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ALOPECIA IN HUMANS AND BALANCE OF TRACE ELEMENTS: CAUSES AND SIGNIFICANCE

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ŽMONIŲ PLIKIMAS IR MIKROELEMENTŲ PUSIAUSVYROS SUTRIKIMAS:
PRIEŽASTYS IR REIKŠMĖ

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1. SYMBOLS AND ABBREVIATIONS

ATPO – antimicrosomal thyroid antibodies
ATSDR – Agency for Toxic Substances and Disease Registry
Ca – calcium
Ca$^{2+}$ – bivalent calcium
Cd – cadmium
Cr – chromium
Cu – copper
ESR – erythrocytes sedimentation rate
FT$_4$ – free thyroxin
Hg – mercury
IARC – International Agency for Research on Cancer
IQ – intelligence quotient
Mg – magnesium
Mn – manganese
P – phosphorus
Pb – lead
PTH – parathyroid hormone
PUVA – psoralen (P) and ultraviolet A (UVA)
SD – standard deviation
TE – trace element
TSH – thyroid stimulating hormone
UV – ultraviolet
WHO – World Health Organisation
Zn – zinc
2. INTRODUCTION

Alopecia is a non–scaring, inflammatory, autoimmune, highly unpredictable hair loss disease that can affect humans and animals. Research into the demographics of alopecia areata suggests that up to 2% of the population will be affected with alopecia at any time. This hair loss disease can affect any hair–bearing area of both genders in various race, ethnic or social groups, and appear at any age of lifetime. The hair re–growth can occur with or without treatment, though remissions are common.

The factors that activate the onset of alopecia and the mechanisms of its development are not fully understood. A number of reasons have been proposed e.g., psychological stress, viral or bacterial infection, physical trauma or local injury, genetic predisposition, cancer, hormonal shifts, allergies, seasonal changes, pharmacological, etc. might trigger the onset of alopecia (McDonald Hull 2003, Bolduc 2002, McDonagh 1996, Price 1991, Perini 1984). It was demonstrated that cells under the stress e.g., inflammation, irradiation, viral infection, malignancy, oxidation, intoxication with heavy metals etc. can produce heat shock proteins. Those heat shock proteins play a housekeeping role in immune system responses. Circumstantial evidences suggest alopecia is an autoimmune disease where cells of an individual's own immune system prevent hair follicles from producing hair fibber. Typically, autoimmune diseases are far more common in females than males.

It has had been suggested that intoxication with heavy metals or chemicals also may cause the onset of alopecia (National Alopecia Areata Foundation, Ptašekas 2002, Skalny 1999, Gailevičius 1995). The imbalance of trace elements that have been induced by the replacement of essential elements with heavy toxic metals may lead to the disorders of zinc metabolism. Consequently, the zinc deficiency (Harrison 2003, Rushton 2002, Sinclair 1999) might provoke the onset of alopecia in sensitive individuals beside the rest factors.

Toxic heavy metals can get into the organism due to environmental pollution through respiratory track, with contaminated nutrition or drinking water. Chronic exposure to nonessential toxic metals and acute poisoning cases are not excluded (US EPA, Elinder 1994, National Research Council 1993). The deficiency of essential elements in an organism might occur due to imbalanced nutrition or nutrition, which is poor in essential trace elements (Oberleas 1999, Goyer 1994). Therefore, the preference, lack or overuse of certain type of foodstuffs in the diet might influence and shift the metabolism of trace elements. The other reasons of trace elements’ imbalance in the organism involve impaired absorption of the metals’ ions through intestine or impaired excretion, as well as higher requirements of essentials trace
elements (e.g., copper, zinc, selenium, iron etc.) and macro–elements (e.g., calcium, magnesium) during critical periods of life.

Every suggested factor might act as a trigger in people predisposed to alopecia onset due to genetic susceptibility, for example. Whatever the initiation factor it needs not to be permanent – rather a short sharp shock may be just enough to tip the balance of the immune system into autoimmunity. Once an autoimmune disease was initiated, it can be self–perpetuating. Moreover, the spontaneous hair re–growth may occur even without treatment and even after many years. Besides that, once alopecia treatment is stopped the relapse and renewed hair loss disease frequently develops.

Although alopecia is not life threatening, the pressures of an image–orientated society can make hair loss psychologically devastating for those affected, their families and friends. Adults who suffer from any types of hair loss can attest to the pain and anxiety associated with their condition. However, a young alopecia patient can sustain far more hardship and emotional scaring than any adult can. While it is culturally "acceptable" for mature men and even women to experience hair loss, the pecking order of a child's peer group can inflict devastating ridicule on any of its group that somehow seems different.

Novelty and practical significance of the research

There were no studies on alopecia in humans in Lithuania so far. There was no hair loss disease registry in the state neither the number of inhabitants affected with alopecia was known. The reference values of the content of the trace elements in Lithuanian population were unknown as well. Therefore, this research is the first one in the country undertaken to assess the alopecia among Lithuanian inhabitants and to investigate the imbalance of trace elements as one of the possible triggers of this disease. In addition, the approach of zinc supplements treatment along with the monitoring of trace elements’ balance in the organism of treated children was used.

The determined mean levels of trace elements in people with alopecia and controls did not exceed the physiologically accepted limits have been in use by Lithuania Ministry of Health (copper, zinc, lead, mercury, manganese, chromium, cadmium), Europe Commission Directives, WHO, IARC, ATSDR and US Environmental Protection Agency recommendations. It may indicate that the total environmental pollution with heavy metals most likely did not pose serious concern for humans’ health in the state. However, this requires more detailed investigations.

The systematic literature search revealed few studies in the world linking intoxication (qualified as poisoning or considering the concentrations much above the physiologically
acceptable levels) with heavy metals and alopecia in humans. However, there are only few data on quantifying the levels of heavy metals and essential elements within permissible and physiologically acceptable ranges in humans with alopecia, what was the case in this research.

Therefore, the results of study revealed that increased concentration of heavy metals ions even within physiologically permissible levels might significantly reduce the level of zinc and lead to zinc deficiency. This research showed that people with alopecia had higher levels of heavy metals (lead, cadmium, copper) ions in their organism if compared to controls, and in the sequel had lower content of zinc ions in their organisms. It might be that relative zinc deficiency may trigger the onset of alopecia in susceptible individuals or vulnerable population groups along with other factors.

The sinister approach of this research was that for the first time the content of trace elements in the organism (hair, blood/plasma, urine) of Lithuania inhabitants was estimated, in particular in the individuals originating from different districts of Lithuania. The findings of this research might be useful further for population based epidemiological studies and establishment of the reference values of trace elements content for Lithuanian population.

Moreover, based on the collected data on the prevalence of hair loss disease in the country the alopecia registry or alopecia patients’ society may be considered, initiated and established.
3. OBJECTIVE AND THE TASKS

The objective

The objective of this research was to assess the content of trace elements (lead, manganese, chromium, cadmium, mercury, copper and zinc) ions in the organism of people with hair loss disease, and to test the hypothesis whether the imbalance of trace elements may cause the alopecia onset in humans.

The tasks

To reach the principal objective of this study the following tasks were set up:
1. To evaluate the alopecia among children and adults in Lithuania, and to assess the content of heavy metals in people with alopecia from different districts of the country;
2. To investigate and evaluate the concentration of heavy metals ions in hair, blood/plasma and urine of children and adults with hair loss disease, and to assess whether imbalance of trace elements may cause alopecia in humans;
3. To assess the content of heavy metals, indices of blood and hormones, and status of thyroid gland in children with alopecia, and to test the hypothesis whether hormonal shifts may cause the onset of hair loss disease in children;
4. To monitor the balance of trace elements ions in the organism of children with hair loss disease after the treatment with zinc supplementation, and to assess the effect of the application of zinc supplements in the treatment of children alopecia.
4. LITERATURE OVERVIEW

4.1 Hair

Hair prevent organism from mechanic, thermal and solar lesions (Gailevičius 1995, Gollinck 1990). They are involved in the functions of touching and perspiration. Hair are elastic, flexible and hygroscopic derivatives of skin horny layer. Gases and various odours can penetrate the hair. Humans and mammalians have different amount and types of hair on their organism. Different species of mammalians also differ in the amount, length, colour, thickness and functional properties of the hair. For example, animals’ hair are shorter (up to 5 cm) and thicker. Thickness of the humans hair is about 0.08 – 0.14 mm while in animals usually it is <0.08 mm or >0.14 mm. The colour of humans’ hair is commonly uniform and homogenous while usually it is uneven and salutatory in animals (Forensic Evidence 2001).

Humans’ scalp, skin, facial, pubic hairiness as well as the length, thickness, location, structure, colour, conformation and shape of hair, age of greying, pattern of boldness etc. usually is inherited though may change during lifetime, and are subject to racial and gender differences. Humans’ hair cover the whole body except surfaces of metacarpi, metatarsi, some parts of digits, prepuce, glans, clitoris and labia (Gailevičius 1995). There are three types of hair: long ones (scalp, axial, pubic, sex parts, male beard and moustache), bristle (eyebrow, eyelashes, hair in nose and ear) and lanugo (thin hair almost without pigments covering the whole body–surface).

Hair have three parts: corpus pili – hair–body protruding above the skin; radix pili – root of hair situated in the skin follicle; and apex pili – the top of hair. Every hair has myofibrils that upon cold lift the body–hair and contract skin thus providing the thermoregulation. Every hair has an oil gland.

The colour of the hair is very diverse and varies from very bright till completely black. There are few main colours of the hair: brunet – dark and black hair; chatain – maroon and chestnut hair; bright – blond hair; and red hair – brown and reddish ones. Other colours of the hair are various mixtures and different expression of pigmentation of those above listed. Therefore, the hair colour depends on the pigmentation and colour of skin, and is a subject to race dependence. Sometimes hair may change their colour due to genetics, hormonal shifts, aging of melanocytes, nutrition, pharmaceuticals, influence of UV and X–rays, chemicals and occasional reasons. For example, blond hair due to occupational exposure to high amounts of copper compounds or its abundant presence in the swimming pools may turn into greenish ones (Goldschmidt 1979, Goette 1978). Cobalt compounds bring the bright blue tone to hair. Due to
indigo hair may become dark blue. With age, especially after forties, hair become brighter since hair looses melanin and due to decreased activity of tyrosinase.

Usually humans have about 100 – 120 thousands of hair. Brighter hair are thicker: black–haired persons have 147, brunets – 167 and blonde–haired people have 182 piece hairs per square centimetre (Gailevičius 1995). Humans usually have about 5 millions of follicles, the number of those declining with age. Hair follicles experience two types of aging: hair loss (alopecia) and greying. Hair usually is growing by flocks of three or four, sometimes of two–piece of hair. Hair grows about 1 – 1.5 cm per month. The velocity of the hair growth depends on climate, season, gender, age and location of the hair. At the age of 30 – 40 years, the growth of the hair is the fastest. The vertex hair rises about 0.44 mm/day, temporal – 0.39 mm/day, jaw – 0.27 mm/day. Female’s hair grow faster than male’s.

Visual organs have three types of hair: eyebrows, eyelashes and hair of teardrops bursa. Only humans have the eyebrows. The upper eyelid has about 100 – 150 longer (11 – 11 mm) eyelashes while lower eyelid has 60 – 75 shorter (7 – 8 mm) hair. Male eyebrows are thicker and eyelashes are thinner if compared to female (Gailevičius 1995).

Every piece of hair and follicle has the phases of germination and tranquillity that interchange with each other. There are three phases (Gailevičius 1995) of this cycle: development (growing, proliferation) or anagen, intermediate or catagen, and tranquillity or telogen. The anagen phase has six steps, the last one metagen may last about 2 – 5 years of scalp hair and only 70 days of eyebrows. The metagen step turns into the catagen. During the last phase of telogen hair get solid structure and remain unchanged for about 3 months for scalp and up to 9 months for eyebrows. Then hair shed and new one piece of hair starts to grow in the same follicle. The rate of anagen to telogen phases is 9:1. Normally the growing phase lasts from 2 to 6 years. At any time, about 10% of the hair on scalp is in a resting phase while about 90% of the hair is in a growing one. In alopecia areata 73% of hair were found to be in the telogen (resting) phase and 27% in the anagen (proliferation) phase (Gailevičius 1995). In long standing alopecia areata the percentage of telogen phase hairs can approach 100%.

Newborns’ hair are in the anagen phase, and their hair growth phases are simultaneous up to 6 month of age. Then hair lose their simultaneity and gets the pace of terminal hair growth and activity specific to mature adults. It is normal to shed some hair each day as part of the cycle. However, some people may experience excessive (more than normal) hair loss. Normally, 35 – 150 hairs fall out each day, this number almost doubles during washing, and increases each fall and decreases each spring.
4.2 Hair diseases

Luxurious scalp hair expresses femininity for women and masculinity for men. The lack of scalp hair or the presence of excessive facial or body hair is often distressing to females as the loss of beard and body hair to males. There is a vast number of hair diseases (Gailevičius 1995, Medicine Encyclopedia 1993, Rook 1986, Astrauskas 1980). Those can be summarised as quantitative and structural hair diseases. Some hair diseases are detached (localised scarring alopecia, traumatic alopecia etc.) and some are systemic e.g., benign and malignant tumours, psychiatric disorders, various syndromes (Down, Menkes, etc.).

Certain part of all hair diseases constitutes various hair losses – alopecias. Abnormal and unusual hair loss that differs from described above the hair lifetime, growing and shedding cycle are called alopecia. The mechanisms of hair loss are various. There are those types of shedding: aplasis of hair follicle, destruction of hair follicles, increased stimulation of telogen, regression of hair follicles, terminal hair conversion into lanugo, plentiful shedding of terminal hair, delayed maturation of follicles and impairment of hair anagen.

Various factors i.e., scorch by heat, alkali, acids, chilblain, X–rays, traumas, fungal diseases, syphilis, herpes zoster, patchy and systemic scleroderma may initiate the scarring alopecia. Hairpins, stretch of hair, particular mode of hairstyle, various cosmetic applications and chemicals may damage hair also.

A number of toxic substances and chemicals may cause the anagen shedding since the matrix of the hair is the most sensitive to toxins and certain physical factors. Inherited hypothyreosis, diseases of thyroid gland, lack of iron and vitamin B₁₂, impaired absorption through intestine, some chronic diseases e.g., malignant tumours, leucosis, lympho–granulomatosis, malignant lymphosis etc. may trigger alopecia. Intoxication with heavy metals like arsenic, bismuth, tin and mercury also may cause hair loss (Gailevičius 1995). Other types of alopecia involve premature and senile hair shedding, psychogenetic or neurotic alopecia, enteropathic acrodermatitis (due to impaired zinc absorption through intestine and subsequently decreased levels of zinc in organism), Kwashiorkor, traumatic, male and female androgenic alopecia, patchy and diffuse alopecias. Sometimes patients cut of (trichotemnomania) or pool out (trichotillomania – repeated hair pulling or nervous hair twisting or twirling) hair for themselves due to various psychogenic disorders.

Various emotional stressful situations may trigger the alopecia and maintain the pathologic hair conditions further on in spite of the initial stress. Sometimes stress due to hair loss may cause even serious sexual problems. Usually women react more sensitive to various hair diseases e.g., severe or diffuse alopecia, hirsute, hypertrichosis, flakes or whatever they are.
Androgenic alopecia – male pattern alopecia – constitutes up to 95% of all male alopecias (Gailevičius 1995). Usually this type of baldness is genes’ conditioned. Since Hippocrates, male baldness was considered as a secondary sexual indication and was not qualified as a disease. Aristotle in IV B.C. for the first time described male baldness in medical terms and considered it as organic and constitutional condition. Up to now, baldness was qualified differently. In ancient Rome, men hesitated the baldness while in the later centuries bald men were rated and appreciated even higher. Recently masculine androgenic alopecia is not treated as a pathologic condition.

Various androgenic female alopecias are conditioned by high fluctuations of hormones e.g., pubertal, puerperal and climacteric. Female androgenic alopecia is the most frequent alopecia type among women. In general, androgenic alopecia affects only adult individuals and indicates sexual maturity. Children never have androgenic alopecias. Androgenic alopecia affects about 19% of females and 52% of males up to the age of thirty. In elderly, those numbers vary around 75% among women and 80% among men. In Lithuania, about one third of all women have androgenic alopecia (Gailevičius 1995). Female androgenic male pattern and androgenic female pattern alopecia in women sometimes are difficult to differentiate from diffuse alopecia.

### 4.3 Alopecia areata

Among above described various hair loss diseases there is patchy alopecia – spot hair loss disease, or alopecia areata. Alopecia areata (gr. alōpekia – fox) is a non–scaring, inflammatory, hair loss disease that can affect humans (Papadopoulos 2000, McDonagh 1996) and animals (McElwee 1998) e.g., cats, cattle, dogs, horses, mice, non–human primates (Chimpanzee, Spider Monkey, Stump Tailed Macaque, White–fronted Capuchin) and rats. Alopecia areata (Picture 1 and Picture 2) is the second the most common hair loss disease after the androgenic alopecia (Gailevičius 1995).
Research into the demographics of alopecia areata suggests that 0.05 – 2 % of the population will be affected with alopecia at any time (Barahamani 2002, Sahn 1995, Safavi 1992, Gollinck 1990). The lifetime risk of developing alopecia areata is estimated to be 1.7%. This hair loss disease can affect any hair bearing–area of both genders in various race, ethnic or social groups (Nanda 2002, Safavi 1995, Safavi 1992, Sharma 1990), and appear at any age of lifetime (Schwartz 1997). The first expression of alopecia is mostly likely to occur in people in
their teenage years or yearly twenties, but individual cases were reported in children younger than 2 years of age or older than 70 years (Gollinck 1990). The hair re-growth can occur with or without treatment, though remissions are common.

Hippocrates first used the term alopecia (literally translated as "fox's disease"), the characteristics of the hair loss disease we now know to be alopecia areata, were first described by Cornelius Celsus in 30 A.D. Celsus described two forms of alopecia. The first he described as complete baldness occurring in people of all ages. The second he called ophiasis literally translated as "snake" due to the winding way the bald region spread across the skin. He suggested ophiasis to be only seen in children. Alopecia areata had been given many different names throughout history. However, the actual term "alopecia areata" was first used by Sauvages in his "Nosologica Medica", published in 1786 in Lyons, France.

The classification of alopecia areata is as follows:

1. *Alopecia areata* can present in many different forms. Most frequently, it develops as single one or few patches of hair loss on scalp. These patches of hair loss may develop in any hair bearing region e.g., eyelashes, eyebrows, arms, legs, axillaries, pubic, beard or moustache in men (*alopecia barbae*).

2. The first one or two patches may expand in size and/or other patches of hair loss may develop. The hair loss may develop into total scalp hair loss – *alopecia totalis*.

3. *Alopecia universalis* occur when hair loss involves total scalp hair loss and even complete (eyelashes, eyebrows, axillaries, pubic etc.) body hair loss.

4. A few individuals experience diffuse alopecia areata – *alopecia diffusa*. It is quite difficult to distinguish that from other diffuse forms of hair loss. However, with time a differential diagnosis can be made, whereas diffuse alopecia areata tends to be progressive.

The factors that activate the onset of alopecia and the mechanisms of its development are not fully understood. There are several suggested factors (Shapiro 1999, Gailevičius 1995, Gollinck 1990, The American Hair Loss Council; The American Academy of Dermatology, National Alopecia Areata Foundation, European Hair Research Society) that may influence the course of alopecia. Those factors are as follows: psychological long term chronic stress, psychological sudden extreme stress, physical trauma, local injury, genetic predisposition, viral, bacterial infection, diet, vitamin or mineral deficiency, vaccination, surgery, insects ticks, cancer, hormonal shifts, pregnancy, contraceptive pills, androgenetic, allergies, pharmacological, chemicals, toxic heavy metals, seasonal changes and others.

Recently it was demonstrated that cells under the stress e.g., inflammation, irradiation, viral infection, malignancy, oxidation, heavy metals, etc., can produce heat shock proteins. Those heat shock proteins play a housekeeping role in immune system responses. Circumstantial
evidence suggests alopecia is an autoimmune disease where cells of an individual's own immune system prevent hair follicles from producing hair fiber (Madani 2000). In general, women are far better than men in fighting off bacterial and viral infection, but a more sensitive immune system will be more likely to develop into autoimmune activity. Typically, autoimmune disease is far more common in females than males (Ollier 1989).

The most widely accepted hypothesis is that alopecia is T–cell mediated autoimmune condition and is most likely to occur in genetically predisposed individuals. The evidences favouring autoimmunity of alopecia suggest that most significant of autoimmune conditions are vitiligo (Handa 2003) and thyroid diseases (Jabbour 2000). Vitiligo is associated with incidence varying about 4% of alopecia cases. Though, Alkhateeb (2003) did not find the significant association of vitiligo and alopecia in Caucasian probands and their families.

The relation between thyroid gland dysfunction and alopecia areata established for 2.8 – 27% affected patients (Puavilai 1994, Milgraum 1987). Other data do not support this relationship. Often alopecia patients are diagnosed with the thyroidtoxicosis and Hashimoto’s thyroiditis. About 80% of alopecia people are diagnosed with endothyroid thyreocèle, of that mostly thyreocèle I type. The presence of microsomal antibodies is found in 3.3 – 16% of the patients. Antibodies can be found with or without signs or symptoms of thyroid disease. Sometimes alopecia people have antibodies against thyroglobulins.

It was suggested the intoxication (Ptašekas 2002, Skalny 1999, Gailevičius 1995) with heavy metals like arsenic, bismuth, tin and mercury also might cause non–specific alopecia. In addition, there are claims that imbalance of trace elements’ have been induced by the replacement of essential elements with heavy toxic metals may lead to the disorders of zinc metabolism (Oberleas 1999). The zinc deficiency (Harrison 2003, Rushton 2002, Sinclair 1999) consequently might provoke the onset of alopecia in vulnerable individuals or sensitive population groups beside the rest factors.

Every mentioned factor is suggested as environmental trigger in people predisposed to alopecia development due to genetic susceptibility for example. Whatever the initiation factor it needs not to be permanent – rather a short sharp shock may be just enough to tip the balance of the immune system into autoimmunity. Once an autoimmune disease initiated it can be self–perpetuating.

The therapy of alopecia depends on the type and severity of hair loss. If the hair loss is a clinical symptom of other diseases, usually the cure of the main diseases diminishes the secondary affected hair damages. There are two main strategies of alopecia therapy (Khandpur 2004, Freyschmidt–Pau 2003, MacDonald Hull 2003, MacDonald 1999, Price 1999, Hoffmann

Pharmacological treatment among the others involves minoxidil (Rogaine solutions or creams 1%, 2% and 5%), finasteride (Propecia for men only), estrogens (for women only), tretinoin, various steroid injections into the scalp next to areas of baldness (steroids suppress the immune system); irritant creams (steroid creams); phototherapy (UV and PUVA therapy) and massages; and topical and oral zinc therapy (zinc sulphate, oxide, aspartate etc.) therapy.

The non–pharmacologic therapy usually involves the cosmetic measures (hairstyle adjustments, wigs, extensions, hair pieces, hair weaves or artificial hair replacement hats, scarves); cessation of wearing tight braids, buns, pins; avoidance of identified sources of chemical/allergic causes; psychotherapy and autotraining; and surgical interventions (grafts, flaps, reductions, transplantation).

Therefore, there is no one the most effective treatment proposed. The success rate of various alopecia therapies are 22 – 71%, while no effects experience about 20 – 30% alopecia patients. In addition, side effects are common. Moreover, the spontaneous hair re–growth may occur even without treatment and even after many years. Besides that, once alopecia treatment is stopped the relapse and renewed alopecia frequently develops. The female alopecia prognosis is better than male. The patchy alopecia of scalp together with alopecia of eyelashes, eyebrows, other hair bearing areas along with fingernails impairments and progressive conversion into total or universe alopecia shows worse prognosis.

Although alopecia is not life threatening, the pressures of an image–orientated society can make hair loss psychologically devastating for those affected, their families and friends. Adults who suffer from any types of hair loss can attest to the pain and anxiety associated with their condition (Gülec 2004, Schmidt 2003). However, a young alopecia patient can sustain far more hardship and emotional scaring than any adult can. While it is culturally "acceptable" for mature men and even women to experience hair loss, the pecking order of a child's peer group can inflict devastating ridicule on any of its group that somehow seems different.

4.4 Trace elements

Metals and metalloids are elements and do not break down (Elinder 1994). They form a natural part of our environment (Selinus 2005). Pollution of the environment (air, soil, water) and humans’ exposure to metals occur as the results of the natural erosion of metal–containing minerals, and as the results of human activities such as mining, smelting, fossil–fuel combustion
and industrial application of metals. Metals can dissolve in water and in this way become available for uptake by vegetation and consumption by animals. As the metals do not break down, they remain in the body until they are excreted. This can take years of decades. In addition, it is quite difficult to get rid the environment of a metal which is contaminated.

Exposure to metals occurs mainly through polluted air, food or drinking water and occupational activities. Significant exposure to metals may occur through food and beverages, which can become contaminated at various stages of production or during preparation.

A few types of elements present in the organism: macro–elements (magnesium, calcium, sodium, potassium, phosphorus, etc), microelements (lead, cadmium, mercury, manganese, chromium, copper, zinc, iron, molybdenum, vanadium, selenium, boron, etc.) and ultra–trace elements (gold, silver, thallium, aluminium etc.).

Microelements which occur in human in trace concentrations also are called trace elements (TE). The most of the trace metals also are named as heavy metals due to their atomic weight >5g/cm$^3$. About 20 metals and metalloids e.g., lead, cadmium, arsenic, mercury, nickel, chromium etc. are known to be toxic. Some heavy metals (iron, zinc, selenium, manganese, copper, molybdenum, cobalt, chromium etc.) are essential for normal functioning of the organism.

Several trace elements are essential (chromium, manganese) though can be toxic at high concentrations. Especially this could be observed in people who have been long–term or short–term occupationally exposed to high or even moderate concentration of certain metals in their workplaces. Therefore, the data on toxicity or essentiality of some heavy metals is still lacking or disputable.

Once the metals get into the organism they are distributed with blood stream in certain organs (kidney, liver, spleen, bone, teeth, brain, etc.) where they accumulate. The half–life of heavy metals varies between few days up to 35 (cadmium) and 50 (lead) years. Upon certain circumstances, stress, hormonal changes, pregnancy, etc. there can occur the redistribution of heavy metals from depots. Those depots sometimes may serve as secondary internal sources of heavy metals exposure (lead, cadmium).

Metals are toxic because they interfere with the biochemical systems of the cells. A toxic metal can compete with an essential metal or a site on an enzyme. Several factors are enhancers or inhibitors of certain metals absorption or may reduce the hazardous effects of some chemicals. For example, the deficiency of zinc, calcium and iron in the organism increase hazardous effects of lead and cadmium by enhancing absorption and toxicity (Oberleas 1999, Goyer 1997, Goyer 1994, Avtsyn 1991).

Trace element is not necessary to be toxic to compete with essential trace elements. For example, both zinc and copper are essential for humans. Therefore, zinc compounds have been
used to eliminate copper excess in Wilson’s disease (Prasad 1993). On the other hand, the zinc supplementation in megadoses may cause copper deficiency anaemia (Fischer 1984). In addition, the effects of metal–metal mixtures on health can differ from those than sole metals may cause due to their synergistic or antagonistic interactions.

Children are more susceptible than adults are to hazardous impact of various chemicals including metals (WHO HECA, WHO EEA). The nervous, respiratory and reproductive systems of children are not yet completely developed. This makes them less able to excrete certain toxins. Children are more exposed to toxins in water, food and air because they consume it more than adults relative to their body volume. Also, they can be more exposed to chemicals than adults can because they have other exposures at levels near to the ground in their exposure settings. Children have different natural behaviour, e.g., by crawling and hand–to–mouth behaviour. Moreover, children often cannot escape from exposure, they are not aware of certain dangers, have not developed coping mechanisms and cannot change the situation, whereas adults may have the power or resources to do so.

Numerous of human biomedia can be used to measure the levels of heavy metals in the organism. Among those are whole blood, erythrocytes, plasma, serum, spot or 24–hours urine, saliva, milk, semen, biopsies of various organs (spleen, liver, kidney, skin etc.) tissues, hair, nails, bone, teeth etc. Therefore, the levels of heavy metals internal dose in biological systems as measured in hair, blood and urine reflect and are proper and reliable indicators of those heavy metals levels people are exposed to in their living and working environments (US EPA, London Laboratory Service Group (LLSG); Morton 2004, Sharp 2003, Taylor 1999, Kruste–Jarres 1998, Ali 1997, Cornelis 1995, Elinder 1994).

4.5 Lead

Lead (Pb) is probably one of the most studied environmental and occupational hazards. Lead and its toxic nature has been well known since 2000 B.C. At one stage, lead was even used to induce abortions. Lead exists in the earth’s crust (on average 10 – 15 mg/kg), and occurs naturally in the environment through a variety of mechanisms including volcanic emissions and geochemical weathering.

Lead accumulates in the environment, is non biodegradable and does not lose its toxicity over time. Humans are exposed to lead through air, dust, water and food. Lead is toxic when inhaled or ingested. Particular exposure route to children is their hand–to–mouth behaviour and pica. The content of lead in the 70 kg human body is approximately 2 mg (Oberleas 1999).
Lead is used in storage batteries, ammunition and type metal, cable sheaths, solder, and the plastics industry. The organolead compounds tetraethyl and tetramethyl lead have also been used extensively as antiknock and lubricating agents in petrol, although their use for these purposes in many countries is being phased out. Lead can also be a threat at home, particularly small bore rifle shooting, sanding old lead-based paints and making diving and fishing weights, and in artists' studios and potteries.

Even though interventions for removing lead have been implementing around the world, its toxic effects are felt everywhere. Study showed that lead content in Greenland snow (pre-Christian era) was less than 0.001 mg/kg. Recently the lead content in snow in Greenland is 0.2 mg/kg (Oberleas 1999). On another hand, data (Rogan 2003, Pirkle 1994) indicate that the USA children with elevated blood lead levels (>10 µg/dl) has plunged from 88.2% in the late 1970s to 4.4% in the early 1990s. Data show that less than 10% of the children have levels above 20 µg/dl, but 99% of them lived in developing regions (WHO HECA). Even in the world’s most developed countries it is estimated that a large proportion of children suffer from lead poisoning (4th Ministerial Conference on Environment and Health, 2004).

A Provisional Tolerable Weekly Intake (PTWI) of 25 µg/kg body weight has been decided for lead. The drinking water directive 1998/83/EC has set 10 µg/l of lead, with 15 years transition period to allow for replacing lead distribution pipes. For lead in ambient air the 1999/30/EC limit was set at 0.5 µg/m³. According to current information, adults in Europe have an average of 42 µg/day intake of lead.

Active smokers have a higher blood lead levels than non-smokers (Willers 1988). Mainstream tobacco smoke contains 60 ng of lead per cigarette, while side stream smoke contains 5 – 10 ng of lead per cigarette (Connelly 1999). Studies have shown mean lead levels in the indoor air of homes where smoking occurs is higher than in non-smoking households (21.8 ng/m³ vs. 7.8 ng/m³) (Bonnano 2001). Moreover, the concentration of lead in wine is often also high (10 – 200 µg/l).

Orally ingested lead is very poorly absorbed (5 – 10%), depending upon solubility. However, inhaled lead is almost completely absorbed. The typical absorption rates of lead after ingestion in adults and infants are 20% and 70%, respectively (Sanborn 2002). Very little inorganic lead is absorbed through the skin, but tetraethyl lead is efficiently absorbed through the skin, lungs and gut. Ingested lead is excreted in both urine and faeces.

There are three metabolic pools in humans (Rabinowitz 1973). The first compartment consisting of blood and interstitial fluids contains about 2 mg of lead and has a biological half-life of 27 days. Up to 90% of lead in blood is circulating in the erythrocytes (Skerfving 1993). The second pool, largely soft tissue, contains 0.7 mg of lead with a half-life of 30 days. The
third pool that is mainly the skeleton has a very long (5 up to 50 years) half-life. The skeleton contains more than 90% of the body burden of lead.

The summary of numerous studies (Opler 2004, Canfield 2003, Fewtrell 2003, Mazur 2003, Roggan 2003, Silbergeld 2003, Mendola 2002, Stein 2002, Carpenter 2001, Wigg 2001, Lanphear 2000, Tong 1998, etc.) indicates that lead poisoning may cause diseases and symptoms as follows: mild mental retardation, reduction in IQ and attention span, reading and learning disabilities, hyperactivity and behavioural problems, antisocial behaviour and delinquency, puberty delays, reduced gestational age, reduced weight at birth, impaired growth, decreased ability to maintain steady posture, impaired visual and motor functioning, hearing loss, impairment of synthesis of the active metabolite 1,25-(OH)$_2$, vitamin D, impaired haemoglobin synthesis, anaemia, reproductive impairments of both women and man, brain, liver, kidney, nerve, stomach damage, cancer, coma, convulsions and death. There are indications that intoxication with lead may cause alopecia.

Importantly, many of lead's health effects may occur without overt signs of toxicity. Usually children, women or some population subgroups are more susceptible to hazardous lead impacts (US CDC 1991, WHO HECA, WHO EEA).

### 4.6 Manganese

Manganese (Mn) was isolated in 1774 by Gahn (Oberleas 1999). It was named after mangania, the Greek word for magic. It is the 12th most abundant element in the earth's crust occurring at about 0.1%. Manganese is both essential and toxic element.

Main exposure is mining of manganese ores and welding of mild steel (Elinder 1994). Manganese is used in paint pigments, dry cell batteries and welding electrodes. Permanganate compounds are used in the glass and ceramics industries and are also used as a powerful oxidising agent. The inorganic manganese released with combustion products from cars or trucks. Organic forms of manganese are used as gasoline additive or in pesticides.

Inhalation of fumes and ingestion with beverages and food are the main exposure routes. The intake ranges from 2 – 9 mg/day up to 20 mg/day among vegetarians (Pennington 1989). Foods particularly rich in manganese are seeds and nuts. Animal products are not rich sources particularly when organ tissues rich in mitochondria are not consumed. Freshwater contains manganese in a range of 1 to 200 µg/l. Well water can be contaminated by natural or anthropogenic sources and contain up to 2000 µg/l manganese. Soil contamination is mainly seen near the industry.
The body of a normal 70 kg man is estimated to contain 12 – 20 mg of manganese (Oberleas 1999). Manganese is a ubiquitous element that is essential for certain enzymatic processes and normal physiologic functioning in all animal species. It is found throughout the body, accumulates in tissues high in mitochondria, with the highest level in the liver. High levels of manganese are toxic and can cause various neurological effects.

Man's daily requirement of manganese is unknown but a recommended intake of 2.5 to 5.0 mg/day is proposed. The proposed intake guideline values in water are 0.4 mg/l (WHO Guidelines for Drinking–water Quality 2004) and air 0.15 µg/m³ (WHO Air Quality Guidelines for Europe 2000).

Manganese absorption from the intestinal tract averages 3% to 5%, and decreases with age (Davidsson 1995). Absorbed manganese is quickly removed by the liver, and is excreted in the faeces (Lonnerdal 1987). Very little manganese is excreted in the urine.

High dietary calcium (Johnson 1991, Lassiter 1970) or phosphate (Oberleas 1981) decrease its absorption. Iron deficiency and low protein intake are associated with increased oral manganese absorption. Ethanol consumption will increase hepatic manganese that appears to be related to an effect of alcohol on manganese absorption (Schafer 1974). The absorption mechanisms of manganese and iron show many similarities that are not shared by their excretion processes (Patterson 1984).

The number of manganese metalloenzymes is very limited whereas the enzymes that can be activated by manganese are numerous. The best–described manganese metalloenzymes are arginase, pyruvate carboxylase, and superoxide dismutase. The enzymes that are manganese activated include hydrolases, kinases, decarboxylases, and transferases. Many of these metal activations are non–specific, so that manganese may be partly replaced by other metal ions, particularly magnesium.

Manganese deficiency is manifested by retardation of growth, structural and chemical anomalies of bone, ataxia of the newborn, female sterility, male impotence, and abnormalities of lipid and carbohydrate metabolism. Behaviour similar to manganese deficiency is seen in mice with the mutant gene "pallid" also associated with hair coat colour (Hurley 1987).

Chronic exposure might lead to the impairments of the central nervous system (Carpenter 2001). Neurological symptoms include psychiatric symptoms and symptoms that mimic Parkinson’s disease. Symptoms of the CNS including behavioural and emotional disturbances are seen after long–term exposure. Other impairments are a metal fume fever with flu like complaints, liver injury and pulmonary effects.
4.7 Chromium

Chromium (Cr) was discovered and purified in 1797 by Vauquelin (Oberleas 1999). The concentration of chromium in the earth’s crust is on average 125 mg/kg (Elinder 1994). Chromium is both essential and toxic element.

Chromium(III) is recognized as a trace element that is essential to both humans and animals. It is an essential element necessary for the formation of glucose tolerance factor, the metabolism of insulin and serum cholesterol homeostasis. It is involved in lipid metabolism and is associated with diabetes type II. Chromium deficiency is characterized by impaired growth, impaired response to the glucose tolerance test, elevated circulating insulin, glycosuria, fasting hyperglycemia, elevated serum cholesterol and triglycerides, increased incidence of aortic plaques, peripheral neuropathy, decreased fertility and sperm count, and shortened life expectancy (Davis 1997, Anderson 1981).

Chromium(VI) compounds are toxic and carcinogenic (US EPA, IARC, Barceloux 1999). The human body is able to reduce the carcinogenic hexavalent chromium to less toxic trivalent chromium. This reduction occurs in bodily fluids such as gastric juice, epithelial lining fluid of the respiratory tract, blood, and other fluids (Paustenbach 2003, De Flora 2000, Jones 1990). Chromium(VI) traverses the placenta and is present in breast milk in low concentrations (Barceloux 1999). Chromium(VI) is a skin and mucous membrane irritant, and powerful oxidising agent, affects kidney and liver. Chromate dusts cause conjunctivitis, lacrimation, nose and throat irritation, rhinitis, epistaxis, ulceration or perforation of the nasal septum, and contact dermatitis.

Chromite ores are the major source of chromium. Chromium is used in the chemical, metallurgical and refractory industries because of its hardness and its resistance to corrosion. Occupational exposure to chromium occurs in wood tantalising, stainless steel welding, chrome plating, the leather tanning industry and the use of lead chromate or strontium chromate paints. Contamination of chromium in soil results from deposition in areas around landfills or in the neighbourhood of chromate manufacturing sites. Chromium in air mainly comes from fossil fuel combustion and from steel production.

Available data, generally expressed as total chromium, show a concentration range of 5–200 ng/m$^3$ in background air. Total chromium concentrations in drinking water are usually less than 2 mg/l although concentrations as high as 120 mg/l have been reported (WHO Guidelines for Drinking–water Quality 2004). In general, food and inhalation appears to be the major source of intake. The sources of organic chromium are namely brewer’s yeast, liver, cheese, meat,
lobster, shrimp, black pepper and whole grain cereals are more bioavailable than inorganic chromium (Hazell 1985).

Information on the speciation of chromium in ambient air is essential since, when inhaled, only hexavalent chromium is carcinogenic in humans. The IARC (IARC 1990, US EPA 1984) has stated that for chromium and certain its compounds there is sufficient evidence of carcinogenicity in humans (Group 1). When assuming a linear dose–response relationship between exposure to chromium(VI) compounds and lung cancer, no safe level of chromium(VI) can be recommended. At an air concentration of chromium(VI) of 1 µg/m³, the lifetime risk is estimated to be $4 \times 10^{-2}$ (WHO Air Quality Guidelines for Europe 2000). The guideline 0.05 mg/l for total chromium value in the drinking water is designated as provisional due to uncertainties in the toxicological database (WHO Guidelines for Drinking–water Quality 2004). Recommended Dietary Allowance for chromium has not been established, however, an estimated safe and adequate intake for chromium is established between 50 – 200 µg/day.

The evidence available suggests that chromium is absorbed in the upper small intestine, though mechanism is unknown. Less than 2% of inorganic chromium is absorbed from the small intestine both by animals and by humans. Once absorbed, trivalent chromium appears to bind primarily to transferring. Albumin also can act as non–specific carrier.

Little is known about chromium disposition. Plasma is quickly cleared of chromium within a few days. Tissue chromium appears to distribute into at least three sub–compartments with biological half–lives of 0.5, 6, and 83 days. Absorbed chromium(VI) usually distributed differently from chromium(III). Chromium(VI) can enter the erythrocytes that are impermeable to chromium(III) (Anderson 1987). Chromium is excreted in both urine and faeces. Hair and skin may also account for relatively sizeable losses (Oberleas 1990).

4.8 Cadmium

Cadmium (Cd) was discovered independently by Strohmeyer and Hermann in 1817 (Oberleas 1999). Cadmium is a natural element in the earth’s crust. It is used in nickel/cadmium batteries, as pigments in paints, glazes and plastics. The welding and gas cutting of cadmium coated steel carries a high risk due to the high volatility of cadmium metal. Cadmium occurs naturally with zinc and is a by–product in the smelting of zinc and some lead ores. Cadmium is also a contaminant in phosphate fertilizers and sewage sludge (Elinder 1994, Nordberg 1992).

The environment is polluted by cadmium through industries, household waste incinerators and tobacco smoking. The concentration in air ranges from non–detectable in areas
without industry to 0.06 g/m$^3$ in industrial areas. Cadmium particles in air can travel long
distances, dissolve to some extent in water, and bind strongly to soil. Cadmium can easily be
taken up by fish, animals and some plants, mostly by leafy vegetables.

To prevent any further increase of cadmium in agricultural soils that likely to increase the
dietary intake of future generations, an air guideline values of 5 ng/m$^3$ (WHO Air Quality
Guidelines for Europe 2000) and water 0.003 mg/l (WHO Guidelines for Drinking–water
Quality 2004) was established. The WHO established Provisional Tolerable Weekly Intake
which (PTWI) is 7 µg/kg body weight of cadmium (JECFA 2003). Cadmium is very slowly
excreted from the human body, so current exposure will still be a burden in the future.

Humans are exposed to cadmium by contaminated air (cigarette smoke, industry),
contaminated water and food. Several studies indicate that smoke inhalation is the greatest
source of body burden for cadmium (Staessen 1999). Smoking 20 cigarettes daily ads 2 – 4
µg/day to the inhalatory intake, about 10% of which is absorbed (Staessen 1999, Menden 1972).
Women with low iron stores and iron deficiency, which occurs more often during pregnancy,
have increased cadmium uptake (Vahter 2002).

Cadmium contained in food up to 5% may be absorbed by the upper small intestine.
Inhaled cadmium is completely absorbed and retained efficiently. Cadmium absorption can be
decreased (ATSDR 1999) by a diet rich in oligo–elements e.g., iron, selenium, zinc.

Only 0.01% of the body load of cadmium is excreted daily. Small amounts of cadmium
are excreted in the urine but animal studies have indicated that the faeces were the major route of
cadmium excretion when consumed orally.

Because of the relatively long biological half–life (10 – 35 years), cadmium accumulates
progressively in the body with age in all people, with a greater retention by males than females
of all species receiving the same exposure. Cadmium accumulates mostly in the liver, kidney and
duodenum which account for 50% of the body burden. It was showed (Anke 1979) the female to
be somewhat more protected from the accumulation of cadmium than the male. Estrogens appear
to promote the excretion of cadmium.

Cadmium is toxic and carcinogenic by the inhalation route, and IARC has classified
cadmium and cadmium compounds in Group 2A. Several studies (Verougstraete 2003, Waalkes
2000) indicate a role for cadmium in human renal, pancreatic, urinary bladder, prostate, stomach,
liver and haematopoietic system cancers.

Kidney and renal dysfunction (proteinuria, glucosuria, aminoaciduria, enzymuria) along
with impaired renal tubular re–absorption has been associated (Satarug 2003) with cadmium
exposure. Cadmium ingestion causes increased salivation, choking, vomiting, abdominal pain,
diarrhoea and lead to shock, renal failure and death. Inhalation cause coughs, headache,
vomiting, chest pain and may lead to pulmonary oedema and death. Cadmium may impair haematopoietic system and cause anaemia due to reduced absorption of iron from the intestines. Relatively large cadmium doses cause haemorrhage and subsequent necrosis. Pre-treatment with selenium and zinc can prevent the necrosis.

In addition, cadmium impairs the bone density and may cause osteoporosis (Jarup 2000). The cadmium concentration in bone tissue is low even at high body burdens, and the evidence for a direct action of cadmium on bone is little. Cadmium accelerates the osteoporotic process probably due to the competitive replacement of zinc with collagen synthesis or in essential enzymes. Cadmium is at least one of the important causative factors in the development of Itai–Itai disease (osteomalacia–type condition which characterised by fractures of softened bones).

There have been two reports suggesting that cadmium may be an essential nutrient (Anke 1978, Schwarz 1977) influencing growth. A slightly slower growth rate was observed in goats fed a diet containing less than 25 μg/kg of cadmium. Cadmium has been shown to activate several enzymes in vitro, tryptophan oxygenase, δ–aminolevulinate dehydrogenase and carboxypeptidase b. All of these are normally zinc containing enzymes. No enzymes have yet been identified which may be considered strictly cadmium–dependent (Kostial 1986). Neither report is totally convincing the essentiality.

4.9 Mercury

Mercury (Hg) was known to ancient Chinese and Hindus (Oberleas 1999). It was found in Egyptian tombs of 1500 B.C. Although mercury, like water is present almost everywhere in nature, it is a rare element and comprises less than 30 billionths of the earth's crust. Mercury is the only metal that is liquid at room temperature.

Mercury can enter the biosphere from a variety of industries (Elinder 1994), burning coal and fossil fuels and released by volcanoes. The EU produces 1.000 tonnes mercury per year of the current global supply of 3.600 tonnes per year. Microorganisms have the ability to methylate mercury that is contained in industrial wastes (Clarkson 1987). Mercury and methyl–mercury are accumulated through food chain of fish and sharks (Grandjean 2003, Storelli 2001, Watts 2001). Mercury is cumulative, extremely toxic in compounded form. The liquid form is not a poison. The guideline value of 0.001 mg/l for total mercury in drinking water was set up (WHO Guidelines for Drinking–water Quality 2004).

The major sources of exposure are through dietary intake (methylmercury or organometallic form in fish), inhalation and dental amalgam filling. Mercury when ingested is
rapidly and completely absorbed. Mercury vapour cannot be absorbed through intact skin but many mercurial compounds can be. Some population groups can be exposed additionally through local pollution, occupation, cosmetics, vaccines, cultural or ritualistic use of mercury.

Less than 2% of ingested inorganic mercury is absorbed. There may be a conversion of inorganic to methyl–mercury by bacteria in the intestines but this has not been confirmed. Organic mercury is rapidly and effectively absorbed and reabsorbed from the gastrointestinal (90–95%) tract in humans (Inskip 1985). The mercury is excreted primary by faeces, via bile and urine (Ishihara 2000). Small amounts are excreted via the kidney in both organic and inorganic forms (Clarkson 1987).

Mercury has been detected in all human tissues from people with no known exposure to mercury. The highest mercury levels were found in skin, nails and hair. Of the internal organs, kidney has the highest content reported at 2.7 µg/g wet weight, other tissues range from 0.05 to 0.30 µg/g. The biological half-life of methyl–mercury in humans is estimated to be 70 days, for inorganic mercury is 40 days; mercury vapour is about 50 days (Clarkson 1987). Methyl–mercury establishes sulphidril bonds with proteins and in this form has a half–life of more than two years. If the contaminated proteins are digested by another animal, methyl–mercury becomes again available for absorption.

The main target organs for mercury are the central nervous system and the kidney. Mechanisms of mercury toxicity include: alteration of calcium homeostasis, cytoskeletal damage, generation of free radicals, inhibition of protein synthesis, neuronal cell damage and death. Chronic exposure to mercury may produce symptoms such as tremors, vertigo, irritability, moodiness, depression, diarrhoea, hypoglycaemia and stomatitis. Ingestion of inorganic mercury causes a necrosis of the kidneys and liver, proteinuria, a lack of coordination (cerebellar damage), necrosis of the lining of the small intestine and diarrhoea. However, there is a high, unexplained inter–individual variability of outcome in persons exposed to the same dose.

Methyl–mercury easily penetrates the placental barrier. The sensitivity of the foetus to methyl–mercury is greater than that of the pregnant mother. Organic mercury (methyl–mercury) penetrates the blood–brain barrier and may result in destruction of cells in the cerebellum (balance and coordination) and the visual and hearing centres. Early symptoms of mercury poisoning are not specific. They may include fatigue, headaches, and general irritability, followed by tremors, numbness of arms and legs, difficulty in swallowing, deafness, blurred vision, emotional disturbances, a slurring of speech and a general psychological withdrawal. Other symptoms include muscle wasting, neurological disorders, and death (Watts 2001).
The presence of selenium in the diet diminishes or delays the toxic effects of inorganic and organic mercury compounds (Clarkson 1987). The mechanism of selenium in reducing toxicity is not understood.

4.10 Copper

It is said copper (Cu) to have been mined at least 2000 B.C. Copper is both an essential nutrient and a drinking–water contaminant. It has many commercial uses. It is used to make pipes, valves and fittings and is present in alloys and coatings. Copper sulphate pentahydrate is sometimes added to surface water for the control of algae. Copper concentrations in drinking water vary widely, with the primary source most often being the corrosion of interior copper plumbing. Levels in running or fully flushed water tend to be low, whereas those in standing or partially flushed water samples can be substantially higher (>1 mg/l). The guideline value for level of copper in drinking water was set at 2 mg/l (WHO Guidelines for Drinking–water Quality 2004).

The healthy human body contains 80 mg of copper (Oberleas 1999). Newborn and young animals normally contain more copper per unit of body weight and maintain these levels throughout the suckling period, followed by a steady decline to adulthood. Copper metabolism is homeostatically well controlled. The levels are influenced by physiological (such as in pregnancy) or pathological conditions e.g., in some liver diseases.

The estimated safe and adequate daily dietary intake range for copper is considered to be 1.5 – 3 mg/day for adults. Only about 30% of this copper being absorbed, and only about 0.4 mg/day of copper is available to be utilized for all copper dependent metabolic activities. The richest sources of copper are animal livers, crustaceans and shellfish with concentrations from 20 – 50 mg/kg, and brazil and hazelnuts with 11 – 14 mg/kg. All other foods must be consumed in large quantities to provide the estimated requirement of 2 mg/day for adults.

In most species, dietary copper is poorly absorbed, the extent of absorption is influenced by the amount and chemical form of the copper ingested, by the dietary level of several other metal ions and organic substances, and by the age. High levels of ascorbic acid, fructose or sucrose, a fructose containing disaccharide, appear to interfere with copper absorption (Reiser 1983). Inorganic elements which interfere with copper absorption, retention and distribution within the body include cadmium, calcium, iron, lead, silver, zinc and in ruminants molybdenum plus sulphur. Intakes of zinc well above the nutritional requirement result in copper deficiency.
The distribution of total body copper among the tissues varies with the age. Ingested copper is excreted via the faeces and bile. Only in cases of biliary obstruction, Wilson's disease (hypercuprosis) and nephrosis, urinary copper increases. Negligible amounts of copper are lost in the sweat and a small amount in menstrual flow.

Copper entering the blood plasma from the intestine binds to serum albumin (12 – 17%), selected amino acids i.e., histidine, threonine, glutamine, and loosely bound to a transport protein transcuprein (12 – 14%) (Weiss 1985). Ceruloplasmin is the major source of circulating form of copper representing 60 – 65% of plasma copper (Bremner 1980).

Liver is the central organ of copper metabolism. Though this does not emulate copper absorption in man and animals, it may approximate copper transfer across plasma membranes and copper distribution and metabolism within human cells. Liver synthesizes the copper transport protein ceruloplasmin that is physiologically important in the regulation and homeostasis of copper (Harris 1991, Davis 1987).

Copper plays a key role in the formation of red blood cells and maintenance of normal brain function. It is a constituent of essential metallo–enzymes required in cytochrome oxidation, free radical detoxification and catecholamine production, and in the cross linking of collagen, elastin and keratin. Several enzymes (ceruloplasmin, lysyl oxidase, cytochrome c oxidase, superoxide dismutase, tyrosinase, dopamine β–monooxygenase, amine oxidases) are known to contain copper and are sensitive to copper depletion (Prohaska 1990).

The manifestations of copper deficiency vary with age, sex, severity and the duration of deficiency. In sheep, pigmentation and keratinisation of wool are first affected by lowered copper status with no other signs of copper deficiency.

Several diseases have been associated with copper deficiencies. Among those are anaemia and impaired iron metabolism, various skeletal disorders and cardiovascular disorders. Some diseases (Menke's syndrome (hypocuprosis), Wilson's disease (hypercuprosis), osteoporosis, amyotrophic lateral sclerosis) are associated with impaired copper metabolism.

Chronic copper poisoning may occur in domestic animals due to excessive nutritional intake of copper and humans due to high occupational exposure. Symptoms of copper intoxication include depressed feed intake, depressed growth rate, hypochromic, microcytic anaemia and jaundice in animals. Symptoms of acute poisoning include nausea, vomiting, diarrhoea, circulatory collapse and intramuscular haemolysis. Chronic copper poisoning can lead to liver diseases.
4.11 Zinc

Zinc (Zn) has been known from the beginning of recorded history (Oberleas 1999). Zinc is generally non-toxic and is essential for normal growth and development, wound healing and immune-competence, being involved in virtually all metabolic pathways. It is an essential nutrient with intake values ranging 5 – 15 mg/day for different age and gender categories. Human organism contains 1.5 – 2 mg of zinc.

Zinc is used in galvanising iron and steel, and as an alloy of brass and bronze. It is also used in smoke screens. Zinc fumes are produced from welding flux, wood preservatives and the manufacture of high quality paper, dyes and deodorants. The levels of zinc in surface water and groundwater normally do not exceed 0.01 and 0.05 mg/l respectively, the concentrations in tap water can be much higher as a result of dissolution of zinc from pipes (WHO Guidelines for Drinking–water Quality 2004).

The routes of exposure are dietary ingestion and inhalation. Zinc is found in all food groups: breads and cereals, fruits and vegetables, dairy products and meats. Most sources from meat, poultry and fish are readily absorbable (Hazell 1985). The requirement for zinc is relative to the dietary intake of phytate expressed as the phytate:zinc molar ratio 10:1 or less (Oberleas 1983, 1993, 1996, 2003, Lo 1981, Morris 1980). Zinc continues to be one of the trace elements consumed in the USA general population in less than optimal amounts.

Regulation of zinc absorption is thought to be controlled by metal free albumin. Zinc absorption decreases in the presence of dietary phytate, high dietary phosphate and excessive calcium. Coffee, dairy products and high fibre bread also reduce zinc absorption. Ingestion of alcoholic beverages can mobilize body stores of zinc and increase the urinary excretion. Acute or chronic infection increases urinary losses of zinc (Beisel 1976). Certain drugs, food additives and preservatives such as EDTA may cause increased zinc excretion.

Increased zinc intake depresses copper absorption and conversely copper absorption is greatly increased in zinc deficiency. Metabolic interactions occur between zinc and cadmium, zinc and iron, and zinc and chromium. Cadmium and iron uptake are depressed by high zinc levels, while chromium and zinc are mutually antagonistic.

Zinc is mainly excreted in the faeces, with small amounts via the kidneys and urine. Large amounts (115 µg/dl) of zinc can also be lost in sweat.

Zinc is widely distributed in the body. Zinc is mainly stored in muscle, with bone, liver and plasma forming a small exchangeable pool. About 20% of body zinc is found in skin, nails and hair. The zinc levels drops significantly after the meals (Kučinskienė 2001). Plasma zinc is affected by circadian variation: the highest level being at 9 a.m. and 6 p.m.
Bone marrow, kidney, testis and thymus are the tissues that are first depleted during the onset of zinc deficiency. Cadmium stimulation of metallothionein in the pancreas is inhibited under the zinc deficient conditions and copper complexation in the pancreas is minimal. Pancreatic fluid, which is rich in both zinc and metallothionein, indicates an important role for these in the maintenance of zinc homeostasis (Onosaka 1988). The seminal fluid is rich in zinc. The prostate contains the highest concentration of zinc.

Zinc is essential for the function of about 300 metalloenzymes (Hambidge 1986). The enzymes affected by zinc are of three different types. The first type are enzymes in which zinc is directly associated with the enzyme as structural or active site entities; second are enzymes in which the enzyme activity is induced and thus regulated by a zinc–finger protein or transcription factor as an adjunct of DNA expression but are not physically associated with the active enzyme; and third are enzymes regulated by zinc via inhibition of activity. Non–enzymatic functions (haemoglobin, zinc–finger proteins, matrixins, and metallothioneins) have also been demonstrated for zinc (De Silva 2001).

Zinc is the primary physiological inducer of the metallothioneins in the body. Metallothioneins donate metal ions to high affinity ligands on other proteins. Detoxification of heavy metals (cadmium, mercury, lead etc.) appears to be a primary function of metallothioneins (Oh 1978). Metallothioneins also serve to ameliorate the effects of several noxious agents such as carbon tetrachloride, paracetanol, chemotherapy, UV and ionizing radiation (Coyle 2002). Metallothioneins exist mostly in zinc form or as mixed–metal proteins. Metallothionein binds 3 and 4 ions of cadmium and zinc, respectively (Zhou 2000).

Zinc deficiency is by far the most prevalent nutritional deficiency worldwide (Oberleas 2003). Zinc deficiency is easy to predict but difficult to diagnose. Zinc deficiency occurs particularly in adults without exhibiting overt clinical symptoms thus making clinical diagnosis difficult. It is significant that many of the symptoms attributed to inanition during the past half century are now attributable to zinc deficiency. More than 75% of the world’s population may benefit from exogenous zinc supplementation.

Deficiency of zinc causes upset stomach, slightly depraved appetite, growth depression, skin rashes, poor wound healing, difficult pregnancies and deliveries, mental retardation in offspring, impaired immune response, impaired cell mediated immunity, failure of sexual maturation, taste abnormalities, accumulation of lactic acid during exercise, the decrease and softening of supporting collagen, and impaired vitamin A metabolism. Any condition of lowered zinc intake (Prasad 1978) or increased metabolic demand for zinc will affect zinc homeostasis.

Most mineral elements are toxic when consumed in sufficient high dose. Inhalation of zinc oxide fumes produced during welding can cause metal fume fever characterised by nausea,
headaches, muscular and joint pain, shortness of breath, thirst and a cough. Zinc chloride fumes are highly corrosive to skin, eyes and mucous membranes. Large doses of zinc taken orally also can cause nausea, vomiting, diarrhoea and abdominal pain.

Some diseases e.g., immune deficits, infections are associated with zinc metabolism. Diseases with acute or chronic bleeding, fever, infection, trauma or stress require additional zinc. Zinc deficiency has been observed in patients with chronic renal disease, cirrhosis of the liver, malabsorption syndrome, and sickle cell anaemia.

Acrodermatitis enteropathica is a genetic disorder of zinc metabolism that manifests as zinc deficiency, with retarded growth, hypogonadism, gastrointestinal disturbances, alopecia and skin lesions. It appears in early infancy when the child is weaned from breast milk to cow’s milk. There is a possibility that the higher calcium content of cow’s milk may be a factor in decreasing zinc absorption (Kiely 1988). If treated early and persistently, normal growth, development and a total recovery of the child occurs.

Supplementation with zinc and its compounds should be carefully controlled. For example, zinc supplementation therapy with megadoses of up to 5 g/day, as well as smaller amounts of 150 mg/day, taken for 1 to 2 years have produced copper deficiency anaemia (Fischer 1984). In a study (Prasad 1978) patients with sickle cell anaemia were supplemented of 150 – 200 mg zinc/day for 2 years. The supplement resulted in copper deficiency; serum copper and plasma ceruloplasmin levels were decreased.

A 10–week study of zinc supplementation in 18 healthy women given zinc gluconate supplements twice daily (50 mg zinc/day, or 1.0 mg/kg–day) resulted in a decrease of erythrocyte superoxide dismutase activity (Yadrick 1989). However, zinc does not appear to have the same effect on females that it has on males.
5. MATERIAL AND METHODS

5.1 Subjects

Children at the age of 2 – 16 years (n = 113) with different dissemination of alopecia from all the country diagnosed by endocrinologists or dermatologists were screened for the content of heavy metals in the organism. The physicians rejected other alopecia initiating factors, e.g., traumatic, fungi – bacterial, pharmacological, stress or hormonal shifts. The control subjects (n = 89) were healthy children at the age of 6 – 15 years (51 boys and 38 girls) have been seen in the outpatient clinics for prophylactic health check–up. During the period of ongoing study on children, the number of grown–ups with hair loss disease was screened for the level of trace elements in their organism. Since it was observed the same pattern of uncertainty in adults’ alopecia pathogenesis, the data on heavy metals’ content in adult people with alopecia at the age of 16 – 72 years (n = 72) were incorporated in this research.

To test the hypothesis whether the content of trace elements differ in alopecia people originating from different places of country, the evaluation of heavy metals level in organism of people with alopecia from all ten districts (Vilnius, Kaunas, Klaipėda, Šiauliai, Panevėžys, Alytus, Marijampolė, Utena, Tauragė and Telšiai) was performed as well.

Upon collection of sufficient data in the second stage of the research, the hypothesis whether there is a relation between heavy metals’ content, hormones and blood indices and status of thyroid gland in children with alopecia was tested. The obtained data of sub–sample of children at the age of 2 – 16 years (n = 80, 42 boys and 38 girls) with different dissemination of alopecia from the Department of Paediatric Endocrinology (Kaunas Medical University Hospital) were compared to those of the control group of children without alopecia.

During entire study, 17 children (11 boys and 6 girls) with low zinc ions content either zinc deficiency in their organism were screened. The endocrinologists or dermatologists prescribed these children the treatment with zinc supplementation i.e., partial chelating and recovery therapy with various commercially available supplements (zinc sulphate, oxide or aspartate) or vitamins with enhanced content of zinc. The dosage and duration of daily zinc intake respectively varied and depended on the age and severity of zinc deficiency and was 10 – 30 mg/day. In addition, after some time these treated children have been seen in the Laboratory for Environmental Health Research (further Laboratory) to monitor the balance of trace elements’ content in their organism. Thus, the double–repeated (n = 17) and triple–repeated (n = 11) analysis of trace elements content in different biomedia of children was accomplished.
5.2 Material and methods

The research accomplished in the Laboratory for Environmental Health Research (previously Laboratory for Anthropogenic Factors Research), Institute for Biomedical Research of Kaunas University of Medicine, Lithuania in 1997 – 2004. The concentration of trace elements’ ions was analysed in hair, blood/plasma and spot urine samples of people with alopecia as well as in relevant biological media of the control subjects. To avoid influence of the usage of vitamins and microelements patients were advised to refrain from intake of any vitamins and food supplements at least 2 – 4 weeks prior analysis. The data on residence area, living and working environment, possible sources of heavy metals emission, occupation, smoking and alcohol intake habits (if relevant), hobbies, dietary habits, usage of medicines and other relevant information of the investigated people were collected. In total, content of the following seven heavy metals’ ions: lead, manganese, chromium, cadmium, mercury, copper and zinc was assessed in this study. The choice of elements to analyse was performed in relevance and contribution of those metals to the hair loss disease, and synergistic or antagonistic interactions with each other. The determination of lead, manganese, chromium, cadmium and mercury ions level performed in whole blood while the concentration of copper and zinc ions was measured in plasma.

The piece up to 150 mg weight of total length of head hair was scissored up from not less than 5 – 7 locations of scalp – occipital, vertex, both temples, frontal region and a few random ones. Only the piece up to 5 cm of proximate hair part was involved into analysis in spite of the length of hair was available. Specimens of hair were clear, without hair spray, gel, mousse for styling etc. Permanent waving, dyeing–hair or decolouration along with natural colour of hair was label and considered if it could influence the trace elements’ analysis in hair. The hair samples were washed, dissolved, stored and analysed.

The volume of 5 – 7 ml ulnar vein blood was obtain with single usage syringes and using metals–free heparin "Biochemie" (Biochemie GmbH, Vienna – Austria) as an anticoagulant. The vast majority of specimens were taken while people were without meals. The plasma was separated by centrifugation 3000 rpm 15 min. The volume of 10 ml spot urine specimens were collected into the nitric acid washed polyethylene tubes. The obtained biomedia was treated immediately, and the prepared samples were stored in the refrigerator (+4º C) till the analysis was carried out. The total duration of analysis between sampling and actual determination time did not exceed the 3 – 7 calendar days.

The modified direct analysis method (Schlemmer 1989, Ryselis 1996) for trace elements’ (lead, manganese, chromium, cadmium, copper and zinc) ions analysis in hair, vein
blood/plasma and urine specimens was applied. The electro–thermal graphite furnace atomic absorption spectrophotometer (ET HGA–600, AS–60) with Zeeman background correction Zeeman–3030 (Perkin–Elmer, USA) was in use. The analysis standard error as evaluated by apparatus statistical calculations did not exceed 9.6%. The detection limits for all analysed elements were not less than 0.001 ppm. The preciseness, reliability and reiteration (International Organization for Standardization 1980) of trace elements analysis were controlled by inclusion of internal and external control materials.

The mercury measurements were carried out by atomic absorption spectrophotometer. The modified methodology of "cold vapour" method on mercury concentration determination in hair (Ryselis 1996), blood and urine (Xavezov 1980, Ribeyre 1995) was involved.

The assessment of common blood indices, blood biochemical and hormones’ parameters and evaluation of thyroid gland performed in sub–sample of 80 children with alopecia. The routine analyses of some blood indices (leucocytes, haemoglobin and erythrocytes sedimentation rate (ESR)), biochemical parameters (calcium (Ca), calcium ions (Ca^{2+}), phosphorus (P), magnesium (Mg), total protein and glucose) as well as hormones and antibodies (cortisol, thyroid stimulating hormone (TSH), free thyroxine (FT_{4}), parathyroid hormone (PTH) and antimicrosomal thyroid antibodies (ATPO)) were performed in the Laboratories of the Kaunas Medical University Hospital. The endocrinologists evaluated the grade of thyreoecele according to WHO classification.

The guidelines (London Laboratory Service Group 2005, Walker 1998, Cornelis 1995, Pineu 1993, Friberg 1988, Iyengar 1986, Katz 1985) for the specimens’ collection along with trace elements analysis and monitoring in biological media to handle and avoid contamination with trace elements of samples and lab–ware were in use. In addition, every batch of syringes and heparin was monitored for heavy metals contamination. Metals’ contamination–free, repeatedly super–pure 2.4M nitric acid washed and followed by repeated deionised pure water rinse mini–sorption plastic tubes and lab–ware were used. To avoid contamination and shifts in methodological techniques the biomedia collection was performed by single laboratory worker and episodically by instructed nursing staff as well as the samples treatment and relevant analysis were done by the same personnel. Therefore, the sample treatment and preparation along with the analyses were controlled by inclusion of internal and external quality control materials.
5.3 Statistical analysis

The descriptive statistics and inferential statistical tests were in use for data analysis. Descriptive statistics, such as mean, standard deviation, minimum, maximum, proportions, were calculated to describe data central tendency and dispersion.

Parametric and nonparametric methods were applied for making inferences about data. Parametric methods were used for quantitative normally distributed data analysis. Quantitative data, which did not meet requirements of normal distribution, were analysed by nonparametric methods. Differences among means in the independent groups were tested using Student’s t test or Mann – Whitney U test, parametric and nonparametric Kruskal – Wallis ANOVA tests (Daniel 1995, Kirkwood 1989). Changes of metals concentration after zinc treatment were assessed performing repeated ANOVA measures with post–hoc evaluation. Relationship among qualitative data evaluated by chi–square or exact Fisher test.

The significance level was set up at p<0.05. The statistical software packages SPSS and STATA were in use for data statistical analysis.

The indices of confidential intervals or standard deviation are not indicated in the figures on purpose do not to overload diagrams and therefore to keep the maximum of visual lucidity and evidence information. The entire results in the figures presented solely as a mean. This was condition by intention to highlight the facts that entire variations of changes in trace elements level present within physiological limits though are different in people with and without alopecia. Therefore, entire statistical indices are present in relevant tables.
6. RESULTS AND DISCUSSION

6.1 Alopecia in Lithuania

6.1.1 Alopecia in different age and genders

In total, the analysis of trace elements ions in children with hair loss disease involved 113 children – 47 girls and 66 boys. The established female to male ratio in children was 1:1.4. In addition, there were investigated 72 adult people with hair loss disease – 45 women and 27 men. The detected women to men ratio was 1.7:1. The established mean of children age was 9.6 years, 10.0 years for girls and 9.3 years for boys, respectively. The ranges of age were 2 – 16 years for both girls and boys as well. The detected mean of age in adult people was 31.1 years, 32.9 years for women and 27.8 years for men, respectively. The ranges of age were 17–55 years for women and 16 – 72 years for men, respectively.

The literature claims that women are far better than men are in fighting off bacterial and viral infection, but a more sensitive immune system will be more likely to develop into autoimmune activity, including onset of alopecia. Typically, autoimmune disease is far more common in females than males (Ollier 1989). Literature (Nanda 2002) reports the boys to girls ratio is to be 1:2.5. On the other hand, girls to boys ratio 1:1.4 also was reported (Tan 2002). Our research showed contradictory results: boys reported about 1.4–fold increase in prevalence of alopecia if compared to girls. Female showed about 1.7–fold higher prevalence of hair loss disease than men did. Literature (Friedman 1985) also claims the female to male ratio is to be 2:1. This is believed to be in part due to the differences in hormone levels between the two genders. Therefore, the summary of literature data available claims the female to male ratio to be 1:1. In total, in our study there were investigated 92 female and 93 males, supporting almost equal incidence and prevalence among different genders.

The contradictory results (higher prevalence of alopecia among boys vs. girls, and women vs. men) of our research remain unclear. It might be that female in adulthood are inclined to show far more concern and look after their appearance in preference than men do. Though, it was reported (Biondo 2004) that women with female pattern androgenic hair loss underestimated the severity of their hair loss compared with their clinicians’ ratings.

Despite head hair being not essential for survival, it seems to play a crucial role in humans’ social society and serve as second sexual tempters (relishes) especially in women. On the other hand, it seems to be normal in humans’ society and quite acceptable for men to have androgenic alopecia unless severe other types of alopecia or alopecia barbae occur. It is
interesting to point out, that men are more inclined to tolerate whatever alopecia except alopecia of moustache and beard. This seems also to be related to the expression of second sexual relishes and could be based on self–image of being a male. Therefore, the overlap of alopecia and androgenic alopecia, and approach to tolerate male hair loss may hide true situation and shift results towards higher number of alopecia in women.

Other possible reasons might be that the metabolism of some trace elements is gender–related and based on the synergistic and antagonistic interaction of elements, e.g., both iron and copper ions are antagonists for zinc ions. Due to peculiarities of iron metabolism in females, it may influence the metabolism of zinc. There are no data indicating that in childhood the metabolism of essential metals are gender related though the physiological levels of copper, zinc and iron vary in different genders in adulthood, besides being different in childhood and adulthood (Zaleskis 2003, Kučinskienė 2001, Oberleas 1999, LLSG). The boys due their physical activities and habits to explore living and playing environments are likely to play outside more frequently. Hence, they may accumulate higher contents of heavy metals (e.g. lead, cadmium, mercury etc.) and subsequently may have relatively lower content of zinc in their organism due to antagonism of those metals.

**Key findings**

The alopecia prevalence was more frequent among children in preference to adults with hair loss disease. In addition, results showed higher prevalence of alopecia in boys vs. girls among those children who were seen in the Laboratory for heavy metals screening in their organism. Completely different results observed in grown–ups: alopecia distribution in women was higher in preference to men among those people investigated in this research.

**6.1.2 Types and symptoms of alopecia**

Hair loss disease has been reported in association with many other medical conditions (Price 1991, Perini 1984). The disorders involving alopecia as a primary (main and first united) symptom were diagnosed for 106 of total 113 children and for 55 of total 72 investigated adult people. The 77 kids among those 106 children had alopecia as the main single symptom. The alopecia as the first symptom accompanying with other diseases e.g., endocrinological, neurological, dermal, digestive tract disorders and allergy, were suffering 29 children, the alopecia as the second symptom followed by other disease was established in 7 children. The 44
adults of those 55 grown–ups had the alopecia as the main single symptom. The alopecia as the first symptom accompanying with other diseases had 11 adult people, the alopecia as the second symptom followed by other disease was found in 17 adults. The diagrams of the proportional distribution of symptomatic presence of hair loss disease in children and adults are presented in Figure 1 and Figure 2, respectively.

Figure 1. Alopecia as a symptom in children

Figure 2. Alopecia as a symptom in adult people
Data show that the majority of alopecia cases were attributed to hair loss disease as the main complaint and primary disease that prompted the patients to see in the Laboratory for the investigation of trace elements’ content. Therefore, it was assumed that the patients with alopecia as a collateral symptom when the main disease which might have triggered the alopecia onset was known were not seen in the Laboratory. This was confirmed by the reports of patients and physicians affirming that people approaching the Laboratory for trace elements’ content screening in their organism had no any observed and documented clinical complaints or alterations in clinical or biochemical tests.

Due to incompatible number of cases for symptomatic evaluation of hair loss disease, the entire alopecia cases were pooled up respectively into different two groups of children and adults with alopecia. Hence, further evaluation of trace elements content performed without specification whether alopecia was primary or secondary symptom.

The research revealed the 71 case of alopecia areata of scalp, 11 cases of alopecia totalis, 10 cases of alopecia diffusa, 9 cases of alopecia areata of scalp together with alopecia of eyebrows, 4 cases of alopecia universalis, 3 cases of alopecia subtotalis, 2 cases of alopecia areata of scalp together with alopecia of eyebrows and eyelashes, and 3 cases of other alopecias in children. The proportional presence of different types of hair loss disease in children is showed in Figure 3.

![Figure 3. Different types of alopecia in children](image-url)

Data show that the majority of cases (64%) were alopecia areata of scalp though about one third of total investigated cases were the other types of alopecia. Therefore, altogether about 20% of all cases were alopecia diffusa and alopecia totalis, and constituted more than half of
those other observed types of hair loss disease or crude one third of entire alopecia areata cases in children.

The investigation revealed 31 case of alopecia areata of scalp, 6 cases of alopecia totalis, 25 cases of alopecia diffusa, 2 cases of alopecia areata of scalp together with alopecia of eyebrows, 2 cases of alopecia universalis, 3 cases of alopecia areata of scalp together with alopecia barbae, 1 case of alopecia areata of scalp together with alopecia of eyebrows and eyelashes, 1 case of alopecia totalis of scalp together with alopecia of eyebrows and eyelashes, and 1 case of other alopecias in adult people. The proportional presence of different types of hair loss disease in adults is showed in Figure 4.

![Figure 4](image)

**Figure 4. Different types of alopecia in adult people**

The data show that the majority of alopecia cases in adults were alopecia areata (43%) and alopecia diffusa (36%). The other types of hair loss were minor. The portion of alopecia of moustache and beard in men was 4% and constituted three cases of all 27 men alopecia cases involved in this study.

As could be see from the diagrams the majority of alopecia cases were alopecia areata of scalp, 64% in children and 43% in adults, respectively. Therefore, adults (36%) more frequently reported the diffuse hair loss if compared to the children (9%) did. It can be that alopecia areata is more prevalent in childhood while alopecia diffusa is more frequent in adulthood. Differently, higher number of women vs. men investigated could shift the results towards the higher number of observed diffuse alopecia since particularly women are more inclined to take care of their
appearance if compared to the men. Other data (Gailevičius 1995) indicate that iron deficiency may cause the alopecia especially in young women. The haemoglobin content in blood may be normal while the iron content in plasma is lower. Usually the iron deficiency triggers diffuse hair loss. This could explain higher prevalence of diffuse hair loss in adulthood. However, as was mentioned earlier, the patients’ records and tests did not indicate any documented deficiencies of iron. Other reasons of higher female alopecia prevalence involve frequent application of various dyeing, permanent waving and other cosmetic techniques. Some of them could damage hair. Among other reasons could be far more social image–driven attitude of adults, or inadequate approach to consider the children’s slight or diffuse hair loss as concern and disease should be consulted the physicians, especially when in early childhood some of individuals did not express profuse amount of scalp hair.

The incompatible number of alopecia types in children and adults did not allow to extract significant variations of heavy metals ions content in relation to hair loss type. Hence, all children and adults alopecia cases were pooled and further analysed as general type of alopecia in children and alopecia in adults.

**Key findings**

The primary complaint and mostly the single reason for the children and adults to be seen in the Laboratory for heavy metals’ investigation in their organism was hair loss of various degrees per se. The majority of observed types of alopecia in both children and adults were alopecia areata of scalp. Therefore, adults more frequently than children were suffering from alopecia diffusa. The other types (alopecia totalis, alopecia universalis, alopecia of eyebrows and eyelashes, alopecia barbae and mixed variations of those listed) of hair loss disease constituted rare cases.

**6.1.3 Trace elements in people with alopecia from different districts**

The assessment of content of trace elements in organism of people with alopecia involved 113 children and 72 adults with different dissemination of alopecia from all ten districts (Vilnius, Kaunas, Klaipėda, Šiauliai, Panevėžys, Alytus, Marijampolė, Utena, Tauragė and Telšiai) of country. The main approach of this evaluation was to test the hypothesis whether the content of heavy metals and trace elements differ in alopecia people originating from different districts of country.
The distribution of control subjects residing in different districts of Lithuania, and children and adults with alopecia have had been investigated in the Laboratory is presented in Figure 5, Figure 6 and Figure 7, respectively.

**Figure 5.** Distribution of control subjects residing in different districts of country

**Figure 6.** Distribution of investigated children with alopecia in different districts of country
The distribution and prevalence of alopecia among children and adults among those who were screened for heavy metals content, was the highest in Kaunas district (40% and 74%, respectively), Šiauliai and Panevėžys (16% and 7%, respectively) and Vilnius district (10% and 5%, respectively). The lowest one was observed in Klaipėda, Telšiai and Tauragė districts (4%) among children, and Marijampolė and Šiauliai (3%) districts among adult people.

The obtained data of heavy metals (lead, manganese, chromium, cadmium, mercury) and essential trace elements’ (copper and zinc) ions concentrations in the organism of children with alopecia from different districts is given in Table 1.
Table 1. Concentration (mean±SD) of heavy metals in hair (µg/g), blood/plasma (µg/dl) and urine (µg/l) of children with alopecia from different districts of Lithuania

<table>
<thead>
<tr>
<th>TE</th>
<th>Biomedia</th>
<th>Vilnius</th>
<th>Kaunas</th>
<th>Klaipėda</th>
<th>Šiauliai</th>
<th>Panevėžys</th>
<th>Alytus</th>
<th>Marijampolė</th>
<th>Utena</th>
<th>Tauragė</th>
<th>Telšiai</th>
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<tr>
<td></td>
<td></td>
<td>1.90±1.12</td>
<td>1.76±1.95</td>
<td>2.11±1.33</td>
<td>1.30±1.46</td>
<td>10.52±5.72</td>
<td>0.87±0.62</td>
<td>1.78±1.50</td>
<td>5.06±5.37</td>
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<td>2.46±0.00</td>
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<tr>
<td>Pb</td>
<td>Blood</td>
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<td>3.00±1.52</td>
<td>2.49±2.05</td>
<td>3.65±1.88</td>
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<tr>
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<td>Urine</td>
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<td>5.30±2.68</td>
<td>7.02±5.89</td>
<td>5.33±3.21</td>
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<td>Mn</td>
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<td>1.04±0.61</td>
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<td>Cr</td>
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<tr>
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<td>Plasma</td>
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<td>Urine</td>
<td>18.27±0.18</td>
<td>11.11±7.32</td>
<td>16.95±0.00</td>
<td>9.52±5.63</td>
<td>42.63±60.60</td>
<td>11.43±6.14</td>
<td>19.62±28.36</td>
<td>15.61±4.38</td>
<td>13.18±2.35</td>
<td>7.82±6.21</td>
</tr>
<tr>
<td>Zn</td>
<td>Hair</td>
<td>152.7±35.6</td>
<td>146.3±48.7</td>
<td>251.9±120.4</td>
<td>174.7±46.7</td>
<td>153.3±29.7</td>
<td>149.1±55.2</td>
<td>173.3±204.3</td>
<td>116.9±33.5</td>
<td>126.6±0.00</td>
<td>324.2±0.00</td>
</tr>
<tr>
<td></td>
<td>Plasma</td>
<td>105.02±28.5</td>
<td>106.48±35.6</td>
<td>107.36±37.80</td>
<td>113.9±50.0</td>
<td>112.77±29.66</td>
<td>127.02±41.0</td>
<td>116.43±71.4</td>
<td>99.02±60.5</td>
<td>99.62±23.6</td>
<td>112.22±26.0</td>
</tr>
<tr>
<td></td>
<td>Urine</td>
<td>1867.1±554.5</td>
<td>527.0±346.4</td>
<td>986.9±0.00</td>
<td>381.7±232.6</td>
<td>1211.3±607.6</td>
<td>558.3±409.4</td>
<td>363.2±317.1</td>
<td>902.5±960.4</td>
<td>620.7±195.2</td>
<td>371.2±169.3</td>
</tr>
</tbody>
</table>
The detected mean concentration of heavy metals in hair, blood/plasma and urine of children with different dissemination of alopecia did not exceed the permissible values. Data showed that the highest concentration of lead ions in hair and blood was in children from Panevėžys district while the lead concentration excreting with urine was the highest in children from Vilnius district. The highest amount of mercury ions in hair, blood and urine detected in children with hair loss disease from Alytus, Klaipėda and Marijampolė districts. The highest content of cadmium ions established in hair of children with alopecia from Telšiai and Marijampolė districts, and in blood and urine of children from Klaipėda and Panevėžys districts. Moreover, the lowest concentration of zinc ions found in hair and plasma of children from Utena and Tauragė districts, and in urine of children from Marijampolė, Telšiai and Šiauliai districts. However, the highest level of zinc ions lost with urine detected in Vilnius, Panevėžys and Utena districts.

The obtained data of heavy metals (lead, manganese, chromium, cadmium, mercury) and essential trace elements’ (copper and zinc) ions concentrations in organism of adults with alopecia from different districts is given in Table 2.
Table 2. Concentration (mean±SD) of heavy metals in hair, blood/plasma and urine of adults with alopecia from different districts of Lithuania

<table>
<thead>
<tr>
<th>TE</th>
<th>Biomedia</th>
<th>Vilnius</th>
<th>Kaunas</th>
<th>Panevėžys</th>
<th>Alytus</th>
<th>Marijampolė</th>
<th>Utena</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>µg/g</td>
<td>µg/dl</td>
<td>µg/g</td>
<td>µg/dl</td>
<td>µg/l</td>
<td>µg/l</td>
</tr>
<tr>
<td>Pb</td>
<td>Hair</td>
<td>1.23±0.73</td>
<td>1.30±2.16</td>
<td>1.14±1.28</td>
<td>0.65±0.09</td>
<td>6.66±7.50</td>
<td>1.69±0.92</td>
</tr>
<tr>
<td></td>
<td>Blood</td>
<td>1.93±1.06</td>
<td>3.31±2.21</td>
<td>4.12±2.10</td>
<td>2.89±0.71</td>
<td>5.45±5.43</td>
<td>3.51±2.29</td>
</tr>
<tr>
<td></td>
<td>Urine</td>
<td>2.47±1.48</td>
<td>6.88±13.89</td>
<td>14.66±16.67</td>
<td>1.14±0.82</td>
<td>13.68±4.99</td>
<td>1.37±1.23</td>
</tr>
<tr>
<td>Mn</td>
<td>Hair</td>
<td>2.25±2.44</td>
<td>1.74±1.95</td>
<td>1.02±0.74</td>
<td>1.10±1.09</td>
<td>2.46±2.78</td>
<td>1.39±0.81</td>
</tr>
<tr>
<td></td>
<td>Blood</td>
<td>0.87±0.35</td>
<td>0.89±0.63</td>
<td>0.99±0.72</td>
<td>1.32±0.18</td>
<td>0.80±0.36</td>
<td>1.11±0.89</td>
</tr>
<tr>
<td></td>
<td>Urine</td>
<td>1.83±1.89</td>
<td>1.33±1.45</td>
<td>1.82±1.98</td>
<td>1.84±0.86</td>
<td>0.73±0.90</td>
<td>0.38±0.32</td>
</tr>
<tr>
<td>Cr</td>
<td>Hair</td>
<td>0.22±0.10</td>
<td>0.23±0.21</td>
<td>0.21±0.18</td>
<td>0.43±0.30</td>
<td>0.34±0.03</td>
<td>0.23±0.18</td>
</tr>
<tr>
<td></td>
<td>Blood</td>
<td>~</td>
<td>0.19±0.16</td>
<td>~</td>
<td>~</td>
<td>~</td>
<td>0.12±0.05</td>
</tr>
<tr>
<td></td>
<td>Urine</td>
<td>~</td>
<td>0.52±0.54</td>
<td>0.56±0.38</td>
<td>~</td>
<td>~</td>
<td>0.81±0.04</td>
</tr>
<tr>
<td>Cd</td>
<td>Hair</td>
<td>0.12±0.16</td>
<td>0.06±0.08</td>
<td>0.06±0.07</td>
<td>0.02±0.01</td>
<td>0.53±0.20</td>
<td>0.13±0.10</td>
</tr>
<tr>
<td></td>
<td>Blood</td>
<td>0.09±0.05</td>
<td>0.08±0.08</td>
<td>0.04±0.03</td>
<td>0.07±0.06</td>
<td>0.07±0.05</td>
<td>0.06±0.06</td>
</tr>
<tr>
<td></td>
<td>Urine</td>
<td>0.17±0.18</td>
<td>0.31±0.33</td>
<td>0.42±0.31</td>
<td>0.11±0.09</td>
<td>0.87±0.90</td>
<td>0.15±0.12</td>
</tr>
<tr>
<td>Hg</td>
<td>Hair</td>
<td>0.31±0.34</td>
<td>0.09±0.08</td>
<td>0.07±0.04</td>
<td>0.09±0.04</td>
<td>0.04±0.01</td>
<td>0.07±0.02</td>
</tr>
<tr>
<td></td>
<td>Blood</td>
<td>0.45±0.29</td>
<td>0.31±0.09</td>
<td>0.27±0.14</td>
<td>0.25±0.08</td>
<td>0.11±0.03</td>
<td>0.30±0.12</td>
</tr>
<tr>
<td></td>
<td>Urine</td>
<td>3.03±1.77</td>
<td>1.86±0.87</td>
<td>0.91±0.93</td>
<td>1.68±0.21</td>
<td>1.08±0.40</td>
<td>1.70±0.79</td>
</tr>
<tr>
<td>Cu</td>
<td>Hair</td>
<td>15.08±8.89</td>
<td>17.67±11.23</td>
<td>15.21±7.00</td>
<td>14.02±4.30</td>
<td>18.35±5.03</td>
<td>10.44±1.60</td>
</tr>
<tr>
<td></td>
<td>Plasma</td>
<td>96.07±16.94</td>
<td>94.73±26.99</td>
<td>106.27±17.88</td>
<td>104.83±20.54</td>
<td>106.17±15.36</td>
<td>87.00±27.58</td>
</tr>
<tr>
<td></td>
<td>Urine</td>
<td>4.05±2.50</td>
<td>11.76±13.71</td>
<td>15.02±6.84</td>
<td>2.83±0.70</td>
<td>17.82±4.93</td>
<td>7.47±3.59</td>
</tr>
<tr>
<td>Zn</td>
<td>Hair</td>
<td>165.30±30.81</td>
<td>199.77±63.18</td>
<td>189.70±56.42</td>
<td>197.43±17.03</td>
<td>135.00±46.10</td>
<td>198.50±28.10</td>
</tr>
<tr>
<td></td>
<td>Plasma</td>
<td>133.30±93.95</td>
<td>104.79±39.78</td>
<td>121.96±46.52</td>
<td>110.97±9.94</td>
<td>91.50±2.97</td>
<td>96.33±38.42</td>
</tr>
<tr>
<td></td>
<td>Urine</td>
<td>487.33±614.49</td>
<td>427.23±340.94</td>
<td>386.80±162.74</td>
<td>241.57±103.21</td>
<td>551.20±590.15</td>
<td>687.43±345.21</td>
</tr>
</tbody>
</table>
The detected mean concentration of heavy metals in hair, blood/plasma and urine of adults with different dissemination of alopecia did not exceed the permissible values. Data showed that the highest level of lead ions in hair, blood and urine of adults with alopecia was respectively in Marijampolė and Panevėžys district while the highest excreting with urine lead content was observed in Panevėžys district. The highest cadmium content in hair and urine established in Marijampolė district and the highest urinal cadmium content observed in alopecia adults from Vilnius district. The lowest zinc ions content in hair and plasma of adults with alopecia observed in Marijampolė district while the lowest urinal zinc level established in Alytus district.

The results showed that the difference of lead and chromium ions concentration in hair of children with alopecia from different districts was p<0.001 and p<0.01, respectively. Also, blood lead concentration (p<0.01) and content of zinc in urine (p<0.001) of those children was different, respectively. In addition, the established difference of cadmium in hair and copper in urine of adults with alopecia from different districts was p<0.05 and p<0.001, respectively. Therefore, those solitary and isolated differences of trace elements content in separate biomedia of alopecia people did not allow to make sound conclusions that content of trace elements in the entire organism (altogether in hair, blood/plasma and urine) differed in alopecia people from various regions of the country. In addition, the established fluctuations of trace elements ions concentration in children and adults did not have relationship with observed distribution and prevalence of hair loss disease in different districts of the country. The correlation (data not shown) between the alopecia prevalence in the different districts and the content of the heavy metals’ in alopecia patients were not established.

In this research, the relationship between detected concentration of heavy metals in the organism of children and adults and alopecia distribution in different districts was not established. Thus, some reasons for uneven distribution of alopecia among Lithuanian inhabitants remain unclear though. Heavy metals content as measured in topsoil is proper and good indicator of general environmental pollution (Lanphear 1998), or may indicate the potential pollution and accumulation of other chemicals in this site. Topsoil is the major source contributing to metals amount in house dust (Meyer 1999, Mielke 1998) and subsequently to higher levels inhaled with air. Data roughly indicate that the content of toxic heavy metals are the highest as follows: lead – in topsoil of Klaipėda, Šiauliai, Kaunas and Tauragė districts; chromium – Alytus and Kaunas districts; manganese – Vilnius, Alytus and Utena districts; copper – Alytus and Kaunas districts, and zinc – Alytus, Šilalė and Kaunas districts (Zinkutė 2002, Kadūnas 1999). Therefore, the content of heavy metals in topsoil in most of the cases did not match the concentration of trace elements has been established in alopecia people.
The Laboratory for Environmental Health Research, that is single one over the state providing the extended analysis of heavy metals content in various biomedia of humans, is located in Kaunas city. In addition, Kaunas is about 70 km away from the geographical centre of country, which is in the nearby village of Ruoščiai (Dotnuva, Kėdainiai subdistrict). This also indicates comfortable and good attainability. So, the highest observed number cases in Kaunas district may be biased by better access for screening in the Laboratory rather than the real the highest prevalence of alopecia, though this needs further detailed analysis.

Both Vilnius and Šiauliai cities are not so far away from Kaunas and easy reachable via highways. Besides that, Kaunas Medical University Hospital provides the highest health care service, so-called third level, for inhabitants of Šiauliai. Once, the alopecia people were seen the physicians in the Hospital, they requested to make the heavy metals analysis. Therefore, the environmental conditions in Šiauliai favour the probable imbalance of heavy metals in people organism. The quality of drinking water in Šiauliai is unsatisfactory since it contains high quantity of iron that known to be zinc antagonist. In addition, the soviet military airport is left along with plenty remains of soviet missile bases and previous military camps around the city. We need to admit that Kaunas, Šiauliai and Vilnius cities are highly industrial. Thus, it could be that the observed high prevalence of alopecia among Šiauliai and Vilnius children could be related to site pollution with lead and other toxic heavy metals, though it requires further research that is more detailed.

Klaipėda is the harbour city and has well-developed health care system. Since it is in a quite distance from Kaunas, probably plenty of alopecia cases were left behind the screening. The observed lower prevalence of alopecia in other regions and rural areas gives an idea about relatively smaller accumulation of heavy metals in those districts. Data (Fewtrell 2003) indicate the lower pollution in rural areas with heavy metals and various chemicals except pesticides. In addition, the contribution of possible occupational exposure to heavy metals could not be excluded though the data on potential sources of heavy metals emissions in working environment of adults were collected. Among other reasons of uneven distribution of hair loss disease the different living and working environments, different diet preferences and nutritional habits of investigated individuals should be noted.

Extending into the details of alopecia distribution among children and adults the geographical, social, economical conditions, health care policy and management, along with the personal contribution of physicians and motivation of patients should not be excluded though. These seem to be sinister but important factors for screening of alopecia incidence or prevalence along with search for possible triggers of this disease.
Key findings

The mean content of the trace elements in the organism of alopecia patients from different districts of the country did not differ between each other. The imbalance of trace elements’ ions concentration in children and adults organism was not related to the distribution and prevalence of children and adults alopecia in different districts of the country. The distribution and prevalence of children and adults alopecia among those investigated in the Laboratory from different districts of Lithuania is unequal, though reasons for it remain unclear and require detailed further research. The observed higher prevalence of alopecia among children and adults in Kaunas, Šiauliai and Vilnius districts could be stronger associated with geographical, environmental, social, economical, health care system policy and management rather than with the disease initiating factors, though this requires further research.

6.2 Trace elements and humans’ alopecia

6.2.1 Heavy metals in organism of people with hair loss disease

The content of heavy metals ions in children and adults with alopecia were determined, and obtained results compared to those of the group of control subjects. The extended statistical indices of established content of heavy metals ions in hair are presented in Table 3.
### Table 3. Concentration of heavy metals ions in hair

<table>
<thead>
<tr>
<th>TE</th>
<th>Group</th>
<th>Mean (µg/g)</th>
<th>Min</th>
<th>Max</th>
<th>95% CI of mean</th>
<th>Difference, p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pb</td>
<td>alopecia children</td>
<td>2.72</td>
<td>0.00</td>
<td>20.10</td>
<td>1.92 – 3.51</td>
<td>between children and adults with alopecia, p&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>alopecia adults</td>
<td>1.43</td>
<td>0.06</td>
<td>13.72</td>
<td>0.87 – 2.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>control group</td>
<td>1.36</td>
<td>0.16</td>
<td>5.61</td>
<td>0.76 – 1.96</td>
<td></td>
</tr>
<tr>
<td>Mn</td>
<td>alopecia children</td>
<td>1.29</td>
<td>0.80</td>
<td>8.71</td>
<td>0.96 – 1.62</td>
<td>between children with alopecia and controls, p&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>alopecia adults</td>
<td>1.68</td>
<td>0.18</td>
<td>9.41</td>
<td>1.23 – 2.13</td>
<td></td>
</tr>
<tr>
<td></td>
<td>control group</td>
<td>0.77</td>
<td>0.09</td>
<td>3.78</td>
<td>0.35 – 1.18</td>
<td></td>
</tr>
<tr>
<td>Cr</td>
<td>alopecia children</td>
<td>0.37</td>
<td>0.01</td>
<td>3.30</td>
<td>0.26 – 0.47</td>
<td>between children with alopecia and controls, p&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>alopecia adults</td>
<td>0.25</td>
<td>0.01</td>
<td>0.91</td>
<td>0.20 – 0.30</td>
<td></td>
</tr>
<tr>
<td></td>
<td>control group</td>
<td>0.13</td>
<td>0.05</td>
<td>0.50</td>
<td>0.08 – 0.18</td>
<td></td>
</tr>
<tr>
<td>Cd</td>
<td>alopecia children</td>
<td>0.09</td>
<td>0.00</td>
<td>0.47</td>
<td>0.07 – 0.11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>alopecia adults</td>
<td>0.08</td>
<td>0.01</td>
<td>0.67</td>
<td>0.05 – 0.11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>control group</td>
<td>0.09</td>
<td>0.02</td>
<td>0.23</td>
<td>0.06 – 0.12</td>
<td></td>
</tr>
<tr>
<td>Hg</td>
<td>alopecia children</td>
<td>0.11</td>
<td>0.02</td>
<td>0.57</td>
<td>0.08 – 0.13</td>
<td>between children with alopecia and controls, p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>alopecia adults</td>
<td>0.10</td>
<td>0.02</td>
<td>0.76</td>
<td>0.07 – 0.13</td>
<td></td>
</tr>
<tr>
<td></td>
<td>control group</td>
<td>0.25</td>
<td>0.02</td>
<td>1.87</td>
<td>0.06 – 0.44</td>
<td></td>
</tr>
<tr>
<td>Cu</td>
<td>alopecia children</td>
<td>16.73</td>
<td>1.88</td>
<td>133.55</td>
<td>13.14 – 20.33</td>
<td>between children with alopecia and controls, p&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>alopecia adults</td>
<td>17.03</td>
<td>7.12</td>
<td>65.05</td>
<td>14.53 – 19.52</td>
<td></td>
</tr>
<tr>
<td></td>
<td>control group</td>
<td>11.44</td>
<td>3.38</td>
<td>31.40</td>
<td>8.23 – 14.65</td>
<td></td>
</tr>
<tr>
<td>Zn</td>
<td>alopecia children</td>
<td>151.4</td>
<td>36.6</td>
<td>389.8</td>
<td>138.6 – 164.1</td>
<td>between children and adults with alopecia, p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>alopecia adults</td>
<td>188.9</td>
<td>76.00</td>
<td>394.2</td>
<td>174.7 – 203.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>control group</td>
<td>179.3</td>
<td>62.7</td>
<td>363.6</td>
<td>147.1 – 211.4</td>
<td></td>
</tr>
</tbody>
</table>

Due to vast of data the obtained results were splitted up and are presented in the form of diagrams of heavy metals ions (lead, manganese, chromium, cadmium and mercury) and trace elements (copper and zinc) content in hair of investigated subjects, respectively in Figure 8 and Figure 9.
Figure 8. Concentration of heavy metals (lead, manganese, chromium, cadmium and mercury) ions in hair.

* – p<0.05 between children with alopecia and control group

** – p<0.01 between children with alopecia and control group

xx – p<0.01 between children with alopecia and adults with alopecia

Figure 9. Concentration of trace elements (copper and zinc) ions in hair.

** – p<0.01 between children with alopecia and control group

xx – p<0.01 between children with alopecia and adults with alopecia
The detected mean content of trace elements in hair did not exceed the permissible levels. The concentration of lead ions in hair of children with alopecia vs. adults with alopecia was significantly higher (p<0.01) while the concentration of zinc ions in hair of children with alopecia vs. adults with alopecia was significantly lower (p<0.01). The amount of copper, chromium and manganese ions in hair of children with alopecia vs. control group was significantly higher (respectively, p<0.01, p<0.01 and p<0.05). The concentration of mercury ions in hair of children with alopecia vs. control group was significantly lower (p<0.01).

The increased concentration of heavy metals as lead, chromium, copper and manganese due to physical antagonism of those metals may lead to zinc decline (Oberleas 1999, Elinder 1998, Goyer 1994). On another hand, there are claims that the copper concentration increase in falling out or alopecic hair might be the consequence of hair loss (Anke 2003, personal communication). Actually, it is quite difficult to assess whether those hair were cut off and taken for the analysis could be considered as the alopecic or potentially falling ones. Therefore, we failed to find relevant literature data to provide analysis that is more detail to evaluate whether the enhanced copper ions content in hair might be attributed as cause or outcome of alopecia.

Other data (Jin 1998) indicate that in men with alopecia manganese and zinc concentrations in the hair were found to be lower than those for healthy men but copper concentrations were higher. The study in Byelorussia (Gress at al. 2002, European Hair Research Society) reported the deficiency of manganese to be found in 87% of children with alopecia areata. Potassium deficiency reported in 69%, calcium – 62%, magnesium – 50%, selenium and iron – 25% alopecia children. The most significant was excess of mercury (18%), arsenic (19%) and aluminium (13%). Approximately 1/5 of all cases of intoxication with lead as measured in hair correlated with alopecia of scalp and eyelashes (Ptašekas 2002).

The tendencies that the observed lead ions content was the highest in children with alopecia while the amount of zinc ions was the lowest suggest clear antagonistic pattern between lead and zinc ions. In addition, it should be noted that hair is one of the ways of elimination (Oberleas 1999) of metals. This gives an idea that total lead burden in children with alopecia might be the highest compared to adults and control subjects. Otherwise, it indicates that the resources of zinc ions in organism could be lower in humans with alopecia, particularly in children.

The extended indices of statistical analysis of measured concentration of heavy metals ions in blood and plasma are showed in Table 4.
Table 4. Concentration of heavy metals ions in blood/plasma

<table>
<thead>
<tr>
<th>TE</th>
<th>Group</th>
<th>Mean (µg/dl)</th>
<th>Min</th>
<th>Max</th>
<th>95% CI of mean</th>
<th>Difference, p</th>
</tr>
</thead>
<tbody>
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<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Pb</td>
<td>alopecia children</td>
<td>3.65</td>
<td>0.13</td>
<td>16.43</td>
<td>3.21 – 4.08</td>
<td>between children with alopecia and controls, p&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>alopecia adults</td>
<td>3.38</td>
<td>0.71</td>
<td>9.29</td>
<td>2.86 – 3.90</td>
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</tr>
<tr>
<td></td>
<td>control group</td>
<td>2.15</td>
<td>0.37</td>
<td>5.67</td>
<td>1.25 – 3.05</td>
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</tr>
<tr>
<td>Mn</td>
<td>alopecia children</td>
<td>1.08</td>
<td>0.08</td>
<td>3.11</td>
<td>0.98 – 1.18</td>
<td>between children and adults with alopecia, p&lt;0.01</td>
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<tr>
<td></td>
<td>alopecia adults</td>
<td>0.92</td>
<td>0.11</td>
<td>3.27</td>
<td>0.78 – 1.07</td>
<td>between children with alopecia and controls, p&lt;0.05</td>
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<tr>
<td></td>
<td>control group</td>
<td>1.37</td>
<td>0.47</td>
<td>2.42</td>
<td>1.08 – 1.66</td>
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</tr>
<tr>
<td>Cr</td>
<td>alopecia children</td>
<td>0.09</td>
<td>0.02</td>
<td>0.19</td>
<td>0.08 – 0.11</td>
<td>between children and adults with alopecia, p&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>alopecia adults</td>
<td>0.17</td>
<td>0.01</td>
<td>0.61</td>
<td>0.08 – 0.26</td>
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</tr>
<tr>
<td></td>
<td>control group</td>
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<td>--</td>
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</tr>
<tr>
<td>Cd</td>
<td>alopecia children</td>
<td>0.08</td>
<td>0.00</td>
<td>0.43</td>
<td>0.07 – 0.09</td>
<td></td>
</tr>
<tr>
<td></td>
<td>alopecia adults</td>
<td>0.08</td>
<td>0.01</td>
<td>0.41</td>
<td>0.06 – 0.10</td>
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</tr>
<tr>
<td></td>
<td>control group</td>
<td>0.07</td>
<td>0.01</td>
<td>0.20</td>
<td>0.04 – 0.10</td>
<td></td>
</tr>
<tr>
<td>Hg</td>
<td>alopecia children</td>
<td>0.31</td>
<td>0.12</td>
<td>1.20</td>
<td>0.28 – 0.33</td>
<td></td>
</tr>
<tr>
<td></td>
<td>alopecia adults</td>
<td>0.31</td>
<td>0.09</td>
<td>0.76</td>
<td>0.28 – 0.34</td>
<td></td>
</tr>
<tr>
<td></td>
<td>control group</td>
<td>0.32</td>
<td>0.21</td>
<td>0.45</td>
<td>0.28 – 0.37</td>
<td></td>
</tr>
<tr>
<td>Cu</td>
<td>alopecia children</td>
<td>103.43</td>
<td>6.42</td>
<td>183.3</td>
<td>98.05 – 108.82</td>
<td>between children and adults with alopecia, p&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>alopecia adults</td>
<td>96.06</td>
<td>33.70</td>
<td>174.99</td>
<td>90.09 – 102.02</td>
<td>between children with alopecia and controls, p&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>control group</td>
<td>86.15</td>
<td>61.10</td>
<td>130.60</td>
<td>74.70 – 97.60</td>
<td></td>
</tr>
<tr>
<td>Zn</td>
<td>alopecia children</td>
<td>109.4</td>
<td>41.5</td>
<td>313.8</td>
<td>101.4 – 117.3</td>
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</tr>
<tr>
<td></td>
<td>alopecia adults</td>
<td>106.9</td>
<td>31.2</td>
<td>241.6</td>
<td>97.2 – 116.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>control group</td>
<td>117.2</td>
<td>71.8</td>
<td>158.7</td>
<td>99.7 – 134.6</td>
<td></td>
</tr>
</tbody>
</table>

Due to numerous data the obtained results were splitted up and are given in the form of diagrams of heavy metals ions (lead, manganese, cadmium and mercury) level in blood and trace elements (copper and zinc) content in plasma of investigated subjects, respectively in Figure 10 and Figure 11.
**Figure 10.** Concentration of heavy metals (lead, manganese, cadmium and mercury) ions in blood

**Figure 11.** Concentration of trace elements (copper and zinc) ions in plasma
The detected mean content of trace elements in blood/plasma did not exceed the permissible levels. The level of copper in plasma and manganese in blood of children with alopecia vs. adults with alopecia was significantly higher (respectively, p<0.05 and p<0.01), and the amount of chromium ions in blood of children with alopecia vs. adults with alopecia was significantly lower (p<0.05). The level of lead in blood and copper ions in plasma of children with alopecia vs. control group was significantly higher (respectively, p<0.01 and p<0.05). In addition, the content of manganese ions in blood of children with alopecia vs. control group was significantly lower (p<0.05).

Mean blood levels in Western European countries are reported to be below 5.0 µg/dl, in Eastern European countries between 5.1 and 10 µg/dl (WHO 2004). The study in Byelorussia indicated (Gress et al. 2002, European Hair Research Society) the blood lead levels exceeded the permissible level (10 µg/dl) in 65% of children with alopecia. Moreover, in 10% of them blood lead levels was 5 – 8 times higher than 10 µg/dl. Moderate mercury excess in blood was found up to 60% of children. Iron and copper deficiency was revealed in 50%, zinc deficiency – in 37% (p<0.05) of children. In addition, the severity and rate correlated to the zinc content decline in blood.

It is important to note that children with alopecia had the highest lead ions content that was lower in adults with alopecia and the lowest in control group. The blood lead content is generally accepted biomarker of lead exposure (US EPA). Numerous literature data (WHO HECA, US EPA, Fewtrell 2003) indicates children are more susceptible to exposure to lead due genetic, physiological, behavioural, social and other reasons. Hence, it might be that at the same level of lead exposure the hazardous effects induced by lead could be pronounced higher in vulnerable or susceptible individuals.

In addition, the obtained data suggest that child’s organism is tender to accumulate the ions of lead and other toxic metals what subsequently may reduce the content of zinc due to physical antagonism of those elements. The established level of zinc ions in alopecia subjects was lower as compared to control subjects though not significant. The provisional blood zinc levels in Lithuania are to be 130 µg/dl (International Zinc Nutrition Consultative Group (IZiNCG) 2004). It was concluded that the risk for zinc deficiency is unlikely.

The findings of Finland alopecia study (Mussalo–Rauhamaa 1986) reported that the statistical significant difference was found between the copper content of serum in alopecia areata and alopecia universalis patients and also between the copper content of serum in alopecia areata plus alopecia totalis and alopecia universalis patients. Due to incompatible number of patients with alopecia areata, alopecia totalis and alopecia universalis in our research we could
not stratify the alopecia groups and evaluate whether the concentration of trace elements would differ in patients with different type and severity of alopecia.

The extended indices of statistical analysis of heavy metals ions concentration in urine are presented in Table 5.

### Table 5. Concentration of heavy metals ions in urine

<table>
<thead>
<tr>
<th>TE</th>
<th>Group</th>
<th>Mean (µg/l)</th>
<th>Min</th>
<th>Max</th>
<th>95% CI of mean</th>
<th>Difference, p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pb</td>
<td>alopecia children</td>
<td>4.67</td>
<td>0.04</td>
<td>29.34</td>
<td>3.34 – 5.60</td>
<td></td>
</tr>
<tr>
<td></td>
<td>alopecia adults</td>
<td>6.97</td>
<td>0.01</td>
<td>91.24</td>
<td>3.89 – 10.06</td>
<td></td>
</tr>
<tr>
<td></td>
<td>control group</td>
<td>3.18</td>
<td>0.01</td>
<td>14.50</td>
<td>1.28 – 5.09</td>
<td></td>
</tr>
<tr>
<td>Mn</td>
<td>alopecia children</td>
<td>1.34</td>
<td>0.01</td>
<td>8.63</td>
<td>0.98 – 1.70</td>
<td></td>
</tr>
<tr>
<td></td>
<td>alopecia adults</td>
<td>1.35</td>
<td>0.02</td>
<td>7.26</td>
<td>1.01 – 1.68</td>
<td></td>
</tr>
<tr>
<td></td>
<td>control group</td>
<td>0.96</td>
<td>0.01</td>
<td>6.17</td>
<td>0.27 – 1.65</td>
<td></td>
</tr>
<tr>
<td>Cr</td>
<td>alopecia children</td>
<td>0.52</td>
<td>0.02</td>
<td>2.29</td>
<td>0.29 – 0.75</td>
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<tr>
<td></td>
<td>alopecia adults</td>
<td>0.58</td>
<td>0.01</td>
<td>1.63</td>
<td>0.35 – 0.81</td>
<td></td>
</tr>
<tr>
<td></td>
<td>control group</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Cd</td>
<td>alopecia children</td>
<td>0.22</td>
<td>0.00</td>
<td>1.26</td>
<td>0.16 – 0.27</td>
<td></td>
</tr>
<tr>
<td></td>
<td>alopecia adults</td>
<td>0.32</td>
<td>0.01</td>
<td>1.53</td>
<td>0.24 – 0.40</td>
<td></td>
</tr>
<tr>
<td></td>
<td>control group</td>
<td>0.16</td>
<td>0.01</td>
<td>0.66</td>
<td>0.09 – 0.24</td>
<td></td>
</tr>
<tr>
<td>Hg</td>
<td>alopecia children</td>
<td>2.21</td>
<td>0.48</td>
<td>8.79</td>
<td>1.82 – 2.59</td>
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</tr>
<tr>
<td></td>
<td>alopecia adults</td>
<td>1.88</td>
<td>0.28</td>
<td>4.29</td>
<td>1.65 – 2.11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>control group</td>
<td>3.10</td>
<td>0.84</td>
<td>9.81</td>
<td>2.04 – 4.16</td>
<td></td>
</tr>
<tr>
<td>Cu</td>
<td>alopecia children</td>
<td>15.36</td>
<td>0.51</td>
<td>164.50</td>
<td>10.00 – 20.71</td>
<td></td>
</tr>
<tr>
<td></td>
<td>alopecia adults</td>
<td>11.59</td>
<td>1.58</td>
<td>100.43</td>
<td>8.58 – 14.60</td>
<td></td>
</tr>
<tr>
<td></td>
<td>control group</td>
<td>15.20</td>
<td>5.66</td>
<td>49.00</td>
<td>10.30 – 20.11</td>
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<tr>
<td>Zn</td>
<td>alopecia children</td>
<td>629.1</td>
<td>77.2</td>
<td>2310.9</td>
<td>503.4 – 754.8</td>
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<tr>
<td></td>
<td>alopecia adults</td>
<td>436.0</td>
<td>34.7</td>
<td>1585.2</td>
<td>356.4 – 515.5</td>
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</tr>
<tr>
<td></td>
<td>control group</td>
<td>820.9</td>
<td>299.2</td>
<td>1814.2</td>
<td>578.4 – 1063.5</td>
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</tr>
</tbody>
</table>

Due to numerous data the obtained results were splitted up and are given in the form of diagrams of heavy metals ions (lead, manganese, cadmium and mercury) and trace elements (copper and zinc) content in urine of investigated subjects, respectively in Figure 12 and Figure 13.
Figure 12. Concentration of heavy metals (lead, manganese, cadmium and mercury) ions in urine.

Figure 13. Concentration of trace elements (copper and zinc) ions in urine.

* – p<0.05 between children with alopecia and control group

x – p<0.05 between children with alopecia and adults with alopecia
The determined content of urinal excretion of heavy metals ions did not exceed the permissible levels. The significant differences between alopecia and control subjects were not established. The content of lead and cadmium excretion in urine of alopecia subjects was higher as compared to control group though not significant. It probably indicates the total lead and other toxic heavy metals burden in organism. The concentration of zinc ions in urine of children with alopecia vs. adults with alopecia was significantly higher (p<0.05) and significantly lower (p<0.05) vs. control group. As was discussed previously, the low excretion of zinc with urine of alopecia people probably indicates low resources of zinc ions available in the organism.

Therefore, we found merely one study in our literature database investigating the content of trace elements in urine of alopecia people. The research in Finland (Mussalo–Rauhamaa 1986) found no differences of copper, zinc, cadmium, chromium and selenium concentrations’ in the urine samples of alopecia patients if compared to those of the normal population could be found. However, we found that alopecia patients had lower urinal zinc content if compared to the control group.

**Discussion and key findings**

The higher concentrations of lead, copper and cadmium ions in hair, blood/plasma and urine in people with alopecia were found if compared to the control group while the content of zinc ions in the organism of people with alopecia was lower. The increased intake of lead, cadmium, iron and copper may lead to zinc deficiency due to zinc physical antagonism of those elements (Oberleas 2002, Miller 1990, Petering 1978, Petering 1974). Lead replaces zinc on haeme enzymes and cadmium replaces zinc on metallothioneins (Goyer 1994). Lead competes with calcium, inhibiting the release of neurotransmitters, and interferes with the regulation of cell metabolism by binding to second–messenger calcium receptors, blocking calcium transport by calcium channels and calcium–sodium ATP pumps, and by competing for calcium–binding protein sites and uptake by mitochondria (Goyer 1995).

Metabolic interactions occur between zinc and cadmium, zinc and iron, zinc and copper, and zinc and chromium. Cadmium and iron uptake are depressed by high zinc levels, while chromium and zinc are metabolised by a common pathway in the intestine and are mutually antagonistic (Oberleas 1999). Otherwise, the zinc deficiency increases the hazard from lead and cadmium exposure by enhancing absorption and toxicity (Skalny 1999, Avtsyn 1991).

Moreover, the genetic polymorphism has been recognised as the important determinants of the biologic consequences of lead exposure (Kelada 2001). The delta–aminoleavulinic acid dehydratase (d–ALAD) genotype d–ALAD 1–2/2–2 individuals are more susceptible to the
hazardous effects of lead. The prevalence of the d–ALAD 2 allele ranges 0 – 20 % depending on the population. Caucasians have the highest frequency of the d–ALAD–2 allele, with approximately 18% of it being d–ALAD 1–2 heterozygotes and 1% being 2–2 homozygotes.

In addition, undernourished children are more susceptible to lead toxicity because their bodies absorb more lead if other nutrients, such as calcium, iron or zinc, are lacking (Lidsky 2003, Gilbert 2002, Bradman 2001, Finkelstein 1998).

The levels of trace elements as measured in hair in different East European countries was summarised in the literature (Skalny 2003). It was concluded that generally Lithuania differs from the other East European countries (Russia, Croatia, Macedonia, Ukraine, Byelorussia and Latvia) by lower mean concentration of virtually all elements in hair of population. However, there are no data indicating whether there is the difference in alopecia incidence and prevalence in those countries. Therefore it was reported that outbreak of alopecia areata in Chernivtsi (Western Ukraine) in 1988 likely was due to heavy metals poisoning (Rich 1996).

It should be kept in mind that the investigation focused the scope on the trace elements’ research and did not investigate the metabolism of macro–elements’ ions. The regulation mechanisms of uptake, excretion and homeostasis are different in metabolism of microelements and macro–elements though. While exist mechanisms for keeping balance for macro–elements the toxic heavy metals once get into the organism usually deposit and accumulate in the organism. Plenty of factors are involved in this process: levels of metals in the living, playing and working environments, nutrition, abilities of organism to metabolite and excrete hazardous agents, interaction of substances with each other etc. Hence, the hazardous effects usually amplify giving quite clear picture of exposure – dose and internal dose – effects relationship.

The essential thing that deserves to discuss is the physiological ranges of toxic heavy metals and essential trace elements. The established mean concentration of trace elements in this research did not exceed the allowed limitations levels had have been set up by Lithuania Ministry of Health, EU Directives, WHO, IARC, ATSDR, US Environmental Protection Agency and other institutions. Thus, those established amounts of trace elements in humans could not be qualified as intoxication or poisoning cases, what are usually mentioned as possible trigger of the alopecia (Ptašekas 2002, Skalny 1999, Gailevičius 1995, Avtsyn 1991).

Still the discussion is focused rather on threshold limits of chemicals actions and the heavy metals internal dose that still did not produce clinically expressed symptoms or was supposed do not to cause any health impairments. Although there might be some lower limits of exposure at which toxicity may not be detected (threshold), there may be no level at the molecular level that does not have an adverse effect (Goyer 1994).
It seems likely even slight increase of toxic metals within permissible levels may shift the balance of essential trace elements. This research on balance of heavy metals demonstrates that relative increase (within physiological reference values) of toxic heavy metals may lead to imbalance of essential elements and subsequently prompt to relative deficiency (within physiological) of zinc in the organism. The deficiency of essential elements influences health effects upon toxic metals exposure and might cause the immune system imbalance or course of disease in vulnerable or susceptible individuals.

6.2.2 Trace elements in different gender and age of people with hair loss disease

The concentration of heavy metals’ ions in children and adults with hair loss disease was compared according to the gender and age. The obtained data of trace elements content in organism of alopecia subjects are presented in Table 6.
Table 6. Content of trace elements in organism of people with alopecia

<table>
<thead>
<tr>
<th>TE</th>
<th>Group</th>
<th>Biomedia</th>
<th>Mean</th>
<th>Min</th>
<th>Max</th>
<th>95% CI of mean</th>
<th>Difference, p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Girls</td>
<td>hair (μg/g)</td>
<td>2.25</td>
<td>0.00</td>
<td>15.12</td>
<td>1.19 – 3.30</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>blood (μg/dl)</td>
<td>3.20</td>
<td>0.17</td>
<td>7.63</td>
<td>2.71 – 3.68</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>urine (μg/l)</td>
<td>4.78</td>
<td>0.04</td>
<td>29.34</td>
<td>1.58 – 7.98</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Boys</td>
<td>hair (μg/g)</td>
<td>3.09</td>
<td>0.28</td>
<td>20.10</td>
<td>1.91 – 4.28</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>blood (μg/dl)</td>
<td>3.96</td>
<td>0.13</td>
<td>16.43</td>
<td>3.30 – 4.63</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>urine (μg/l)</td>
<td>4.62</td>
<td>0.06</td>
<td>23.90</td>
<td>3.20 – 6.04</td>
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</tr>
<tr>
<td></td>
<td>Women</td>
<td>hair (μg/g)</td>
<td>1.24</td>
<td>0.06</td>
<td>11.94</td>
<td>0.64 – 1.82</td>
<td>between women and men, p&lt;0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>blood (μg/dl)</td>
<td>3.24</td>
<td>0.71</td>
<td>9.20</td>
<td>2.55 – 3.94</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>urine (μg/l)</td>
<td>7.28</td>
<td>0.02</td>
<td>45.64</td>
<td>4.32 – 10.24</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Men</td>
<td>hair (μg/g)</td>
<td>1.89</td>
<td>0.28</td>
<td>13.72</td>
<td>0.53 – 3.24</td>
<td>between women and men, p&lt;0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>blood (μg/dl)</td>
<td>3.60</td>
<td>1.04</td>
<td>9.29</td>
<td>2.78 – 4.40</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>urine (μg/l)</td>
<td>6.47</td>
<td>0.01</td>
<td>91.24</td>
<td>0.37 – 13.32</td>
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</tr>
<tr>
<td></td>
<td>Girls</td>
<td>hair (μg/g)</td>
<td>1.85</td>
<td>0.24</td>
<td>8.71</td>
<td>1.17 – 2.52</td>
<td>between girls and boys, p&lt;0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>blood (μg/dl)</td>
<td>1.16</td>
<td>0.08</td>
<td>2.86</td>
<td>1.00 – 1.32</td>
<td>between girls and women, p&lt;0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>urine (μg/l)</td>
<td>1.21</td>
<td>0.01</td>
<td>3.27</td>
<td>0.76 – 1.66</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Boys</td>
<td>hair (μg/g)</td>
<td>0.84</td>
<td>0.08</td>
<td>2.59</td>
<td>0.65 – 1.04</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>blood (μg/dl)</td>
<td>1.02</td>
<td>0.16</td>
<td>3.11</td>
<td>0.90 – 1.15</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>urine (μg/l)</td>
<td>1.39</td>
<td>0.05</td>
<td>8.63</td>
<td>0.90 – 1.88</td>
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</tr>
<tr>
<td></td>
<td>Women</td>
<td>hair (μg/g)</td>
<td>2.07</td>
<td>0.29</td>
<td>9.41</td>
<td>1.47 – 2.68</td>
<td>between women and men, p&lt;0.01</td>
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<td></td>
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<td>0.03</td>
<td>7.26</td>
<td>1.10 – 2.01</td>
<td>between women and men, p&lt;0.01</td>
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<tr>
<td></td>
<td>Men</td>
<td>hair (μg/g)</td>
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<td>0.18</td>
<td>2.35</td>
<td>0.48 – 1.08</td>
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<td>blood (μg/dl)</td>
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<td>0.11</td>
<td>2.48</td>
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<td>0.02</td>
<td>5.73</td>
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<td>Girls</td>
<td>hair (μg/g)</td>
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<td>between girls and boys, p&lt;0.05</td>
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<td>blood (μg/dl)</td>
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<td>1.35</td>
<td>0.24 – 0.43</td>
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<td>blood (μg/dl)</td>
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<td>0.02</td>
<td>0.19</td>
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<td>0.02</td>
<td>1.10</td>
<td>0.16 – 0.53</td>
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<td>Women</td>
<td>hair (μg/g)</td>
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<td>0.01</td>
<td>0.74</td>
<td>0.17 – 0.28</td>
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<td>0.61</td>
<td>0.03 – 0.42</td>
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<td>0.01</td>
<td>1.63</td>
<td>0.21 – 0.93</td>
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<td>Men</td>
<td>hair (μg/g)</td>
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<td>0.04</td>
<td>0.91</td>
<td>0.18 – 0.39</td>
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<td></td>
<td>blood (μg/dl)</td>
<td>0.15</td>
<td>0.05</td>
<td>0.24</td>
<td>0.09 – 0.20</td>
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<td>urine (μg/l)</td>
<td>0.60</td>
<td>0.11</td>
<td>1.26</td>
<td>0.28 – 0.93</td>
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<tr>
<td></td>
<td>Girls</td>
<td>hair (μg/g)</td>
<td>0.09</td>
<td>0.00</td>
<td>0.36</td>
<td>0.06 – 0.13</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>blood (μg/dl)</td>
<td>0.07</td>
<td>0.01</td>
<td>0.36</td>
<td>0.05 – 0.09</td>
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<td>urine (μg/l)</td>
<td>0.24</td>
<td>0.01</td>
<td>1.26</td>
<td>0.11 – 0.38</td>
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<tr>
<td></td>
<td>Boys</td>
<td>hair (μg/g)</td>
<td>0.08</td>
<td>0.01</td>
<td>0.47</td>
<td>0.05 – 0.11</td>
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<td>blood (μg/dl)</td>
<td>0.08</td>
<td>0.00</td>
<td>0.43</td>
<td>0.07 – 0.10</td>
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<td></td>
<td></td>
<td>urine (μg/l)</td>
<td>0.20</td>
<td>0.00</td>
<td>0.97</td>
<td>0.15 – 0.26</td>
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<tr>
<td></td>
<td>Women</td>
<td>hair (μg/g)</td>
<td>0.08</td>
<td>0.01</td>
<td>0.67</td>
<td>0.04 – 0.12</td>
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<tr>
<td></td>
<td></td>
<td>blood (μg/dl)</td>
<td>0.07</td>
<td>0.01</td>
<td>0.26</td>
<td>0.05 – 0.09</td>
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<tr>
<td></td>
<td></td>
<td>urine (μg/l)</td>
<td>0.29</td>
<td>0.01</td>
<td>1.53</td>
<td>0.20 – 0.39</td>
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<tr>
<td></td>
<td>Men</td>
<td>hair (μg/g)</td>
<td>0.08</td>
<td>0.01</td>
<td>0.39</td>
<td>0.04 – 0.13</td>
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</tr>
<tr>
<td></td>
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<td>blood (μg/dl)</td>
<td>0.09</td>
<td>0.01</td>
<td>0.41</td>
<td>0.05 – 0.13</td>
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<tr>
<td></td>
<td></td>
<td>urine (μg/l)</td>
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<td>0.01</td>
<td>1.51</td>
<td>0.21 – 0.51</td>
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### Continuance Table 6. Content of trace elements in organism of people with alopecia

<table>
<thead>
<tr>
<th>TE</th>
<th>Group</th>
<th>Biomedia</th>
<th>Mean</th>
<th>Min</th>
<th>Max</th>
<th>95% CI of mean</th>
<th>Difference, p</th>
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<tr>
<td></td>
<td></td>
<td>hair (µg/g)</td>
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<td>0.02</td>
<td>0.57</td>
<td>0.09 – 0.19</td>
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<td></td>
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<td>blood (µg/dl)</td>
<td>0.34</td>
<td>0.12</td>
<td>1.20</td>
<td>0.28 – 0.39</td>
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</tr>
<tr>
<td></td>
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<td>urine (µg/l)</td>
<td>2.30</td>
<td>0.67</td>
<td>8.79</td>
<td>1.29 – 3.32</td>
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</tr>
<tr>
<td>Hg</td>
<td>Girls</td>
<td>hair (µg/g)</td>
<td>0.08</td>
<td>0.03</td>
<td>0.44</td>
<td>0.06 – 0.11</td>
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<td>blood (µg/dl)</td>
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<td>0.13</td>
<td>0.49</td>
<td>0.26 – 0.31</td>
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<td>urine (µg/l)</td>
<td>2.17</td>
<td>0.48</td>
<td>6.86</td>
<td>1.76 – 2.58</td>
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<tr>
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<td>Women</td>
<td>hair (µg/g)</td>
<td>0.11</td>
<td>0.02</td>
<td>0.76</td>
<td>0.07 – 0.15</td>
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<td></td>
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<td>blood (µg/dl)</td>
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<td>0.13</td>
<td>0.50</td>
<td>0.27 – 0.33</td>
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<td>urine (µg/l)</td>
<td>1.86</td>
<td>0.35</td>
<td>4.28</td>
<td>1.56 – 2.16</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Men</td>
<td>hair (µg/g)</td>
<td>0.09</td>
<td>0.02</td>
<td>0.41</td>
<td>0.05 – 0.13</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>blood (µg/dl)</td>
<td>0.33</td>
<td>0.09</td>
<td>0.76</td>
<td>0.27 – 0.38</td>
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<tr>
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<td></td>
<td>urine (µg/l)</td>
<td>1.91</td>
<td>0.28</td>
<td>4.29</td>
<td>1.52 – 2.29</td>
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<tr>
<td>Cu</td>
<td>Girls</td>
<td>hair (µg/g)</td>
<td>16.88</td>
<td>8.91</td>
<td>57.40</td>
<td>13.75 – 20.02</td>
<td>between boys and men, p&lt;0.001</td>
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<td></td>
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<td>plasma (µg/dl)</td>
<td>102.53</td>
<td>6.42</td>
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<td>92.69 – 112.36</td>
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<td>urine (µg/l)</td>
<td>13.08</td>
<td>0.51</td>
<td>84.78</td>
<td>4.64 – 15.2</td>
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<td>Boys</td>
<td>hair (µg/g)</td>
<td>18.54</td>
<td>7.74</td>
<td>133.53</td>
<td>13.84 – 22.75</td>
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<td>plasma (µg/dl)</td>
<td>104.08</td>
<td>58.50</td>
<td>157.10</td>
<td>97.84 – 110.32</td>
<td>between women and men, p&lt;0.01</td>
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<td>urine (µg/l)</td>
<td>16.32</td>
<td>1.670</td>
<td>164.50</td>
<td>9.42 – 23.23</td>
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<td>Women</td>
<td>hair (µg/g)</td>
<td>13.56</td>
<td>6.30</td>
<td>27.48</td>
<td>10.77 – 16.34</td>
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<tr>
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<td>plasma (µg/dl)</td>
<td>103.71</td>
<td>33.70</td>
<td>124.88</td>
<td>75.35 – 91.23</td>
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<td>urine (µg/l)</td>
<td>10.42</td>
<td>3.58</td>
<td>150.43</td>
<td>8.08 – 12.77</td>
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<tr>
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<td>Men</td>
<td>hair (µg/g)</td>
<td>136.53</td>
<td>48.00</td>
<td>207.80</td>
<td>124.83 – 148.23</td>
<td>between boys and men, p&lt;0.001</td>
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<tr>
<td></td>
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<td>plasma (µg/dl)</td>
<td>120.69</td>
<td>41.50</td>
<td>313.80</td>
<td>105.42 – 135.96</td>
<td>between girls and boys, p&lt;0.05</td>
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<tr>
<td></td>
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<td>urine (µg/l)</td>
<td>556.08</td>
<td>189.80</td>
<td>2259.30</td>
<td>328.21 – 783.95</td>
<td>between girls and women, p&lt;0.01</td>
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<tr>
<td>Cu</td>
<td>Girls</td>
<td>hair (µg/g)</td>
<td>192.65</td>
<td>76.00</td>
<td>292.60</td>
<td>173.75 – 211.54</td>
<td>between girls and women, p&lt;0.001</td>
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<tr>
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<td>plasma (µg/dl)</td>
<td>101.31</td>
<td>58.40</td>
<td>219.40</td>
<td>93.24 – 109.39</td>
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<td>urine (µg/l)</td>
<td>660.11</td>
<td>77.20</td>
<td>2310.90</td>
<td>504.81 – 815.42</td>
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<td>Boys</td>
<td>hair (µg/g)</td>
<td>188.28</td>
<td>89.10</td>
<td>292.60</td>
<td>161.04 – 199.53</td>
<td>between girls and women, p&lt;0.001</td>
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<tr>
<td></td>
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<td>plasma (µg/dl)</td>
<td>114.59</td>
<td>31.20</td>
<td>241.60</td>
<td>95.06 – 134.12</td>
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<tr>
<td></td>
<td></td>
<td>urine (µg/l)</td>
<td>666.78</td>
<td>68.10</td>
<td>1585.20</td>
<td>518.11 – 815.46</td>
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<td>Women</td>
<td>hair (µg/g)</td>
<td>297.46</td>
<td>34.70</td>
<td>885.40</td>
<td>230.98 – 363.94</td>
<td>between boys and men, p&lt;0.001</td>
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<td></td>
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<td>plasma (µg/dl)</td>
<td>297.46</td>
<td>34.70</td>
<td>885.40</td>
<td>230.98 – 363.94</td>
<td>between girls and women, p&lt;0.05</td>
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<tr>
<td></td>
<td></td>
<td>urine (µg/l)</td>
<td>114.59</td>
<td>31.20</td>
<td>241.60</td>
<td>95.06 – 134.12</td>
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</tr>
<tr>
<td>Zn</td>
<td>Men</td>
<td>hair (µg/g)</td>
<td>180.28</td>
<td>89.10</td>
<td>292.60</td>
<td>161.04 – 199.53</td>
<td>between girls and women, p&lt;0.001</td>
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<tr>
<td></td>
<td></td>
<td>plasma (µg/dl)</td>
<td>114.59</td>
<td>31.20</td>
<td>241.60</td>
<td>95.06 – 134.12</td>
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<tr>
<td></td>
<td></td>
<td>urine (µg/l)</td>
<td>666.78</td>
<td>68.10</td>
<td>1585.20</td>
<td>518.11 – 815.46</td>
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</tr>
</tbody>
</table>

The content of manganese and zinc ions in hair and zinc ions in plasma of girls vs. boys was significantly higher (p<0.05) in children with hair loss disease. The content of lead in hair and zinc ions in urine of men vs. women was significantly higher (respectively, p<0.05 and p<0.01) in the group of adults with alopecia. Therefore, the amount of manganese in hair, copper in plasma and, lead and manganese ions in urine of men vs. women was significantly lower (p<0.01) in adults. In addition, the amount of copper ions in plasma of boys with alopecia vs. men with alopecia was significantly higher, (p<0.01).
The extended results of zinc ions concentration in the organism of subjects with hair loss disease are showed in Figure 14.

![Bar chart showing zinc concentration in different body parts for girls, boys, women, and men](chart.png)

**Figure 14. Content of zinc ions in organism of people with alopecia**

The results showed that the level of zinc ions in the hair of boys and girls with alopecia vs. the level of zinc ions in men and women hair was subsequently significantly lower (respectively, p<0.01 and p<0.05). However, the level of zinc ions in the girls plasma and urine of children with alopecia vs. women with alopecia was significantly higher (p<0.05).

The findings revealed that somewhat children with alopecia had lower resources of zinc in their organism than adults with hair loss disease, what reflect in lower zinc concentration in hair and subsequently high excretion in urine. Therefore, it should be kept in mind as it was already discussed previously, that children with alopecia also had higher levels of lead and cadmium, both antagonists of zinc, in their organism than adults did. Hence, this could explain lower zinc resources in children organism if compared with adults. In addition, as it was mentioned earlier, the tendencies of higher amount of lead in boys’ organism somewhat to be higher was observed. This antagonistic lead–zinc interaction subsequently could give rough explanation of higher zinc levels in girls since girls had lower lead contents in organism what probably resulted in higher zinc level.

Similar results were reported (Meng 1998, Zachwieja 1995) indicating that the contents of zinc in female hair were higher than in male, but the hair lead concentrations of males were slightly higher than those of females. In addition, it was reported that children up to 5 years of
age are especially prone to higher cadmium and lead exposure and to lower the zinc status if compared to the older children (Wilhelm 1994).

The highest zinc ions excretion with urine of male (both boys and men) is in good agreement of the observed lower zinc ions level in hair of male (both boys and men). This indicates worse male organism saturation with zinc ions. Besides that, the metabolism of zinc due to linkage to iron metabolism is gender–related, especially pronounced in adulthood. This could prompt some explanation of the highest observed zinc levels in women hair and the lowest urinal zinc excretion.

Contradictory results were reported indicating no difference of the age and gender on the content of trace elements in humans’ hair (Bertazzo 1996). The gender does not influenced the copper and zinc contents for both men (1 – 85 years old) and women (1 – 92 years old). While the age influenced the copper and zinc concentrations, but only significantly in females: copper levels decrease over 60 years of age; whereas zinc levels increased significantly from age groups 2 – 5 to 20 – 40 years.

Among other possible explanations, it could be the differences in gender and age of alopecia cases available in this study. Number of enrolled children was 1.6–fold higher compared to adults. However, this simple statistical approach do not explain the observed differences in zinc levels in alopecia people in relation to different gender since the total ratio of female–male alopecia cases was almost equal to one (92 females and 93 males).

Moreover, as was discussed previously, it seems to be that certain individuals or even population sub-groups (i.e. children, women) may be more vulnerable and susceptible to the hazardous effects of heavy metals even at the same level of exposure. These observations and findings support the idea that the main probable reasons for differences in observed content of trace elements, particularly lead and zinc, in patients with alopecia are higher susceptibility of certain individuals to these slight shifts, though within permissible levels, in metabolism of trace elements. The imbalance of trace elements and particularly the zinc deficiency may provoke the course of hair loss disease in sensitive individuals or population groups.

**Key findings**

The imbalance of zinc metabolism, have been caused by heavy metals interaction with essential trace elements, subsequently may provoke alopecia onset in both genders at every age in higher susceptible certain individuals or sensitive population groups.
6.3 Heavy metals and hormonal status in sub–sample of children with alopecia

6.3.1 Trace elements, hormones and blood indices in children with alopecia

Among other reasons besides heavy metals involvement in etiopathogenesis of alopecia there is quite widely accepted hypothesis that alopecia is T–cell mediated autoimmune condition and is most likely to occur in genetically predisposed individuals. The evidences favouring autoimmunity of alopecia suggest that the most significant of autoimmune conditions are vitiligo (Handa 2003) and thyroid diseases (Jabbour 2000). Hence, the hypothesis whether dysfunction of thyroid gland or hormonal shifts may cause the onset of alopecia in children was tested. In addition, the contribution of heavy metals balance and endocrinological status towards the alopecia onset was evaluated. This assessment included the sub–sample of 80 children (of total 113 alopecia children enrolled in this study) with different degree and dissemination of hair loss disease from the Department of Paediatric Endocrinology. The obtained data of trace elements content in alopecia children were compared to those of control group of children without hair loss disease. The analysis of blood and blood biochemical indices of sub–sample of children with alopecia are presented in Table 7.

<table>
<thead>
<tr>
<th>Indices</th>
<th>Mean</th>
<th>Min</th>
<th>Max</th>
<th>95% CI of mean</th>
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<tr>
<td>Leucocytes (× 10^9 l)</td>
<td>7.39</td>
<td>3.40</td>
<td>12.30</td>
<td>6.38 – 8.39</td>
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<tr>
<td>Haemoglobin (g/l)</td>
<td>129.70</td>
<td>120.00</td>
<td>144.00</td>
<td>126.24 – 133.15</td>
</tr>
<tr>
<td>ESR (mm/h)</td>
<td>7.41</td>
<td>2.00</td>
<td>20.00</td>
<td>5.10 – 9.71</td>
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<tr>
<td>Ca (mmol/l)</td>
<td>2.44</td>
<td>2.15</td>
<td>2.75</td>
<td>2.35 – 2.52</td>
</tr>
<tr>
<td>Ca(^{2+}) (mmol/l)</td>
<td>1.17</td>
<td>0.94</td>
<td>1.67</td>
<td>1.11 – 1.22</td>
</tr>
<tr>
<td>P (mmol/l)</td>
<td>1.56</td>
<td>1.07</td>
<td>1.94</td>
<td>1.48 – 1.65</td>
</tr>
<tr>
<td>Mg (mmol/l)</td>
<td>0.82</td>
<td>0.68</td>
<td>0.98</td>
<td>0.74 – 0.89</td>
</tr>
<tr>
<td>Total protein (g/l)</td>
<td>69.88</td>
<td>62.40</td>
<td>77.50</td>
<td>67.03 – 72.73</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>4.97</td>
<td>3.45</td>
<td>6.63</td>
<td>4.68 – 5.26</td>
</tr>
<tr>
<td>Cortisol (mmol/l)</td>
<td>315.54</td>
<td>131.00</td>
<td>679.00</td>
<td>257.02 – 374.05</td>
</tr>
<tr>
<td>TSH (× 10(^{-6}) IU/ml)</td>
<td>1.74</td>
<td>0.88</td>
<td>4.28</td>
<td>1.53 – 1.95</td>
</tr>
<tr>
<td>FT(_4) (pmol/l)</td>
<td>17.16</td>
<td>10.60</td>
<td>26.20</td>
<td>15.80 – 18.52</td>
</tr>
<tr>
<td>PTH (pmol/l)</td>
<td>2.20</td>
<td>1.70</td>
<td>3.20</td>
<td>1.12 – 3.28</td>
</tr>
<tr>
<td>ATPO (IU/ml)</td>
<td>44.98</td>
<td>33.30</td>
<td>81.28</td>
<td>19.18 – 70.78</td>
</tr>
</tbody>
</table>

The obtained data of analysed blood and blood biochemical indices showed normal mean values of leucocytes, haemoglobin, ESR, Ca, Ca\(^{2+}\), P, Mg, total protein and glucose mean values in children with alopecia. The determined mean levels of hormones and antibodies did not
indicate the abnormalities of cortisol, TSH, FT4, PTH and ATPO indices in children with alopecia. Moreover, no single case even of increase or decrease of analysed blood, hormonal and antibodies indices we observed.

Available literature data (Bolduc 2002, Puavilai 1994, European Hair Research Society) indicate the presence of positive microsomal antibodies is found in 3.3 – 16% of patients, often with (Sharma 1999) or without signs and symptoms of thyroid diseases.

Sometimes alopecia people have antibodies against thyroglobulins. However, data are contradictory: some research (Puavilai 1994, Lutz 1987) did not find increased incidence of antibodies against thyroglobulin or thyroid microsomes. The increase of basal TSH levels were present in 13.3% and hypothyroid–type stimulated secretion (TRH test) in two (out of 12) children (Kurtev 2004). Antithyroglobulins were increased in 39.5% and antimicrosomal antibodies in 33.3% children with alopecia.

Alopecia areata investigation in Byelorussia (Yanovich et al. 2002, European Hair Research Society) showed that the proportion of children positive for anti–thyroid antibodies (both AB–TG and AB–TPO) was higher (p<0.05) in the group from the region that have been radio contaminated due to Chernobyl accident as compared to the alopecia children from uncontaminated area.

Unfortunately, due to technical reasons, the hormonal indices and the evaluation of thyroid gland status were not available for the control group and adults with alopecia in our research. Hence, it was impossible to trace any changes of hormonal shifts in relation to alopecia onset in adults. On the other hand, it might be that other hormones that have not been analysed here also may have some relation to onset of alopecia in children. This requires further detailed research.

Only data of trace elements ions content in sub–sample of children with alopecia was assessed and compared to the results of the control group. The extended statistical indices of heavy metals content in hair children are given in Table 8.
### Table 8. Content of heavy metals ions in organism of sub–sample of children with alopecia and control group

<table>
<thead>
<tr>
<th>TE</th>
<th>Group</th>
<th>Biomedia</th>
<th>Mean</th>
<th>Min</th>
<th>Max</th>
<th>95% CI of mean</th>
<th>Difference, p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pb</td>
<td>children with alopecia</td>
<td>hair (µg/g)</td>
<td>1.20</td>
<td>0.12</td>
<td>5.27</td>
<td>1.07 – 2.92</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>blood (µg/dl)</td>
<td>3.20</td>
<td>0.21</td>
<td>16.10</td>
<td>2.73 – 3.67</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>urine (µg/l)</td>
<td>3.55</td>
<td>0.67</td>
<td>23.90</td>
<td>1.74 – 5.37</td>
<td></td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>hair (µg/g)</td>
<td>1.36</td>
<td>0.16</td>
<td>5.61</td>
<td>0.76 – 1.96</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>blood (µg/dl)</td>
<td>2.15</td>
<td>0.37</td>
<td>5.67</td>
<td>1.25 – 3.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>urine (µg/l)</td>
<td>3.18</td>
<td>0.01</td>
<td>14.50</td>
<td>1.28 – 5.09</td>
<td></td>
</tr>
<tr>
<td>Mn</td>
<td>children with alopecia</td>
<td>hair (µg/g)</td>
<td>0.88</td>
<td>0.09</td>
<td>2.64</td>
<td>0.49 – 1.26</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>blood (µg/dl)</td>
<td>1.06</td>
<td>0.16</td>
<td>2.86</td>
<td>0.96 – 1.16</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>urine (µg/l)</td>
<td>1.28</td>
<td>0.16</td>
<td>7.79</td>
<td>0.50 – 2.06</td>
<td></td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>hair (µg/g)</td>
<td>0.77</td>
<td>0.09</td>
<td>3.78</td>
<td>0.35 – 1.88</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>blood (µg/dl)</td>
<td>1.37</td>
<td>0.47</td>
<td>2.42</td>
<td>1.08 – 1.66</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>urine (µg/l)</td>
<td>0.96</td>
<td>0.01</td>
<td>6.17</td>
<td>0.27 – 1.65</td>
<td></td>
</tr>
<tr>
<td>Cr</td>
<td>children with alopecia</td>
<td>hair (µg/g)</td>
<td>0.46</td>
<td>0.11</td>
<td>1.35</td>
<td>0.27 – 0.65</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>blood (µg/dl)</td>
<td>0.10</td>
<td>0.05</td>
<td>0.19</td>
<td>0.06 – 0.14</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>urine (µg/l)</td>
<td>0.39</td>
<td>0.15</td>
<td>0.63</td>
<td>0.01 – 3.44</td>
<td></td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>hair (µg/g)</td>
<td>0.13</td>
<td>0.05</td>
<td>0.50</td>
<td>0.88 – 0.18</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>blood (µg/dl)</td>
<td>0.13</td>
<td>0.05</td>
<td>0.50</td>
<td>0.88 – 0.18</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>urine (µg/l)</td>
<td>0.13</td>
<td>0.05</td>
<td>0.50</td>
<td>0.88 – 0.18</td>
<td></td>
</tr>
<tr>
<td>Cd</td>
<td>children with alopecia</td>
<td>hair (µg/g)</td>
<td>0.10</td>
<td>0.01</td>
<td>0.47</td>
<td>0.04 – 0.18</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>blood (µg/dl)</td>
<td>0.07</td>
<td>0.00</td>
<td>0.43</td>
<td>0.05 – 0.08</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>urine (µg/l)</td>
<td>0.16</td>
<td>0.00</td>
<td>0.50</td>
<td>0.11 – 0.21</td>
<td></td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>hair (µg/g)</td>
<td>0.09</td>
<td>0.02</td>
<td>0.23</td>
<td>0.06 – 0.12</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>blood (µg/dl)</td>
<td>0.07</td>
<td>0.01</td>
<td>0.20</td>
<td>0.04 – 0.10</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>urine (µg/l)</td>
<td>0.16</td>
<td>0.01</td>
<td>0.66</td>
<td>0.09 – 0.24</td>
<td></td>
</tr>
<tr>
<td>Hg</td>
<td>children with alopecia</td>
<td>hair (µg/g)</td>
<td>0.12</td>
<td>0.02</td>
<td>0.44</td>
<td>0.04 – 0.19</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>blood (µg/dl)</td>
<td>0.29</td>
<td>0.12</td>
<td>0.50</td>
<td>0.26 – 0.31</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>urine (µg/l)</td>
<td>1.95</td>
<td>0.80</td>
<td>4.61</td>
<td>1.46 – 2.44</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>hair (µg/g)</td>
<td>0.25</td>
<td>0.02</td>
<td>1.87</td>
<td>0.06 – 0.44</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>blood (µg/dl)</td>
<td>0.32</td>
<td>0.21</td>
<td>0.45</td>
<td>0.28 – 0.37</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>urine (µg/l)</td>
<td>3.10</td>
<td>0.84</td>
<td>9.81</td>
<td>2.04 – 4.16</td>
<td></td>
</tr>
<tr>
<td>Cu</td>
<td>children with alopecia</td>
<td>hair (µg/g)</td>
<td>20.26</td>
<td>8.80</td>
<td>65.92</td>
<td>11.25 – 29.27</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>plasma (µg/dl)</td>
<td>99.54</td>
<td>57.51</td>
<td>203.48</td>
<td>93.45 – 105.62</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>urine (µg/l)</td>
<td>17.72</td>
<td>1.60</td>
<td>164.50</td>
<td>4.12 – 31.31</td>
<td></td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>hair (µg/g)</td>
<td>11.44</td>
<td>3.38</td>
<td>31.40</td>
<td>8.23 – 14.65</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>blood (µg/dl)</td>
<td>86.15</td>
<td>61.10</td>
<td>130.6</td>
<td>74.71 – 97.60</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>urine (µg/l)</td>
<td>15.20</td>
<td>5.66</td>
<td>49.00</td>
<td>10.30 – 20.11</td>
<td></td>
</tr>
<tr>
<td>Zn</td>
<td>children with alopecia</td>
<td>hair (µg/g)</td>
<td>173.73</td>
<td>48.00</td>
<td>609.40</td>
<td>104.35 – 243.11</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>plasma (µg/dl)</td>
<td>109.60</td>
<td>41.50</td>
<td>313.80</td>
<td>98.84 – 120.37</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>urine (µg/l)</td>
<td>565.45</td>
<td>82.60</td>
<td>2270.20</td>
<td>364.84 – 766.07</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>hair (µg/g)</td>
<td>179.28</td>
<td>62.70</td>
<td>363.60</td>
<td>147.13 – 211.43</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>blood (µg/dl)</td>
<td>117.46</td>
<td>71.80</td>
<td>158.70</td>
<td>99.67 – 134.65</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>urine (µg/l)</td>
<td>820.92</td>
<td>299.20</td>
<td>1814.20</td>
<td>578.36 – 1063.47</td>
<td></td>
</tr>
</tbody>
</table>

Due to plenty of data the obtained data of heavy metals (lead, manganese, chromium, cadmium and mercury) and trace elements (copper and zinc) ions content in hair of children were splitted up and presented in Figure 15 and Figure 16, respectively.
Figure 15. Content of heavy metals (lead, manganese, chromium, cadmium and mercury) ions in hair of children

Figure 16. Content of trace elements (copper and zinc) ions in hair of children
The level of chromium and copper ions in hair was significantly higher (p<0.01) while the content of mercury ions was lower (p<0.05) in hair of children with alopecia compared to control group, respectively. As indicated earlier, the tendencies of higher toxic heavy metals (lead, cadmium, manganese, chromium) ions content and declined level of zinc ions in children with alopecia in preference to control group can be observed.

Due to numerous data, the splitted up results of heavy metals (lead, manganese, cadmium and mercury) ions in blood and trace elements (copper and zinc) ions in plasma of children are shown in Figure 17 and Figure 18, respectively.

Figure 17. Content of heavy metals (lead, manganese, cadmium and mercury) ions in blood of children
The established level of blood lead ions in children with alopecia was significantly higher while amount of blood manganese ions was significantly lower if compared to the control group, respectively (p<0.05). The same patterns of increased levels of metals (e.g., lead and copper) which are antagonistic to zinc observed. The established amount of zinc ions in the organism of alopecia children was lower though not significant.

Due to numerous data, the splitted up results of heavy metals (lead, manganese, cadmium and mercury) and trace elements (copper and zinc) ions content in urine of sub–sample of children with alopecia are given in Figure 19 and Figure 20, respectively.

Figure 18. Content of trace elements (copper and zinc) ions in plasma of children
**Figure 19.** Content of heavy metals (lead, manganese, cadmium and mercury) ions in urine of children

**Figure 20.** Content of trace elements (copper and zinc) ions in urine of children
The detected concentration of heavy metals in urine revealed the lower excretion of zinc and mercury ions amount with urine in children with alopecia if compared to the control group, \( p<0.05 \) and \( p<0.05 \), respectively. The pattern to excrete higher levels of toxic heavy metals (e.g., lead and manganese) remains the same as in the total group of investigated children with alopecia in spite those tendencies are not significant.

**Key findings**

The obtained results showed no abnormal changes of blood and hormonal indices in sub-sample of children with alopecia. However, the trends of copper and lead ions increase in sub-sample of children with alopecia and decline content of zinc ions as compared to those of the control group were established. The findings of this research support and favour the hypothesis of the imbalance of trace elements rather than hormonal shifts as the possible triggers of the onset of the hair loss disease in children.

### 6.3.2 Heavy metals and thyroid gland in children with alopecia

Despite of the fact that no abnormal changes of hormonal indices or dysfunction of thyroid gland were observed in this study, some of the children with alopecia had mild diffuse thyreocole. It was diagnosed 8 cases of thyreocole grade II, 38 cases of grade IA and IB in children with alopecia, and 34 children had normal (O) thyroid gland.

Literature data indicate (Bolduc 2002, Nanda 2002b, Suditu 2001) that about 17 – 80% of alopecia people are diagnosed with endothyroid thyreocole, of that mostly thyreocole I type. Our research showed that about 47% alopecia children had thyreocole grade I. Other data (Lutz 1987) indicate 18% no goiters, 7% diffuse goiters and 12% nodula goiters in alopecia subjects.

Therefore, the evaluation of trace elements’ content in children with different thyreocole and alopecia performed. The obtained data of metals ions (lead, manganese, and mercury) and trace elements (copper and zinc) content distribution in children with alopecia and different thyreocole grade are presented in Figure 21 and Figure 22, respectively.
Figure 21. Content of heavy metals (lead, manganese, and mercury) ions in children with different thyreocele and alopecia

Figure 22. Content of trace elements (copper and zinc) in children with different thyreocele and alopecia
The investigation did not extract any significant variations of trace elements content in children with alopecia and thyreocele. Therefore, the trends of heavy metals distribution in children with alopecia and thyreocele are very interesting to note. Analysis revealed the trends of copper and lead (both antagonist of zinc) accumulation as well as processes of mercury excretion whereas the amount of zinc in hair of children with alopecia had trends to decline along with expanding size of thyroid gland.

Our findings support the results (Utenina 2002) on alopecia and non–toxic goiter performed in Russia. All examined children with nontoxic goiter and alopecia were found to have lower concentrations of essential elements (copper, zinc iron) and higher levels of toxic trace elements (strontium, nickel, chromium), but more pronounced in children with alopecia.

Particular attention requires the observed tendencies of mercury ions content distribution in children with alopecia and different dimension of thyroid gland. It seems that mercury content is leaned to demonstrate clean off processes of the children organism (declined in hair and increased with urine) along with the enlargement of thyroid gland. It should be noted, that mercury has huge affinity to mercapto HS– groups. Hair has the most affinity to mercury ions and is one of the best biomarkers of long–term mercury exposure (Morton 2004, Grandjean 2003). Otherwise it can be that enlarging thyroid gland reduces the mercury affinity to hair mercapto groups or somewhat shapes way of mercury elimination with hair towards that one excretes with urine. However, this needs further research and detailed investigations.

It should be reminded that this investigation observed only size changes of thyroid gland but no functional and hormonal changes of this gland. Therefore, due to the lack of other supporting data it would be too bold to discuss whether the enhanced excretion of mercury with urine may be considered as protective effects of enlarging thyroid gland towards elimination of mercury from depots of organism.

Heavy metals can influence the function of thyroid gland, however the entire established concentration of heavy metals did not exceed the permissible levels and cannot be attributed as a principal cause of thyreocele in this research. However, more extended investigations require to determine whether thyreocele influenced the imbalance of trace elements or vice versa, and what is attributed part of every factor that can trigger the onset of alopecia in children.

**Key findings**

The investigation did not extract any significant differences of trace elements content in children with alopecia and thyreocele. The observed trends of heavy metals content changes still
favour the hypothesis of imbalance of heavy metals rather than hormonal shifts and dysfunction of thyroid gland as the possible triggers of onset of hair loss disease in children. However, more detailed investigation should be performed to estimate whether thyreoecele influenced the imbalance of heavy metals or vice versa, and what is attributable part of every factor that can trigger the onset of alopecia in children.

6.4 Treatment of children alopecia with zinc supplements

During investigation, it were screened 17 children with lower zinc ions content either zinc deficiency in their organism. The endocrinologists or dermatologists prescribed these children the treatment with zinc supplementation (partial chelating or recovery therapy with various commercially available in the pharmacies zinc supplements (i.e. zinc sulphate, oxide or aspartate) or vitamins with enhanced content of zinc and other microelements). In addition, after some time these treated children twice have been seen in the Laboratory for the monitoring of the changes of trace metals’ content in their organism. The results of double–repeated analysis of trace elements ions content in different biomedia of children are shown in Table 9.
Table 9. Content of trace elements (lead, manganese chromium, cadmium, mercury, copper and zinc) in organism of children with alopecia before and after the zinc supplementation

<table>
<thead>
<tr>
<th>Biomedia</th>
<th>TE</th>
<th>Before treatment</th>
<th>After treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>95% CI of mean</td>
</tr>
<tr>
<td>Hair (µg/g)</td>
<td>Pb</td>
<td>6.04</td>
<td>2.92 – 9.16</td>
</tr>
<tr>
<td></td>
<td>Mn</td>
<td>0.84</td>
<td>0.55 – 1.14</td>
</tr>
<tr>
<td></td>
<td>Cr</td>
<td>0.35</td>
<td>0.25 – 0.45</td>
</tr>
<tr>
<td></td>
<td>Cd</td>
<td>0.09</td>
<td>0.05 – 0.13</td>
</tr>
<tr>
<td></td>
<td>Hg</td>
<td>0.06</td>
<td>0.04 – 0.08</td>
</tr>
<tr>
<td></td>
<td>Cu</td>
<td>22.74</td>
<td>17.49 – 27.99</td>
</tr>
<tr>
<td></td>
<td>Zn</td>
<td>117.38</td>
<td>96.34 – 138.43</td>
</tr>
<tr>
<td>Blood (µg/dl)</td>
<td>Pb</td>
<td>5.32</td>
<td>3.34 – 7.31</td>
</tr>
<tr>
<td></td>
<td>Mn</td>
<td>1.29</td>
<td>0.94 – 1.65</td>
</tr>
<tr>
<td></td>
<td>Cd</td>
<td>0.18</td>
<td>0.06 – 0.31</td>
</tr>
<tr>
<td></td>
<td>Hg</td>
<td>0.33</td>
<td>0.27 – 0.39</td>
</tr>
<tr>
<td>Plasma (µg/dl)</td>
<td>Cu</td>
<td>112.94</td>
<td>95.90 – 129.98</td>
</tr>
<tr>
<td></td>
<td>Zn</td>
<td>131.06</td>
<td>93.14 – 168.98</td>
</tr>
<tr>
<td>Urine (µg/l)</td>
<td>Pb</td>
<td>9.42</td>
<td>5.80 – 13.04</td>
</tr>
<tr>
<td></td>
<td>Mn</td>
<td>1.97</td>
<td>0.56 – 3.38</td>
</tr>
<tr>
<td></td>
<td>Cr</td>
<td>0.53</td>
<td>0.35 – 0.71</td>
</tr>
<tr>
<td></td>
<td>Cd</td>
<td>0.20</td>
<td>0.14 – 0.26</td>
</tr>
<tr>
<td></td>
<td>Hg</td>
<td>1.47</td>
<td>1.11 – 1.83</td>
</tr>
<tr>
<td></td>
<td>Zn</td>
<td>809.10</td>
<td>579.61 – 1038.60</td>
</tr>
</tbody>
</table>

* – p<0.05 between the level before treatment and after treatment

The double–repeated analysis extracted the significant decrease (p<0.05) of blood lead level in children after the treatment with zinc supplements, 5.32 µg/dl and 3.26 µg/dl respectively. There were established trends of the decreased lead content in hair and urine, and cadmium in hair and blood, though not significant. The declined elimination of zinc ions’ content with urine resulted in the increased content in hair though not significant.

Analysis of data of triple–repeated measures showed the decrease of the content of lead ions in hair and blood, chromium, manganese and cadmium ions in blood, and chromium ions in urine though insignificant (data not shown). The earlier discussed competitive interactions of metals conform to the observed tendencies of lead (Figure 23) clear off processes as a positive lateral effect of zinc supplementation.
The data showed the decline of the total lead burden in organism of children due to zinc supplementation: declining blood lead levels resulted in decreased lead levels in hair and increased lead elimination with urine though not significant. The extracted results of monitoring of zinc ions’ content changes in urine, plasma and hair of children due to treatment with zinc supplementation are shown in Figure 24.

**Figure 23.** Changes of lead content in hair, blood and urine of children with alopecia due to zinc supplementation

**Figure 24.** Changes of zinc content in urine of children with alopecia due to zinc supplementation
The results show that declined level of zinc in plasma resulted in the declined urinal zinc content excretion, and subsequently in the increased content of zinc ions in hair of children who received zinc supplements, though enrichment was insignificant. Probably theses tendencies reveal the organism saturation with zinc ions. Therefore, the main finding is that a few cases of initiation of the hair re–growth were reported. Thus, the effectiveness of zinc supplementation in the treatment of certain alopecia type in particular individuals was demonstrated. There are claims (Gailevičius 1995) that due to zinc sulphate 0.005 therapy 2 – 3 times per day during 3 – 4 months the recovery or the improvement of the conditions were demonstrated in about of 60% of alopecia patients. However, five patients with total and universal alopecia did not respond to the zinc treatment. Therefore, the literature data are contradictory. Some authors (Freyschmidt–Paul 2001) denies the effectiveness of alopecia therapy with zinc supplements.

Zinc sulphate was found to be effective in the treatment of alopecia areata, and this may be due to immunomodulation, especially through the increase of CD8+ cells (Lutz 1990). Hair count results showed a modest and sustained improvement (p<0.05) in hair growth with the daily use of a 1% pyrithione zinc shampoo over a 26–week treatment period (Berger 2003).

Zinc is involved in the metabolism of cystein that is essential for the keratinisation of hair (Gailevičius 1995, Lindelof 1979, Somnichen 1984, Goyer 1984). Data indicate that sometimes the treatment with zinc supplements require half up to 2 years. Though the more detailed investigation should be carried out further on to evaluate what dosage, duration and types of zinc supplements would be the most effective in alopecia treatment.

This investigation demonstrated the antagonistic interaction of heavy metals (lead and cadmium) with zinc ions. In this research, the observed competitive principles and interactions of heavy metals clearly come with the tendencies of lead elimination and the processes of organism clear off as the positive lateral effects of zinc supplementation.

The findings of this research demonstrated the effectiveness of zinc supplementation in certain alopecia treatment. Therefore, there is a need to perform further studies and search if other trace elements besides the zinc ions could be considered as the proper chelators or agents eliminating the hazardous effects, sometimes without any clinical manifestation, of toxic heavy metals.

The efforts of the researchers to understand the etiopathogenesis of fair loss disease and propose the most effective treatment are enormous. Therefore, the progress since Thomson (1996) stated “Alopecia areata is only predictable in its unpredictability“, is not so great and unsatisfactory so far. Thus, the wide research space remains for further investigations in the alopecia.
Key findings

The obtained results showed that declined level of zinc in plasma resulted in decreased urinal zinc content excretion, and subsequently in increased content of zinc ions in hair of children who received zinc supplements, though insignificant. As collateral effects of zinc treatment, the significant decline of lead ions content in blood of children with alopecia was observed. Our research demonstrated the effectiveness of zinc supplementation in the treatment of alopecia in certain individuals resulting in few cases of initiation of hair re–growth.

Though more detailed investigation should be undertaken further to evaluate effective dosage, duration and compounds of zinc supplements in alopecia treatment.
7. CONCLUSIONS

1. The onset of alopecia areata in children and both the alopecia areata and alopecia diffusa in adults with unknown etiopathogenesis was the primary and mostly single complaint of people were undertaking the screening of trace elements in their organism.

2. The content of trace elements in children and adults organism was not related to the distribution and prevalence of children and adults alopecia in different districts of Lithuania. The distribution and prevalence of children and adults alopecia in different districts of the country is uneven.

3. The established mean content of heavy metals in the organism of people with alopecia did not exceed the physiologically permissible limits. The levels of lead, copper and cadmium in hair, blood/plasma and urine were significantly higher if compared to the control group, while the content of essential element zinc in the organism of people with alopecia was significantly lower.

4. The zinc deficiency and imbalance (even within physiological limits) of trace elements’ metabolism, induced and conditioned by the heavy metals interaction with the essential trace elements, subsequently may provoke alopecia onset in both genders at any age in high susceptible individuals or vulnerable population groups.

5. No abnormal changes of blood and hormones indices in the sub–sample of children with alopecia were established. Research showed no significant differences of trace elements’ content in children with alopecia and thyreocele of different grade.

6. The concentration of heavy metals lead and copper was higher in the sub–sample of alopecia children if compared to the control group while the zinc content was lower. Those findings favour the hypothesis of the imbalance of the trace elements rather than hormonal shifts and dysfunction of the thyroid gland as the possible triggers of alopecia onset in children.

7. Significantly reduced blood lead levels in alopecia children who were treated with the zinc supplements were detected during monitoring, though no other significant changes of trace elements content were observed.

8. The treatment with zinc supplements increased zinc level in children though the organism saturation with zinc was insignificant. The observed few case of the initiation of the hair re–growth demonstrated the effectiveness of zinc supplements in the treatment of children alopecia.
8. REFERENCES


107. London Laboratory Service Group (LLSG). Trace Elements Laboratory. [http://www.lhsc.on.ca/lab/metals/panels.htm]


181. Ribeyre F, Amiard–Triquet C, Boudou A, Amiard JC. Experimental study of interactions between five trace elements – Cu, Ag, Se, Zn and Hg – toward their bioaccumulation by Fish (Brachydanio rerio) from the direct route. Ecotox Environ Safety. 1995; 32: 1–11.


268. WHO HECA. Healthy Environment for children Alliance. www.who.int/heca


9. PUBLICATIONS ON THE TOPIC OF DOCTORAL DISSERTATION

Articles and conference proceedings


Other publications


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