Hard tissue samples as markers of occupational exposure in a phosphate fertiliser plant

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Abstract: This paper describes the results of study on testing of hair and nails samples as possible markers of occupational exposure in a phosphate fertiliser plant. The key objectives were to verify if: (1) elements originating from fertiliser production may accumulate in hard tissue and hence allow preliminary occupational exposure risk assessment; (2) linear discriminant function analysis (LDA) may be applied as a diversification tool in preliminary assessment of occupational exposure risk based on hard tissue samples analysis; and (3) both hairs and nails can be used successfully as an efficient biological sample in biomonitoring studies and as markers of occupational exposure in various types of plant. The complex data matrix (2025 observations) obtained by the determination of 25 elements by neutron activation analysis in hard tissue samples was treated by LDA. The obtained results indicated the presence of two discriminant functions (DFs). The data variance explained by the first DF is 78%, and that by the second DF is 22%. The first DF is highly related to S, W, Cu, K and Mg concentrations and separates hair from nails samples, and the second DF, being related to phosphate fertiliser precursors, separates the control group from the employees. Sm, Al, Mo and As accumulate in nails and hairs of fertiliser plant employees, whereas Cl and Ti are removed from hard tissues. The mean concentrations of Na, In, I, Au, Dy, Ca, La, U, Sb, V, Zn, Mn and Co in hard tissue samples

do not discriminate employees and control group members and hence prove their ineffectiveness in occupational exposure assessment in phosphate fertiliser plants.

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1   Introduction

Industrial technologies may contaminate various constituents of the environment (air, water and soils) and impact the quality of life and human health. The aspect of environment pollution has been intensively studied for decades (Namieśnik, 2000; Namieśnik, 2001; Namieśnik and Wardencki, 2002). The majority of studies has been carried out in connection with the currently known most dangerous sources of contaminants despite the existence of less studied sources, which seem to be less dangerous. The technology of phosphate fertilisers belongs to the latter group (usually only fluorine impact is under monitoring and investigation).

Use of phosphate rock in phosphoric acid and fertiliser production may be hazardous, owing to the release of dust and polluted water containing stable and radioactive elements like uranium and Rare Earth Elements (REEs) (Ogunleye et al., 2002). As Goering et al. (1991) and Papaefthymiou et al. (2003) found, long-term inhalation of air containing radon and its daughters (the decay products of natural uranium) produces a risk of lung cancer. Very little is known about lanthanide elements and their toxicity. These elements are probably not nutritionally essential for humans; however, many replace calcium in a number of calcium-mediated biological processes, which may be of

certain risk in several functions (Goering et al., 1991). Chen et al. (2001) suggested that some of REEs and Fe might have potentially similar metabolic pathways, which implied that REEs might not only affect biological functions of Ca, but also of Fe. The biggest part of REEs which entered the human body is excreted, but a small amount may get into blood stream and can be deposited in various organs (Koeberl and Bayer, 1992). It was also found by Sabbioni et al. (1982) that prolonged occupational and environmental exposure to REEs may negatively influence human health.

The potential risk to the health and safety of workers can be assessed based on the occupational exposure. For this reason two approaches are usually performed: direct monitoring or biological monitoring. Direct monitoring is based on measurements of the stressors (toxic substances) in the workplace air. Many worldwide known institutes for occupational safety and health established the highest permissible exposure limits for both organic and inorganic substances (i.e. Cl, Ti, Co, Cu, As, Br, Mo, Sb, I, W and U) expressed in $mg \cdot m^{-3}$ of indoor air and dependent on shift's exposure time (NIOSH, 1992; Laws newscast, 2002; ACGIH, 2007; OSHA, 2008) (Table 1). Nevertheless, the results obtained can be misleading due to the various mechanisms of intake and absorption of substances in human body. Kučera et al. (2004) suggests that more reliable results can be obtained using biological monitoring. There are many types of samples used in biomonitoring: body fluids (urine, whole blood, milk, sweat, saliva), soft tissue (liver, kidney, placenta, adipose tissue) and hard tissue (bones, teeth, hair, nails). Because of serious health concern the most investigated metals in body fluids as well as in soft and hard tissue samples are Cd (Horng et al., 2002; Mortada et al., 2002), Cr (Miksche and Lewalter, 1997), Hg (Cianciola et al., 1997), Pb (Vural and Duydu, 1995; Lee, 1999) and Ni (Horng et al., 2002). Phosphate rock-related elements were rarely investigated and this is why only in case of several of them the highest recommended (not obligatory) concentration values in biological samples are established by national legislation. Besides Cr, Cd, Pb and Hg, Polish legislation specifies that the highest permissible concentration of As in urine after weekly exposure should be lower than 35 $\mu g \cdot l^{-1}$ (CIOP, 2007). German legislation specifies that the highest permissible concentration of Al in urine after weekly exposure should not exceed 200 $\mu g \cdot l^{-1}$ (DFG, 2004). The highest permissible concentrations of As and Co in urine, specified by American Conference of Governmental and Industrial Hygienists (ACGIH), should be lower than 35 $\mu g \cdot l^{-1}$ and 1 $\mu g \cdot l^{-1}$, respectively (ACGIH, 2007). ACGIH documentation of the threshold limit values for chemical substances and physical agents as well as biological exposure indices also specifies the highest permissible concentration of Co and V (in the form of vanadium pentoxide) in blood for 15 $\mu g \cdot l^{-1}$ and 50 $\mu g \cdot g^{-1}$ (creatinine) (ACGIH, 2007). It has to be emphasised, that various, both EU and US, legislations are not coherent because they precise the highest permissible concentration values of, in general, incomparable elements.

Body fluids reflect short-term exposure, while soft and hard tissues are good indicators of long-term exposure (Iyengar et al., 1998; Hać et al., 2000; Petersen et al., 2000; Gomes et al., 2004). Hair or nails analysis is a non-invasive investigation method and has been used for many years to calculate exposure to toxic metals in occupational exposure and environmental pollution studies in both individuals and population groups (Bencko et al., 1982; Wilhelm and Idel, 1986; Cheng et al., 1994; Nowak and Chmielnicka, 2000; Rodushkin and Axelsson, 2000; Violante et al., 2000;

Biziuk and Wrońska, 2001; Murao et al., 2002; Sera et al., 2002; Strumylaite et al., 2004; Özden et al., 2007). Determination of trace elements concentration in hair and nails serves as an indicator tool for tracking deficiency states in nutrition and poisoning states in forensic medicine (Kintz, 2004).

Considering the above facts, this study was undertaken to determine an impact of Gdańsk phosphate fertiliser plant 'Fosfory' on its employees. A key objective was to verify hypothesis assuming that excluding diversity, which reflects variability of factors affecting element concentration (such as dietary and smoking habits, lifestyle, geochemical environment, age, sex, hair colour), the elements originating from fertiliser production may accumulate in hair or nails and hence make preliminary occupational exposure risk assessment possible. The study also demonstrates the efficiency of discriminant function (DF) analysis as a distinction tool in assessment of occupational exposure risk based on determination of 25 elements in hair and toenails collected from 21 employees and a control group (20 persons). The last objective was to compare both hard tissues as samples used in various biomonitoring studies and investigate possibility of limitation of variables determined in occupational exposure assessment in case of fertiliser plant employees.

Table 1   The highest permissible exposure limits (indoor air) established by Polish and selected worldwide known institutes for occupational safety and health

Element	NDS (Laws newscast, 2002) ($mg \cdot m^{-3}$)	PEL-TWA (OSHA, 2008) ($mg \cdot m^{-3}$)	REL-TWA (NIOSH, 1992) ($mg \cdot m^{-3}$)	TWA-STEL (ACGIH, 2007) ($mg \cdot m^{-3}$)
Al	–	5	5	10
As	0.01	0.01	–	0.01
Au	–	–	–	–
Br	0.7	0.7	0.7	–
Ca	–	–	–	–
Cl	1.5	1	0.5	0.5
Co	0.05	0.1	0.05	0.02
Cu	1^1	1^5; 0.1^6	1^5; 0.1^6	0.1^5; 0.2^6
Dy	–	–	–	–
I	1	–	–	–
In	–	–	0.1	–
K	–	–	–	2
La	–	–	–	–
Mg	–	15^7	–	10^7
Mn	0.3	–	$1^{8, 9}$	0.2^8
Mo	4	15^{10}; 5^{11}	–	10^{10}; 0.5^{11}

Table 1 The highest permissible exposure limits (indoor air) established by Polish and selected worldwide known institutes for occupational safety and health (continued)

Element	NDS (Laws newscast, 2002) ($mg \cdot m^{-3}$)	PEL-TWA (OSHA, 2008) ($mg \cdot m^{-3}$)	REL-TWA (NIOSH, 1992) ($mg \cdot m^{-3}$)	TWA-STEL (ACGIH, 2007) ($mg \cdot m^{-3}$)
Na	–	–	–	–
S	–	–	–	–
Sb	0.5	0.5	0.5	0.5
Sm	–	–	–	–
Ti	10	15[12]	–	10[12]
U	0.8[2]	0.25[13]; 0.05[14]	0.2[13]; 0.05[14]	0.2[13, 14]
V	–	–	–	0.05
W	6[3]; 5[4]	–	5[15]; 1[16]	–
Zn	–	5	5	1

Notes: NDS (CIOP) – the highest permissible concentration value, PEL-TWA (OSHA) – permissible exposure limit-time weighted average (Occupational Safety & Health Administration), REL-TWA (NIOSH) – recommended exposure limit-time weighted average (US National Institute for Occupational Safety and Health); TWA-STEL (ACGIH) – time weighted average-short term exposure limit (American Conference of Governmental and Industrial Hygienists).

[1]Cu as a sum for oxides dust and insoluble salts.

[2]U as a sum of soluble and insoluble compounds.

[3]W as a sum of soluble and insoluble compounds.

[4]W in the form of smoke and dust.

[5]Cu metal dust.

[6]Cu metal fumes.

[7]Mg oxide.

[8]Mn compounds and dust.

[9]Mn fume.

[10]Mo metal and insoluble compounds.

[11]Mo soluble compounds.

[12]Ti dioxide.

[13]U metal and insoluble compounds.

[14]U soluble compounds.

[15]W metal and insoluble compounds.

[16]W soluble compounds.

2 Materials and methods

2.1 Sampling

Hair and toenails clippings were collected using stainless steel scissors. Hair (labelled as h) was collected from the head – part above the neck – from 20 employees, while toenails (labelled as n) from 21. Both hair and toenails clippings were washed in accordance with

IAEA recommended procedure consisting of five successive washes: acetone – water – water – water – acetone each for 10 min in 25 ml of each solvent (Ryabukhin, 1978). No strict rules exist concerning the control group size. In similar projects realised by others the control group size varies in the range between 30% (Gerhardsson et al., 2002) (comparing to investigated population) and 300% (Georgescu et al., 1997). In our case, because of cost-time optimisation, control samples of hair (labelled as c_h) and nails (labelled as c_n) were collected from 20 persons not connected with the plant in any way, but of similar age and the same sex. The characteristics of the investigated group of workers (age, period of service in phosphate fertiliser plant, working place – section and localisation) and of the control group (age range) are listed in Table 2.

Table 2   Characteristic of investigated employees and control group

No.	Section	Localisation	Age (years)	Period of service (months)
1	M, PAP, FP	whole shops	60	391
2	FP	dispatch room, final product store	52	437
3	FP	dispatch room, crushers	42	205
4	FP	final product belt convey or flights, final product store	44	179
5	FP	final product store	35	80
6	FP	mixers	38	63
7	SH	loader cabin	48	341
8	M	raw material belt conveyor flights	53	351
9	M	material for milling belt conveyor flights, ball mill	56	361
10	SH	loader cabin	31	150
11	PAP	dispatch room, Prayon filter	56	332
12	PAP	dispatch room, Prayon filter, reactors	41	217
13	PAP	whole shop	58	389
14	PAP	locksmith shop, phosphogypsum belt conveyor flights	58	366
15	PAP	locksmith shop	52	167
16	PAP	locksmith shop, phosphogypsum belt conveyor flights	43	35
17	FP	whole shop	33	135
18	FP	whole shop	48	76
19	FP	whole shop	51	342
20	M	locksmith shop	56	397
21	M	locksmith shop	45	182
Control group				
22–41	–	–	28–59	–

Note: M – mill; SH – store house; FP – fertilisers production; PAP – phosphoric acid production.

2.2 *Analytical technique*

Neutron Activation Analysis (NAA) is a sensitive analytical technique useful for performing both qualitative and quantitative multi-element analysis of major, minor and trace elements in samples from almost every conceivable field of scientific or technical interest (Bode and De Goeji, 1998; Orvini and Speziali, 1998; Weise et al., 2001). Determination of concentration of 25 analytes (Na, Mg, Al, S, Cl, K, Ca, Ti, V, Mn, Co, Zn, Cu, As, Br, Mo, In, Sb, I, La, Sm, Dy, W, Au and U) in hair and nails was performed at Frank Laboratory of Neutron Physics, Joint Institute for Nuclear Research (FLNP JINR), Dubna, Russia, at IBR-2 pulsed fast reactor. The sample masses of hair and nails clippings varied from 0.02 g to 0.2 g and from 0.02 to 0.08 g, respectively. Two kinds of analysis were performed: long irradiations for 100 hours by epithermal neutrons to determine the elements associated with long-lived radionuclides and short irradiations for 5 min for short-lived radionuclides. The samples were packed in aluminium cups for long-term irradiation and put into polyethylene foil bags for short-term irradiation. In every case, a blank capsule was processed simultaneously for internal quality control. Gamma-ray spectra were recorded four times; after decay periods of 5 min and 12 min following the short irradiation and after 4 days and 20–24 days following the long irradiation. Data processing and element concentration determination was performed using software developed in FLNP JINR described before by Ostrovnaya et al. (1993). The QC of NAA results was maintained by carrying out a concurrent analysis of the certified reference materials: GBW-07601 (human hair) and NIST-1577B (bovine liver). The accuracy of results of testing (as a determined/certified concentration ± uncertainty/certified uncertainty) along with detection limit range for the investigated elements are presented in Table 3. Detection limit range varies between 10^{-1} µg·g^{-1} for Al, Cl, Mg and S and even 10^{-5} µg·g^{-1} for V, Sm, Au and As. Better detection limits of the analytes can be achieved by optimising irradiation parameters (type, energy, fluency rate of the neutrons, irradiation time), while single-established validation parameters are not expected due to variability of many of them like beam energy, radiation dose rate, etc. and this is why each single analysis requires internal quality control step (analysis of CRM blank capsule). This procedure is applied in FLNP JINR (Dubna) (Ermakova et al., 2004; Frontasyeva et al., 2004) as well as in accredited laboratories of INNA facilities at Delft (Bode, 1994, 2000; Bode and Van Dalen, 1994). Moreover, NAA is a self-validating technique: two or more analytical γ–lines can be used for the determination of one element allowing a crosscheck of the process. For GBW-07601 certified material, 13 elements (As, Au, Br, Cl, I, K, La, Mg, S, Sb, Sm, U and Zn) fit within certified ranges or information values. Mn concentration determined in hair sample fitted within an interval of ±20% from the certified value. The results obtained for Na and Ca in hair show poor accuracy, due to the fact that these elements were very close to the detection limit (high value of uncertainty). For NIST-1577B certified material, nine elements (Br, Co, Cu, K, Mg, Mn, Mo, Na and Zn) match certified ranges or information values, while for Cl and Co concentrations determined in bovine liver sample fit within an interval of ±5% from the certified value.

Table 3　Results of GBW-07601 and NIST-1577B reference materials analysis as well as detection limit range for investigated elements

	GBW – 07601 (hair) ($\mu g \cdot g^{-1}$)		NIST-1577B (bovine liver) ($\mu g \cdot g^{-1}$)		
Element	Determined concentration ± uncertainty N = 6	Certified concentration ± certified uncertainty	Determined concentration ± uncertainty N = 6	Certified concentration ± certified uncertainty	Detection limit range ($\mu g \cdot g^{-1}$)
Al	13800 ± 1400	–	10.30 ± 0.38	3[a]	10^{-1}
As	0.269 ± 0.029	0.280 ± 0.040	–	–	10^{-5}
Au	0.00250 ± 0.00027	0.0025[a]	–	–	10^{-5}
Br	0.370 ± 0.021	0.36[a]	9.788 ± 0.066	9.7[a]	10^{-3}
Ca	0.150 ± 0.016	0.290 ± 0.020	126 ± 16	116 ± 4	10^{-3}
Cl	39.7 ± 5.6	35[a]	2900 ± 20	2780 ± 6	10^{-1}
Co	–	–	0.2408 ± 0.0057	0.25[a]	10^{-4}
Cu	74 ± 70	10.60 ± 0.70	164.7 ± 1.4	160 ± 8	10^{-1}
K	0.065 ± 0.043	0.017[a]	9957 ± 110	9940 ± 2	10^{-3}
La	0.640 ± 0.082	0.8[a]	–	–	10^{-4}
Mg	21.0 ± 8.5	20[a]	637 ± 24	601 ± 28	10^{-1}
Mn	0.0600 ± 0.0090	0.0490 ± 0.0080	10.61 ± 0.25	10.5 ± 1.7	10^{-2}
Mo	289.0 ± 70.2	360 ± 29.8	3.50 ± 0.16	3.5 ± 0.3	10^{-3}
Na	5.02 ± 0.48	6.30 ± 0.48	2420 ± 24	2420 ± 6	10^{-2}
S	213.0 ± 7.7	152.0 ± 10.0	9278 ± 832	7850 ± 6	10^{-1}
Sb	4.7 ± 1.2	4.3 ± 0.2	<LOD	0.003[a]	10^{-3}
Sm	0.078 ± 0.011	0.095 ± 0.012	–	–	10^{-5}
V	0.01030 ± 0.00063	0.012[a]	0.1105 ± 0.0072	0.123[a]	10^{-5}
Zn	0.0720 ± 0.0068	0.09[a]	123.1 ± 1.1	127 ± 16	10^{-4}

Notes:　LOD – limit of detection; *N* – number of repetitions.
　　　　[a]information value.

2.3　Linear discriminant function analysis

Linear discriminant function analysis (LDA) is used to determine the variables that are best discriminate between a two or more naturally occurring groups in the data set (Fisher, 1936; Jordán et al., 1998; Singh et al. 2006) and to describe the relationship between them (Manley, 1994). The LDA differs from analysis of variance not in the underlying procedure, but in that different types of statistics are computed. The LDA-computed statistics include significance of the contribution of each variable to the discrimination between groups whose contributions to the discrimination do not overlap, performance of a canonical correlation analysis to determine the successive DFs and viewing and plotting of individual discriminate scores. Usually, technique accuracy is assessed by classification matrix, which is a measure of the prediction accuracy of the DF, and its classification scores are calculated with classification

functions, determining to which group each most likely belongs (Massart et al., 1980; Scarponi et al., 1982; Wold et al., 1987; Auf der Heyde, 1990; Brereton, 1990; Einax et al., 1997; Diáz-Flores et al., 2004; Mikkonen et al., 2006). Concerning the contribution of independent variables to the discrimination of groups, this can be appreciated either by the assay of the classes homogeneity using F statistic as in the case of ANOVA/MANOVA method, or by using Wilks' lambda for each variable. Wilks' lambda is the standard statistics used to express the significance of the overall discriminatory power of variables in the model (Fisher, 1936; Atchley and Bryant, 1975; Klecka, 1980; Huberty, 1994). A value of 1.0 indicates no discriminatory power, whereas 0 indicates a perfect discriminatory power. The partial Wilks' lambda describes a unique contribution of each variable to the discriminatory power of the model. The closer the partial lambda to 0, the better the discriminatory force of the variable. In addition, the tolerance value gives information regarding the redundancy of the respective variable in the model and is computed as 1 minus R-squares of the respective variable, with all other variables included in the model. In other words, it is the proportion of the variance contributed by a respective variable. If a variable is completely redundant, the squared tolerance value approaches zero. This kind of information can be obtained from value of discriminant coefficients associated with the descriptive variables x_i, and also from the correlation coefficients between each variable x_i and the vector score. The higher the discriminant coefficient (absolute value) and the closer the correlation coefficient to one, respectively, the more important the variable is for the sample separation in defined groups. Also, the standardised discriminant coefficients, like beta weights in regression, are used to asses the relative classifying importance of independent variables. The theory of LDA was widely described before and this is why, the readers are referred to the referenced literature (Tatsuoka, 1970; Lachenbruch, 1975; Klecka, 1980; Massart et al., 1980; Scarponi et al., 1982; Brereton, 1990; McLachlan, 1992; Huberty, 1994; Manley, 1994; Einax et al., 1997; Diáz-Flores et al., 2004; Mikkonen et al., 2006).

3   Results and discussion

Table 4 shows the statistical characteristics (mean, median, range and standard deviation) of hair and nails samples for employees as well as for control group. The results of element concentration in $PM_{2.5}$, both for investigated and control group used in this paper as discussion background were published before by Raińska et al. (2005). For the majority of investigated analytes (As, Br, Co, Cu, I, Mn, Sb, U and W) mean concentration and maximum concentration determined in $PM_{2.5}$ samples collected within one shift were 10^4–10^6 lower than permissible exposure limits. An occupational exposure assessment based only on measurements of stressors in the workplace air can be misleading, thus application of hard tissues as potential indicators of long-term exposure becomes necessary. It may be easily observed that the mean concentration for the majority of elements in hair as well as in nails appears to be higher for employees' group, especially for elements typically originating from phosphate rocks, such as REEs and uranium. It was also found that the element concentrations have higher values of standard deviation and range in case of employees' group, which suggests a large spread of concentration within this group. Our data revealed that the employees who were exposed to phosphate fertiliser precursors showed the mean hair concentration of Na, Al, Ca, V, Mn, Mo, In, I, La, Sm, Au and U two or more times higher than in case of unexposed

persons. For Mg, S, Cu and Dy the concentration for employees was higher too, but did not exceed 100% of concentration limit while for Ti, Co, Zn, As and Sb comparable values were determined in both groups. A comparison of nails samples indicates two or more times higher mean concentrations for Al, K, As, Br, Sb, La, Sm and U in case of employees than in control group. This spread can results from unknown influence of factors affecting element concentration such as dietary habits, lifestyle, geochemical environment, age, sex, hair colour and smoking habits. At present stage of our research, a complete comparison with the highest permissible concentration values in biological samples established by Polish law is difficult due to different kind of biological matrix. Nevertheless, i.e. for As the maximum determined concentration value was 0.36 $\mu g \cdot g^{-1}$ and 1.04 $\mu g \cdot g^{-1}$ in hair and nails, respectively, while the highest permissible concentration value for urine after a weekly exposure was established at 35 $\mu g \cdot l^{-1}$. It has to be emphasised, that such comparison should be treated as preliminary, because the analysis of body fluids analysis reflects short-term exposure while hard tissues are good indicators of long-term exposure (Iyengar et al., 1998; Hać et al., 2000; Petersen et al., 2000; Apostoli, 2002; Gomes et al., 2004). Several reports on concentration of selected variables in hair and nails with the division for both various professions and non-occupational populations have been published before (Saad and Hassanien, 2001; Gerhardsson et al., 2002; Wilhelm et al., 2002; Strumylaite et al., 2004). Gerhardsson et al. (2002) presented results of median concentration of Cu and Zn in hair and nails samples for copper smelter workers. For Cu it was 14.80 $\mu g \cdot g^{-1}$ (range 9.20–80.30 $\mu g \cdot g^{-1}$) while in nails 4.70 $\mu g \cdot g^{-1}$ (range 1.70–43.00 $\mu g \cdot g^{-1}$). For control group, 16.60 $\mu g \cdot g^{-1}$ (range 10.60–55.80 $\mu g \cdot g^{-1}$) and 4.90 $\mu g \cdot g^{-1}$ (range 1.80–22.00 $\mu g \cdot g^{-1}$) were detected in hair and nails, respectively. The median value and range of Zn were as follows: employees – hair: 212 $\mu g \cdot g^{-1}$ (110–313 $\mu g \cdot g^{-1}$), nails: 79 $\mu g \cdot g^{-1}$ (29–196 $\mu g \cdot g^{-1}$); control group – hair: 233 $\mu g \cdot g^{-1}$ (180–405 $\mu g \cdot g^{-1}$), nails: 79 $\mu g \cdot g^{-1}$ (27–183 $\mu g \cdot g^{-1}$). In case of investigated fertiliser plant employees, median concentration of Cu in hair samples was lowered both for workers and control group: 9.06 $\mu g \cdot g^{-1}$ (4.97–42.58 $\mu g \cdot g^{-1}$) and 10.44 $\mu g \cdot g^{-1}$ (5.82–11.69 $\mu g \cdot g^{-1}$), respectively. Similar dependence was observed also for Zn: employees – 160.10 $\mu g \cdot g^{-1}$ (10.42–229.10 $\mu g \cdot g^{-1}$) and control group – 130.60 $\mu g \cdot g^{-1}$ (119.90–201.40 $\mu g \cdot g^{-1}$). The concentration of Cu and Zn in nails samples were characterised by lower median concentration value for fertiliser plant employees than copper smelter ones (Table 4). Interesting results bring the comparison to the results presented for concentration of selected elements in human hair for 31–80 years old population for a non-industrialised region in Poland. We observed approximately comparable results for K (73.12 $\mu g \cdot g^{-1}$), Ca (857.06 $\mu g \cdot g^{-1}$), Mn (2.88 $\mu g \cdot g^{-1}$), Cu (6.88 $\mu g \cdot g^{-1}$), Co (0.47 $\mu g \cdot g^{-1}$) and Zn (128.21 $\mu g \cdot g^{-1}$) (Nowak, 1998).

To confirm a major hypothesis assuming that elements originating from fertiliser production may accumulate in hair or nails and to confirm statistically preliminary observations forward stepwise linear discriminant analysis was applied. LDA was used to quantitative selection of variables that enhance discrimination of the groups established by the dependent variable defining the origin and nature of samples. For the mathematical evaluation, statistical packages Statistica 7.0 for Windows and SPSS 15.0 Evaluation version were applied. The computed data set included 41 investigated samples (20 hair samples and 21 nails samples) and 40 control samples (20 hair samples and 20 nails samples), and the following characteristics (25): Na, K, Ca, Mg, Al, Ti, V, Mn, Co, Zn, Cu, As, Mo, In, Sb, La, Sm, Dy, W, Au, U, Cl, Br, I and S. Prior to chemometric analysis the data were first pre-processed by the use of standardisation

procedure to scaling to unit standard deviation. Standardisation was applied because in case of many data sets the measured parameters are usually in different units or in different levels of magnitude, which is also the case here (Bro and Smilde, 2003). The summary of the stepwise analysis ($\lambda = 0.110$; F = 5.56, p < 0.001) presented in Table 5 reveals the significant variables retained in the model. The statistics in Table 5 illustrate their contribution to the discrimination (separation) of samples according to their origin and nature. It is apparent that the greatest contribution is given by S ($\lambda^* = 0.696$; $F = 7.867$), the next highest are Cl ($\lambda^* = 0.768$; $F = 5.433$), Ti ($\lambda^* = 0.778$; $F = 5.148$), K ($\lambda^* = 0.832$; $F = 3.636$), Mg ($\lambda^* = 0.873$; $F = 2.619$), Mo ($\lambda^* = 0.882$; $F = 2.415$) and W ($\lambda^* = 0.885$; $F = 2.349$). It is interesting to note that the contributions of Cu and As are quite similar: the smallest contribution was obtained for Sm ($\lambda^* = 0.945$; $F = 1.051$). The values of tolerance (R^2) and 1 minus R-square, respectively (Table 5) provide information of the correlation of the respective variable with all other variables included in the model. The most redundant variables appear to be K ($R^2 = 0.725$), Ag ($R^2 = 0.691$), W ($R^2 = 0.603$), Br ($R^2 = 0.596$) and As ($R^2 = 0.570$) and the most informative variables seem to be Ti ($R^2 = 0.191$) and Al ($R^2 = 0.272$).

Table 4 Statistical characteristics for both hair and nails samples as well as for control group

	Mean ($\mu g \cdot g^{-1}$)	Median ($\mu g \cdot g^{-1}$)	Range ($\mu g \cdot g^{-1}$)	Standard deviation ($\mu g \cdot g^{-1}$)
Hair samples – employees: n = 20 (control group: n = 20)				
Age	47.60 (50.20)	49.50 (55.00)	31.00–60.00 (28.00–59.00)	8.93 (12.60)
Na	105.71 (54.29)	92.37 (39.98)	30.37–410.80 (19.89–89.00)	85.32 (30.22)
Mg	109.15 (93.30)	98.99 (84.64)	36.27–509.30 (50.07–168.90)	101.12 (46.43)
Al	39.24 (21.15)	31.45 (16.10)	11.96–194.90 (12.69–43.82)	38.82 (12.85)
S	5925.15 (5471.40)	5912.50 (5278.00)	3265.00–10710.00 (4445.00–6628.00)	1763.13 (924.91)
Cl	486.93 (1208.22)	356.95 (1196.00)	29.12–1233.00 (537.10–1852.00)	397.10 (492.15)
K	53.60 (91.12)	41.30 (103.00)	12.50–131.70 (20.07–148.70)	31.86 (47.58)
Ca	877.74 (251.72)	687.35 (173.90)	83.52–2893.00 (101.00–586.80)	685.33 (193.13)
Ti	19.18 (19.21)	12.04 (13.22)	5.89–80.38 (8.61–42.71)	18.49 (13.97)
V	0.41 (0.08)	0.30 (0.09)	0.02–2.18 (0.00–0.18)	0.49 (0.07)
Mn	2.39 (0.73)	1.66 (0.81)	0.52–13.99 (0.43–1.02)	2.89 (0.25)
Co	0.17 (0.19)	0.12 (0.23)	0.01–0.53 (0.02–0.41)	0.15 (0.16)
Zn	148.18 (147.58)	160.10 (130.60)	10.42–229.10 (119.90–201.40)	51.03 (34.42)
Cu	12.30 (9.65)	9.06 (10.44)	4.97–42.58 (5.82–11.69)	9.97 (2.42)
As	0.11 (0.07)	0.08 (0.04)	0.01–0.36 (0.03–0.18)	0.09 (0.06)
Br	1.80 (2.76)	0.95 (2.94)	0.27–9.34 (1.99–3.37)	2.13 (0.65)
Mo	0.21 (0.10)	0.12 (0.09)	0.00–1.13 (0.03–0.17)	0.25 (0.05)
In	0.17 (0.07)	0.04 (0.08)	0.00–0.80 (0.02–0.12)	0.26 (0.05)
Sb	0.07 (0.06)	0.04 (0.02)	0.01–0.53 (0.01–0.15)	0.11 (0.06)
I	0.58 (0.25)	0.37 (0.28)	0.09–2.56 (0.11–0.47)	0.56 (0.14)
La	0.23 (0.10)	0.13 (0.05)	0.05–0.92 (0.04–0.28)	0.22 (0.10)

Table 4   Statistical characteristics for both hair and nails samples as well as for control group (continued)

	Mean ($\mu g \cdot g^{-1}$)	Median ($\mu g \cdot g^{-1}$)	Range ($\mu g \cdot g^{-1}$)	Standard deviation ($\mu g \cdot g^{-1}$)
Hair samples – employees: n = 20 (control group: n = 20)				
Sm	0.04 (0.01)	0.03 (0.01)	0.00–0.20 (0.01–0.01)	0.04 (0.00)
Dy	0.13 (0.09)	0.08 (0.09)	0.01–0.46 (0.05–0.13)	0.11 (0.03)
W	2.39 (3.01)	1.81 (2.65)	0.34–6.32 (1.03–5.36)	1.89 (1.76)
Au	0.02 (0.01)	0.01 (0.01)	0.00–0.14 (0.00–0.03)	0.03 (0.01)
U	0.17 (0.03)	0.10 (0.03)	0.02–0.78 (0.01–0.08)	0.18 (0.02)
Nails samples – employees: n = 21 (control group: n = 20)				
Age	47.62 (50.20)	48.00 (55.00)	31.00–60.00 (28.00–59.00)	8.71 (12.60)
Na	458.04 (550.48)	402.90 (518.60)	225.60–829.00 (282.90–902.50)	183.42 (240.28)
Mg	134.32 (170.12)	115.60 (140.90)	61.77–304.30 (72.69–368.90)	60.15 (115.13)
Al	36.41 (26.85)	29.72 (18.66)	7.08–104.70 (14.42–65.03)	24.00 (21.49)
S	3582.86 (4565.80)	3358.00 (3937.00)	2505.00–5617.00 (1770.00–9023.00)	868.18 (2690.74)
Cl	989.44 (949.10)	857.50 (930.80)	357.00–2478.00 (407.90–1486.00)	461.75 (404.95)
K	503.71 (521.78)	437.50 (305.90)	166.90–1095.00 (210.00–975.10)	268.92 (370.24)
Ca	971.02 (831.62)	878.50 (807.00)	405.60–1966.00 (434.10–1316.00)	421.30 (357.71)
Ti	16.35 (33.54)	14.75 (27.71)	4.24–34.07 (19.31–55.69)	8.65 (16.28)
V	0.22 (0.23)	0.17 (0.20)	0.05–0.67 (0.09–0.49)	0.18 (0.16)
Mn	1.39 (1.40)	0.96 (1.27)	0.47–4.64 (0.72–2.26)	1.04 (0.61)
Co	0.21 (0.19)	0.20 (0.24)	0.01–0.58 (0.01–0.38)	0.14 (0.15)
Zn	138.48 (124.78)	127.00 (127.30)	70.67–379.40 (107.90–145.90)	63.43 (16.26)
Cu	7.50 (15.43)	6.46 (9.77)	2.03–22.13 (6.06–30.48)	4.64 (10.20)
As	0.21 (0.10)	0.10 (0.07)	0.02–1.04 (0.03–0.25)	0.25 (0.09)
Br	1.58 (1.55)	1.31 (1.51)	0.63–4.85 (0.97–2.42)	0.94 (0.55)
Mo	0.11 (0.08)	0.09 (0.08)	0.03–0.25 (0.05–0.12)	0.07 (0.03)
In	0.08 (0.19)	0.02 (0.05)	0.00–0.61 (0.00–0.77)	0.15 (0.33)
Sb	0.11 (0.08)	0.06 (0.04)	0.01–0.68 (0.02–0.26)	0.15 (0.10)
I	0.24 (0.18)	0.17 (0.18)	0.07–0.72 (0.11–0.32)	0.18 (0.09)
La	0.23 (0.10)	0.22 (0.09)	0.01–0.80 (0.05–0.18)	0.18 (0.05)
Sm	0.04 (0.01)	0.03 (0.01)	0.00–0.15 (0.00–0.01)	0.03 (0.00)
Dy	0.09 (0.13)	0.08 (0.12)	0.01–0.25 (0.09–0.20)	0.05 (0.04)
W	0.82 (0.69)	0.56 (0.68)	0.22–3.43 (0.23–1.05)	0.76 (0.35)
Au	0.02 (0.01)	0.00 (0.00)	0.00–0.30 (0.00–0.01)	0.06 (0.00)
U	0.26 (0.02)	0.08 (0.03)	0.02–2.16 (0.01–0.03)	0.49 (0.01)

Table 5   Variables in the model (the most discriminant variables)

Variable	Wilks' Lambda	Partial	F-remove	p-level	Tolerance	1-Tolerance
K	0.133	0.832	3.636	0.036	0.725	0.275
S	0.159	0.696	7.867	0.001	0.498	0.502
Cl	0.144	0.768	5.433	0.009	0.532	0.468
Sm	0.117	0.945	1.051	0.360	0.392	0.608
W	0.125	0.885	2.349	0.110	0.603	0.397
Br	0.117	0.941	1.134	0.333	0.596	0.404
Age	0.118	0.937	1.220	0.307	0.691	0.309
As	0.120	0.919	1.593	0.217	0.570	0.430
Mg	0.126	0.873	2.619	0.087	0.283	0.717
Cu	0.120	0.918	1.612	0.213	0.456	0.544
Ti	0.142	0.778	5.148	0.011	0.191	0.809
Mo	0.125	0.882	2.415	0.104	0.313	0.687
Al	0.122	0.906	1.873	0.168	0.272	0.728

The results concerning the DFs and the canonical DFs are presented in Table 6, while pooled within-groups correlations between discriminating variables and standardised canonical DFs in the form of structure matrix are presented in Table 7. It has to be clarified that a positive value of DF coefficient means that particular analyte concentrations increase simultaneously with an increasing DF values, while a negative value of DF coefficient means that particular analyte concentrations decrease simultaneously with an increasing DF value. The eigenvalue of the first axis is 3.54 and explains more than 78% of the variation in samples distribution along this axis. The eigenvalue drops to 0.99 for the second axis and the variation explained represents less than 22%. The highest standardised discriminant coefficients correspond to: S (0.792), Mg (−0.708), K and Cl (−0.545) and Ti and W (0.493) in DF1; Ti (−1.400), Mo (0.839), Al (0.666), Cl (−0.639) and As (0.518) in DF2. Pooled within-groups correlations between discriminating variables and standardised canonical DFs indicate that Sm, Al, Mo and As accumulate in nails and hair of fertiliser plant employees while Cl and Ti are removed from hard tissues. Significantly higher levels of Sm, Al, Mo and As can be easily explained by occupational exposure of the plant employees to these elements. These metals enter the human body mainly after inhalation and also via gastrointestinal absorption and due to solubility can easily be incorporated into various tissues. For example, As is known to be deposited in hair both from blood stream and from external contamination due to either arsenic containing dusts or washing with water contaminated by arsenic (Hindermarsh and McCurdy, 1986). This phenomenon indicates a possible threat to human health caused by long-term exposure, even though the highest permissible concentration values (NDS) determined in $PM_{2.5}$ sample collected during one work shift were not exceeded.

The explanation of decreased concentration of Ti and Cl in hard tissue comparing to control group is difficult due to lack of literature examples and is probably connected with inter-elemental relations. Comparison of both hard tissues as samples used in various biomonitoring studies proves their applicability and indicates that S, W, Cu, K and Mg concentration is crucial in discrimination of these matrices, while for W and Cu only the highest permissible exposure limits in workplace air were established by Polish

legislation (Table 1). As suggested by Chojnacka et al. (2005) Mg and W create an antagonistic pair and this is the reason why their concentrations were negatively correlated. From 25 characteristics Na, In, I, Au, Dy, Ca, La, U, Sb, V, Zn, Mn and Co appear to be ineffective as discriminant factors and do not correspond to occupational exposure assessment in case of fertiliser plant employees, although the permissible exposure limits in workplace air were established U, Sb, Mn and Co. The classification matrix presented in Table 6 indicates a satisfactory separation of samples (subjects) in a good agreement with their origin and nature. The group of hair, for example, showed that 95% of subjects were well classified and only one subject was erroneously included in the group of nails (95.24%). The poorest classification was obtained for subjects from control group (60%). The total classification was more than 88%. This statement is well supported by the two-dimensional scatter plot using the discriminant scores of the samples along DF1 and DF2 as can be seen in Figure 1.

Table 6   The standardised canonical discriminant function (DF) coefficients

Variable	DF1	DF2
K	−0.545	−0.036
S	0.792	−0.496
Cl	−0.545	−0.639
Sm	0.419	0.087
W	0.493	0.065
Br	0.339	−0.142
Age	0.043	−0.426
As	−0.106	0.518
Mg	−0.708	0.339
Cu	0.464	0.159
Ti	0.493	−1.400
Mo	−0.186	0.839
Al	−0.400	0.666
Eigenvalue	3.539	0.995
Cumulative proportion	0.781	1.000

Table 7   Structure matrix

	Function	
	DF1	DF2
Na[a]	−0.544[*]	0.062
K	−0.484[*]	0.081
S	0.384[*]	0.047
W	0.261[*]	0.052
In[a]	0.176[*]	0.068
Cu	0.156[*]	0.131
I[a]	0.127[*]	−0.125
Au[a]	0.115[*]	−0.021
Dy[a]	0.084[*]	0.057

Table 7   Structure matrix (continued)

	Function	
	DF1	*DF2*
Mg	−0.072$^{(*)}$	0.053
Ca$^{(a)}$	−0.049	−0.373$^{(*)}$
Sm	0.023	−0.355$^{(*)}$
Cl	−0.276	0.340$^{(*)}$
La$^{(a)}$	−0.014	−0.327$^{(*)}$
Ti	0.053	0.237$^{(*)}$
U$^{(a)}$	−0.166	−0.223$^{(*)}$
Sb$^{(a)}$	0.081	−0.194$^{(*)}$
Al	0.019	−0.191$^{(*)}$
Mo	0.144	−0.187$^{(*)}$
V$^{(a)}$	0.104	−0.176$^{(*)}$
As	−0.147	−0.166$^{(*)}$
Zn$^{(a)}$	0.081	−0.149$^{(*)}$
Mn$^{(a)}$	0.059	−0.135$^{(*)}$
Br	0.038	0.123$^{(*)}$
Age	0.002	0.112$^{(*)}$
Co$^{(a)}$	0.041	0.101$^{(*)}$

Notes: [*] largest absolute correlation between each variable and any DF.
[a] variables not used in the analysis.

Figure 1   Scatter plot of canonical scores on the plan described by DF1 and DF2 for selected variables (n: nails, h: hair, c_n: nails-control group, c_h: hair-control group)
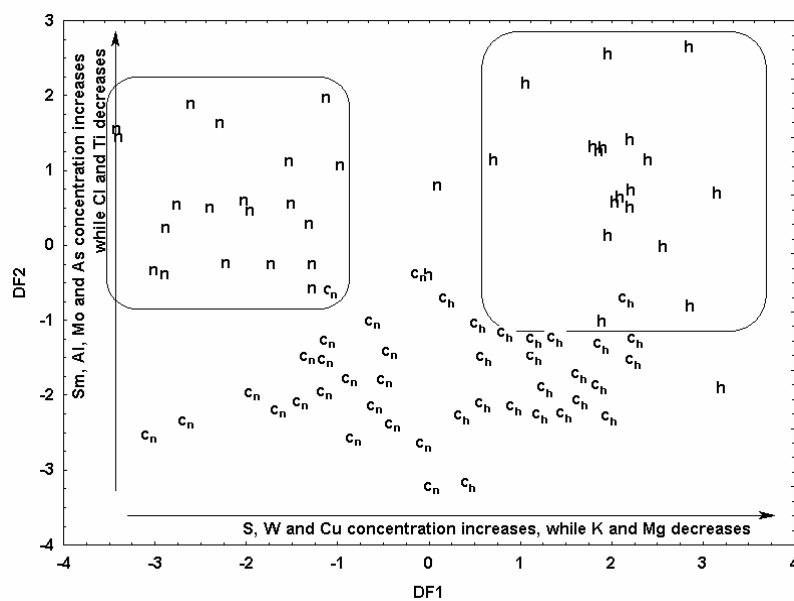
The next step was to include all variables in the model, in order to examine if the ability to discriminate between the samples (subjects) improves. The eigenvalue of the first axis was 4.44 and explained more than 75% of the variation in the subject distribution along this axis. The eigenvalue corresponding to the second axis increased to 1.43 and the variation accounted for by the second axis represented less than 25%. The classification matrix presented in Table 8 indicates a better separation of subjects. The group of hair, for example, showed that 100% of subjects were very well classified. The poorest classification was obtained again for subjects from the control group (80%). The most important finding is that both investigated samples (hair and nails of employees) as well as both control samples (hair of control group and nails of control group members) can be satisfactory differentiated. The total classification was more than 94%. A two-dimensional scatter plot using the discriminant scores of samples along DF1 and DF2 with all the variables included in the model is presented in Figure 2.

Table 8   Classification matrix with selected variables included to LDA and with all variables in the model (validation procedure)

| | *With variables included to LDA* | | | | | *All variables in the model* | | | |
	Percent (%)	Hair	Control group	Nails		Percent (%)	Hair	Control group	Nails
Hair	95.0	19	0	1	hair	100.0	20	0	0
Control group (nails)	60.0	0	12	8	control group (nails)	80.0	0	16	4
Control group (hair)	60.0	8	12	0	control group (hair)	80.0	4	16	0
Nails	95.2	1	0	20	nails	95.2	1	0	18
Total	88.2	22	6	23	total	94.1	23	8	18

Figure 2   Scatter plot of canonical scores on the plan described by DF1 and DF2 with all variables included in the model (n: nails, h: hair, c_n: nails-control group, c_h: hair-control group)
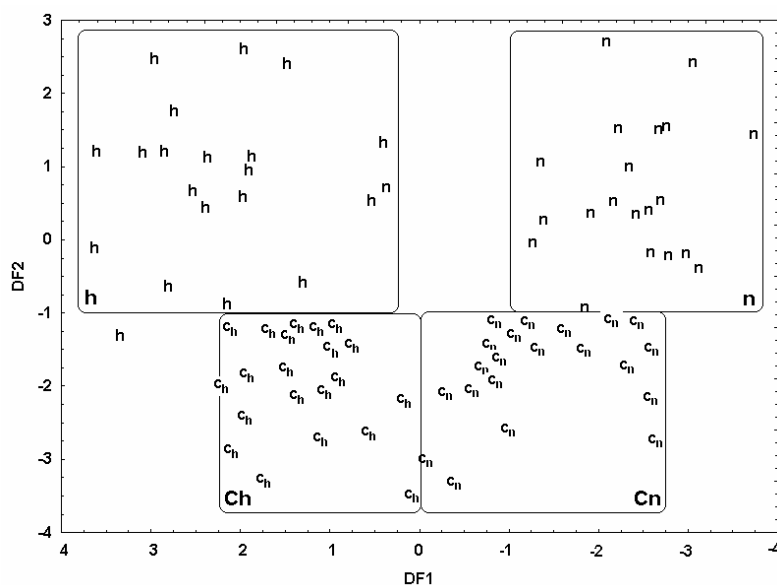
4   Conclusions

Analysed samples were collected from the occupationally exposed group of fertiliser plant employees. The elemental composition (Na, K, Ca, Mg, Al, Ti, V, Mn, Co, Zn, Cu, As, Mo, In, Sb, La, Sm, Dy, W, Au, U, Cl, Br, I and S) of their hair and nails was measured and compared with a control group. As the multivariate method of this study, discriminant analysis was used to examine which of the chemicals are responsible for the similarities or differences observed between hair and nails samples of the exposed group and control group. The concentrations of Ti, Mo, Al, Cl, As, S, Mg, K and W appeared to have significant effects on the differentiation of hair and nails for the investigated groups. The obtained results showed that employees of the Gdańsk phosphate fertiliser plant are occupationally exposed to elements than can be harmful to their health due to long-time exposure, especially for As and Al. Comparison of both hard tissues as samples proves their applicability and indicates that S, W, Cu, K and Mg concentrations constitute a crucial factor in discrimination of these matrices. These results indicate that human biosubstrates are useful in the preliminary elevation of the plant impact on humans. It has to be stressed that full occupational exposure assessment should be associated with the determination of the highest permissible concentration of analytes in workplace air as well as the highest permissible concentration values in urine which reflects short-term exposure. From 25 characteristics, Na, In, I, Au, Dy, Ca, La, U, Sb, V, Zn, Mn and Co appear to be ineffective as discriminant factors and do not correspond to occupational exposure assessment in case of fertiliser plant employees. The best classification results were reached while incorporating all data in the model. Based on the group of hair 100% of subjects were very well classified. The poorest classification was obtained for subjects from control group (80%). The total classification was more than 94%.

Acknowledgements

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