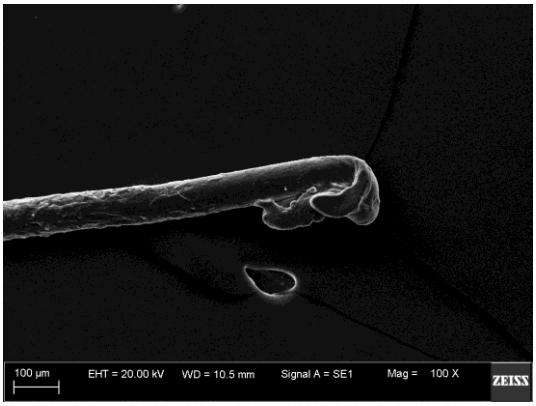


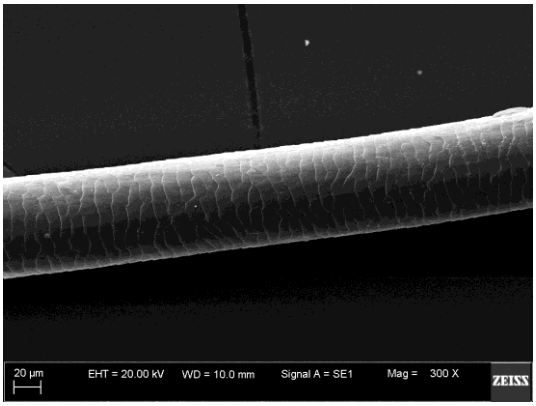


Mane hairs

Appendix – all results

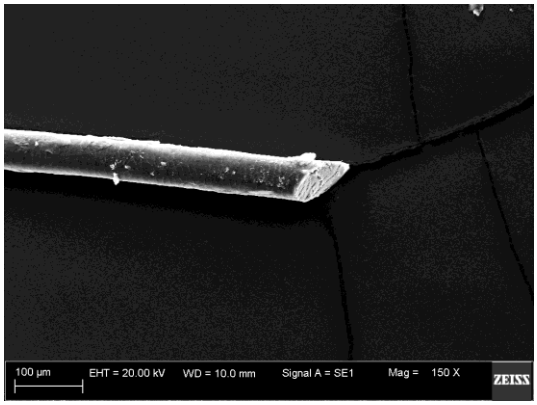
3.1. Before treatment: SEM analysis. Some of hair shafts coming from the side of the body were covered with excess of keratin debris.

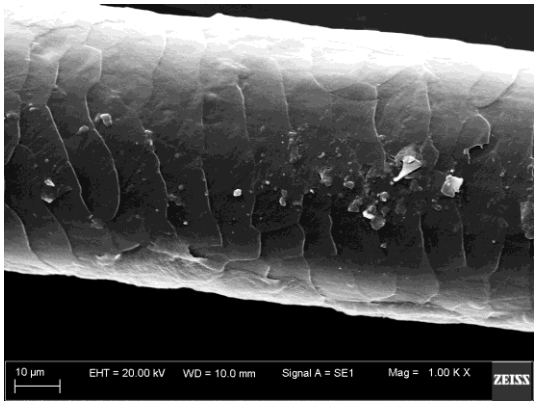


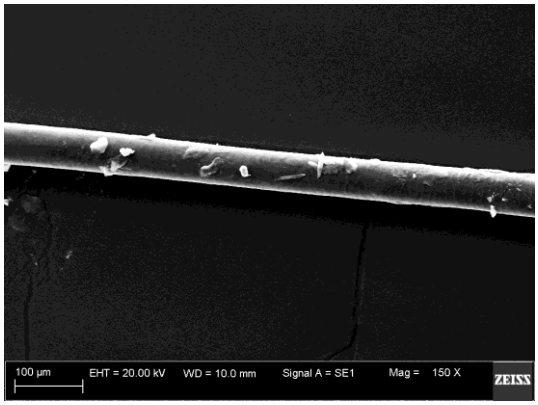


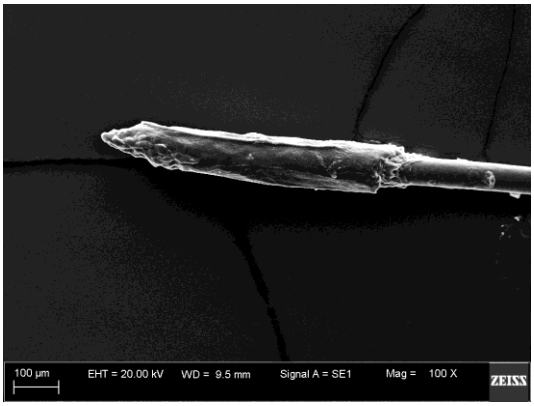
body side hairs

3.2. After treatment:









Extremity hairs

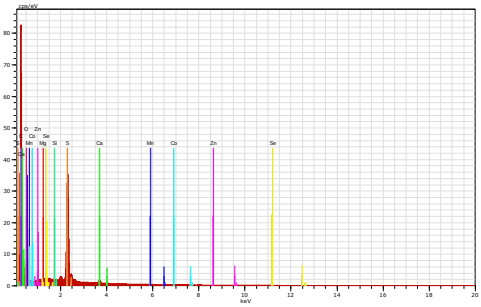
Elemental hair and skin analysis

Elemental analysis of the hairs was performed in peribulbal region of the given hair and on the skin surface. As far as mane hairs are concerned there were deficits of Mn observed. Within hairs coming from affected area of the skin there were low levels of S and Mn noticed. .

Mane hairs

Spectrum: Acquisition

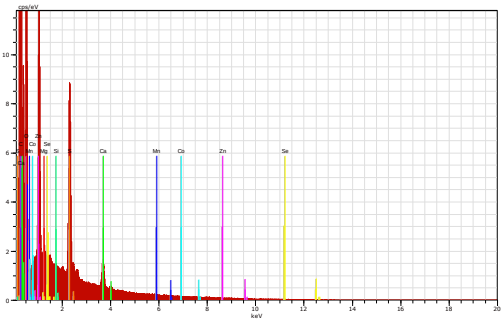
El	AN	Series	Net	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error [%]
C	6	K-series	443314	57.44	57.44	66.55	17.8
O	8	K-series	52185	34.70	34.70	30.18	11.1
S	16	K-series	345908	6.58	6.58	2.85	0.3
Ca	20	K-series	10175	0.32	0.32	0.11	0.0
Se	34	K-series	683	0.30	0.30	0.05	0.0
Si	14	K-series	11439	0.19	0.19	0.09	0.0
Mg	12	K-series	6037	0.16	0.16	0.09	0.0
Zn	30	K-series	1000	0.14	0.14	0.03	0.0
Co	27	K-series	1266	0.11	0.11	0.03	0.0
Mn	25	K-series	1075	0.06	0.06	0.02	0.0
		Total:		100.00	100.00	100.00	



Extremity hairs

Spectrum: Acquisition

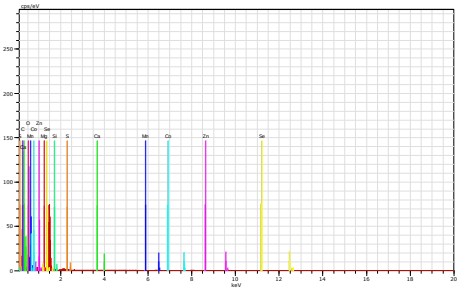
El	AN	Series	Net	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error [%]
C	6	K-series	887872	57.47	57.47	65.96	17.6
O	8	K-series	110938	37.15	37.15	32.01	11.6
S	16	K-series	85387	2.60	2.60	1.12	0.1
Se	34	K-series	495	0.91	0.91	0.16	0.1
Ca	20	K-series	13774	0.68	0.68	0.24	0.0
Mg	12	K-series	12483	0.53	0.53	0.30	0.1
Si	14	K-series	10456	0.28	0.28	0.14	0.0
Zn	30	K-series	587	0.17	0.17	0.04	0.0
Co	27	K-series	786	0.13	0.13	0.03	0.0
Mn	25	K-series	682	0.08	0.08	0.02	0.0
		Total:		100.00	100.00	100.00	



Skin surface

Spectrum: Acquisition

El	AN	Series	Net	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error [%]
O	8	K-series	111449	50.92	50.92	45.05	15.7
C	6	K-series	367242	45.86	45.86	54.05	14.1
Se	34	K-series	1730	0.86	0.86	0.15	0.1
Zn	30	K-series	4096	0.75	0.75	0.16	0.0
S	16	K-series	16135	0.65	0.65	0.29	0.0
Co	27	K-series	3176	0.40	0.40	0.10	0.0
Mn	25	K-series	2231	0.22	0.22	0.06	0.0
Si	14	K-series	5851	0.21	0.21	0.11	0.0
Ca	20	K-series	1873	0.12	0.12	0.04	0.0
Mg	12	K-series	4	0.00	0.00	0.00	0.0
		Total:		100.00	100.00	100.00	



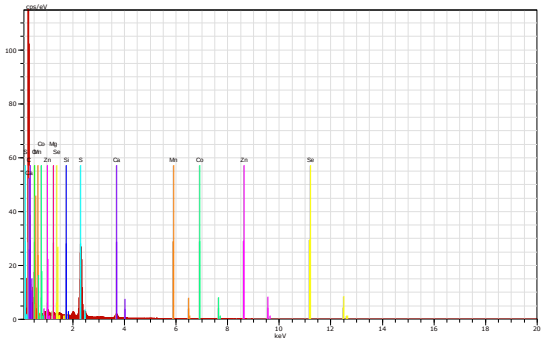
Elemental hair and skin analysis

Elemental analysis of the hairs was performed in peribulbal region of the given hair . As far as mane hairs are concerned there was deficit of, Mn observed . Within side body hairs the levels of Mn an Ca was low.

Mane hairs

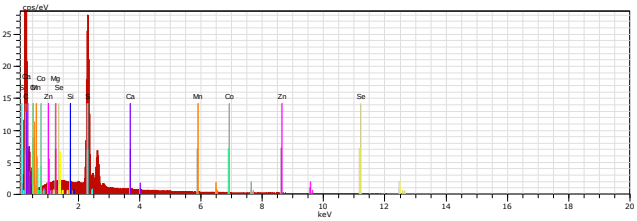
Spectrum: Acquisition

El	AN	Series	Net	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error [%]
C	6	K-series	662409	49.20	49.20	59.05	15.3
O	8	K-series	133636	40.72	40.72	36.69	12.9
S	16	K-series	265079	7.11	7.11	3.20	0.3
Ca	20	K-series	19257	0.93	0.93	0.33	0.1
Se	34	K-series	398	0.75	0.75	0.14	0.1
Mg	12	K-series	14301	0.54	0.54	0.32	0.1
Si	14	K-series	14630	0.34	0.34	0.17	0.0
Zn	30	K-series	760	0.21	0.21	0.05	0.0
Co	27	K-series	869	0.14	0.14	0.03	0.0
Mn	25	K-series	619	0.06	0.06	0.02	0.0
		Total:		100.00	100.00	100.00	



Extremity hairs

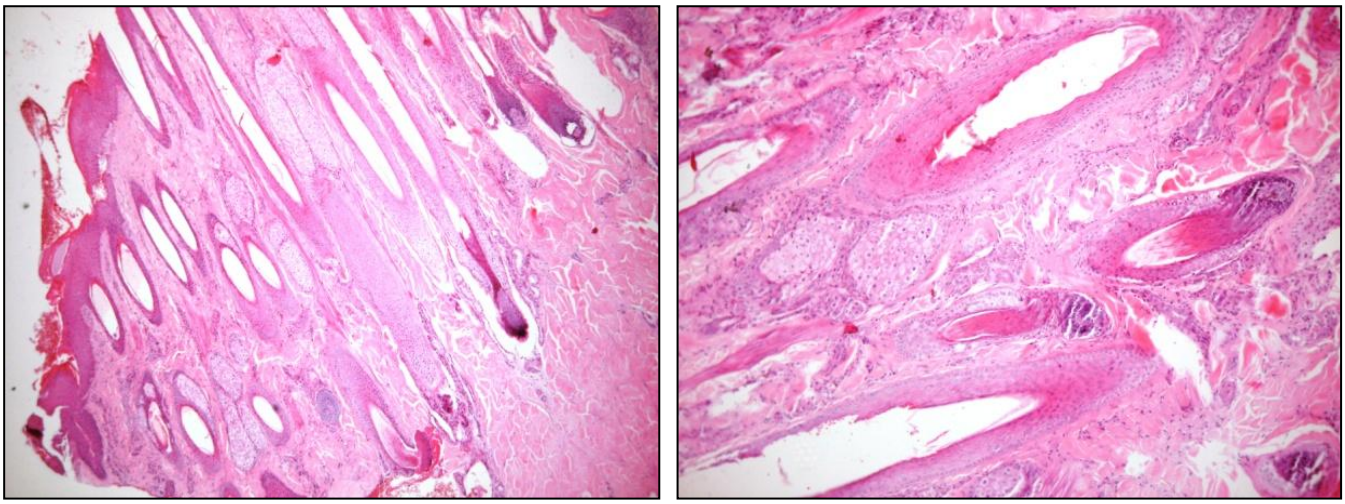
El	AN	Series	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error [%]
C	6	K-series	59.90	59.90	68.74	18.5
O	8	K-series	32.72	32.72	28.19	10.4
S	16	K-series	6.43	6.43	2.76	0.3
Se	34	K-series	0.24	0.24	0.04	0.0
Si	14	K-series	0.18	0.18	0.09	0.0
Mg	12	K-series	0.17	0.17	0.09	0.0
Zn	30	K-series	0.15	0.15	0.03	0.0
Co	27	K-series	0.10	0.10	0.02	0.0
Mn	25	K-series	0.07	0.07	0.02	0.0
Ca	20	K-series	0.04	0.04	0.01	0.0
		Total:	100.00	100.00	100.00	



3.1. Before treatment

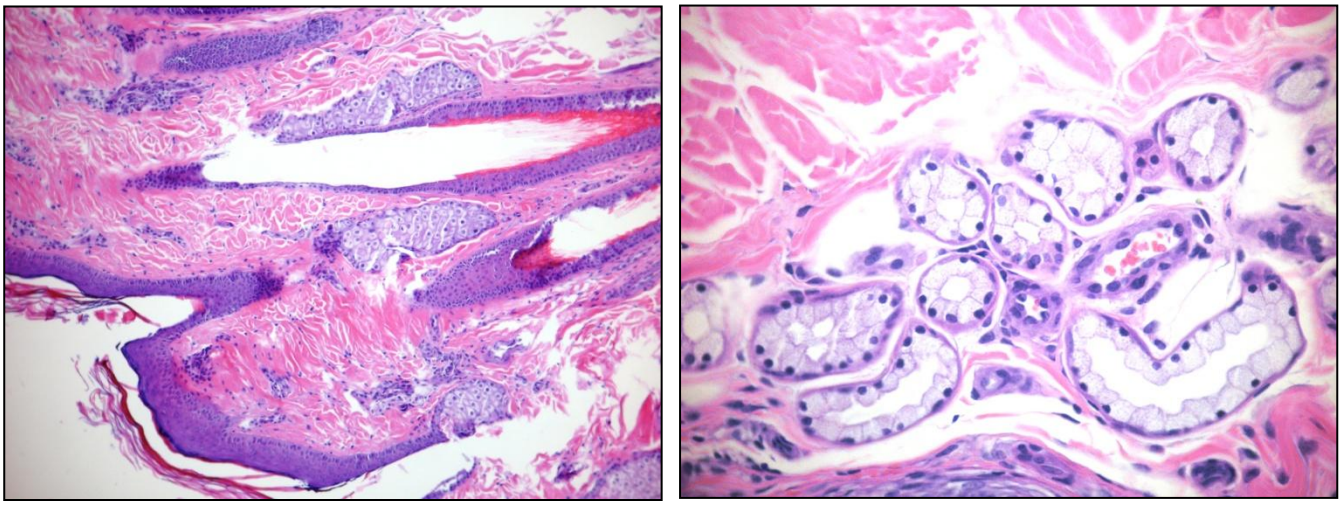
Dermatopathological skin examination

3.1. Before treatment: Dermatopathological examination of the skin reviled hyperkeratosis with focal marked parakeratosis. There were inflammatory infiltrate noticed around skin appendixes (hair follicles and glands) consisted mainly of fibroblasts and mast cells.



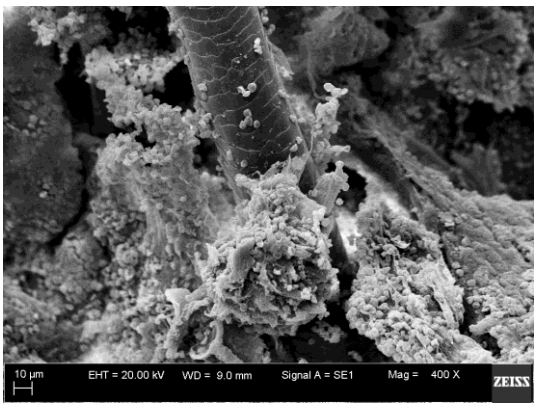
3.2. After treatment: Dermatopathological examination of the skin reviled mild hyperkeratosis of the epidermis. In the dermis area there was mild inflammatory infiltrate both in perivascular and subepidrmal compartment composed mainly from mononuclear cells. The periadnexal compartment showed no significant abnormalities.

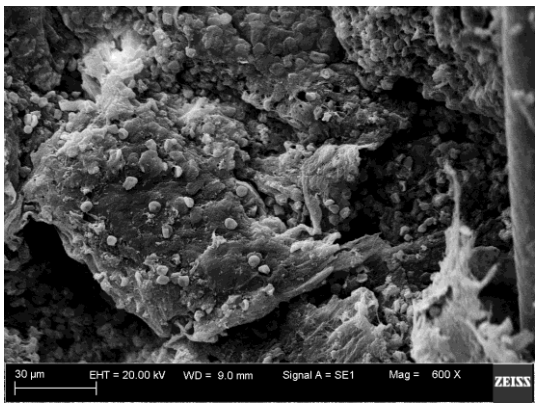
3.2. After treatment

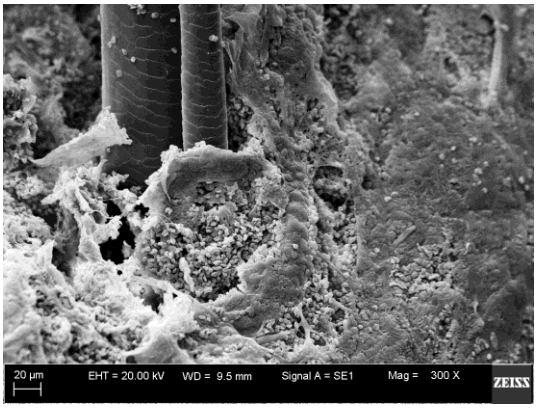


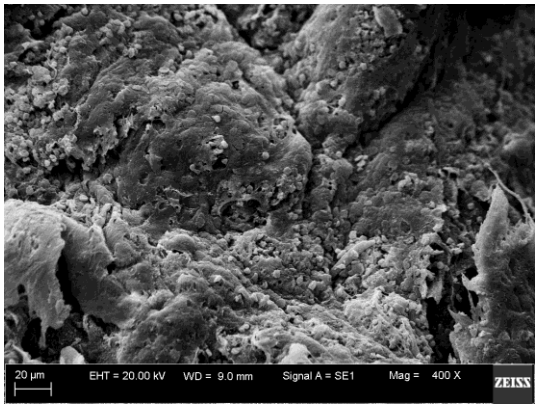
Scanning microscopy examination of the skin

3.1. Before treatment: SEM pictures of the surface of the skin coming from affected areas reviled yeasts organisms located both , close to the hair shafts and in some distance from them. There are also some red blood cells observed spread on the skin surface.

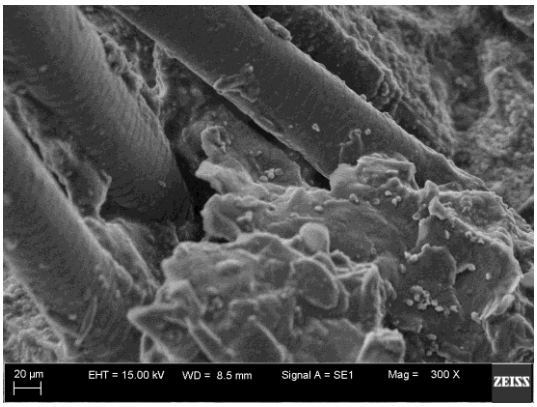


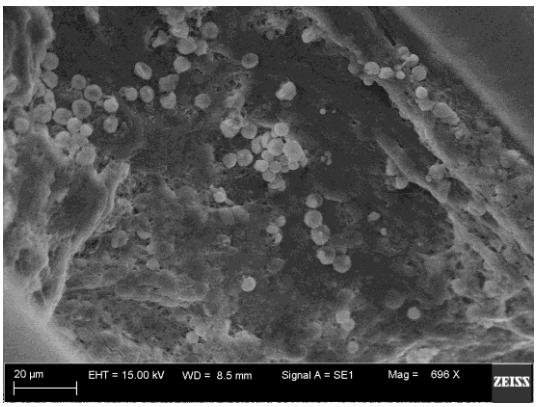


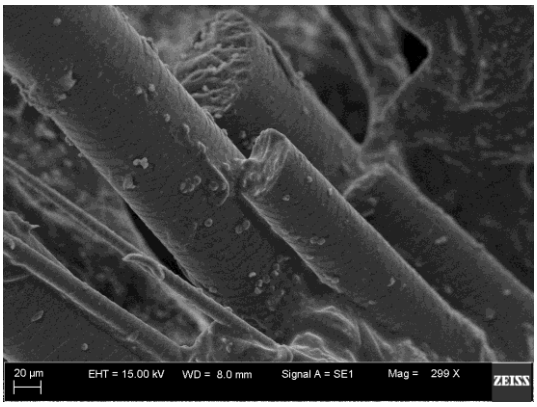


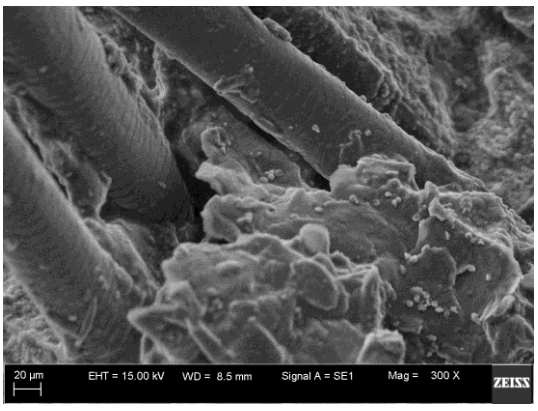


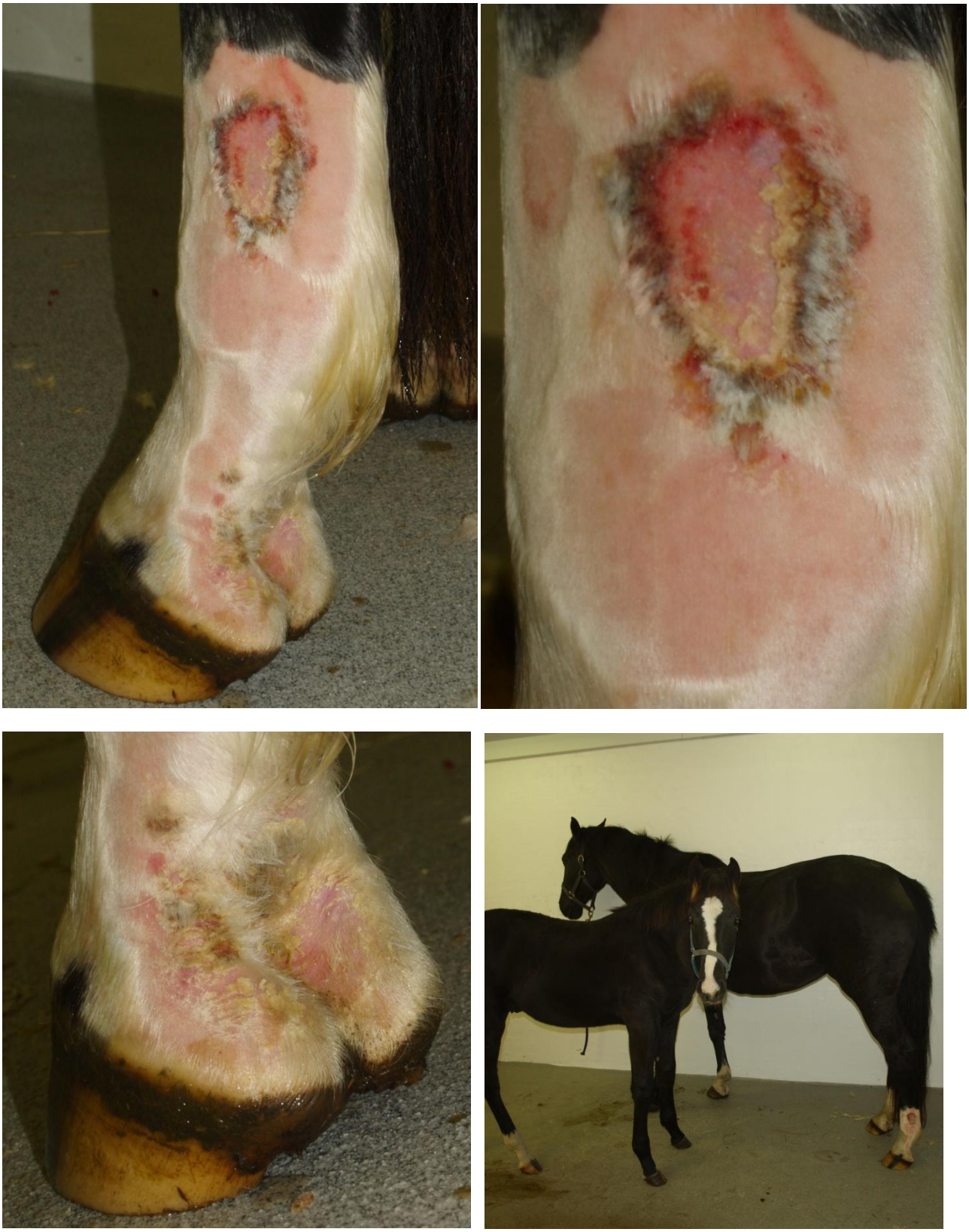
3.2. After treatment: SEM pictures of the surface of the skin coming from affected areas reviled small to moderate amounts of yeasts organisms located close to the hair shafts



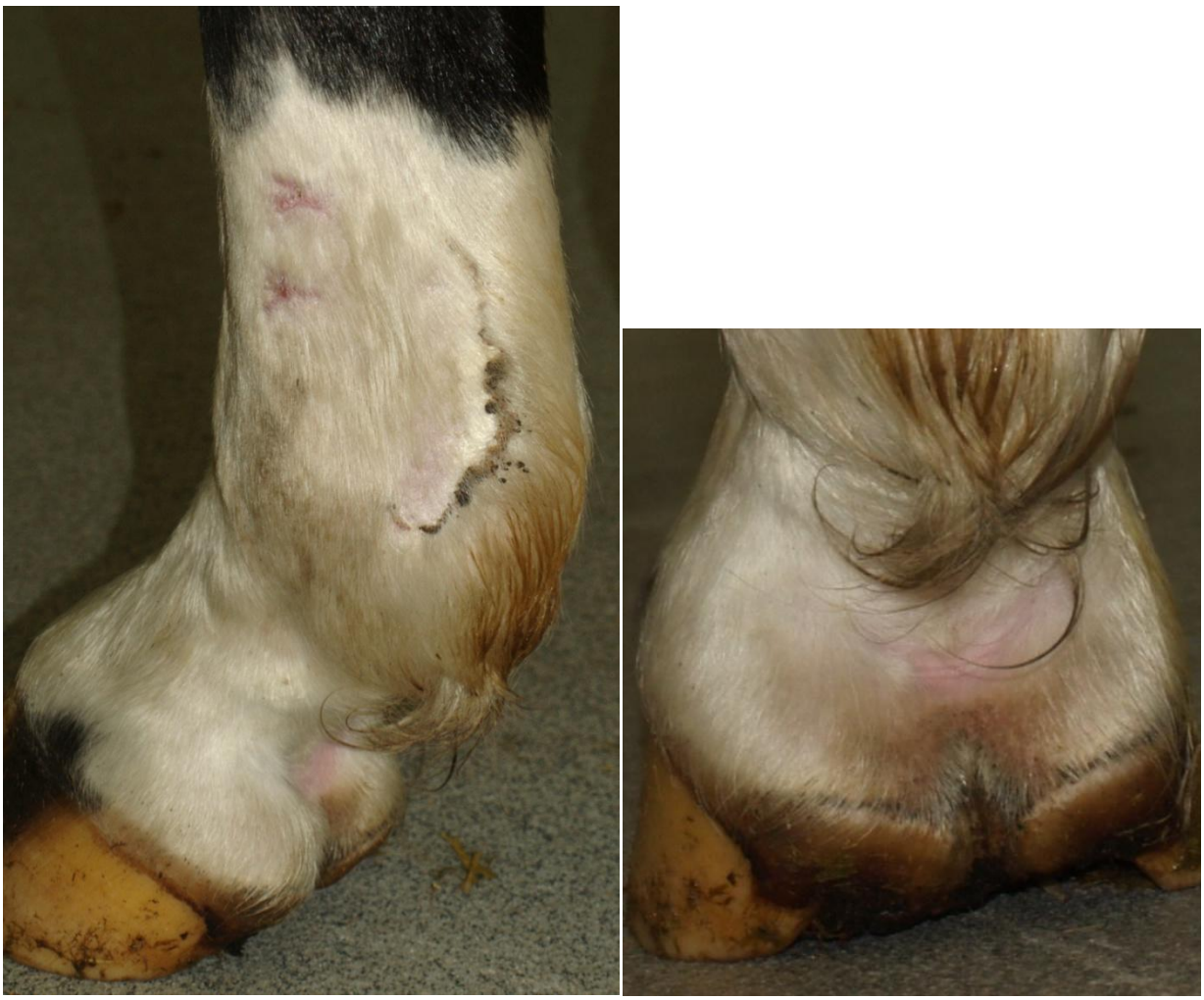








3.1. Clinical appearance before treatment. There is some swelling locally in areas were hyperkeratosis and superficiel dermatitis with central ulceration was visible.



3.2. Clinical appearance after treatment. The swelling has diminished and the profound dermatitis is healed. Only a border line of hyperkeratosis is visible.

Appendix – all results

Pracownia Mikroskopii Elektronowej
Uniwersytetu Przyrodniczego we Wrocławiu

50-375 Wrocław
ul. Kożuchowska 5b

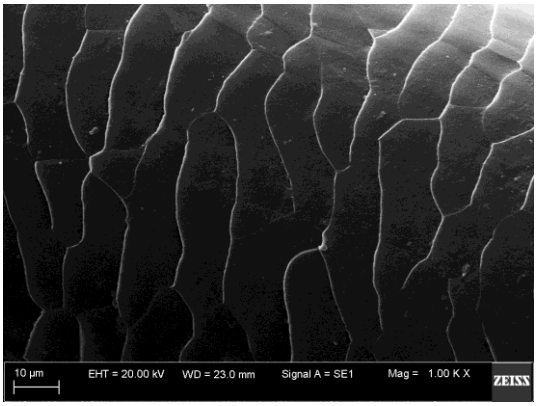
http://microscopy.ar.wroc.pl/

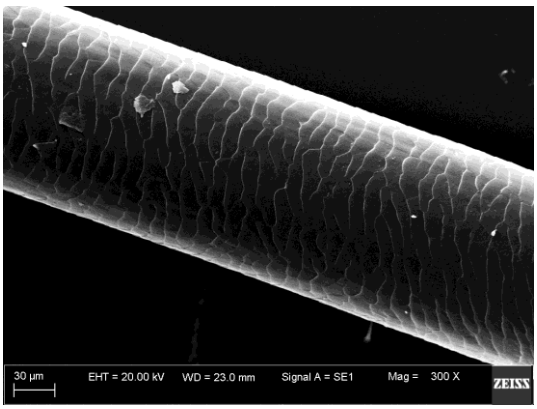
Patient: 4.1.horse, Mare
Responsible: dr Lene

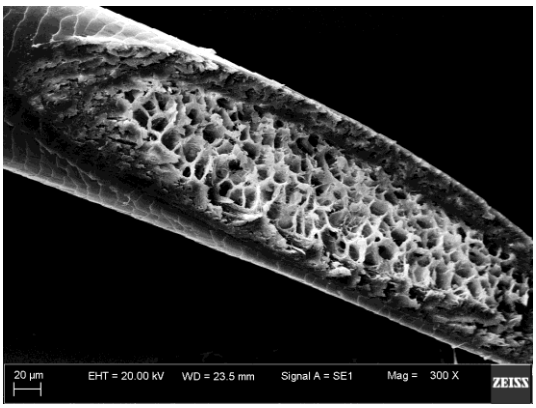
Scanning trichoscopy

The surface of the hair shafts coming from body side and mane of examined horse exhibited on scanning microscopy examination not regular distribution and focal absence of keratin scales of the hair cuticle. Moreover some of the body side hairs were unequal in their diameter. The medulla/hair shaft ratio of examine mane hairs were relatively high.

mane hairs before



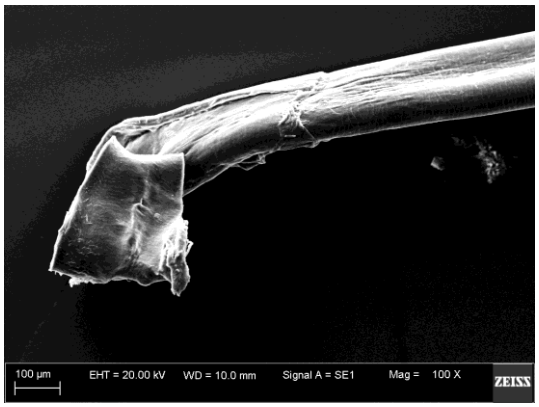


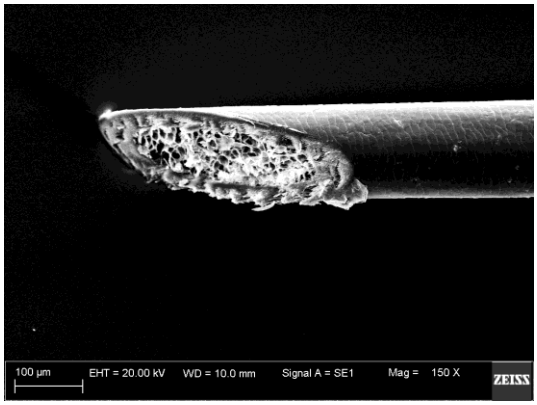


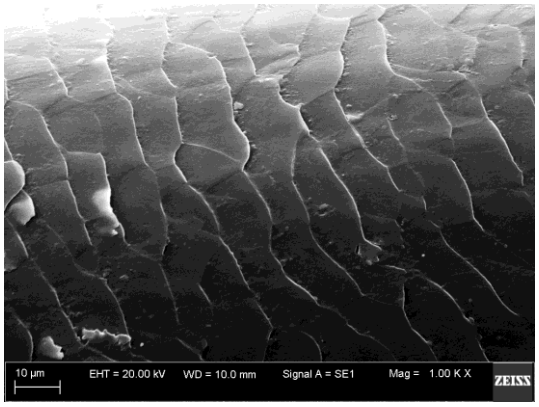
Appendix – all results

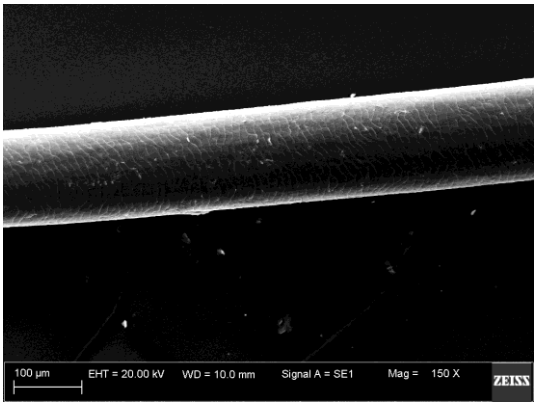
4.2. After treatment: SEM analysis of the hairs coming from mane reviled no serious changes of hair shafts shape but a reasonable amount of hair bulbs was in telogen phase. As far as body hairs are concerned there was irregular pattern of hair shaft cuticle keratin scales noticed.

Mane hairs



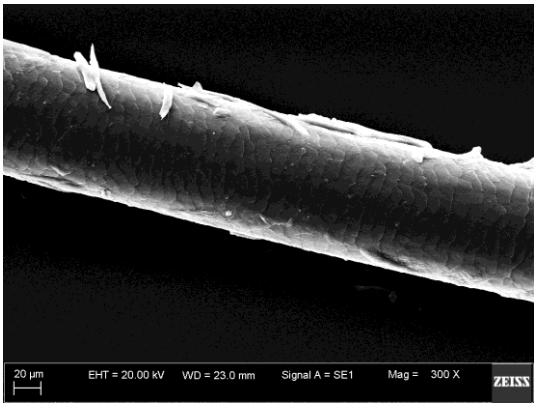


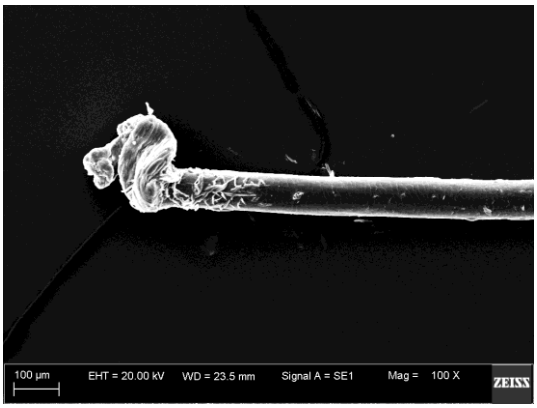


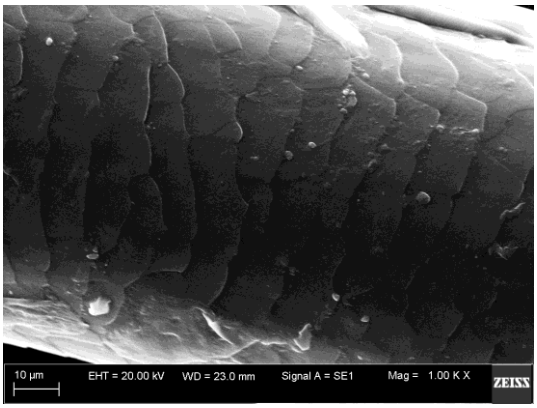


4.1. Before treatment

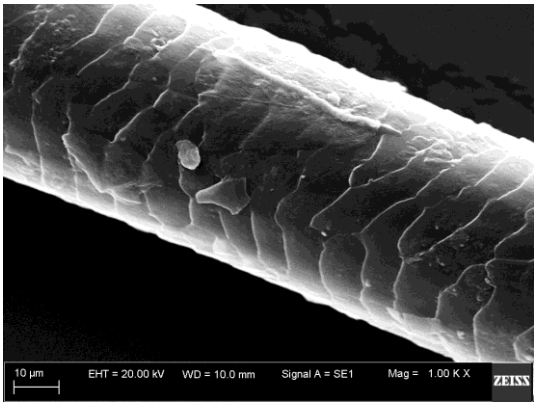
Extremity hairs

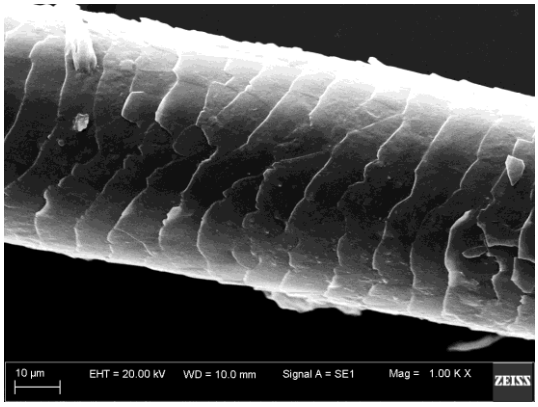


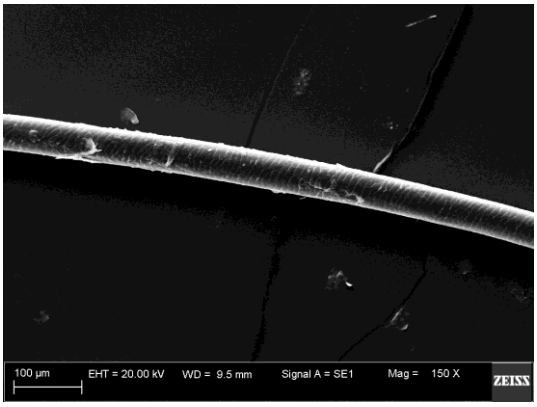




4.2. After treatment



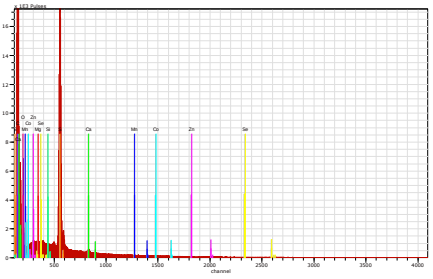




Elemental hair and skin analysis

Examination of mane hairs elemental composition revealed low levels of Mn. In case of hairs coming from lesional skin neighborhood there were low concentration of Mn, Co, Ca, Mg.

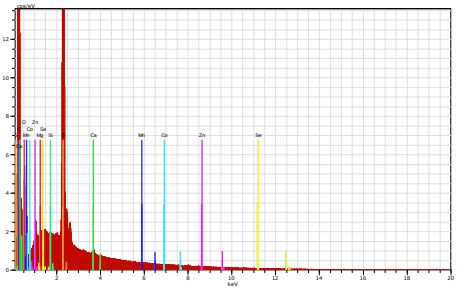
Mane



Spectrum: Acquisition

El	AN	Series	Net	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error [%]
C	6	K-series	680211	58.06	58.06	67.23	17.9
O	8	K-series	75790	33.71	33.71	29.31	10.7
S	16	K-series	347676	7.05	7.05	3.06	0.3
Se	34	K-series	547	0.30	0.30	0.05	0.0
Mg	12	K-series	8884	0.25	0.25	0.14	0.0
Ca	20	K-series	5902	0.22	0.22	0.08	0.0
Si	14	K-series	8654	0.15	0.15	0.07	0.0
Zn	30	K-series	687	0.11	0.11	0.02	0.0
Co	27	K-series	789	0.08	0.08	0.02	0.0
Mn	25	K-series	914	0.07	0.07	0.02	0.0
		Total:		100.00	100.00	100.00	

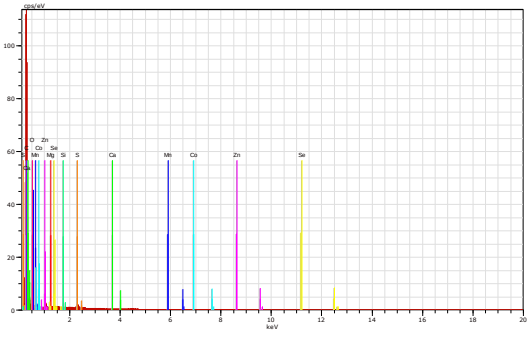
Extremity hairs



Spectrum: Acquisition

El	AN	Series	Net	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error [%]
C	6	K-series	485799	66.80	66.80	74.66	20.5
O	8	K-series	29903	27.48	27.48	23.06	8.8
S	16	K-series	250143	4.89	4.89	2.05	0.2
Se	34	K-series	650	0.27	0.27	0.05	0.0
Zn	30	K-series	1070	0.15	0.15	0.03	0.0
Si	14	K-series	6911	0.11	0.11	0.05	0.0
Ca	20	K-series	2699	0.08	0.08	0.03	0.0
Mg	12	K-series	3096	0.08	0.08	0.05	0.0
Co	27	K-series	954	0.08	0.08	0.02	0.0
Mn	25	K-series	838	0.05	0.05	0.01	0.0
		Total:		100.00	100.00	100.00	

Skin surface



Spectrum: Acquisition

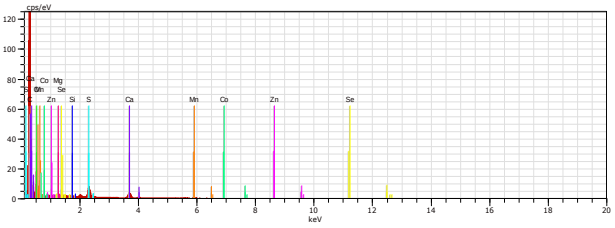
El	AN	Series	Net	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error [%]
C	6	K-series	628284	58.72	58.72	65.98	17.9
O	8	K-series	80440	39.73	39.73	33.51	12.3
S	16	K-series	13492	0.47	0.47	0.20	0.0
Se	34	K-series	464	0.31	0.31	0.05	0.0
Mn	25	K-series	1644	0.18	0.18	0.04	0.0
Mg	12	K-series	3411	0.17	0.17	0.09	0.0
Zn	30	K-series	600	0.14	0.14	0.03	0.0
Co	27	K-series	881	0.13	0.13	0.03	0.0
Si	14	K-series	3137	0.09	0.09	0.05	0.0
Ca	20	K-series	1046	0.06	0.06	0.02	0.0
		Total:		100.00	100.00	100.00	

Elemental hair and skin analysis

Elemental analysis of the hairs was performed in peribulbal region of the given hair and on the skin surface. As far as mane are concerned there were observed low level of S and slightly low Mn concentration In case of hairs collected from lesional skin there was low level of Ca and Mn noticed. With respect to skin samples analysis relatively high contains of S and Ca and low level of Si were indicated comparing with the beginning of the research.

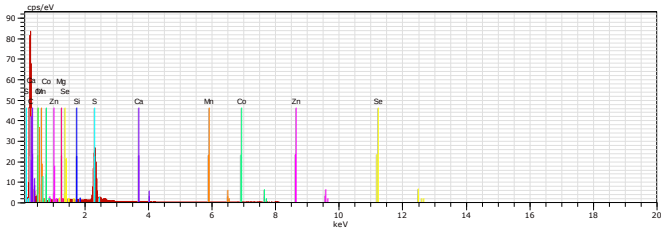
Mane hairs

El	AN	Series	net	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error [%]
C	6	K-series		57.03	57.03	65.91	17.5
O	8	K-series		36.45	36.45	31.62	11.5
Ca	20	K-series		2.11	2.11	0.73	0.1
S	16	K-series		1.94	1.94	0.84	0.1
Se	34	K-series		0.91	0.91	0.16	0.1
Mg	12	K-series		0.82	0.82	0.47	0.1
Si	14	K-series		0.38	0.38	0.19	0.0
Zn	30	K-series	999	0.16	0.16	0.03	0.0
Co	27	K-series		0.11	0.11	0.03	0.0
Mn	25	K-series		0.08	0.08	0.02	0.0
		Total:		100.00	100.00	100.00	



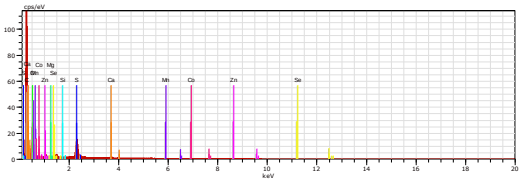
Extremity hairs

El	AN	Series	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error [%]
C	6	K-series	59.73	59.73	68.83	18.5
O	8	K-series	32.05	32.05	27.73	10.3
S	16	K-series	7.18	7.18	3.10	0.3
Se	34	K-series	0.26	0.26	0.05	0.0
Mg	12	K-series	0.19	0.19	0.11	0.0
Si	14	K-series	0.18	0.18	0.09	0.0
Zn	30	K-series	0.16	0.16	0.03	0.0
Co	27	K-series	0.11	0.11	0.02	0.0
Mn	25	K-series	0.07	0.07	0.02	0.0
Ca	20	K-series	0.07	0.07	0.02	0.0
		Total:	100.00	100.00	100.00	



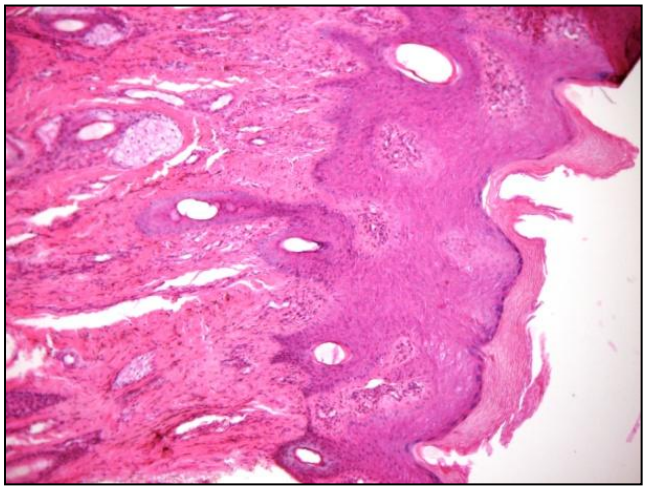
Skin surface

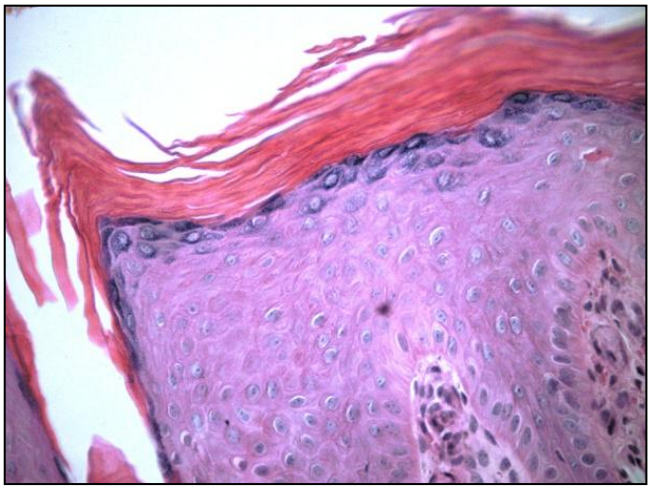
El	AN	Series	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error [%]
C	6	K-series	48.53	48.53	56.59	14.9
O	8	K-series	48.02	48.02	42.04	14.8
S	16	K-series	2.69	2.69	1.18	0.1
Ca	20	K-series	0.25	0.25	0.09	0.0
Se	34	K-series	0.23	0.23	0.04	0.0
Zn	30	K-series	0.13	0.13	0.03	0.0
Co	27	K-series	0.08	0.08	0.02	0.0
Mn	25	K-series	0.04	0.04	0.01	0.0
Mg	12	K-series	0.01	0.01	0.01	0.0
Si	14	K-series	0.00	0.00	0.00	0.0
		Total:	100.00	100.00	100.00	



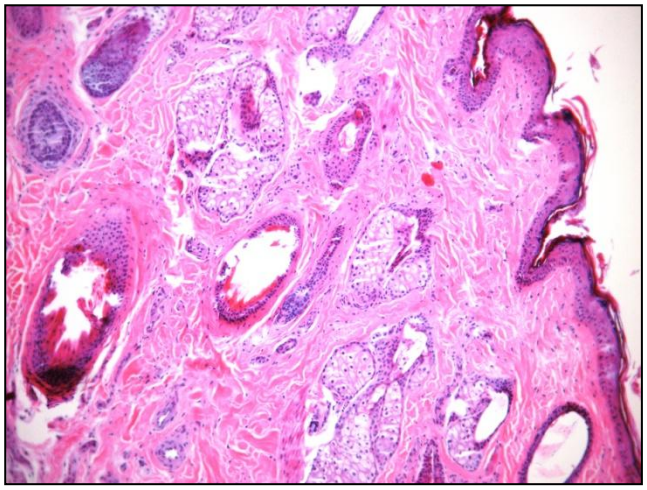
Dermatopathological skin examination

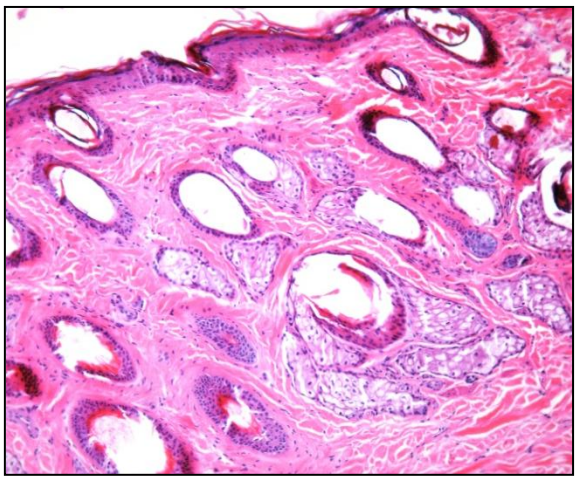
4.1. Before treatment: Histopatological examination of the skin samples revealed marked hyperkeratosis (compact to lamellar) and hyperpigmentation of the epidermis. In dermis area, particularly in subepidermal compartment there were focal fibrosis observed. Skin inflammatory infiltrate was not very abundant and consisted mainly of lymphocytes and fibroblasts.





4.2. After treatment: Dermatopathological examination of the skin reviled mild hyperkeratosis of the epidermis. There was also features of hyper pigmentation observed in some areas of the sample. In the dermis inflammatory cell infiltrate was minimal.

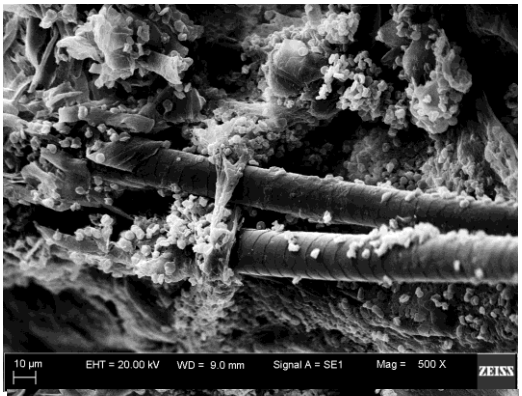


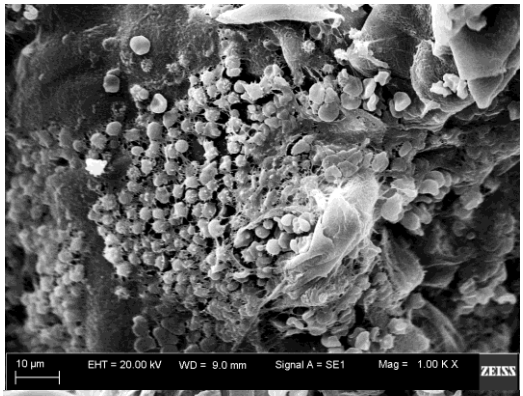


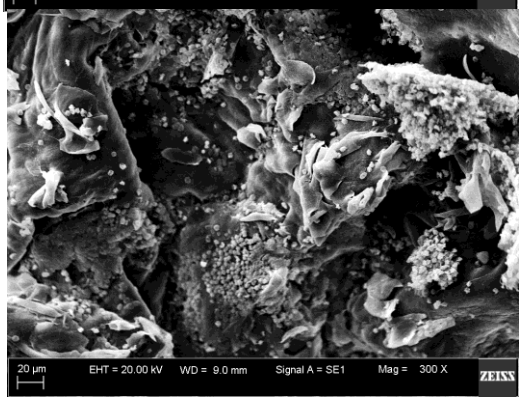
Appendix – all results

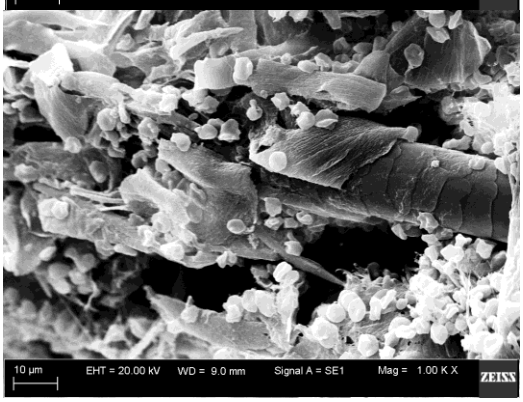
Scanning microscopy examination of the skin

4.1. Before treatment: Scanning electron examination of the lesional skin surface was performed and revealed considerable quantities of yeasts organism mainly in the close proximity to hair shafts areas. There were also blood cells observed but not very abundant.

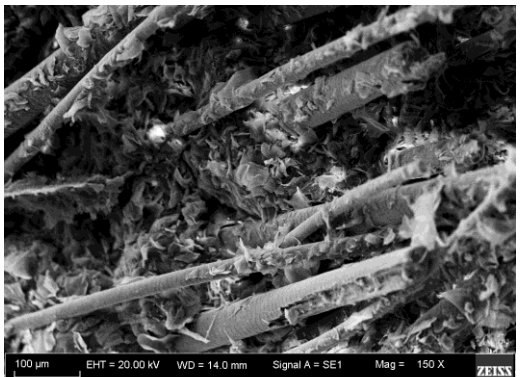


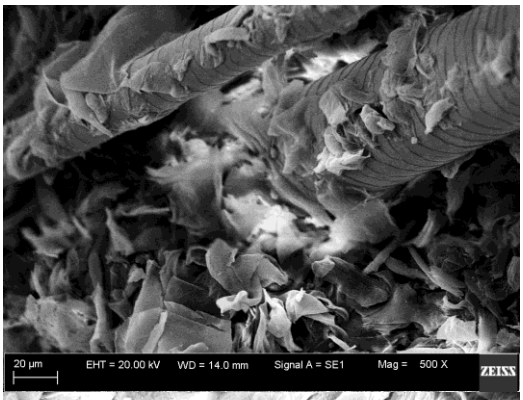


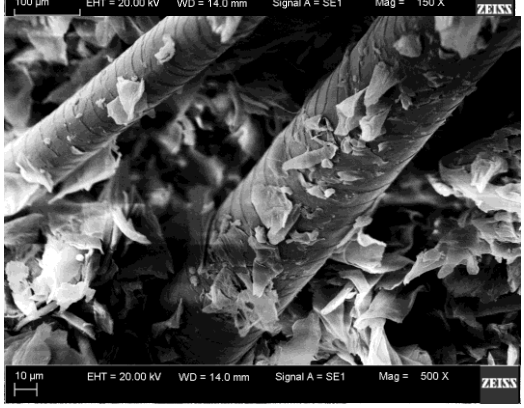


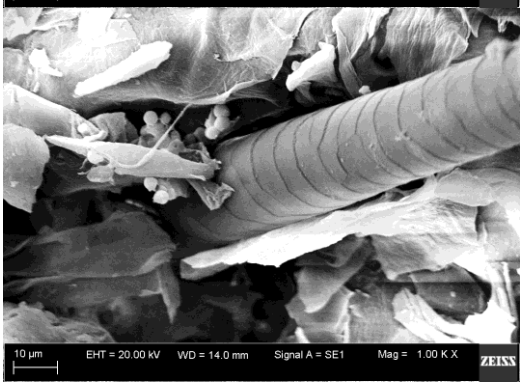


4.2. After treatment: SEM pictures of the surface of the skin coming from affected areas reviled moderate to small quantities of yeasts organisms located mainly in some distance from the hair shafts within desquamated epithelium.



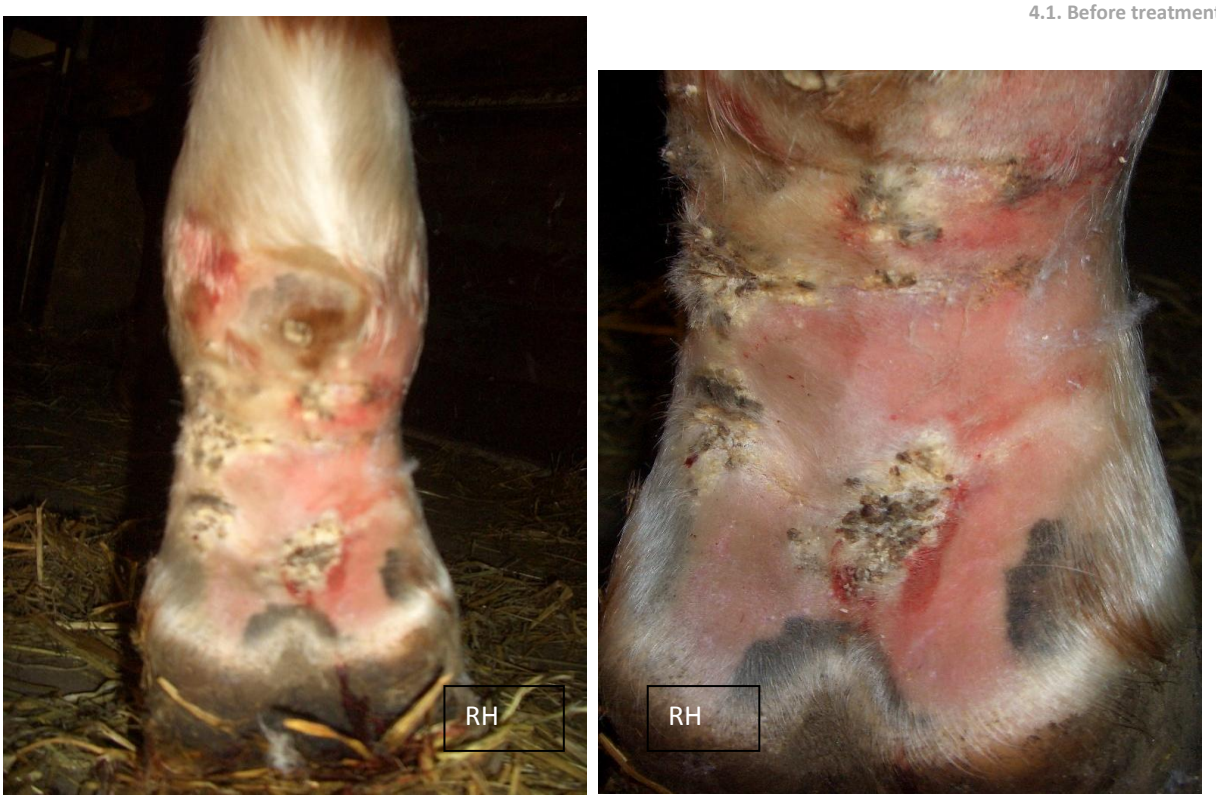




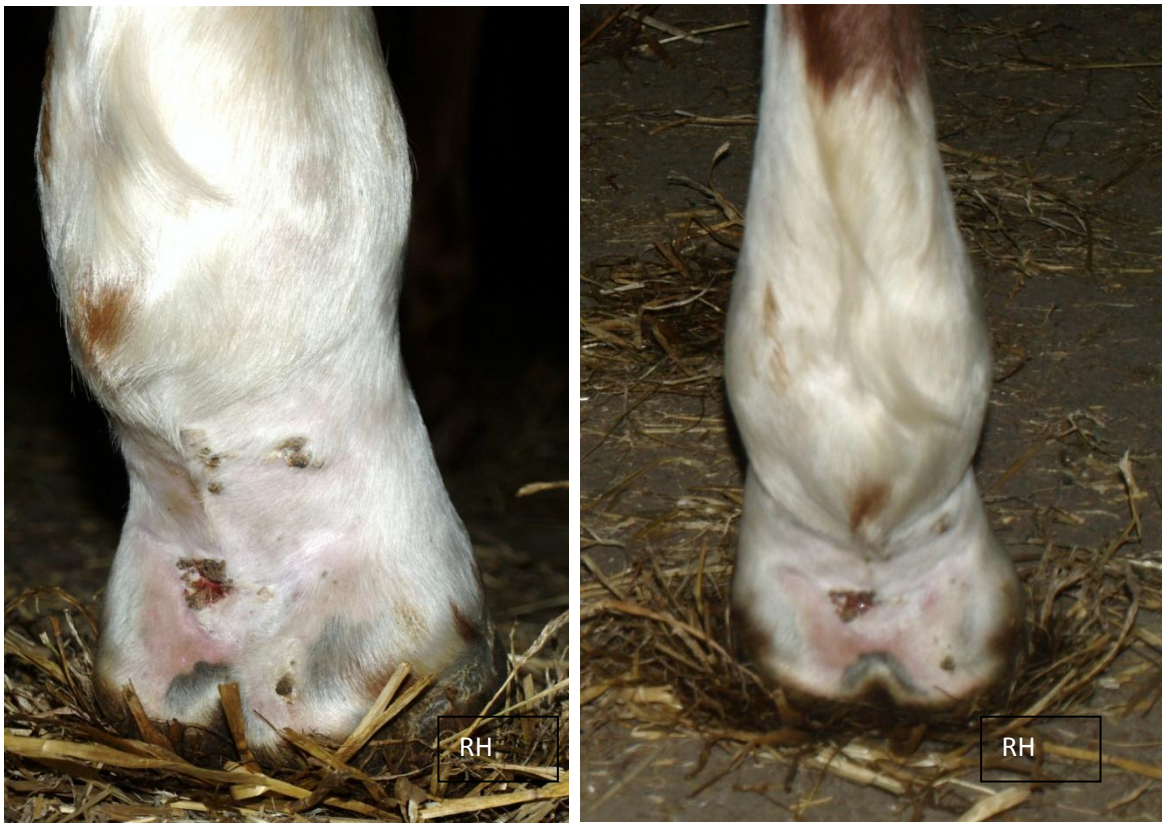


Appendix – all results

4.1. Before treatment



4.1. Clinical appearance before treatment. There is some swelling locally in areas were hyperkeratosis and profound dermatitis was visible.



4.2. Clinical appearance after treatment. The swelling has disappeared but there still are small remaining areas with hyperkeratosis and superficial dermatitis.

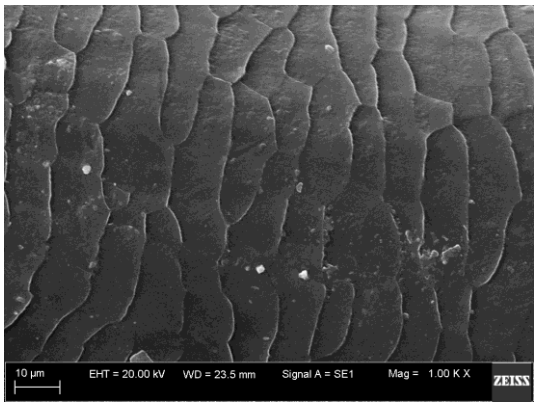
Pracownia Mikroskopii Elektronowej
Uniwersytetu Przyrodniczego we Wrocławiu
50-375 Wrocław
ul. Kożuchowska 5b
http://microscopy.ar.wroc.pl/

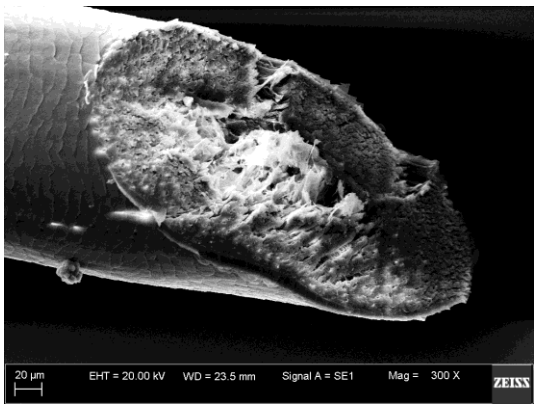
Patient: 5.1. Horse, mare
Responsible: dr Lene

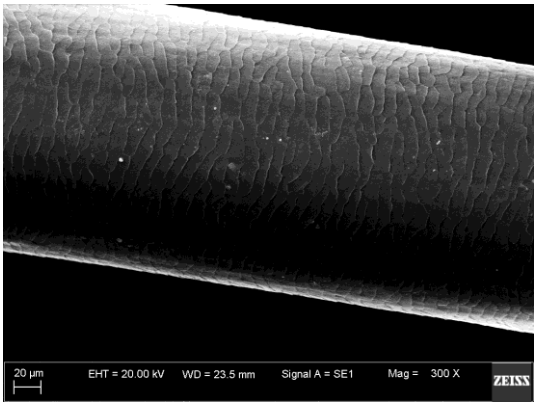
Scanning trichoscopy

5.1. Before treatment: SEM examination of the hair shafts and hair cross-sections revealed some abnormalities. With respect to both, mane and side body hairs there were irregular arrangement of keratin scales of hair cuticle observed. Additionally in case of mane hairs cross-sections there was relatively low medulla/hair shaft ratio noticed.

Mane hairs



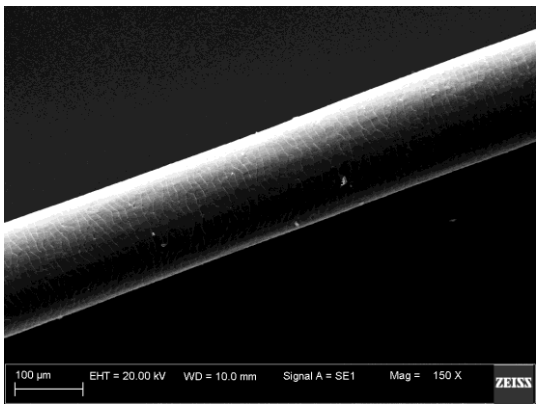


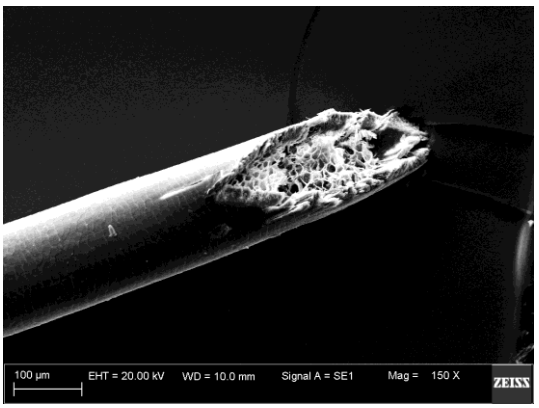


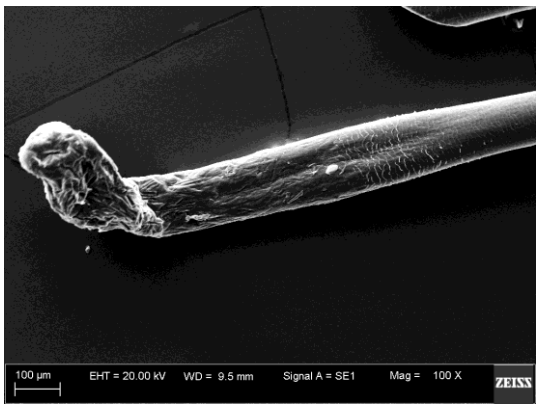
Appendix – all results

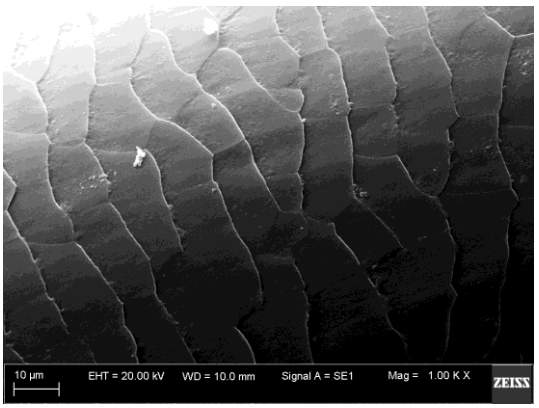
5.2. After treatment: SEM analysis of the hairs coming from mane reviled no serious changes of hair shafts shape but a reasonable amount of hair bulbs was in telogen phase. As far as body hairs are concerned there was irregular pattern of hair shaft cuticle keratin scales noticed.

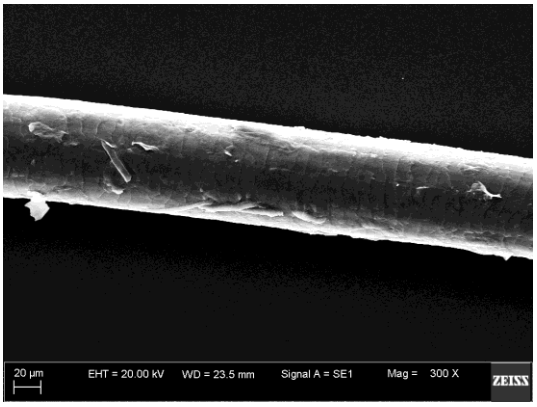
Mane hairs

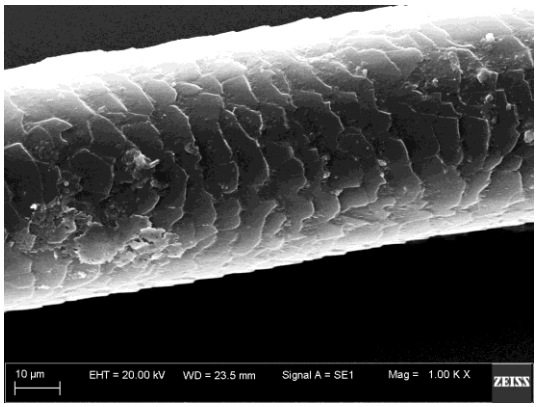


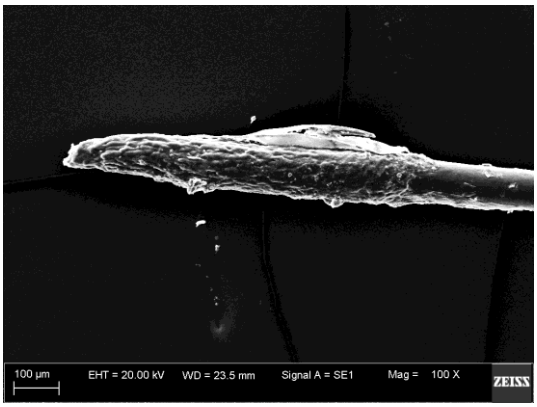








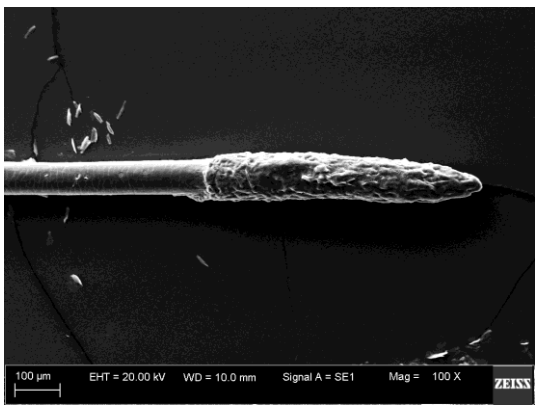


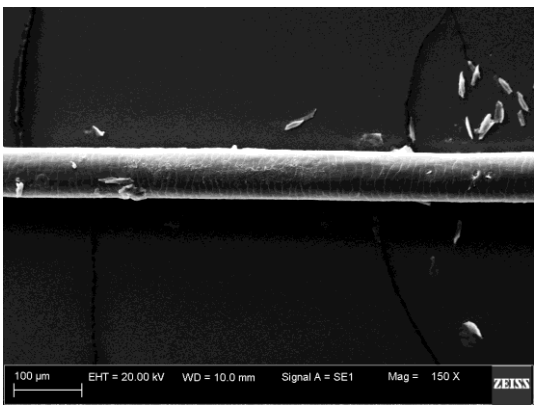


Extremity hairs

5.1. Before treatment

5.2. After treatment





Appendix – all results

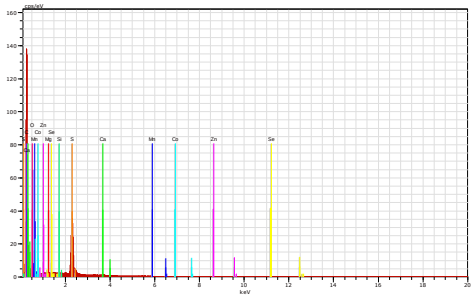
Elemental hair and skin analysis

Elemental analysis of peribulbar areas of horse hairs coming from the mane revealed low level of Mn. With respect to body side hairs there were low levels of Mn and Ca observed.

Mane

Spectrum: Acquisition

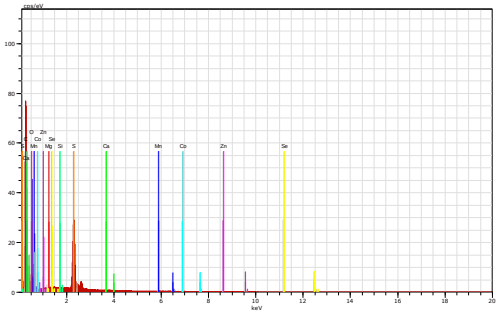
El	AN	Series	Net	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error [%]
C	6	K-series	754266	67.64	67.64	75.74	20.8
O	8	K-series	41626	25.62	25.62	21.54	8.2
S	16	K-series	344979	5.71	5.71	2.40	0.2
Se	34	K-series	597	0.33	0.33	0.06	0.0
Ca	20	K-series	6761	0.19	0.19	0.06	0.0
Mg	12	K-series	7405	0.16	0.16	0.09	0.0
Si	14	K-series	9986	0.14	0.14	0.07	0.0
Zn	30	K-series	704	0.10	0.10	0.02	0.0
Co	27	K-series	812	0.07	0.07	0.02	0.0
Mn	25	K-series	917	0.05	0.05	0.01	0.0
		Total:		100.00	100.00	100.00	



Extremity hairs

Spectrum: Acquisition

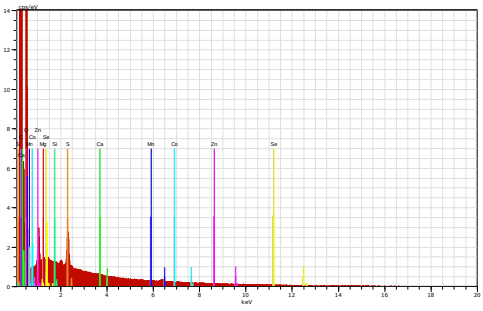
El	AN	Series	Net	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error [%]
C	6	K-series	405357	58.89	58.89	67.74	18.2
O	8	K-series	43918	33.91	33.91	29.29	10.8
S	16	K-series	299995	6.30	6.30	2.72	0.3
Se	34	K-series	627	0.35	0.35	0.06	0.0
Zn	30	K-series	817	0.13	0.13	0.03	0.0
Si	14	K-series	7239	0.13	0.13	0.06	0.0
Mg	12	K-series	3284	0.10	0.10	0.05	0.0
Co	27	K-series	900	0.09	0.09	0.02	0.0
Mn	25	K-series	988	0.07	0.07	0.02	0.0
Ca	20	K-series	1141	0.04	0.04	0.01	0.0
		Total:		100.00	100.00	100.00	



Skin surface

Spectrum: Acquisition

El	AN	Series	Net	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error [%]
C	6	K-series	757130	56.28	56.28	63.60	17.1
O	8	K-series	115741	42.35	42.35	35.93	13.1
S	16	K-series	17462	0.49	0.49	0.21	0.0
Se	34	K-series	550	0.23	0.23	0.04	0.0
Mn	25	K-series	2366	0.18	0.18	0.05	0.0
Mg	12	K-series	3611	0.14	0.14	0.08	0.0
Zn	30	K-series	683	0.11	0.11	0.02	0.0
Co	27	K-series	955	0.10	0.10	0.02	0.0
Si	14	K-series	2899	0.07	0.07	0.03	0.0
Ca	20	K-series	1211	0.05	0.05	0.02	0.0
		Total:		100.00	100.00	100.00	

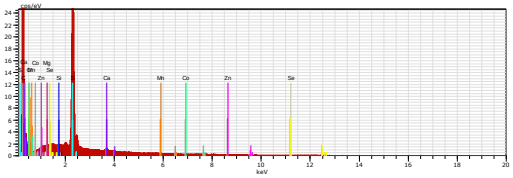


Elemental hair and skin analysis

Elemental analysis of the hairs was performed in peribulbal region of the given hair and on the skin surface. As far as mane hairs are concerned there were observed low levels of Mn and Mg .In case of hairs from extremities low levels of Ca and Mn were detected .

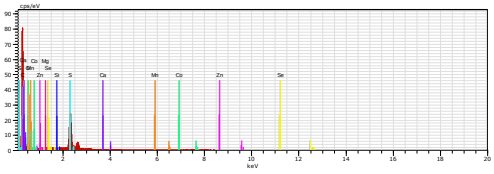
Mane hairs

El	AN	Series	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error [%]
C	6	K-series	61.65	61.65	70.30	19.0
O	8	K-series	31.31	31.31	26.80	10.1
S	16	K-series	6.18	6.18	2.64	0.2
Se	34	K-series	0.24	0.24	0.04	0.0
Ca	20	K-series	0.16	0.16	0.05	0.0
Zn	30	K-series	0.12	0.12	0.03	0.0
Si	14	K-series	0.11	0.11	0.06	0.0
Co	27	K-series	0.09	0.09	0.02	0.0
Mg	12	K-series	0.07	0.07	0.04	0.0
Mn	25	K-series	0.07	0.07	0.02	0.0
		Total:	100.00	100.00	100.00	



Extremity hairs

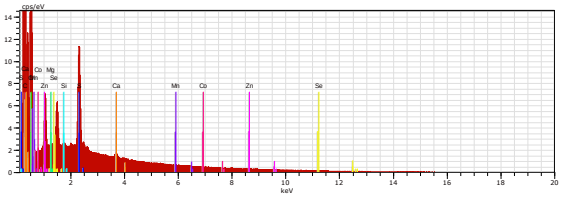
El	AN	Series	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error [%]
C	6	K-series	61.63	61.63	70.32	19.0
O	8	K-series	31.22	31.22	26.74	10.0
S	16	K-series	6.15	6.15	2.63	0.2
Se	34	K-series	0.32	0.32	0.05	0.0
Si	14	K-series	0.18	0.18	0.09	0.0
Mg	12	K-series	0.17	0.17	0.09	0.0
Zn	30	K-series	0.12	0.12	0.03	0.0
Co	27	K-series	0.10	0.10	0.02	0.0
Mn	25	K-series	0.08	0.08	0.02	0.0
Ca	20	K-series	0.03	0.03	0.01	0.0
		Total:	100.00	100.00	100.00	



Skin surface

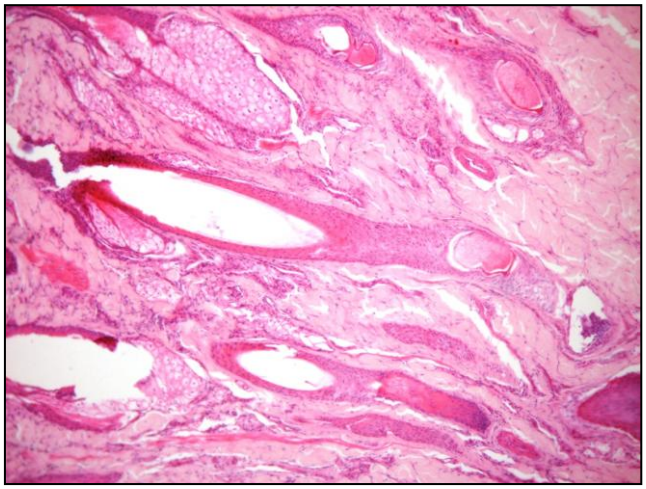
As far as skin elemental composition is concerned reduction of Mg, Zn, Si and Se and rise of S and Ca was noticed comparing to the first examination.

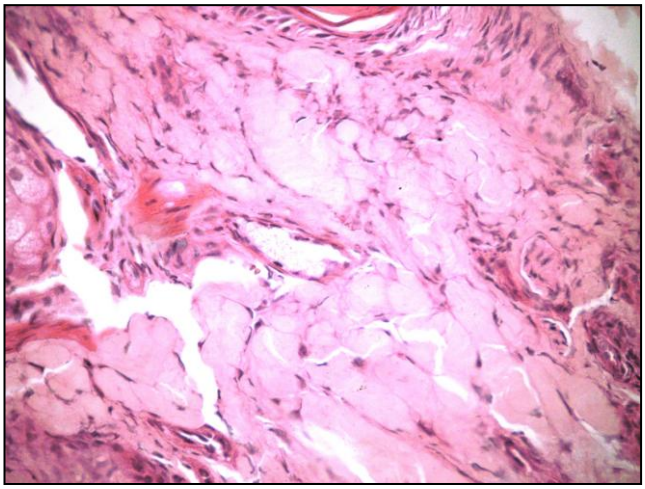
El	AN	Series	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error [%]
C	6	K-series	55.07	55.07	62.52	16.7
O	8	K-series	43.24	43.24	36.85	13.3
S	16	K-series	1.21	1.21	0.51	0.1
Se	34	K-series	0.18	0.18	0.03	0.0
Ca	20	K-series	0.10	0.10	0.03	0.0
Zn	30	K-series	0.07	0.07	0.01	0.0
Co	27	K-series	0.06	0.06	0.01	0.0
Mn	25	K-series	0.04	0.04	0.01	0.0
Mg	12	K-series	0.02	0.02	0.01	0.0
Si	14	K-series	0.01	0.01	0.00	0.0
		Total:	100.00	100.00	100.00	



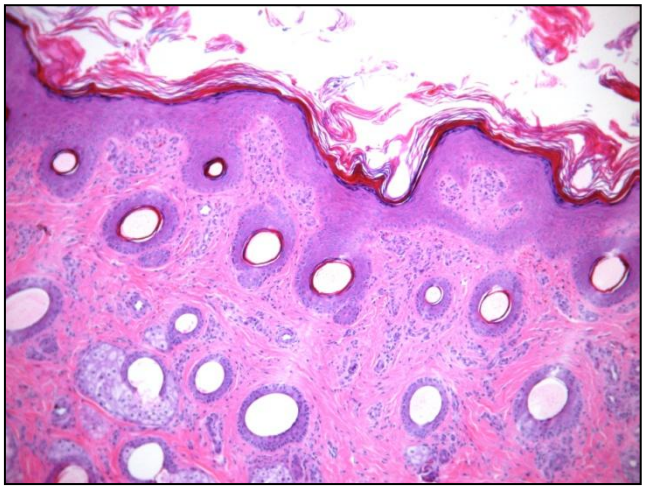
Dermatopathological skin examination

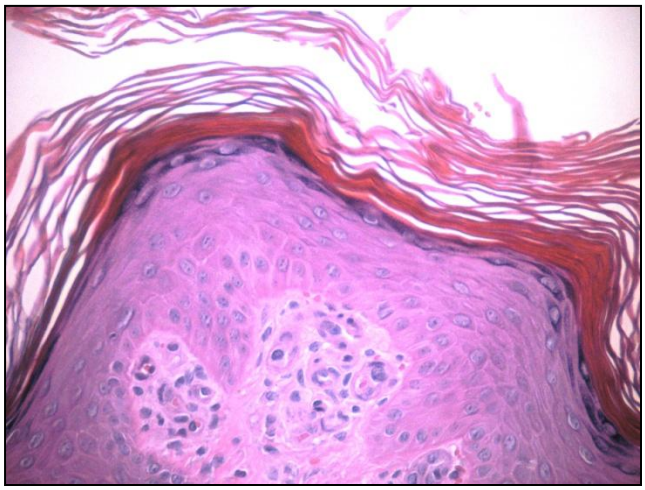
5.1. Before treatment: During dermatopathological examination of the lesional skin moderate hyperkeratosis and hyperpigmentation was observed. Inflammatory infiltrates moderate to significant in their intensity were observed within perivascular and periadnexal dermis compartments. There was also some dermal edema noticed. Sebaceous glands were focally hypertrophic.





5.2. After treatment: Dermatopathological examination of the skin reviled mild hyperkeratosis of the epidermis. There were also features of hyperpigmentation observed. In the dermis inflammatory cell infiltrate was absent.

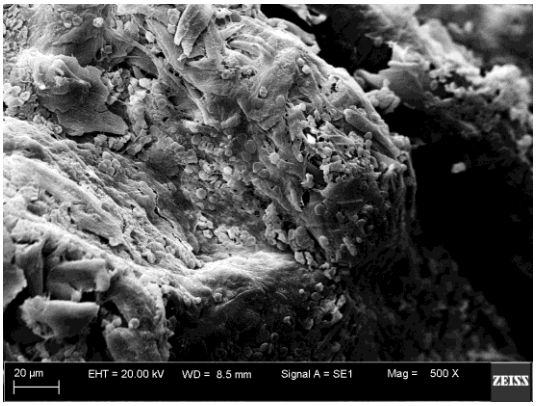


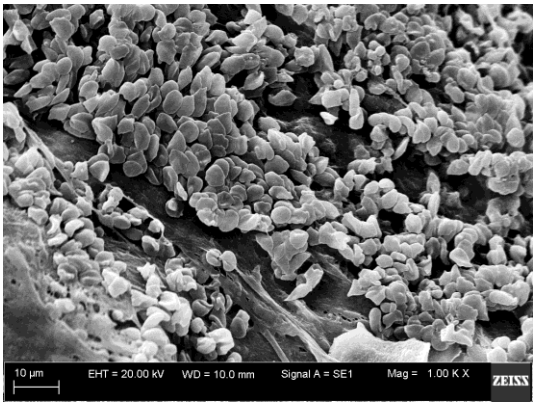


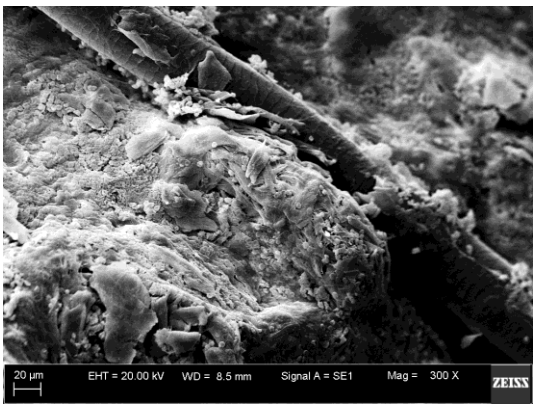
Appendix – all results

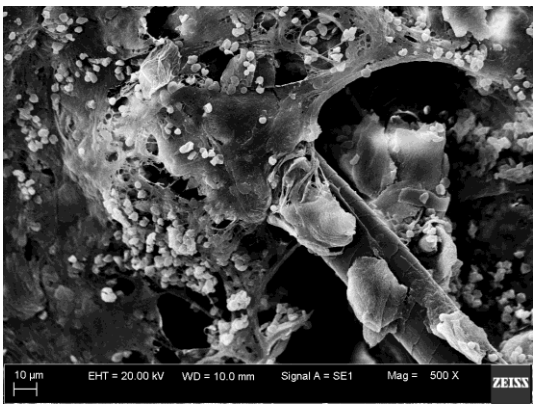
Scanning microscopy examination of the skin

5.1. Before treatment: Scanning electron examination of the skin sample reviled significant quantities of yeasts organisms both in the close proximity to hair shafts as well as over all skin surface .

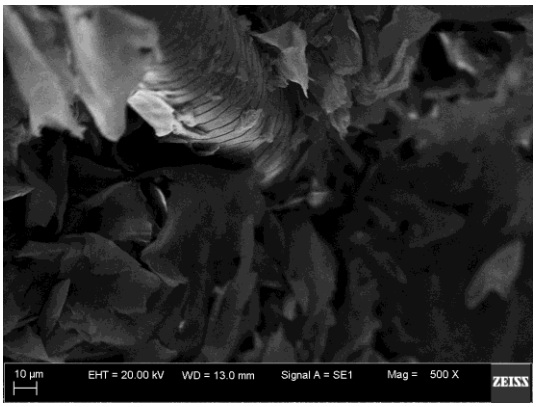


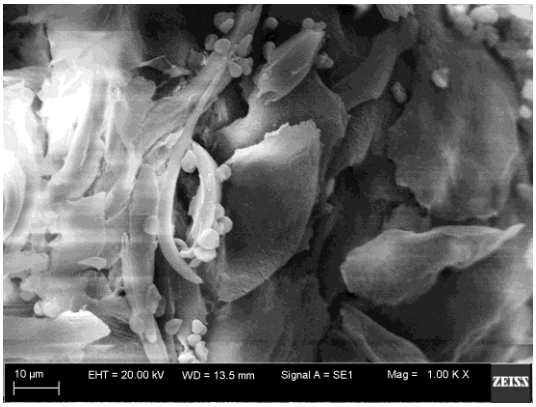


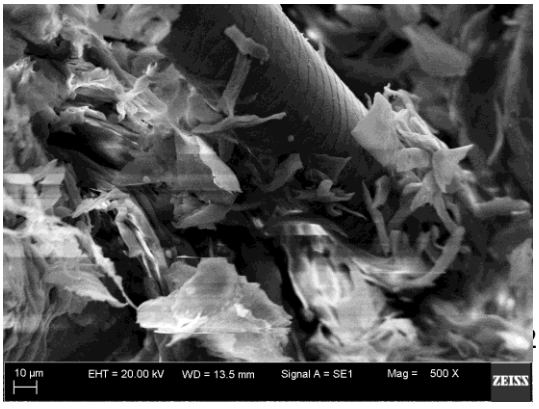


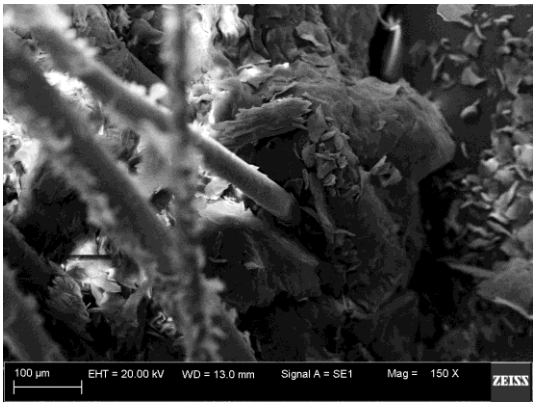


5.2. After treatment: SEM pictures of the surface of the skin coming from affected areas reviled moderate to small quantities of yeasts organisms located mainly in some distance from the hair shafts.

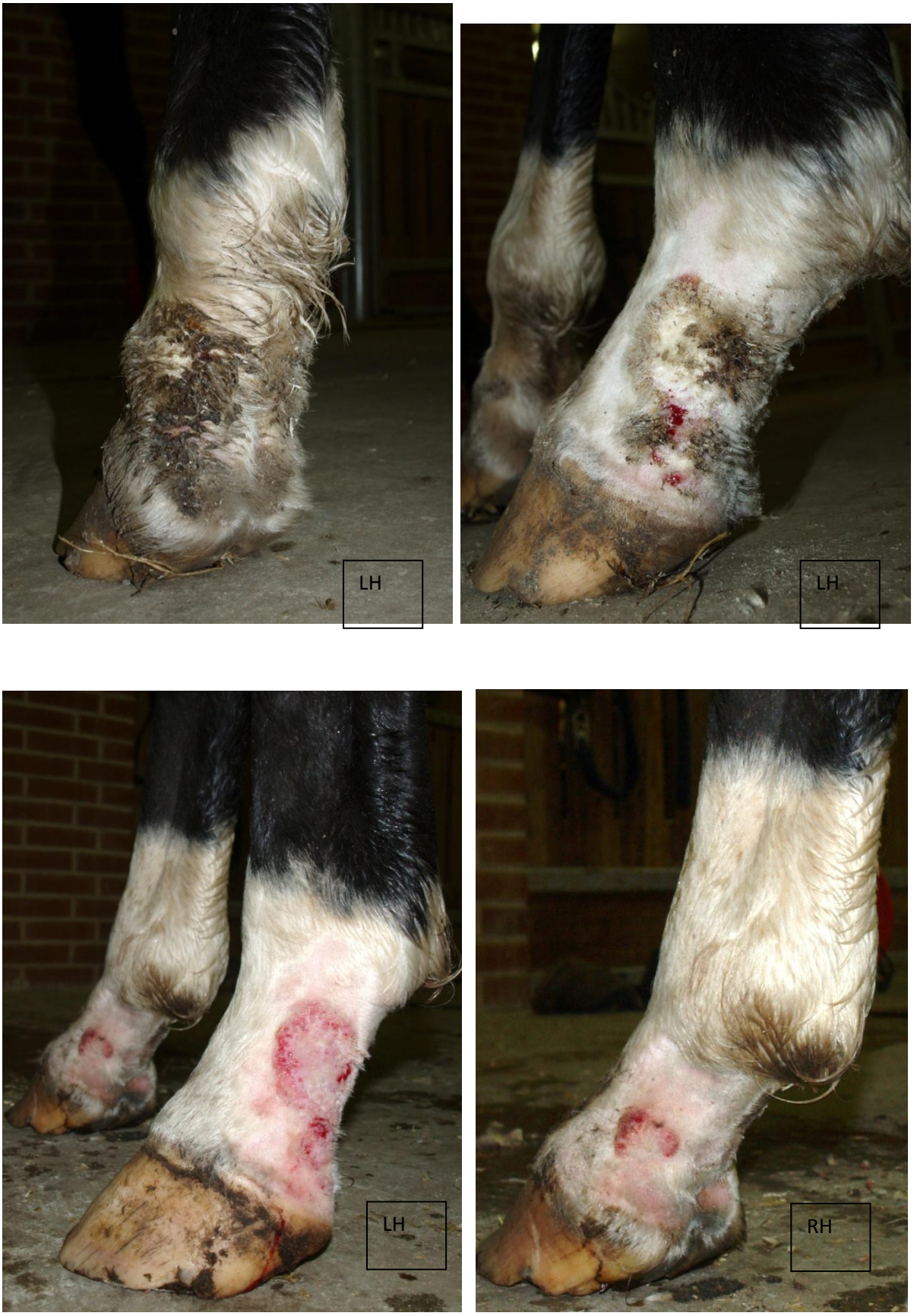






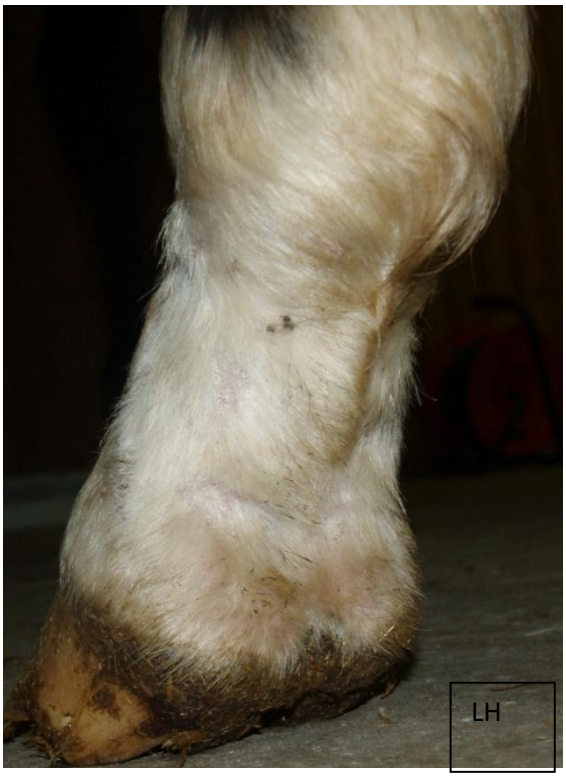


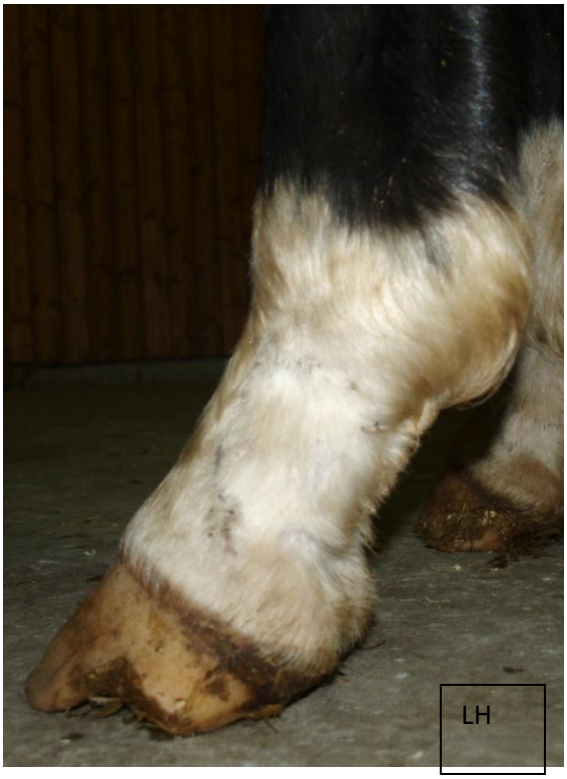
Appendix – all results

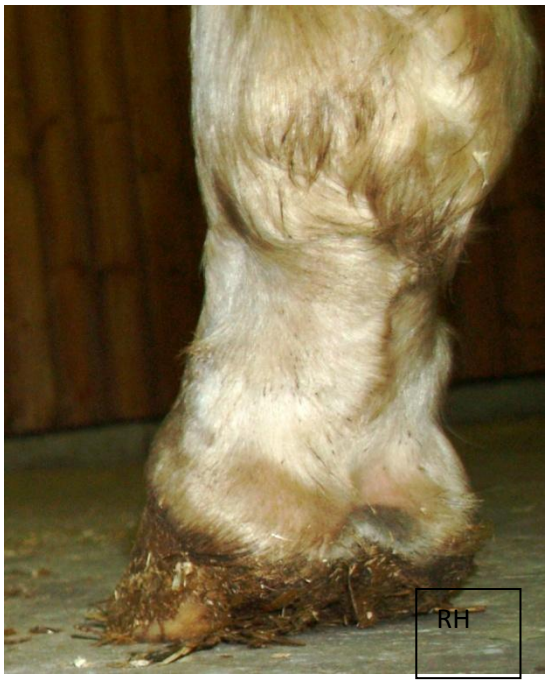


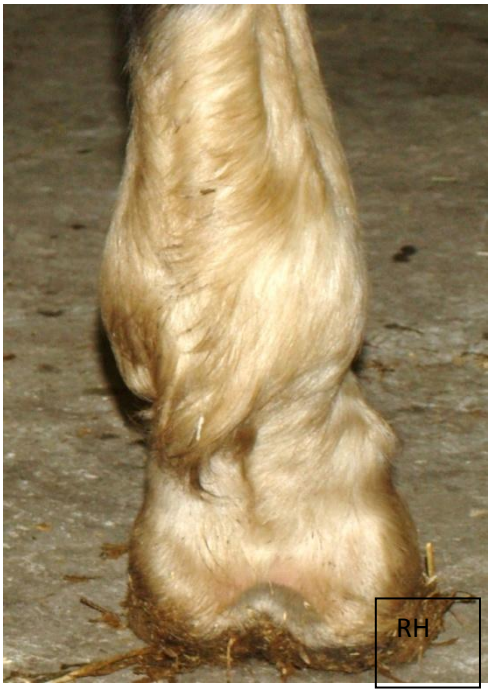
5.1. Clinical appearance before treatment. There is some swelling locally in areas were hyperkeratosis and profound dermatitis was visible.

Appendix – all results









5.2. Clinical appearance after treatment. The previous areas with profound dermatitis were now healed and bordered with hyperkeratosis superficial dermatitis.

Appendix – all results

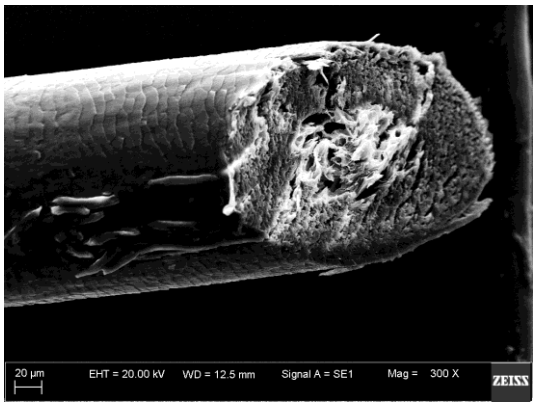
Pracownia Mikroskopii Elektronowej
Uniwersytetu Przyrodniczego we Wrocławiu
50-375 Wrocław
ul. Kożuchowska 5b
http://microscopy.ar.wroc.pl/

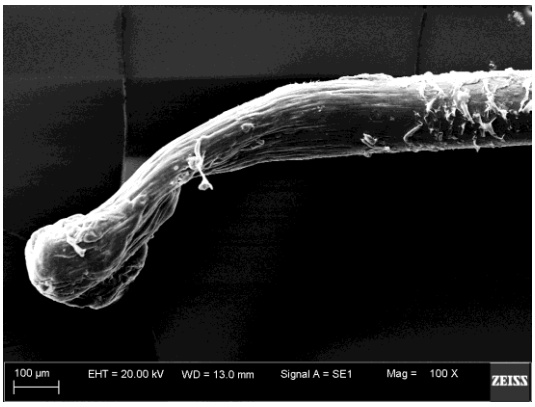
Patient: 6.1. Horse, pregnant mare
Responsible: dr Lene

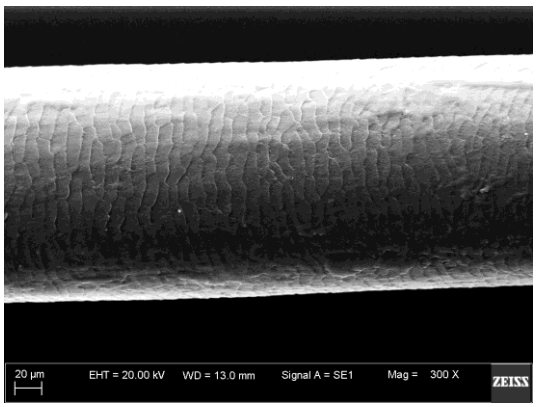
Scanning trichoscopy

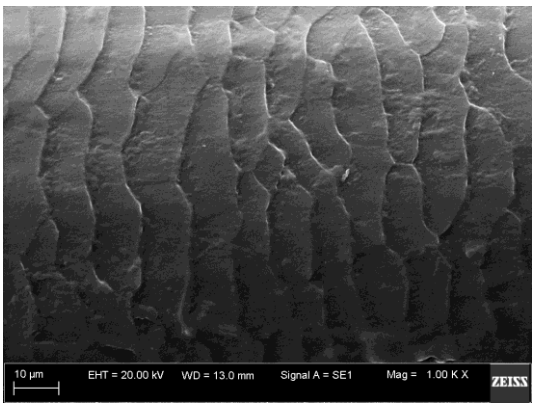
Scanning electron examination of mane hairs sample reviled some morphological abnormalities of the hair structure. The hair medulla /shaft ratio was relatively low. Moreover keratin scales of hair cuticle were arranged in irregular way and focally were covered with keratin debris excess.

Mane hairs





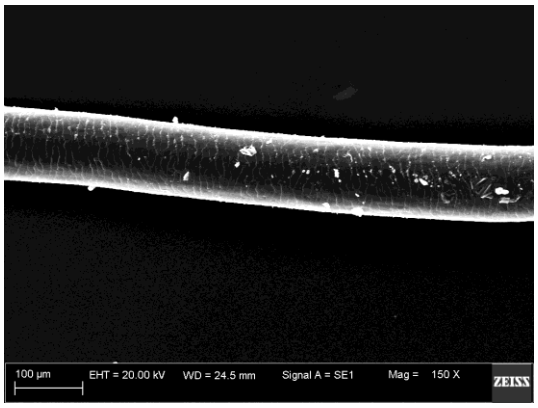


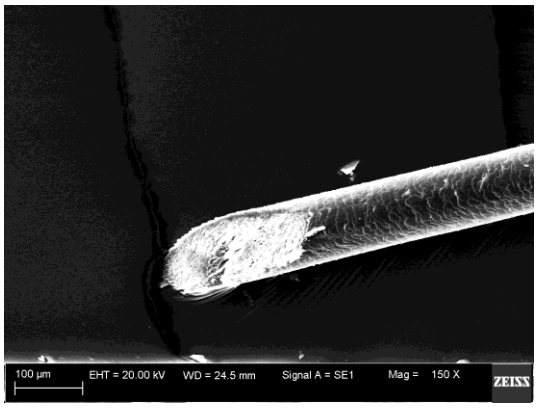


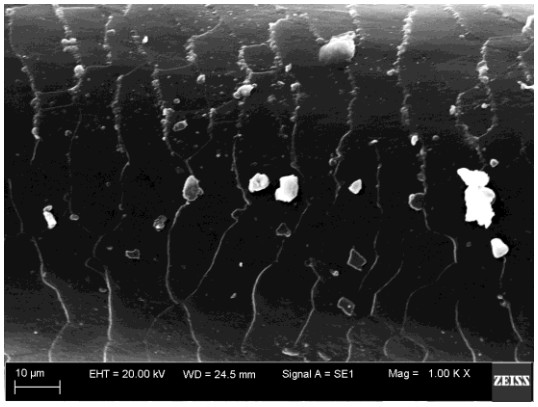
Scanning trichoscopy

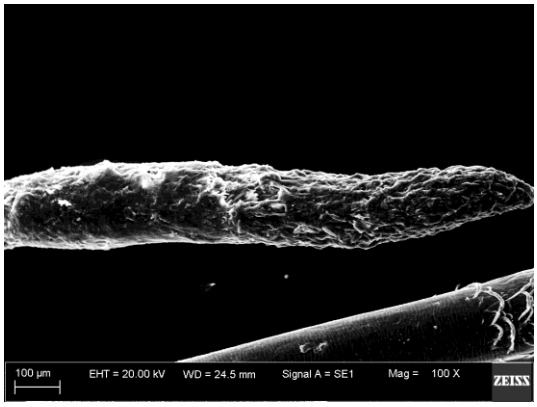
SEM analysis of the hairs coming from mane and extremity of the horse reviled no marked morphological abnormalities. There were only some quantities of keratin debris observed on the surface of examined hairs. Majority of observed hair bulbs were in telogen phase.

Mane hairs









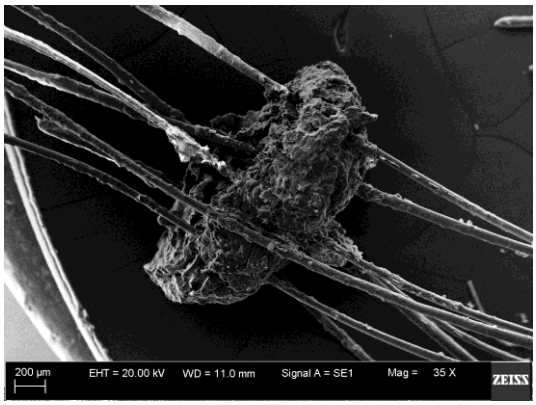
Appendix – all results

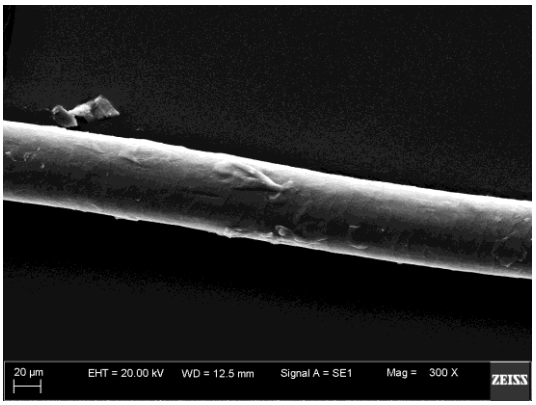
6.1. Before treatment

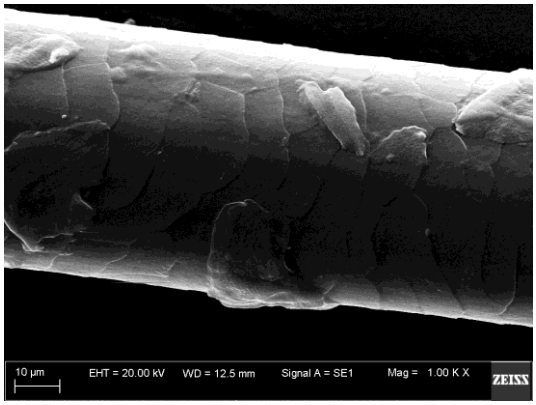
Scanning examination of extremity hairs

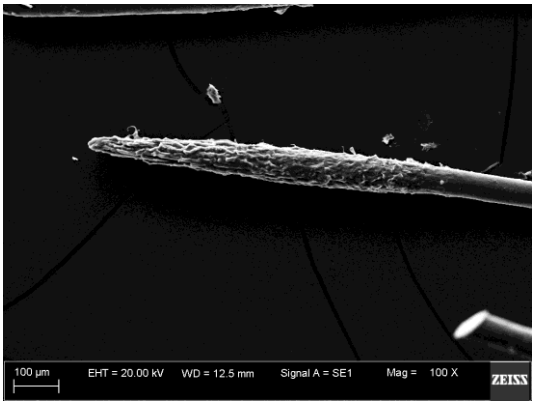
Body hairs, in general displayed no abnormalities with respect to the shape of hair shafts and hair bulb. Marked quantities of keratin-lipid masses were apparent both, on the hair surface and between particular hair shafts.

Extremity hairs



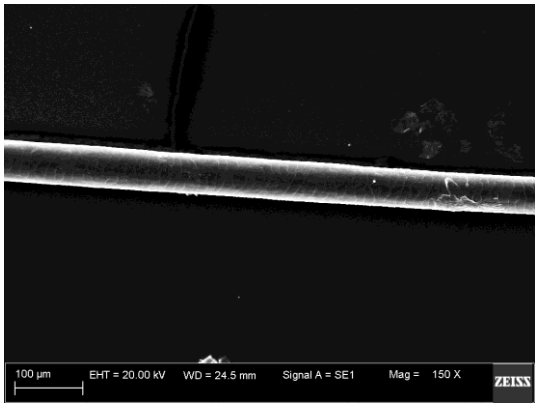


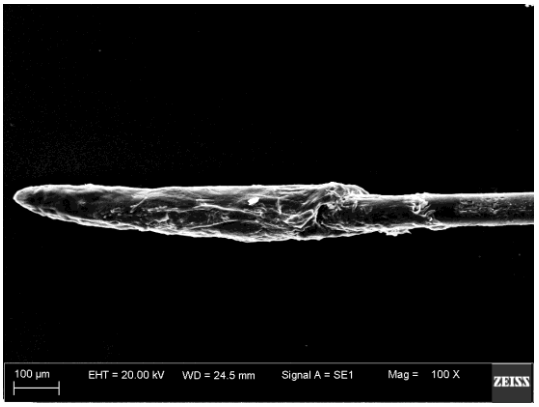


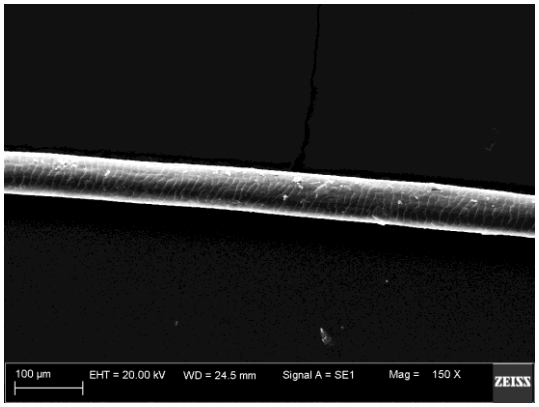


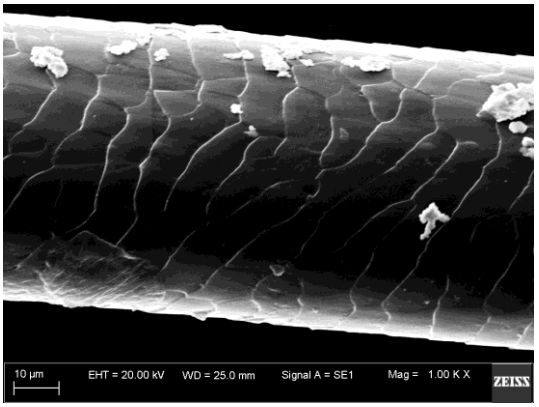
Extremity hairs

6.2. After treatment









Appendix – all results

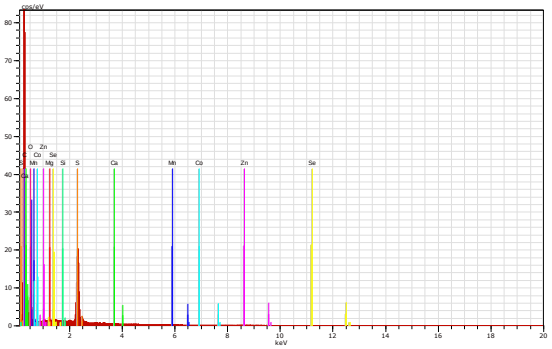
Elemental hair and skin analysis

Mane hairs examination by means of SEM-EDS indicated low level of Mn and Ca. With respect to hairs coming from lesional skin low concentration of Mn and relatively low of S was indicated.

Mane

Spectrum: Acquisition

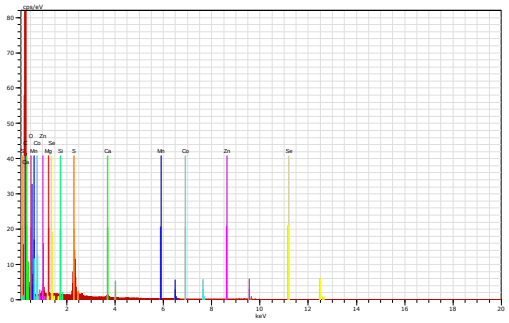
El	AN	Series	Net	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error [%]
C	6	K-series	511277	58.57	58.57	67.70	18.1
O	8	K-series	55765	33.71	33.71	29.25	10.8
S	16	K-series	198261	5.97	5.97	2.59	0.2
Se	34	K-series	590	0.98	0.98	0.17	0.1
Si	14	K-series	6332	0.16	0.16	0.08	0.0
Mg	12	K-series	3925	0.16	0.16	0.09	0.0
Zn	30	K-series	563	0.15	0.15	0.03	0.0
Co	27	K-series	734	0.12	0.12	0.03	0.0
Ca	20	K-series	1863	0.09	0.09	0.03	0.0
Mn	25	K-series	795	0.08	0.08	0.02	0.0
		Total:		100.00	100.00	100.00	



Extremity hairs

Spectrum: Acquisition

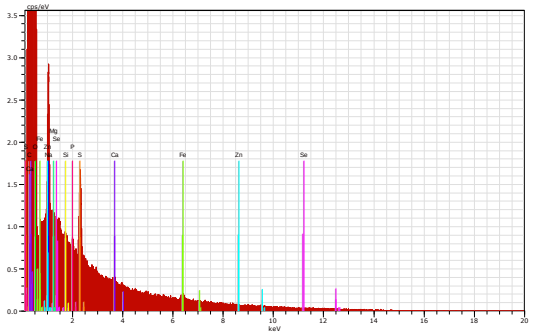
El	AN	Series	Net	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error [%]
C	6	K-series	731667	59.15	59.16	67.55	18.1
O	8	K-series	81041	35.39	35.39	30.34	11.1
S	16	K-series	136929	3.55	3.55	1.52	0.2
Se	34	K-series	473	0.45	0.45	0.08	0.1
Ca	20	K-series	10200	0.44	0.44	0.15	0.0
Zn	30	K-series	1864	0.40	0.40	0.08	0.0
Mg	12	K-series	7615	0.28	0.28	0.16	0.0
Si	14	K-series	7151	0.16	0.16	0.08	0.0
Co	27	K-series	871	0.11	0.11	0.03	0.0
Mn	25	K-series	754	0.07	0.07	0.02	0.0
		Total:		100.00	100.00	100.00	



Skin surface

Spectrum: Acquisition

El	AN	Series	Net	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error [%]
C	6	K-series	933403	66.99	67.00	73.71	20.6
O	8	K-series	63868	30.76	30.76	25.41	9.8
S	16	K-series	10114	0.45	0.45	0.18	0.0
Na	11	K-series	4467	0.40	0.40	0.23	0.2
Se	34	K-series	372	0.35	0.35	0.06	0.0
Mg	12	K-series	5712	0.35	0.35	0.19	0.1
Fe	26	K-series	1352	0.22	0.22	0.05	0.0
Zn	30	K-series	493	0.15	0.15	0.03	0.0
Si	14	K-series	3555	0.13	0.13	0.06	0.0
P	15	K-series	2219	0.10	0.10	0.04	0.0
Ca	20	K-series	1174	0.09	0.09	0.03	0.0
		Total:		100.00	100.00	100.00	



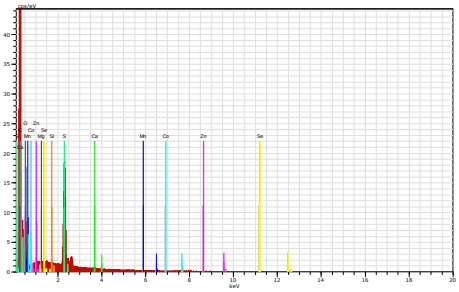
Elemental hair and skin analysis

Elemental analysis of the hairs was performed in peribulbal region of the given hair and on the skin surface. With respect to mane and extremity hairs there was low concentration of Mn and relatively low contents of Ca observed.

Mane hairs

Spectrum: Acquisition

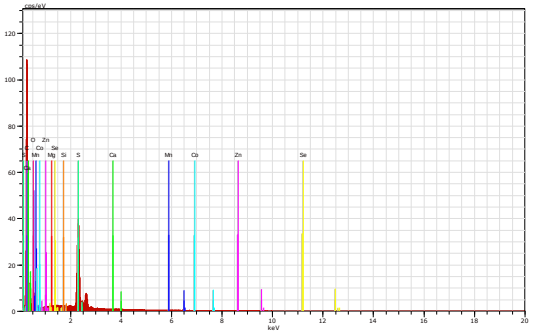
El	AN	Series	Net	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error [%]
C	6	K-series	633367	60.72	60.72	69.42	18.7
O	8	K-series	60002	32.27	32.27	27.70	10.3
S	16	K-series	194580	5.61	5.61	2.40	0.2
Se	34	K-series	420	0.40	0.40	0.07	0.1
Mg	12	K-series	7209	0.29	0.29	0.16	0.0
Si	14	K-series	10532	0.26	0.26	0.13	0.0
Zn	30	K-series	746	0.18	0.18	0.04	0.0
Ca	20	K-series	2066	0.10	0.10	0.03	0.0
Co	27	K-series	665	0.10	0.10	0.02	0.0
Mn	25	K-series	763	0.07	0.07	0.02	0.0
		Total:		100.00	100.00	100.00	



Extrimety hairs

Spectrum: Acquisition

El	AN	Series	Net	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error [%]
C	6	K-series	570191	59.55	59.55	68.72	18.4
O	8	K-series	56613	32.04	32.04	27.76	10.2
S	16	K-series	431413	7.49	7.49	3.24	0.3
Se	34	K-series	595	0.34	0.34	0.06	0.0
Si	14	K-series	10441	0.15	0.15	0.08	0.0
Mg	12	K-series	5689	0.14	0.14	0.08	0.0
Zn	30	K-series	849	0.12	0.12	0.03	0.0
Co	27	K-series	1017	0.09	0.09	0.02	0.0
Mn	25	K-series	931	0.05	0.05	0.01	0.0
Ca	20	K-series	1232	0.04	0.04	0.01	0.0
		Total:		100.00	100.00	100.00	

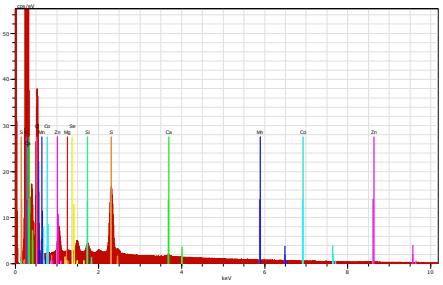


Skin surface

As far as skin surface is concerned there was higher level of S and Si and lower concentration of Zn observed after the treatment.

Spectrum: Acquisition

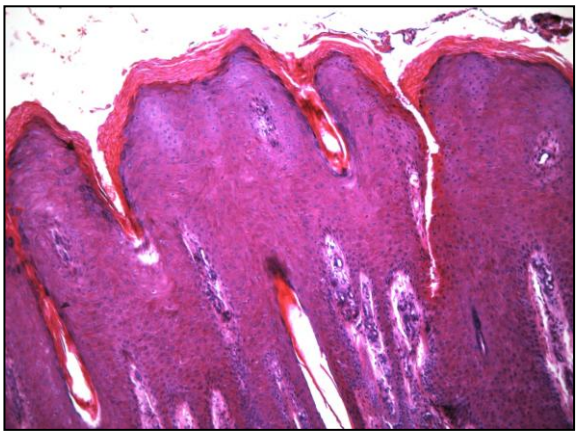
El	AN	Series	Net	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error [%]
C	6	K-series	1410722	54.60	54.60	62.29	16.6
O	8	K-series	235813	42.83	42.84	36.69	13.2
Mg	12	K-series	4425	0.08	0.08	0.04	0.0
Si	14	K-series	16382	0.17	0.17	0.09	0.0
S	16	K-series	150050	1.85	1.85	0.79	0.1
Ca	20	K-series	4916	0.08	0.08	0.03	0.0
Mn	25	K-series	2071	0.05	0.05	0.01	0.0
Co	27	K-series	1728	0.06	0.06	0.02	0.0
Zn	30	K-series	1356	0.08	0.08	0.02	0.0
Se	34	K-series	839	0.19	0.19	0.03	0.0
		Total:		100.00	100.00	100.00	

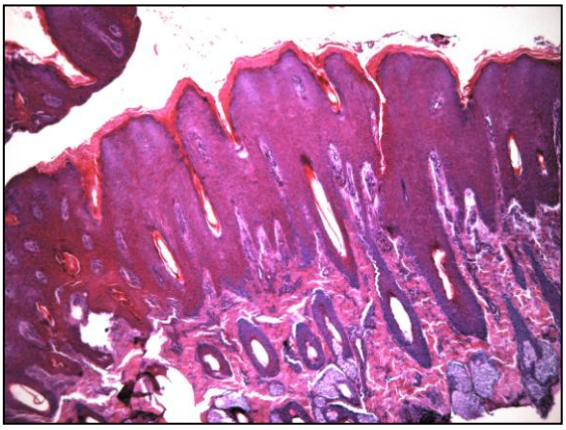


61. Before treatment

Dermatopathological skin examination

Histopatological examination of the skin samples revealed significant hyperkeratosis (mainly compact) of the epidermis. Dermal inflammatory infiltrates were not abundant and localized mainly in subepidermal compartment. They consisted mainly of mast cells and fibroblasts.

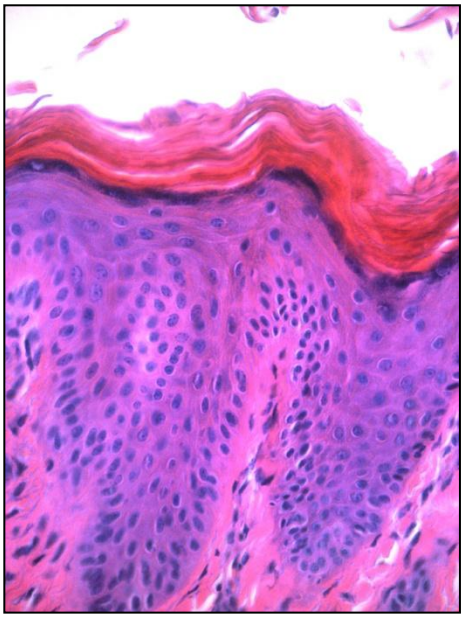


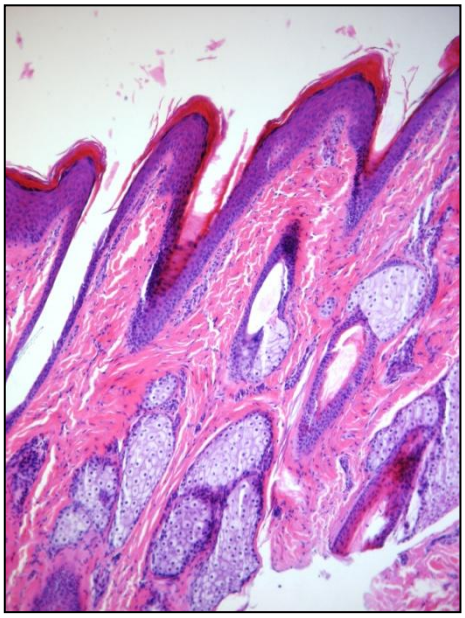


Dermatopathological examination

6.2. After treatment

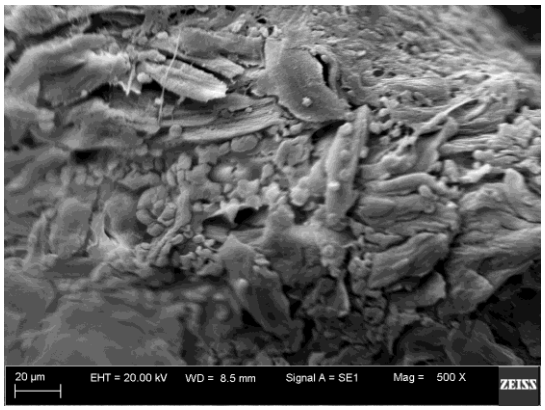
Dermatopathological examination of the skin reviled hyperkeratosis of the epidermis and sparse inflammatory infiltrates in subepidermal compartment (mainly fibroblasts and mononuclear cells).

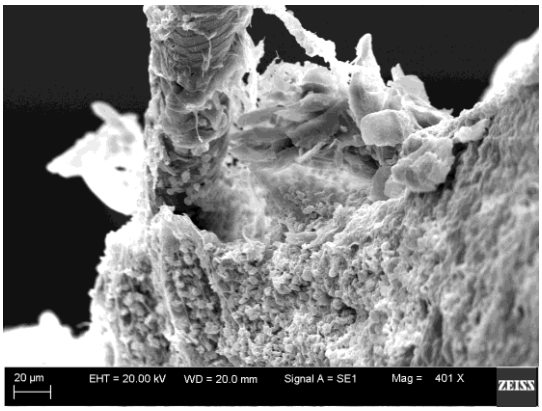


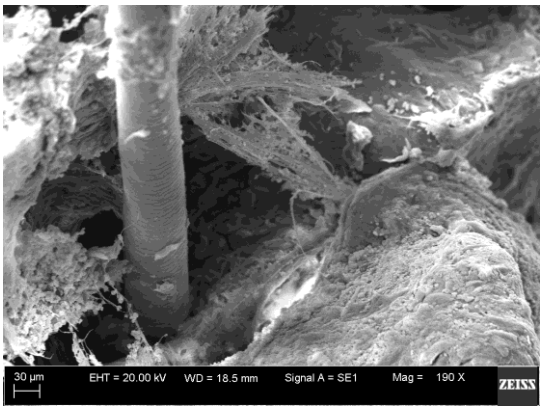


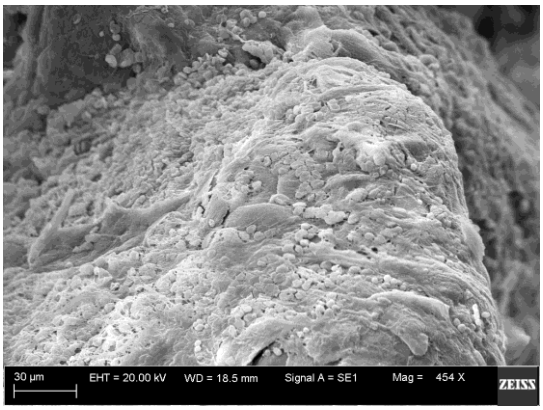
Scanning microscopy examination of the skin

Scanning electron examination of the lesional skin surface revealed a lot of desquamated keratin scales lining the surface of the skin and the hair shaft. Among keratin masses there were significant quantities of yeast organisms observed.





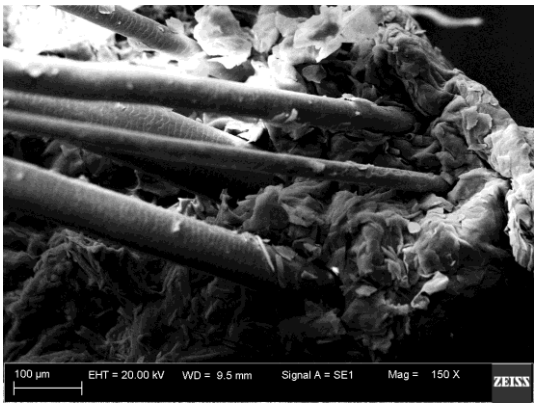


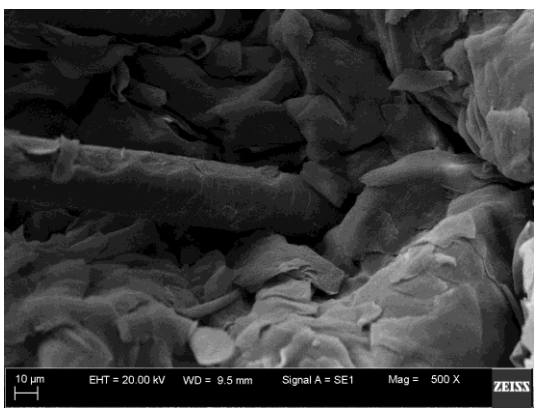


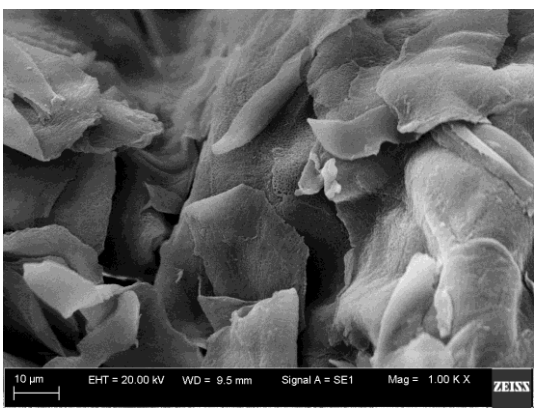
Scanning microscopy examination of the skin

6.2. After treatment

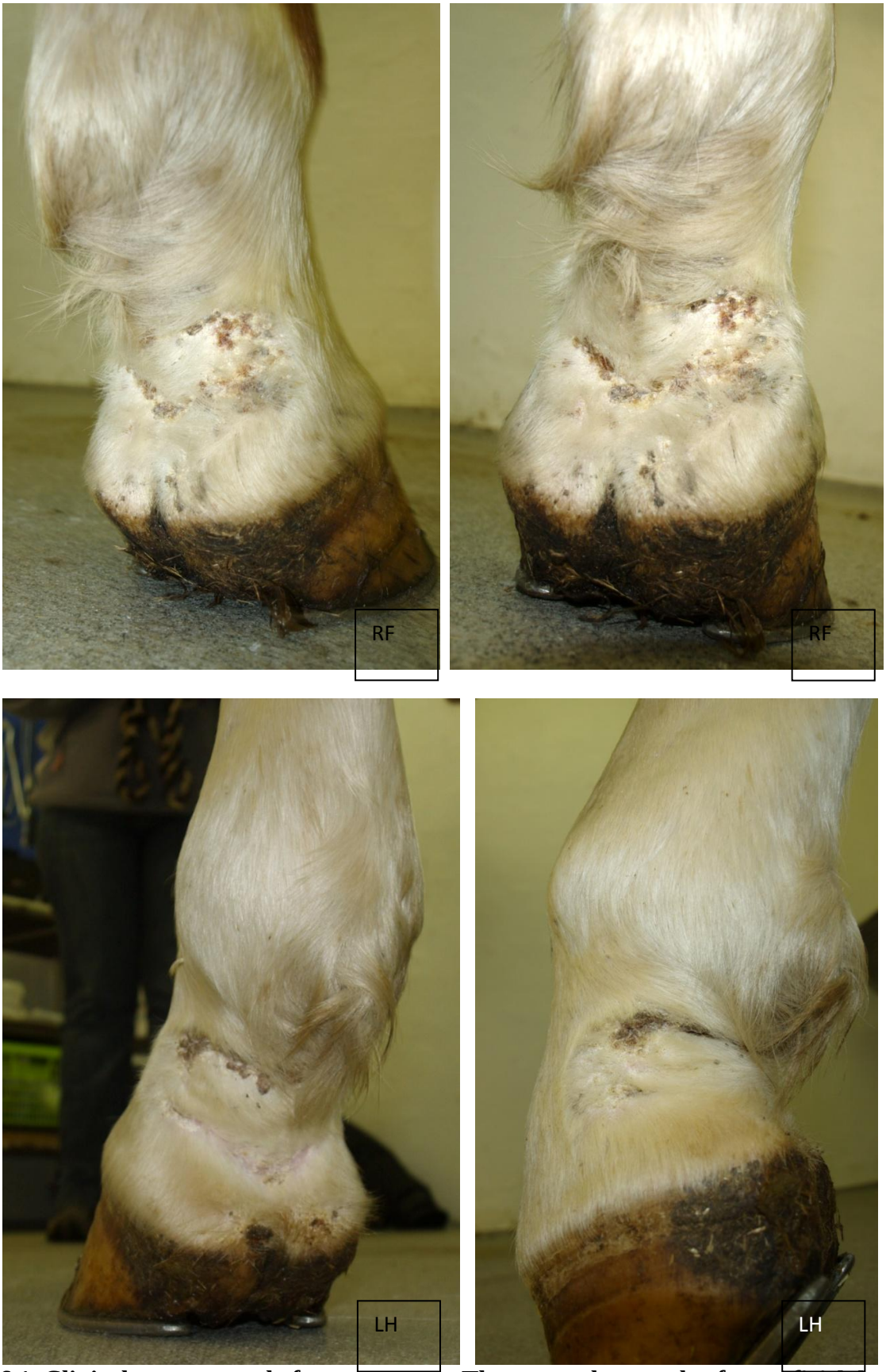
SEM pictures of the surface of the skin coming from affected areas reviled mainly relatively big keratin scales covering surface of the epidermis and some parts of the hair shafts.





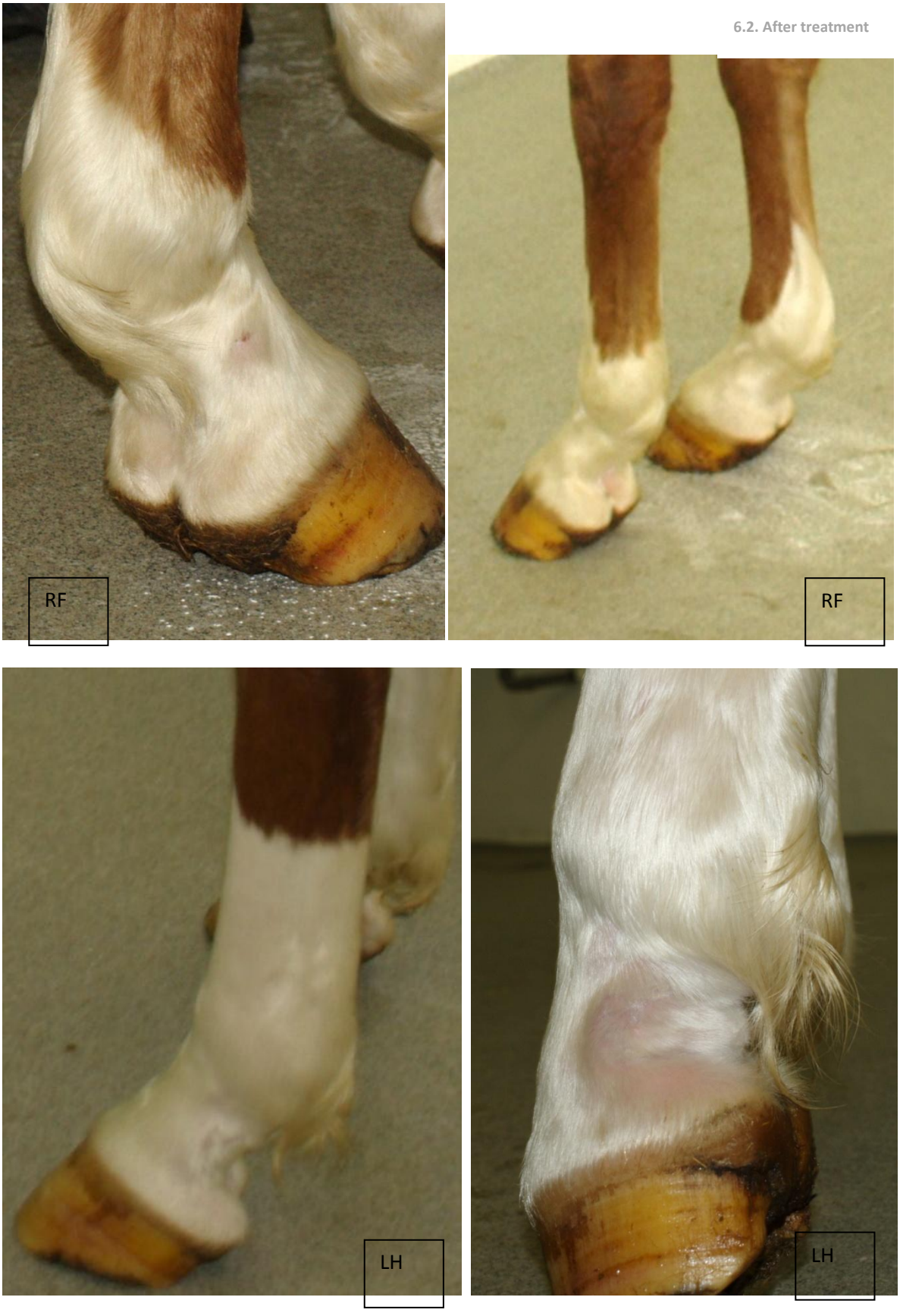


Appendix – all results



6.1. Clinical appearance before treatment. There were low grade of superficiel dermatitis with hyperkeratosis and alopeci.

Appendix – all results



6.2. Clinical appearance after treatment. Totally healing.

Appendix – all results

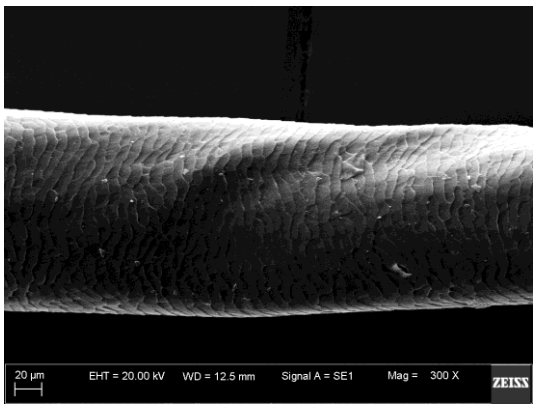
Pracownia Mikroskopii Elektronowej
Uniwersytetu Przyrodniczego we Wrocławiu
50-375 Wrocław
ul. Kożuchowska 5b
http://microscopy.ar.wroc.pl/

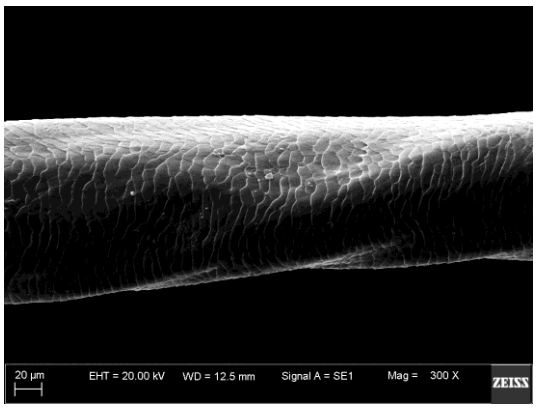
Patient: 7.1. Horse, gelding
Responsible: dr Lene

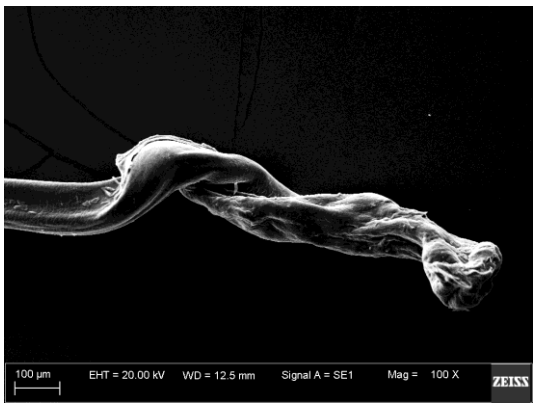
Scanning trichoscopy

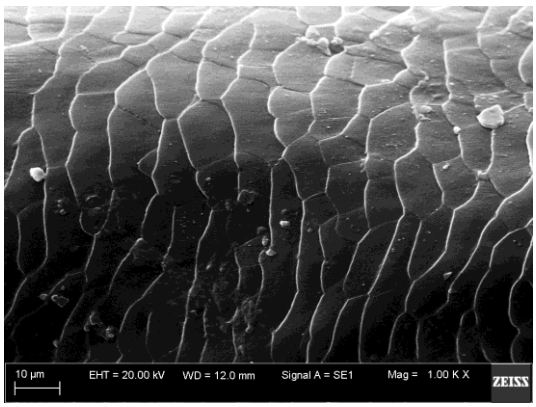
Scanning electron microscopic examination of analyzed hairs coming from the mane reviled some shape abnormalities of the hair shafts in the form of distortions and unequal diameter. There is also irregular arrangement of keratin scales of hair cuticle noticed.

mane hairs









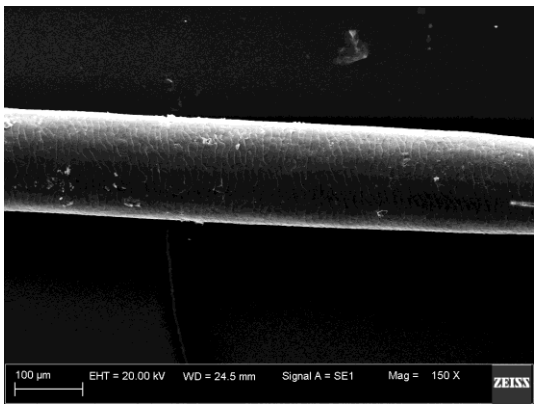
Appendix – all results

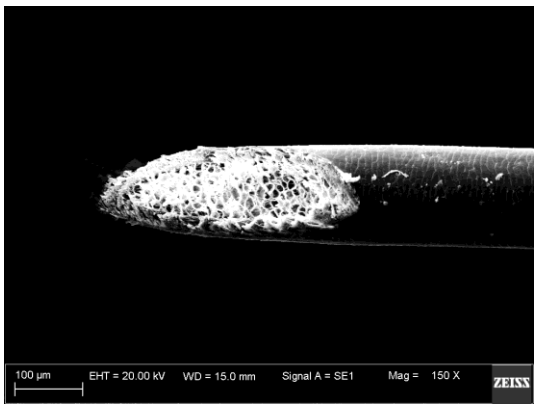
Patient: 7.2. horse, gelding

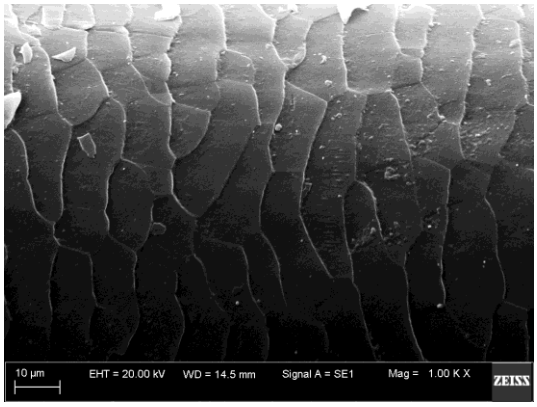
Scanning trichoscopy

SEM analysis of the hairs coming from mane reviled no marked abnormalities except slightly misshaped keratin scales of the hair shafts cuticle.

Mane hairs





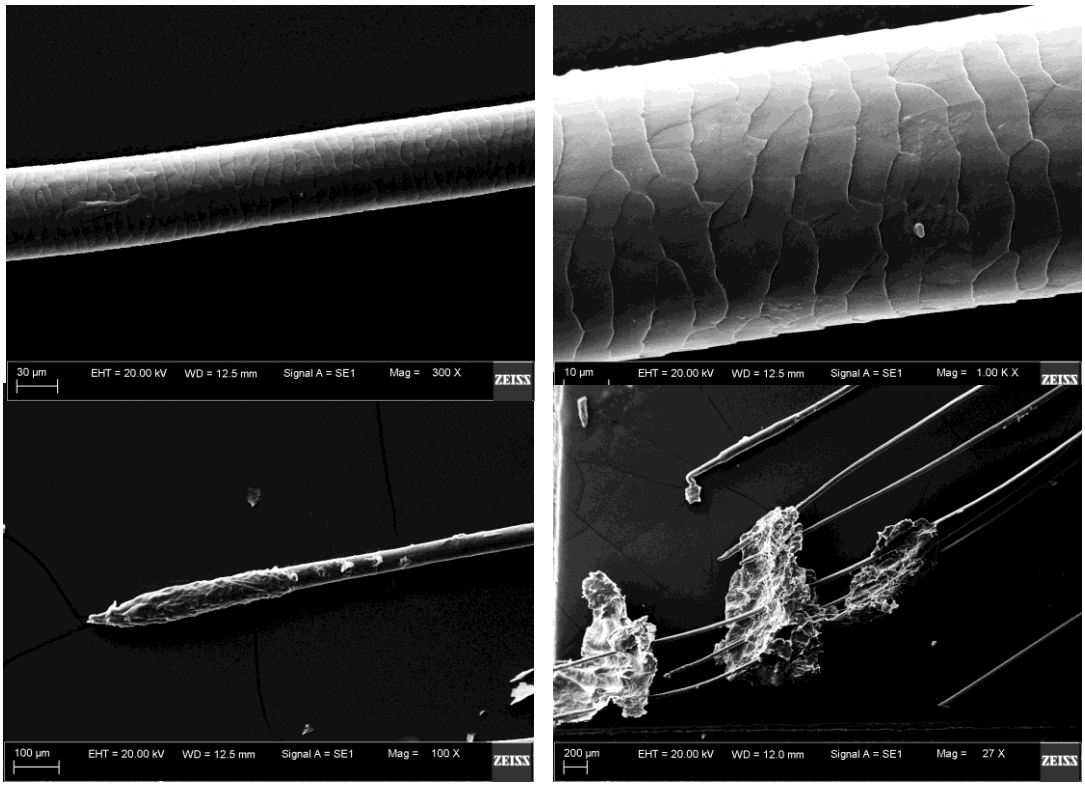


Appendix – all results

Extremity hairs

7.1. Before treatment

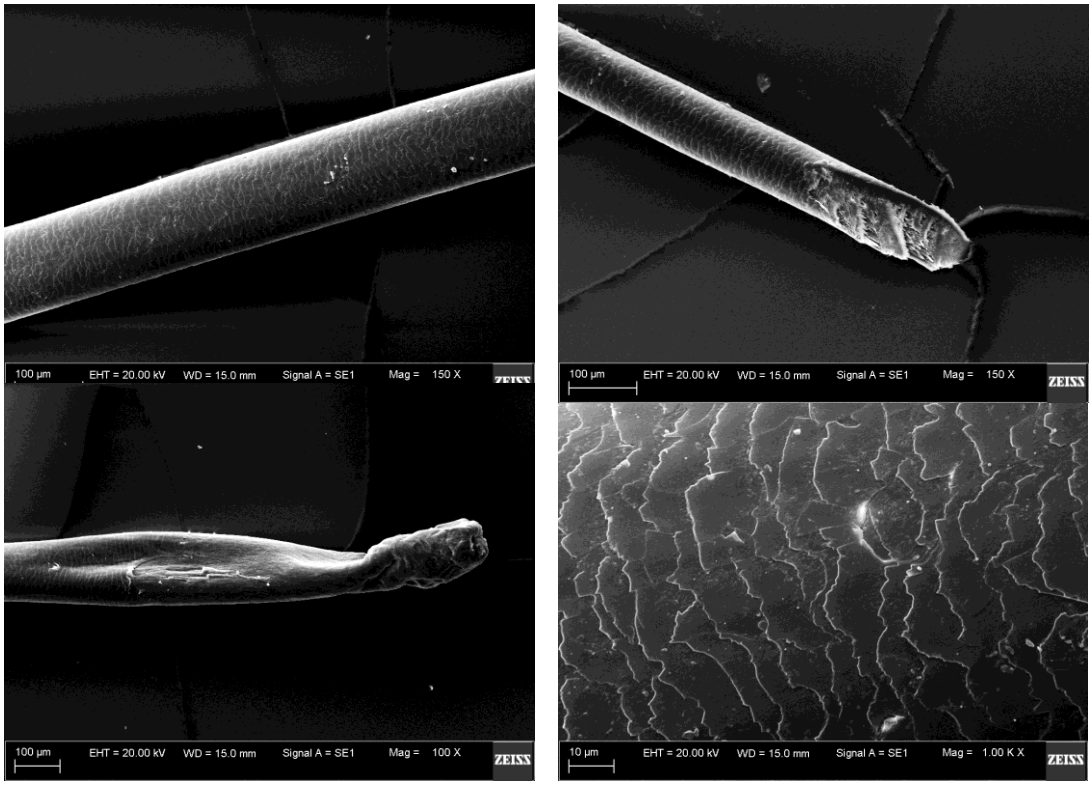
Body hairs examined by means of SEM reviled no gross abnormalities of hairs shape but significant amount of keratin and lipid debris was observed between hairs sticking them together



Extremitv hairs

7.2. After treatment

Ultra structural examination of hairs coming from horse extremity revealed significantly irregular shapes of keratin scales of hair shaft cuticle. The majority of observed hair bulbs were in Telogen Phase.



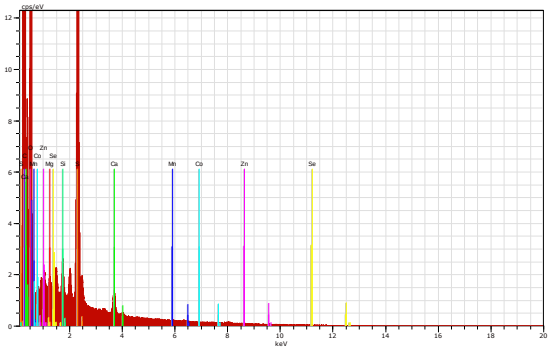
Elemental hair and skin analysis

Mane hairs exhibited deficits of Mn, while hairs coming from the close proximity of the lesional skin reviled low levels of S and Si.

Mane

Spectrum: Acquisition

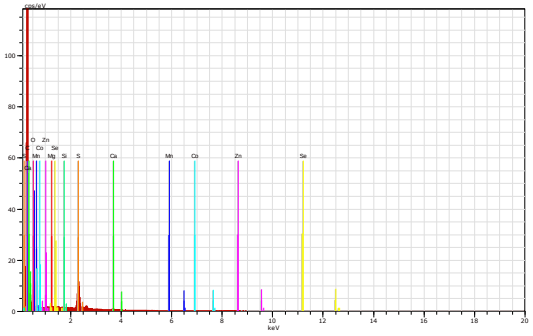
El	AN	Series	Net	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error [%]
C	6	K-series	655047	46.60	46.61	55.71	14.5
O	8	K-series	170020	45.71	45.71	41.02	14.3
S	16	K-series	153858	5.10	5.10	2.28	0.2
Ca	20	K-series	11172	0.64	0.64	0.23	0.0
Si	14	K-series	20231	0.58	0.58	0.30	0.1
Se	34	K-series	395	0.50	0.50	0.09	0.1
Mg	12	K-series	9546	0.45	0.45	0.27	0.1
Zn	30	K-series	737	0.22	0.22	0.05	0.0
Co	27	K-series	662	0.11	0.11	0.03	0.0
Mn	25	K-series	642	0.07	0.07	0.02	0.0
		Total:		100.00	100.00	100.00	



Extremity hairs

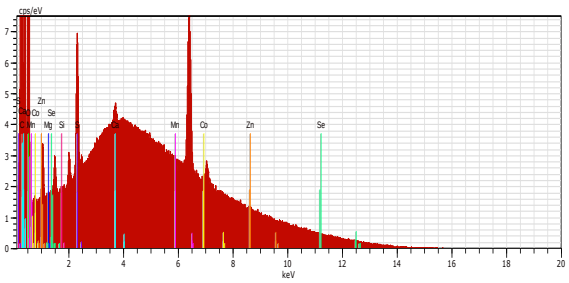
Spectrum: Acquisition

El	AN	Series	Net	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error [%]
C	6	K-series	843107	64.73	64.73	72.12	19.8
O	8	K-series	65506	31.72	31.72	26.53	10.0
S	16	K-series	113468	2.57	2.57	1.07	0.1
Se	34	K-series	406	0.31	0.31	0.05	0.0
Ca	20	K-series	4959	0.21	0.21	0.07	0.0
Mg	12	K-series	5407	0.16	0.16	0.09	0.0
Zn	30	K-series	577	0.12	0.12	0.02	0.0
Mn	25	K-series	945	0.08	0.08	0.02	0.0
Co	27	K-series	674	0.08	0.08	0.02	0.0
Si	14	K-series	777	0.01	0.01	0.01	0.0
		Total:		100.00	100.00	100.00	



skin surface

El	AN	Series	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error [%]
C	6	K-series	53.50	53.50	60.90	16.2
O	8	K-series	45.37	45.37	38.77	13.9
Mn	25	K-series	0.53	0.53	0.13	0.0
S	16	K-series	0.30	0.30	0.13	0.0
Co	27	K-series	0.12	0.12	0.03	0.0
Se	34	K-series	0.11	0.11	0.02	0.0
Ca	20	K-series	0.04	0.04	0.01	0.0
Zn	30	K-series	0.03	0.03	0.01	0.0
Mg	12	K-series	0.00	0.00	0.00	0.0
Si	14	K-series	0.00	0.00	0.00	0.0
		Total:	100.00	100.00	100.00	



Appendix – all results

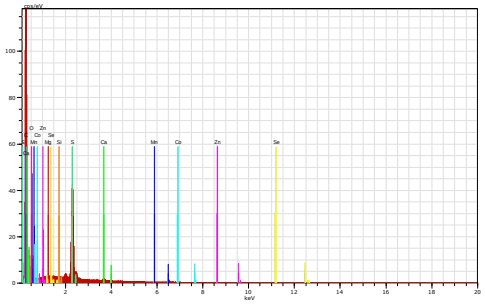
Elemental hair and skin analysis

Elemental analysis of the hairs was performed in peribulbal region of the given hair and on the skin surface. With respect to mane hairs there was low level of Mn indicated. In case of hairs coming from horse extremity there was low concentration of Mn and Co observed. As far as skin elemental analysis is concerned there was higher level S and Si after the treatment than at the beginning of the research.

Mane hairs

Spectrum: Acquisition

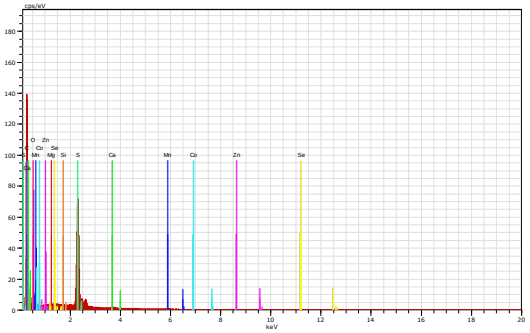
El	AN	Series	Net	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error [%]
C	6	K-series	776106	60.51	60.51	69.36	18.6
O	8	K-series	73453	32.08	32.08	27.60	10.2
S	16	K-series	417929	6.06	6.06	2.60	0.2
Se	34	K-series	566	0.43	0.43	0.08	0.0
Ca	20	K-series	11270	0.28	0.28	0.10	0.0
Mg	12	K-series	10690	0.21	0.21	0.12	0.0
Si	14	K-series	14846	0.18	0.18	0.09	0.0
Zn	30	K-series	763	0.11	0.11	0.02	0.0
Co	27	K-series	1045	0.08	0.08	0.02	0.0
Mn	25	K-series	967	0.05	0.05	0.01	0.0
		Total:		100.00	100.00	100.00	



Extremity hairs

Spectrum: Acquisition

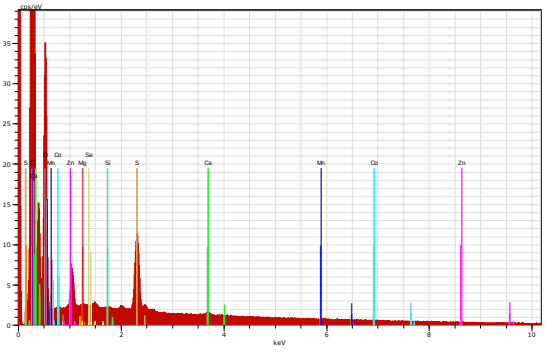
El	AN	Series	Net	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error [%]
C	6	K-series	734733	56.96	56.96	66.90	17.7
O	8	K-series	82512	32.33	32.33	28.50	10.4
S	16	K-series	737478	9.34	9.34	4.11	0.4
Se	34	K-series	819	0.38	0.38	0.07	0.0
Mg	12	K-series	16232	0.28	0.28	0.16	0.0
Ca	20	K-series	10967	0.23	0.23	0.08	0.0
Si	14	K-series	20589	0.22	0.22	0.11	0.0
Zn	30	K-series	1237	0.14	0.14	0.03	0.0
Co	27	K-series	1116	0.07	0.07	0.02	0.0
Mn	25	K-series	1051	0.05	0.05	0.01	0.0
		Total:		100.00	100.00	100.00	



skin surface

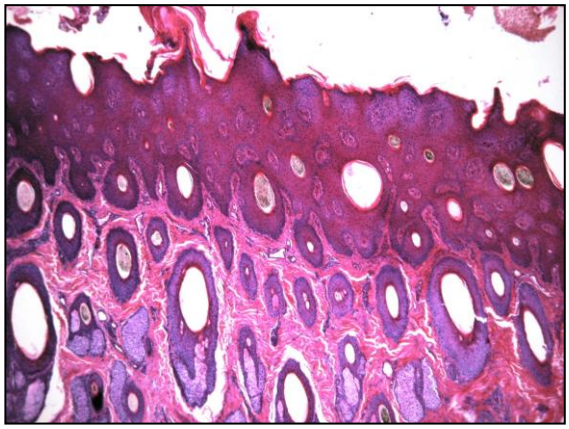
Spectrum: Acquisition

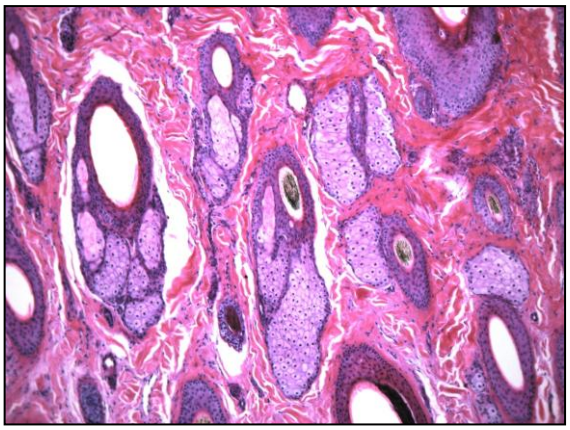
El	AN	Series	Net	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error [%]
C	6	K-series	1257515	54.39	54.39	61.97	16.5
O	8	K-series	215375	43.46	43.46	37.17	13.3
Mg	12	K-series	7654	0.17	0.17	0.09	0.0
Si	14	K-series	2949	0.04	0.04	0.02	0.0
S	16	K-series	101276	1.53	1.53	0.65	0.1
Ca	20	K-series	4458	0.09	0.09	0.03	0.0
Mn	25	K-series	1280	0.04	0.04	0.01	0.0
Co	27	K-series	1469	0.06	0.06	0.01	0.0
Zn	30	K-series	1334	0.08	0.08	0.02	0.0
Se	34	K-series	916	0.16	0.16	0.03	0.0
		Total:		100.00	100.00	100.00	



Dermatopathological skin examination

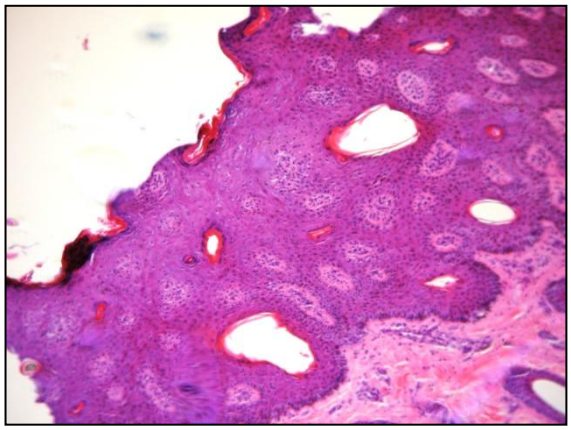
Histopatological examination of the skin samples revealed hyperkeratosis and focal parakeratosis of the epidermis. In some areas of the dermis, there was fibrosis observed. The inflammatory infiltrate were sparse and localized mainly around skin appendixes.

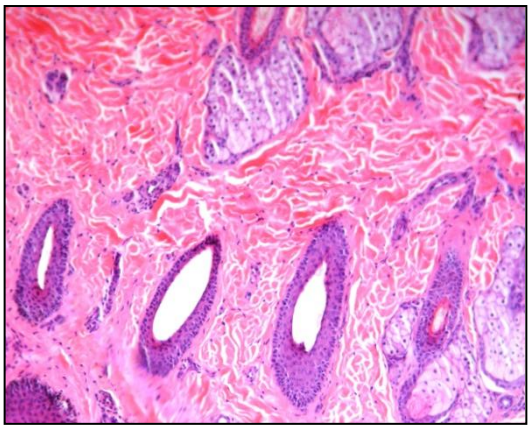




Dermatopathological skin examination

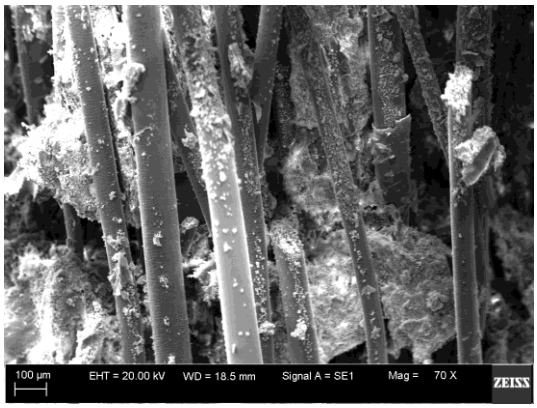
Dermatopathological examination of the skin reviled mild hyperkeratosis of the epidermis. In dermis area there was also sparse inflammatory cell infiltrate noticed mainly in subepidermal location.

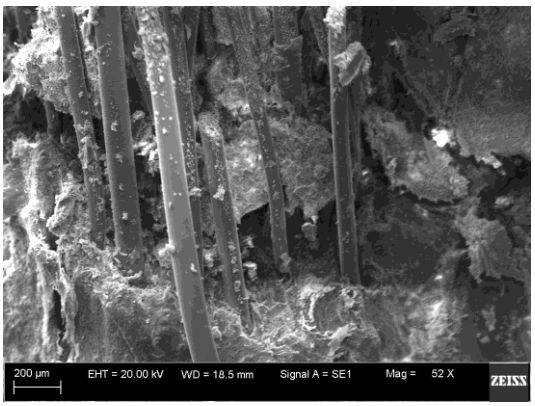


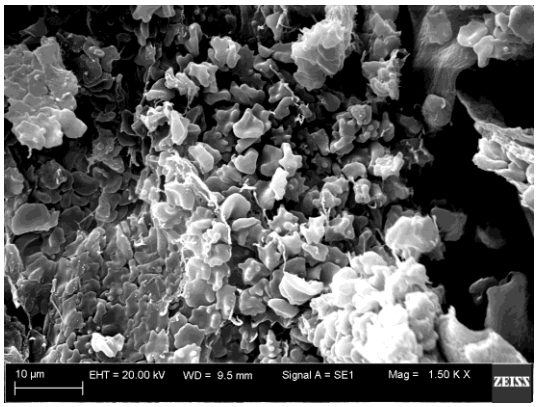


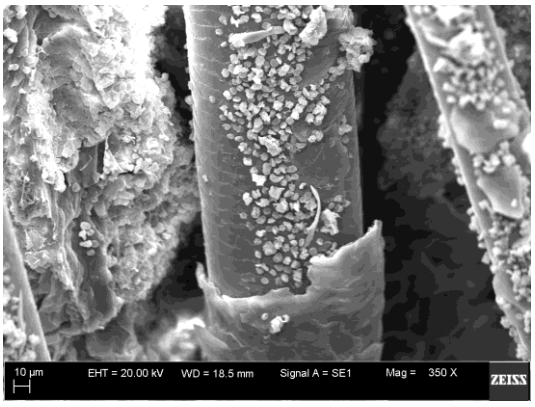
Scanning microscopy examination of the skin

Scanning electron examination of the lesional skin surface revealed yeast organisms mainly on the surface of the hair shafts and in the close proximity to the hair follicles. There were also some erythrocytes observed.



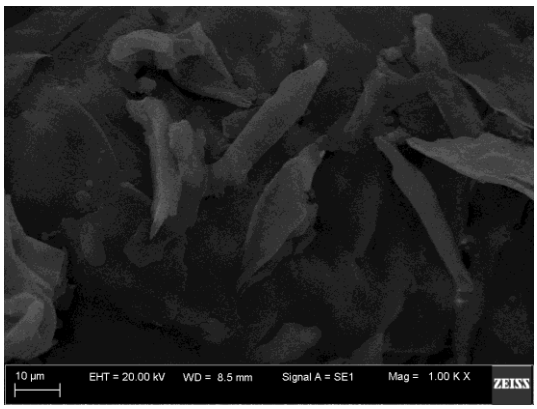


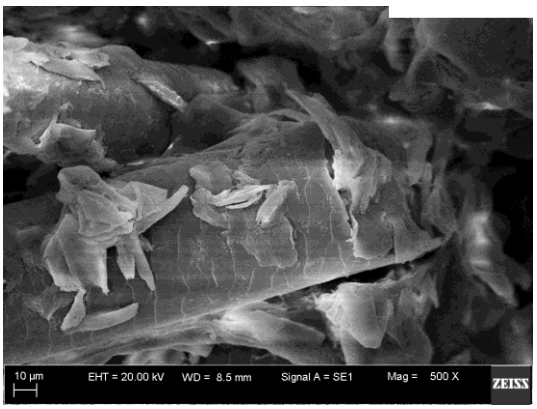


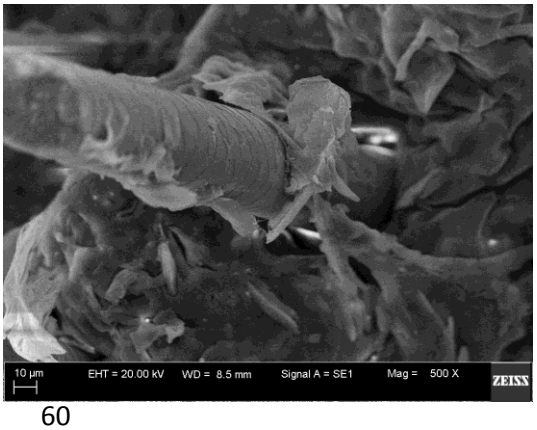


Scanning microscopy examination of the skin

Examination of the skin surface reviled excess of keratin scales at the surface of the epidermis and hair shafts. There are no more visible signs of yeast infection.

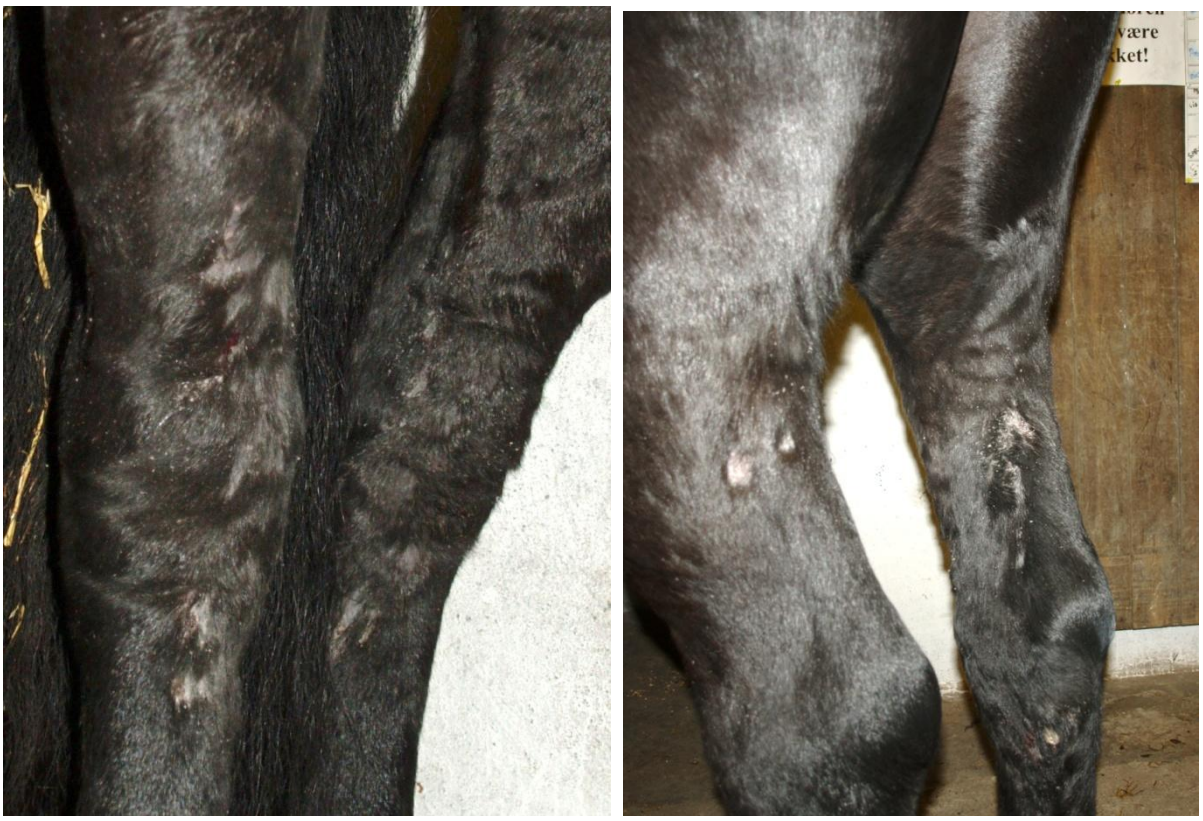




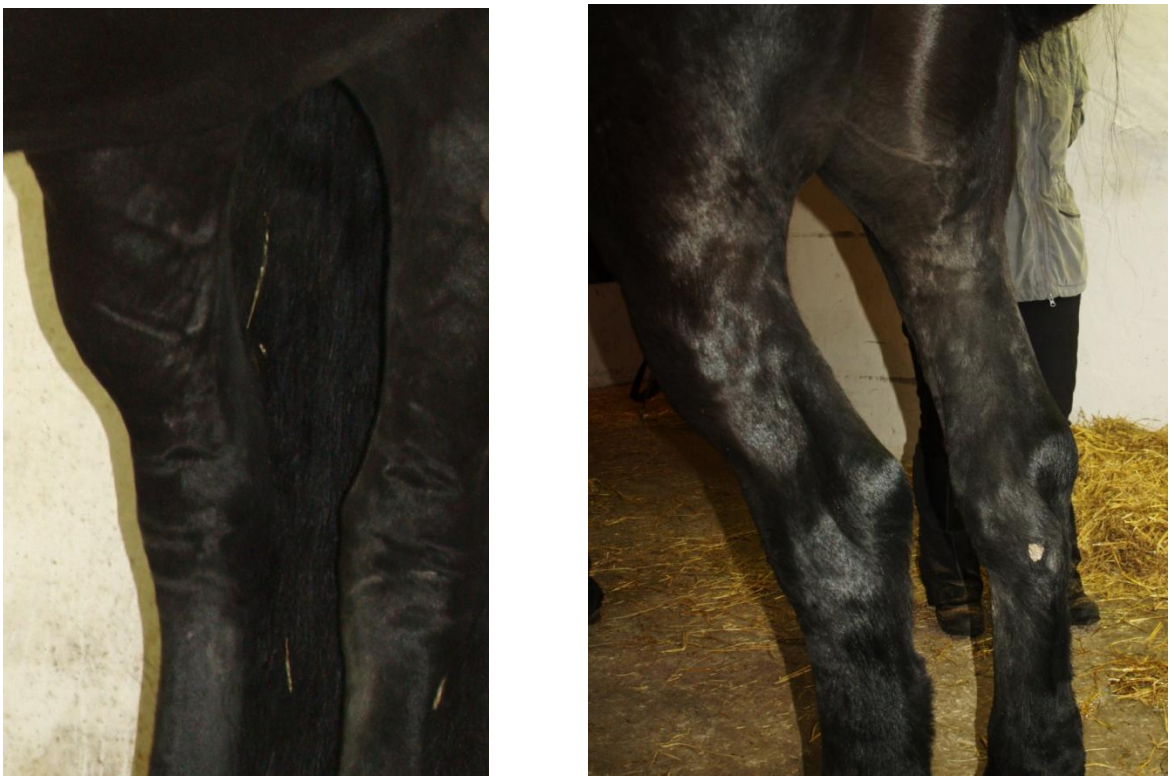


Appendix – all results

7.1. Before treatment



7.1. Clinical appearance before treatment. There were pronounced hyperkeratosis with excoriation superficiel dermatitis and alopeci.



7.2. Clinical appearance after treatment. Low grade of hyperkeratosis and alopeci were left.