

## INVESTIGATIONS OF Ni CONTENT IN HUMAN HAIR

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### Abstract

Effect of nickel (Ni) on human organism is still evaluated, although there are few research papers dedicated to this problem. The participation of Ni in carcinogenesis and allergic reactions is considered. Nickel is absorbed through the alimentary tract, lungs and skin. Concentration of Ni in blood and urine is low (about  $1 \mu\text{g L}^{-1}$ ). More nickel has been determined in tissues such as liver, lungs and osseous tissue. The aim of this work was to assess the Ni level in human hair ( $n = 220$ , 110 women and 110 men) and correlations between Ni and other elements i.e. calcium (Ca), magnesium (Mg), zinc (Zn), iron (Fe), lead (Pb), and cadmium (Cd). Hair washed with acetone and redistilled water was mineralized in mixture of  $\text{HNO}_3$  and  $\text{HClO}_4$  acids. Content of the elements was determined by the atomic absorption spectrometry method AAS. Concentrations of Ca, Mg, Zn, Cu, and Fe was measured by the flame technique (FAAS), while concentration of Pb, Cd and Ni was analyzed by the electrothermal atomic absorption spectrometry in a graphite furnace (GFAAS). The data were the subject of statistical analysis. The mean Ni concentration in the hair samples was  $0.24 \mu\text{g g}^{-1}$  (median  $0.17 \mu\text{g g}^{-1}$ , range  $0.01\text{--}1.77 \mu\text{g g}^{-1}$ ). Slightly more Ni was found in hair of women ( $0.25 \mu\text{g g}^{-1}$ ) than of men ( $0.22 \mu\text{g g}^{-1}$ ). Statistically higher concentrations of Ni were noticed in hair of men  $> 20$  years than in younger men ( $p < 0.05$ ). Statistically significant positive correlations (for Zn, negative correlation) were established between Ni and Cd, Pb, and Cu concentration. Hair is available easily and non-invasively for tests and owing to a higher Ni level in hair than in physiological fluids, such tests help reduce analytical error. Therefore, hair is a very suitable material for monitoring elements in the human body.

**Key words:** nickel, bioelements, toxic metals, hair, atomic absorption spectrometry (AAS).

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## BADANIA NAD ZAWARTOŚCIĄ NIKLU WE WŁOSACH LUDZKICH

### Abstrakt

Oddziaływanie niklu (Ni) na organizm człowieka nadal jest przedmiotem badań, jednak liczba opublikowanych prac poświęconych tej tematyce jest niewielka. Rozpatrywany jest udział tego pierwiastka w procesie kancerogenezy oraz jako czynnika wywołującego alergię. Główne drogi wchłaniania Ni to przewód pokarmowy, płuca i skóra. Stężenie tego pierwiastka we krwi i moczu jest niskie i wynosi ok.  $1 \mu\text{g L}^{-1}$ . Większe jego ilości oznaczono w tkankach (wątrobie, płucach, tkance kostnej). Celem pracy była ocena zawartości Ni we włosach ( $n=220$ , 110 kobiet i 110 mężczyzn) oraz korelacji między jego stężeniem a stężeniem innych pierwiastków: wapniem (Ca), magnezem (Mg), cynkiem (Zn), żelazem (Fe), ołówkiem (Pb) i kadmem (Cd). Włosy umyte w acetonie i wodzie mineralizowano na mokro w mieszaninie kwasów  $\text{HNO}_3$  i  $\text{HClO}_4$ . Zawartość pierwiastków oznaczano metodą spektrometrii absorpcji atomowej (AAS). Stężenie Ca, Mg, Zn, Cu i Fe wykonano techniką płomieniową (FAAS), natomiast Pb, Cd i Ni techniką bezpłomieniową w piecu grafitowym (GFAAS). Otrzymane dane poddano analizie statystycznej. Zawartość pierwiastków podano w zależności od płci i wieku. Średnia zawartość Ni w badanych próbkach włosów wynosiła  $0,24 \mu\text{g g}^{-1}$  (medianą  $0,17 \mu\text{g g}^{-1}$ , rozrzut  $0,01-1,77 \mu\text{g g}^{-1}$ ), nieco więcej tego pierwiastka stwierdzono we włosach kobiet ( $0,25 \mu\text{g g}^{-1}$ ) niż u mężczyzn ( $0,22 \mu\text{g g}^{-1}$ ) i statystycznie więcej we włosach mężczyzn powyżej 20 lat w porównaniu z grupą wiekową poniżej 20. roku życia ( $p<0,05$ ). Istotne statystycznie dodatnie korelacje (z wyjątkiem Zn) wyznaczono między zawartością Ni oraz Cd, Pb i Cu. Włosy są tkanką dostępną w sposób łatwy i nieinwazyjny, a ze względu na znacznie wyższą zawartość Ni w porównaniu z płynami ustrojowymi stanowią dobry materiał badań, co umożliwia zmniejszenie błędów pomiarowych podczas analizy.

**Słowa kluczowe:** nikiel, biopierwiastki, metale toksyczne, włosy, spektrometria absorpcji atomowej (AAS).

## INTRODUCTION

Participation of nickel (Ni) in biochemical processes in the human organism remains the subject of research works. Nickel is absorbed by lungs, skin and the alimentary tract (with water and food). Fish, vegetables, corn, cacao and tea leaves contain high amounts of nickel (CHRISTENSEN 1995, BIEGO et al. 1998, KASPRZAK et al. 2003). In food, nickel may occur as a contaminant or can be added during technological processes. The daily dietary intake of Ni ranges between 170 and 400  $\mu\text{g}$ , although it may be up to nearly 1 mg. Drugs containing Ni are an additional source of this element. The WHO (World Health Organization) recommends 100-300  $\mu\text{g}$  Ni for daily intake (CORNELIS et al. 1995, IYENGAR 1998, KASPRZAK et al. 2003). Gastrointestinal absorption of Ni does not exceed 5% (an average 1-2%). About 80% of Ni is excreted mainly through the alimentary tract and kidneys (10  $\mu\text{g}$  daily). Women retain 14% of consumed Ni, and men – 26%. High amounts of Ni have been found in the skeleton, lungs, skin, muscles, liver, and brain (an average  $7,3 \mu\text{g kg}^{-1}$  body weight). The mean Ni concentration is the

following:  $< 1\text{-}2 \mu\text{g L}^{-1}$  in serum,  $1\text{-}5 \mu\text{g L}^{-1}$  in blood, and  $17 \pm 2 \mu\text{g kg}^{-1}$  in human milk (IYENGAR 1998, DENKHAUS, SALNIKOW 2002). In blood Ni is bound primarily to albumin, histidine and  $\alpha$ -macroglobulin. It can also cross the placenta-blood barrier. Therefore, Ni concentrations in an adults and in a human fetus were found to be similar (CHRISTENSEN 1995). An amount of Ni absorbed via inhalation depends on particle size and solubility of its compounds. It passes through blood to be deposited in the pulmonary tract or eliminated. Citizens may inhale 0.2-1.0  $\mu\text{g}$  of Ni per day. Cigarette smoking may contribute about 4  $\mu\text{g}$  Ni/pack. The primary source of Ni compounds emission is industry related to fly-ash from burning fossil fuels, power plants, and road transport. Occupational exposure occurs e.g. in mining, welding, refining, alloy production, and electroplating. Soluble Ni compounds produced due to Ni-alloys corrosion may be the cause of contact dermatitis and local or systemic allergic reactions (CHRISTENSEN 1995, CORNELIS et al. 1995, KASPRZAK et al. 2003). Until present, no enzymes or cofactors containing Ni have been found in higher organisms. Such enzymes, however, have been identified in plant and bacterial cells (e.g. *Helicobacter pylori*) including urease, hydrogenase, CO-dehydrogenase, cis-trans isomerase, and Ni-superoxide dismutase. Thus, Ni may be indirectly required for normal functions of the digestive system. Animal experiments with Ni-deficient diet have demonstrated that Ni deficiency may be manifested as increased perinatal mortality, decreased growth and impaired iron (Fe) absorption from the intestine (anemia symptoms). Decreased activity of enzymes taking part in metabolism of carbohydrates, aminoacids and lipids (e.g. phospholipid synthesis) was also observed (DENKHAUS, SALNIKOW 2002). In humans, Ni toxicity may be a result of interactions of nickel with other elements, e.g. calcium (Ca), magnesium (Mg), zinc (Zn), manganese (Mn) and iron (Fe). Ni forms stronger bonds with ligands than bioelements i.e. Ca and Mg. Therefore, it can replace them in biologically active compounds (DENKHAUS, SALNIKOW 2002, KASPRZAK et al. 2003, SIDHU et al. 2004). In animals which were given  $\text{NiSO}_4$  in drinking water the concentration of Zn, Cu, and selenium (Se) in liver significantly decreased, but concentration of Ni and Fe significantly increased. Zinc (as  $\text{ZnSO}_4$ ) supplementation to these animals brought back the concentrations of elements to normal values. The authors suggest that Ni may promote excretion of elements from tissues. Increase in Fe concentration may be an implication of using the same absorption and transport mechanisms by both elements (Ni and Fe) (SIDHU et al. 2004). However, in animals which were given Fe-deficient diet, significantly increase in Ni concentration in serum, kidneys, skin, liver, lungs and testes was reported (TALLKVIST, TJÄLVE 1997). Increased Ni level was also noted in blood of patients with renal failure, rheumatoid arthritis, after acute myocardial infarction, in blood of patients taking some drugs and persons drinking wine or beer. The participation of Ni in allergic skin reactions is well documented now and it affects about 10% women and fewer men. This element is present in coins, jewellery,

prostheses and utensils. In 1990 IRAC (the International Agency for Research of Cancer) has concluded that Ni and its compounds are carcinogenic to humans. High amounts of Ni were found in the serum of patients with some types of cancer (e.g. lung and larynx), in the lung tissue of refinery workers and welders (even some hundred fold higher compared to the control group) and in breast cancer tissue (CHRISTENSEN 1995, CORNELIS et al. 1995, DENKHAUS, SALNIKOW 2002, KASPRZAK et al. 2003).

The Ni concentration in physiological fluids is low and may fluctuate during a 24-hour period. Therefore, we aimed to estimate the concentration of Ni in human hair depending on gender and age, and also the correlation between Ni and chosen elements (Ca, Mg, Zn, Cu, Fe, Pb, and Cd).

## MATERIALS AND METHODS

Hair samples were taken from 220 persons (110 women and 110 men). They were cut at 6 different points of head (3-4 cm counting from the scalp) in amounts of about 0.5 g. The hair washed with redistilled water and acetone was mineralized in mixture of acids:  $\text{HNO}_3$  and  $\text{HClO}_4$ . After mineralization, any excess of acids was removed, while the post-mineralization mixture was quantitatively transferred to volumetric flasks, which were filled up with deionized water ( $0.06 \text{ mS cm}^{-1}$ ).

Measurements were performed by the atomic absorption spectrometry method using an atomic absorption spectrometer AAS AVANTA  $\Sigma$  (GBC) equipped with an air-acetylene burner, graphite furnace GF3000 system with Ultra-Pulse background correction and autosampler PAL3000.

Concentrations of Ca, Mg, Zn, Cu, and Fe were determined by the flame technique FAAS under standard conditions, but concentrations of Pb, Cd, and Ni were measured by the flameless technique (GFAAS). The analyses were made in argon atmosphere as purging gas, in pyrolytic graphite tubes. The sample volume injected to the furnace was  $20 \mu\text{L}$ . The peak area mode was used in calculations.

The instrumental parameters and analytical characterization of the methods are listed in Table 1. Table 2 presents results of the reference material analysis NCS 81002 (human hair). The data were verified by the standard addition method and analysis of the reference material.

Our results were expressed in terms of means and medians. The following methods were applied in statistical analyses: Shapiro-Wilks test of normality, Fisher test, *t*-Student test, Cochran-Cox, Kolmogorov-Smirnov test (for non-parametric distributions), Pearson test (for correlations). The level of statistical significance was assumed with  $p < 0.05$ .

Table 1

Instrumental parameters and analytical characterization of the methods

Element	Wavelenght (nm)	Pyrolysis/atomization temperature (°C)	Calibration range (µg mL <sup>-1</sup> ) (ng mL <sup>-1</sup> )*	Limit of detection <sup>3)</sup> (µg mL <sup>-1</sup> ) (ng mL <sup>-1</sup> )*	Sensitivity (µg mL <sup>-1</sup> ) (pg)*	Precison (%)
Ca	422.7		0.5-3.5	0.03	0.07	8.7
Mg	285.2		0.05-0.35	0.003	0.004	5.8
Zn	213.9		0.1-1.5	0.010	0.015	4.3
Cu	324.7		0.1-1.5	0.016	0.030	4.5
Fe	248.3		0.1-1.5	0.02	0.08	5.0
Pb <sup>1)</sup>	283.3	900/2000	1.5-15.0*	0.81*	5.8*	6.8
Cd <sup>2)</sup>	228.8	600/1800	0.15-1.50*	0.06*	0.36*	6.7
Ni	232.0	900/2400	1.5-15.0	0.66*	4.82*	8.7

Modifiers used: <sup>1)</sup>NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub>, <sup>2)</sup>NH<sub>4</sub>NO<sub>3</sub>, <sup>3)</sup>Limit of detection (LOD) defined as 3SD (*n* = 10)<sup>4)</sup>Characteristic mass defined as amount of element giving an absorbance of 0.0044.

Table 2

Results of the certified material NCS ZC 81002 analysis,  $x \pm s$ ,  $n = 6$  (µg g<sup>-1</sup>)

Element	Certified value	Found value	Accuracy (%)
Ca	1090 ± 72	1051 ± 91	104.0
Mg	105 ± 6	103 ± 6	109.1
Zn	189 ± 8	185 ± 8	106.7
Cu	23.0 ± 1.4	22 ± 1.1	102.7
Fe	71.2 ± 6.6	65.2 ± 5.1	99.2
Pb	7.2 ± 0.7	7.4 ± 0.5	93.4
Cd	0.095 ± 0.012	0.105 ± 0.007	107.5

## RESULTS AND DISSUSION

The content of Ni and the other examined elements in women's and men's hair is presented in Table 3. Concentrations of these elements in female hair depending on age (below and above the age of 20 years) are listed in Table 4, and in male hair – in Table 5. The mean Ni concentration in hair of 220 volunteers was  $0.24 \pm 0.24$  µg g<sup>-1</sup>, (median  $0.17$  µg g<sup>-1</sup>, range  $0.01$ - $1.77$  µg g<sup>-1</sup>, percentiles (5%-95%)  $0.04$ - $0.74$  µg g<sup>-1</sup>). In women's hair slightly higher concentration of Ni was found than in men's (Table 3). In

Table 3

Concentration of Ni and other elements in hair of women and men ( $\mu\text{g g}^{-1}$ )

Specification	Ca	Mg	Zn	Cu	Fe	Pb	Cd	Ni
<i>N</i> = 110	women							
Mean	454	19	182	20	12	0.6	0.07	0.25
Median	286	16	177	11	9	0.4	0.05	0.18
S.D.*	509	43	78	45	15	0.6	0.05	0.28
Range	51 3197	2 112	40 514	5 368	4 127	0.1 3.1	0.05 0.40	0.01 1.66
Percentyle 5%-95%	70 1378	5 43	63 303	7 42	5 26	0.1 1.7	0.05 0.17	0.03 0.74
<i>N</i> = 110	men							
Mean	291	18	159	12	11	1.2	0.13	0.22
Median	195	13	161	11	9	0.7	0.05	0.17
S.D.*	326	18	53	7	12	1.8	0.35	0.19
Range	16 1854	2 108	39 394	6 60	4 134	0.1 13.4	0.05 2.9	0.01 1.01
Percentyle 5%-95%	66 1139	3 60	64 244	7 20	5 17	0.2 3.4	0.05 0.42	0.05 0.62

\* S.D. – standard deviation

Table 4

Concentration of Ni and other elements in hair of women depending on the age ( $\mu\text{g g}^{-1}$ )

Specification	Ca	Mg	Zn	Cu	Fe	Pb	Cd	Ni
<i>N</i> = 56	women < 20 years							
Mean	407	17	169	17	13	0.8	0.07	0.27
Median	186	11	146	10	11	0.6	0.05	0.20
S.D.	523	18	93	38	12	0.6	0.04	0.29
Range	51 2523	2 112	52 514	7 296	5 92	0.1 2.8	0.05 0.23	0.02 1.66
Percentyle 5%-95%	64 1514	4 48	56 358	7 29	6 26	0.1 1.7	0.05 0.17	0.04 0.76
<i>N</i> = 110	men > 20 years							
Mean	503	22	195	24	12	0.5	0.08	0.23
Median	345	20	191	11	8	0.3	0.05	0.17
S.D.	494	13	55	51	17	0.6	0.07	0.26
Range	90 3197	7 91	40 328	5 368	4 127	0.1 3.1	0.05 0.40	0.01 1.63
Percentyle 5% - 95%	109 1378	8 41	72 276	7 94	4 33	0.1 1.7	0.05 0.17	0.02 0.53

women aged < 20 years the hair Ni concentration was higher than in older women (Table 4). In contrast, in hair adult men the Ni concentration was significantly higher in comparison with adolescent men ( $p<0.05$ ) – Table 5. Due to a considerable variability range of the results, it seems reasonable to take medians as the best measure of the general tendency. Table 6 contains the Ni concentrations in human hair measured by other authors and proposed reference values for this element. It is concluded that Ni content in hair depends on sex, age, environmental and occupational exposition, nutritional habits, cigarette smoking and even hair colour. In the hair samples examined in the present experiment, the Ni concentration was significantly positively correlated with Cd, Pb, Fe and Cu concentration, but negatively correlated with Zn content. Similar observations, except Zn, are reported by SENOFONTE et al. (1989), NOWAK (1998), CHOJNACKA et al. (2005). Positive correlations may suggest that interactions between elements are synergistic, opposite to the negative direction, which indicates an antagonistic relationship. These interactions take place during absorption, transport, elimination, and cumulation processes. Positive correlations between Ni, Cd, and Pb may be a result of similar environmental exposition to heavy metals or smoking cigarettes. However, in the case of Fe, a possible explanation could be that Fe and Ni use the same absorption and transport processes, e.g. the divalent cation transporter system DMT-1. The negative direction of correlation between Ni and Zn concentrations confirms suggestions that Ni may both participate in Zn metabolism and impair the immune system under conditions excessive exposition to Ni.

Table 5  
Concentration of Ni and other elements in hair of men depending on the age ( $\mu\text{g g}^{-1}$ )

Specification	Ca	Mg	Zn	Cu	Fe	Pb	Cd	Ni
<i>N</i> = 63	women < 20 years							
Mean	248	12	147	13	12	0.9	0.07	0.17
Median	159	10	147	11	10	0.7	0.05	0.15
S.D.*	305	12	61	9	16	0.8	0.04	0.11
Range	16 1756	2 89	39 394	7 60	4 134	0.1 4.8	0.05 0.25	0.01 0.62
Percentile 5%-95%	43 638	2 24	59 246	7 32	5 17	0.2 2.5	0.05 0.14	0.05 0.36
<i>N</i> = 110	men < 20 years							
Mean	395	26	177	11	9	1.5	0.23	0.29 <i>p</i> <0.05
Median	234	18	173	10	9	0.7	0.08	0.23
S.D.*	346	21	35	4	3	2.6	0.52	0.24
Range	66 1854	5 108	101 257	6 29	5 18	0.1 13.4	0.05 2.90	0.04 1.01
Percentile 5%-95%	100 1205	7 68	129 236	7 17	6 14	0.2 4.4	0.05 0.60	0.06 0.87

Table 6

Content of Ni in hair according to other authors ( $\mu\text{g g}^{-1}$ )

Number of samples	Arthmetic Mean	Median	Range	Country	Authors
106 95		0.6 1.3 (environmental exposition)	0.0 - 3.4 0.0 - 207.9	Czech Republic	BENCKO (1995)
44		1.17	0.45 - 12.45	India	SAMANTA et al. (2004)
92	0.868	0.349		Italy	VIOLANTE et al. (2000)
100	0.43	0.32	0.027 - 2.03	Italy	SENOFONTE et al. (1989)
266	0.75	0.30	0.03 - 10.0	Italy	SENOFONTE et al. (2000)
			0.02 - 0.20*	USA	IFYENGAR (1998)
32		0.23	0.08 - 0.90*	France	GOULLE et al. (2005)
266	0.75	0.30		Poland	NOWAK (1998)
83	0.838			Poland	CHOJNACKA et al. (2005)

\*Values reported as reference

Table 7

Pearson's correlation coefficient ( $r_{xy}$ ) between concentration of Ni and other elements in hair

Specification	Ca	Mg	Zn	Cu	Fe	Pb	Cd
Women	-0.082	-0.124	-0.262 $p<0.01$	0.042	0.337 $p<0.001$	0.305 $p<0.00001$	0.498 $p<0.00001$
Women < 20	-0.134	-0.164	-0.243	-0.07	0.002	0.127	0.106
Women > 20	-0.001	-0.031	-0.284 $p<0.05$	0.155	0.606 $p<0.00001$	0.481 $p<0.001$	0.823 $p<0.00001$
Men	-0.005	0.123	-0.070	0.035	0.007	0.193 $p<0.05$	0.354 $p<0.001$
Men < 20	-0.009	0.079	-0.208	-0.048	0.001	0.435 $p<0.001$	0.414 $p<0.001$
Men > 20	-0.096	-0.018	-0.195	0.307 $p<0.05$	0.326 $p<0.05$	-0.094	0.332 $p<0.05$

## CONCLUSIONS

1. Concentration of Ni expressed as a mean and a median found in the tested samples of hair is within the reference range.

2. Age and sex influence Ni concentration in hair. The mean Ni concentration in women's hair is higher in comparison to men's hair. Moreover, it is significantly higher in the group of men above 20 years of age.

3. The correlations between concentrations of Ni and other examined elements such as Ca, Mg, Zn, Cu, Fe, Pb, and Cd reflect their mutual interactions in biochemical processes (absorption, transport, cumulation, elimination). Positive correlations between Ni and Fe, Pb, and Cd can be linked with the synergistic character of these relations, but the negative correlation between Ni and Zn confirms antagonistic relations of both elements.

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