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CONTENTS

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VARIA

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Prezentowane artykuły przeszły normalną procedurę recenzyjną i redakcyjną.

ECOLOGICAL CHEMISTRY AND ENGINEERING A

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Judita BYSTRICKÁ[1], Janette MUSILOVÁ
and Tomáš TÓTH

POSSIBILITES OF CADMIUM UPTAKE LOWERING BY SEEDS OF LEGUMES WITH THE APPLICATION OF Zn^{2+} INTO THE SOIL

MOŻLIWOŚĆ ZMIEJSZENIA POBRANIA KADMU PRZEZ NASIONA ROŚLIN STRĄKOWYCH DZIĘKI APLIKACJI JONÓW Zn^{2+} DO GLEBY

Abstract: Cadmium is toxic, carcinogenic element naturally occurring in soil in concentration of about 1 mg · kg^{-1}. High concentrations of cadmium increase its uptake by the plants and lower the yields. One of the ways how to manage with the phytotoxicity of cadmium could be the antagonistic system of cadmium with cations Zn^{2+}, Ni^{2+} and Mn^{2+}.

The system Cd^{2+} and Zn^{2+} was created and added into the soil. We observed the ability of Zn^{2+} cation to eliminate the negative affecting of cadmium in plant nutrition and to lower the cadmium in the dry matter. The gained results show that the addition of single Cd^{2+} ions into the soil (B variant) had negative effect also on the yield amount as well as on observed qualitative parameters of soya and faba beans. In C variant, when both Cd^{2+} and Zn^{2+} cations were added, there was slight yield increasing in both crops observed. By the assessing of Cd content in dry matter of soya and faba beans by the application of both elements (C variant) there was awaited effect of content lowering in the case of cadmium in both crops. While the single Cd^{2+} addition enhanced the content of this metal in soya beans on the value 3.41 mg · kg^{-1}, by common application of Cd^{2+} and Zn^{2+} ions this value presented 0.2 mg · kg^{-1}. In the case of faba in B variant the value 2.45 mg Cd kg^{-1} was determined, but by the application of both Cd^{2+} and Zn^{2+} ions the content was lowered on 1.33 mg Cd kg^{-1}.

Keywords: soya bean, faba bean, cadmium, zinc, accumulation

Compounds of toxic elements belong to harmful substances which could easily get into the soil. Especially cadmium, mercury and arsenic are the most dangerous toxic elements from ecological standpoint [1]. Biological essential microelements in nature could also have toxic effects if they enhance certain concentration [2]. Ecological risks from cummulation of heavy metals in soil are reflected on soil ability to provide hygienic safe foodstuffs.

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The risky elements content in plants is directly dependent on their concentration and availability in the environment and also on the exposure length. Heavy metals affect plants as stress factors. They induce changes or even species extinction. There are some plants which are able to grow on such soils where the concentration of heavy metals is high [3].

Some methods were suggested for the elimination of negative effects of heavy metals in soils. For example, the lowering of heavy metals solubility by pH value increasing, complex melioration based on re-covering of contaminated soil by the layer of non-contaminated soil.

The obvious attention is focused not only to the content of individual heavy metals in the system soil – plant – food, but also to their mutual interactions among individual heavy metals [4].

Cadmium from the health point of view belongs to the heavy metals group whose toxic properties are manifested at relatively low contents. It is glossy white metal, chemically similar to zinc. The food chain contamination by cadmium is associated mainly with the soil contamination [1]. The soil reaction pH and redox potential are crucial factors for the mobility and thus for biological utilisation for plants. Relative mobility of Cd increases in acid and oxidant environment. The physiological effect of abundant amount of cadmium is connected with damage of the photosynthesis process and changes of nitrogen compounds. The Cd^{2+} cations form compounds with cysteine and proteins with structure of methionine. The thiol group of cysteine helps to bind Cd in plants at 11–34 % (at 71 % in corn), the other form of cadmium in plant is present as the free ions [5]. Cadmium has in higher concentrations carcinogenic, mutagenic and teratogenic effects.

Many authors had focused on the interaction of Cd – Zn and with regard on their chemical relationship and their conclusion was that the addition of zinc into the environment lowers the cadmium uptake by plants.

Our work is focused on the effects of bivalent cations of cadmium and zinc in soil. The solo effects of cadmium on plant growing, yield and cummulation ability of soya bean (*Glycine max*) and faba bean (*Faba equine*) have been compared and the influence of the bivalent zinc cations addition to cadmium cations effect has been evaluated.

Material and methods

Biological material. Soya bean (*Glycine max*, *variety KORADA*) – used in this study is characterized by big seed of yellow to green-yellow color with yellow or brown navel. Soya bean is used mainly for its content of qualite proteins.

Faba bean (*Faba vulgaris subsp. Equina*, *variety Stabil C 1*) belongs to legumes as feed. It is grown mainly for beans. It is valuable for high content of nitrogenous compounds. It contains 32–34 % of crude proteins.

The experiment was realized as a pot trial. The plastic pots were bowl shaped with an average of 20 cm and height of 25 cm with perforated bottom. Into pots 6 kg of soil was weighted and the basic nutrients were applicated in form of NPK fertilizer.

In B variant 10 mg Cd in form of bivalent solution $CdCl_2 \cdot 2^1/_2$ (Merci, Bratislava, Slovak Republic) and in C variant 10 mg cadmium and 80 mg zinc in form bivalent solution $ZnSO_4 \cdot 7H_2O$ (Merci, Bratislava, Slovak Republic) per kg of soil was added.

Table 1

Agrochemical characteristic of the used soil [mg · kg^{-1}], the value of exchangeable pH and of active pH

Locality	K	Ca	Mg	P	N	pH_{KCl}	pH_{H_2O}
Vycapy	212.5	1459.5	265.0	19.86	2975.0	4.36	5.98

Table 2

Heavy metals contents of tested soil in extract of HNO_3 [mg · kg^{-1}]

Locality	Zn	Cu	Cd	Pb	Co	Cr	Ni
Vycapy	5.34	9.12	0.22	8.88	1.84	1.92	6.38

Table 3

Characteristics of pot experiment variants

Variants	
A	NPK – control
B	NPK – 10 mg Cd per kg of soil
C	NPK – 10 mg Cd + 80 mg Zn per kg of soil

We evaluated the weight of the overground biomass and the qualitative composition of soya bean and faba bean from the standpoint of the content of risky elements.

The content of cadmium and zinc was determinated after mineralization by dry way by AAS method on apparaturs PYE UNICAM SP 9.

Results and discussion

The results of pot experiments showed the ability of bivalent cations zinc to decrease the cadmium uptake by plants and simultaneously to eliminate its negative influence oi the soya and the faba beans yield. The single cadmium and also the combination of cadmium with zinc were studied.

The weights of yield of soya bean as well as the content of cadmium and zinc in soya and faba beans from qualitative parameters in mg · kg^{-1} on dry matter were determined. The obtained results show that the addition of solo cadmium application (B variant – 10 mg Cd) had the negative effect on the yield and also on the qualitative parameters in both crops. The yield of soya was 12.95 ± 0.37, while in C variant after the application of cadmium and zinc (10 mg Cd + 80 mg Zn) the yield was mildly increased by 18.02 ± 0.49. The similar situation was in faba bean, where in B variant the yield was 12.07 ± 0.32 and after the application of cadmium and zinc ions the yield was 15.06 ± 0.42 (Figs. 1, 2).

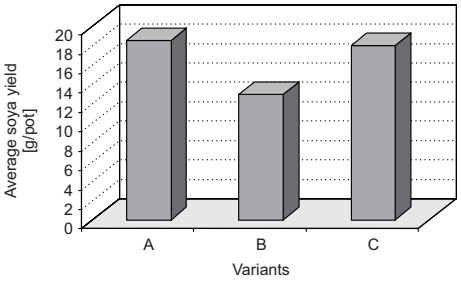

Fig. 1. Soya beans yield after application of Cd and Zn cations

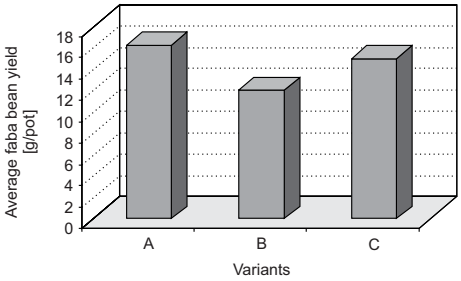

Fig. 2. Faba beans yield after application of Cd and Zn cations

The cadmium content in soya bean and also in faba bean after the application of both ions (C variant) the required effect of cadmium content lowering was observed. While cadmium alone applied had increased its content in soya beans on the value 3.41 ± 0.45, the application of cadmium and zinc ions this value was 0.2 ± 0.045. In Faba bean there was in B variant the value 2.45 ± 0.37 mg Cd · kg^{-1} and in C variant 1.33 ± 0.32 mg Cd · kg^{-1} (Figs. 3, 4).

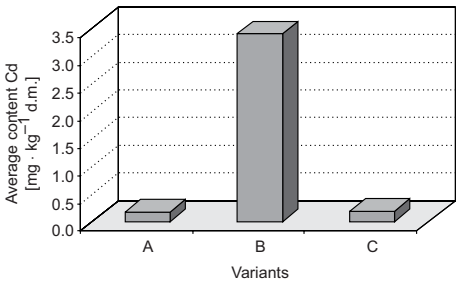

Fig. 3. Content of Cd [mg · kg^{-1} d.m.] in soya bean

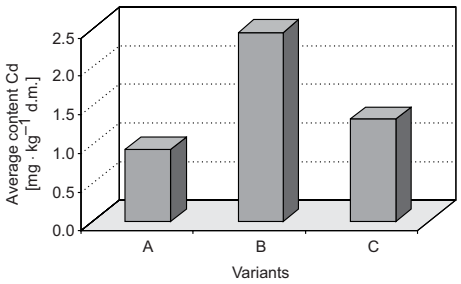

Fig. 4. Content of Cd [mg · kg^{-1} d.m.] in faba bean

Applying zinc ions into the soil to eliminate negative effects we naturally focused on its content in dry matter of plants. The assessed values of zinc present 50.09 ± 1.23 mg Zn · kg^{-1} by application of cadmium alone in soya, in faba bean 39.94 ± 0.9 mg Zn · kg^{-1} and in C variant these values were in soya 79.16 ± 1.88 mg Zn · kg^{-1} and 85.91 ± 2.01 mg Zn · kg^{-1} in faba bean (Figs. 5, 6).

Our results can not be uniformly assigned to the effect of zinc cations, because the plant reaction on zinc presence widely vary and depends on plant species and variety, cadmium content in soil, doses and mutual combination of cadmium and added zinc cations, as well as on many other factors but can indicate cadmium accumulation as well as preventive effect of zinc.

The content of risk elements with high biotoxicity level belong to the most important monitored soil parameters [6]. Monitoring is focused on risk elements mentioned in legislative hygienic directives [7]. Total content includes all forms, in which certain

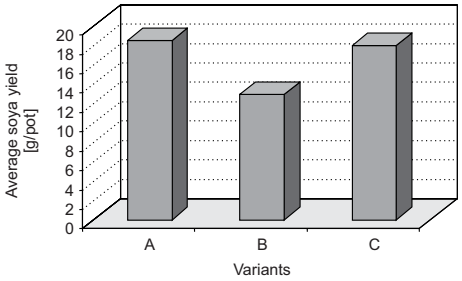

Fig. 5. Content of Zn [mg · kg^{-1} d.m.] in soya bean

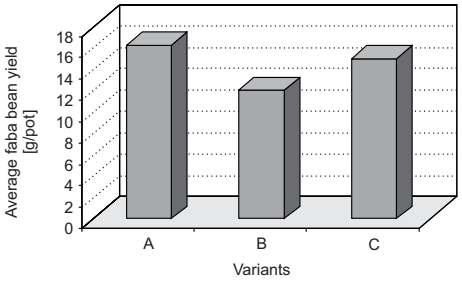

Fig. 6. Content of Zn [mg · kg^{-1} d.m.] in faba bean

element in soil is occurring. It is basic information about natural element content in soil enriched in content aroused from immissions. For evaluating of soil hygienic state it has low importance, because the prevailed part of total risk elements content is formed by other non-soluble and/or less soluble forms [8]. High importance has the determination of total risk elements content only in the case of strongly contaminated or devastated soils, where the highest rate of correlation between their content in plant and in soil takes place.

Many works [9, 10] have been written about the study of interaction of heavy metals, especially the one of Cd – Zn mainly to their chemical relationship. The conclusions of these works are not at all uniform. By [11] the addition of zinc into the environment lowers the Cd uptake by plants, by others [3, 12] the mechanisms of the uptake of zinc and cadmium by plants are depending on each other and it comes to equal uptake of both elements in conditions of their high accumulation in soil [13].

It is believed that the interaction of Cd – Zn is based on competitive inhibition when Cd and Zn compete in active centres of carriers.

Our research has been focused on the interaction of Cd – Zn. While in C variant we increased in both cases the content of potentially available zinc from value 51.5 mg · kg^{-1} (A variant) on the value 131.5 mg · kg^{-1}. This increase was observed for soya as well as for faba bean. It presented 50 % in the soya beans and even 150 % in the horse beans when compared with the control variant.

Zinc uptake by plants is realized in the form of Zn^{2+} and it depends on soil pH reaction. When hydrogens ions concentration increases the zinc availability increases.

In most of plants the zinc content is in range 25–100 mg · kg^{-1}.

Zinc in plants is accumulated mainly in roots, in higher concentrations is phytotoxic. Its mobility mainly in older plant overground biomass is inhibited. Zinc is the activator and stabilizator of many enzymes. Zinc has also an effect on biological active substances forming.

Conclusion

The soil contamination reflects the content of risky elements in vegetation including agricultural soils. It is necessary to find solution which will in some way minimalize the

enter of dangerous heavy metals from agricultural soils into the food chain. Our work was focused on the effects of influence of bivalent cations cadmium and zinc as one of the many ways of elimination of negative effects of cadmium. We found out that the dose 80 mg Zn · kg^{-1} had the positive influence on qualitative and quantitative parameters of soya and faba beans.

Acknowledgement

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MOŻLIWOŚĆ OBNIŻENIA POBRANIA KADMU PRZEZ NASIONA ROŚLIN STRĄKOWYCH DZIĘKI APLIKACJI JONÓW Zn^{2+} DO GLEBY

Abstrakt: Kadm jest toksycznym i rakotwórczym pierwiastkiem występującym w glebie w stężeniu wynoszącym około 1 mg · kg^{-1}. Kadm jest łatwo wychwytywany przez rośliny, co prowadzi do obniżenia plonów. Jednym ze sposobów zmniejszenia wychwytu kadmu przez rośliny może być wykorzystanie antagonizmu między jonami Cd^{2+} a jonami Zn^{2+}, Ni^{2+} i Mn^{2+}. W prezentowanych badaniach obserwowaliśmy zdolność jonów Zn^{2+} do obniżenia kumulacji jonów Cd^{2+} przez rośliny strączkowe oraz zmniejszenia

szkodliwego wpływu Cd^{2+} na odżywianie badanych roślin. Uzyskane wyniki wykazują, że dodanie jonów kadmu do gleby (wariant B) miało negatywny wpływ na ilość plonów oraz badane parametry jakościowe soi oraz bobu. Dodanie do gleby jonów Zn^{2+} oraz Cd^{2+} (wariant C) spowodowało niewielki wzrost plonów w obu obserwowanych uprawach. Dodanie jonów Zn^{2+} do gleby spowodowało zmniejszenie zawartości Cd^{2+} u obydwóch badanych gatunków roślin. Ziarna soi rosnące w glebie z dodatkiem kadmu zawierały w suchej masie kadm w stężeniu 3,41 mg · kg^{-1}. Obecność w glebie jonów Zn^{2+} spowodowało obniżenie zawartości Cd w suchej masie nasion soi do 0.2 mg · kg^{-1}. W przypadku bobu rosnącego w glebie zawierającej kadm (wariant B) zawartość Cd^{2+} w suchej masie nasion wynosiła 2,45 mg · kg^{-1}. Jony Zn^{2+} dodane do gleby zmniejszały zawartość Cd^{2+} w suchej masie nasion bobu do 1,33 mg · kg^{-1}.

Słowa kluczowe: nasiona soi, nasiona bobu, kadm, cynk, kumulacja

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Iwona DOMAGAŁA-ŚWIĄTKIEWICZ[1],
Włodzimierz SADY[2] and Sylwester SMOLEŃ[3]

EFFECT OF NITROGEN FERTILIZATION ON Cd, Cr, Cu, Fe, Mn, Ni, Pb, Sr AND Zn AVAILABILITY FOR COMMERCIALLY GROWN WHITE CABBAGE (*Brassica oleracea* var. *capitata alba*)

WPŁYW NAWOŻENIA AZOTEM NA PRZYSWAJALNOŚĆ Cd, Cr, Cu, Fe, Mn, Ni, Pb, Sr i Zn PRZEZ KAPUSTĘ GŁOWIASTĄ BIAŁĄ (*Brassica oleracea* var. *capitata alba*) UPRAWIANĄ W WARUNKACH PRODUKCYJNYCH

Abstract: The results of three year investigations with 'Galaxy' F_1 cabbage grown commercially in important agricultural region of the southern Poland are presented. The effect of ammonium sulphate and RSM (solution of ammonium nitrate + urea), the method of application (placement and broadcast technique and foliar fertilization) on Cd, Cr, Cu, Fe, Mn, Ni, Pb, Sr and Zn concentrations in edible parts of cabbage were surveyed. In present work all metals concentration in cabbage were below the lower range of content reported for cabbage grown in non-contaminated areas. The low concentration of micro/trace elements were related to soil parent material, with generally low total and extractable levels of metals. Consistently greater concentrations of Cd, Cu, Fe, Mn and Ni were measured in cabbage grown on the site with lower pH compared with concentrations in plants sampled at other soil sites with higher pH. Ammonium sulphate significantly increased Mn and Fe concentrations in cabbage heads. However environmental factors considerably influenced this tendency. The similar trend for Zn was observed. The method of application affected Mn content in 2007 and Ni in 2007 in cabbage. Slightly higher manganese and nickel concentration for placement fertilization was noted. The results obtained would suggest that in commercial cabbage production on over-limed soils using nitrogen ammonium fertilizers may improve Mn, Fe and Zn uptake by plants.

Keywords: white cabbage, micro/trace elements, nitrogen fertilization, bioavailability

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Plant uptake of metals from soil is an important pathway for their entry to the human food chain. Although some elements are essential to plant life, many are toxic in high concentrations [1]. Naturally occurring metals in soils (the pedogeochemical background level) is a direct function of its original natural composition. Over time, the concentration of naturally occurring metals in soil will be influenced by geomorphologic processes such as erosion, weathering, and dissolution of mineral deposit [2]. Background concentrations can be also "anthropogenic" when the concentration of chemical in the environmental is due to human activities, but is not the result of use or release of waste or products, or industrial activity. Anthropogenic inputs from agricultural practices increase metals concentrations in soils as a result of using pesticides/herbicides, lime and fertilizers.

The bioavailability and the potential toxicity of metals in the environmental depend on their speciation in the soil and the soil solution [3]. The pH of the soils is often the most important chemical property governing metals sorption, precipitation, solubility, and availability [4, 5]. Cationic trace element adsorption by oxide surfaces increases to almost 100 % with increasing pH [4]. Sorption by metal oxides is major mechanism for removal of trace element cations: Pb, Cu, Zn, Cd, Cr, and Ni and trace element oxyanions ie CrO_4^{2-} [6]. Liming has often been shown to increase the negative surface charge thereby increase the retention of nutrient ions and toxic heavy metals. It has been widely investigated as a mean of controlling the reaction with nutrients [7–9].

In intensive agricultural systems overapplication of lime may have detrimental effects on factors affecting crop yield, particularly nutrient availability. Alkalinity as result of overlime treatment can subject planting to nutritional deficiencies. But another hand high pH may decrease heavy metals uptake by plants. In a cabbage production liming has been used as a control means for club root (*Plasmodiophora brassiceae*) since the early 19th century. There is a close relationship between soil pH and club root, with acidic soils generally favoring development of the disease.

Application of fertilizers, lime and other materials to soils can affect bioavailability by introducing trace elements into the soil and/or adsorptive phases causing redistribution of trace elements into different chemical pools or chemical "species" [4]. Nitrogen fertilizers induce some direct and/or indirect changes impacting the dynamics availability of metals in soils [7]. Mineral N fertilizers contain ammonium can acidify the soil solution and decrease rhizosphere pH [10]. In neutral or alkaline soils rhizosphere acidification in plants fed with ammonium can enhance the uptake of micronutrients such as iron, manganese, copper and zinc [11–13].

The aim of the present research was to assess the effect of ammonium sulphate and RSM (solution of the ammonium nitrate + urea) applied by placement and broadcast technique on the micro/trace elements accumulation in 'Galaxy' F_1 white cabbage grown commercially under field conditions.

Material and methods

The field experiment was carried out in 2005–2007 with 'Galaxy' F_1 white cabbage on a silty clay soil containing 0.91–1.02 % organic carbon and soil acidity pH_{H_2O}

7.18–8.21 (Table 1). The plots were located at a farm in Zagorzyce (50°23′ and 20°04′) near Miechow. Farms of this area specialize in cabbage production in continuous or highly frequent cropping. In short-term crop rotation systems liming is commonly used as a control measure for club root (*Plasmodiophora brassicae*) potential damage. The calcium oxide application of one month prior to planting is a practical mean of controlling the fungal disease. This land area has an ideal climate for growing a wide range vegetable crop, but there is very little information on the metals content of vegetables produced in the important agriculture region.

Table 1

Organic carbon content [%], soil pH and soil texture in 2005–2007

Year	Sand 0–0.1 mm	Silt 0.1–0.02 mm	Clay < 0.02 mm	% C	CEC cmol kg^{-1}	pH_{KCl}	pH_{H_2O}	Ca mg dm^{-3}
2005	15	47	38	0.91	11.85	7.70	8.21	3000
2006	8	50	41	1.02	7.72	6.17	7.18	1465
2007	9	55	36	0.98	10.49	7.09	7.90	2972

Two factors were examined: the type of N fertilizer ammonium sulphate and RSM (solution ammonium nitrate and urea 1:1), and method of N application. The treatments with both fertilizers were as follows:

1) Control – 100 % N rate (120 kg ha^{-1}) broadcasted at planting of seedlings,
2) 75 % N rate broadcasted at planting of seedlings + 25 % N during plant grow,
3) 75 % N rate broadcasted at planting of seedlings + foliar fertilization,
4) 75 % N placement at seedlings planting,
5) 75 % N placement at seedlings planting + 25 % N during plant growth,
6) 75 % N placement at seedlings planting + foliar fertilization.

Treatments were assigned following the randomized complete block in split-plot arrangement with four replications. Seedlings were transplanted at the beginning of June. Nitrogen fertilizer was applied at the rate of 120 kg N ha^{-1} (100 % N). With the placement fertilization method, fertilizer was located 10 cm depth and 10 cm distance on each plant (plant were spaced 67.5 × 67.5 cm) at transplanting seedlings times. Foliar sprayings started at the beginning of intensive leaves growth and were conducted at growing season in two weeks interval. The foliar nutrition of 2 % urea was carried out 3 times and one time with 1 % Supervit K (% w/v: N-NH_2 – 4.4, N-NO_3 – 0.8, K – 3.1, Mg – 0.6, Mn – 0.05, Ti – 0.05, B – 0.03, Fe – 0.025, Mo – 0.005). Mineral fertilization of phosphorus, potassium and magnesium was based on the results of chemical analysis of the soil samples. The content of soil P, K and Mg was supplemented to level of 50, 200 and 60 mg dm^{-3}, respectively before seedlings planting.

Plant procedures

The harvest was conducted in the last decade of October. Edible parts (disintegrated cabbage head) were dried at 70 °C for 48 h. The Cd, Cr, Cu, Fe, Mn, Ni, Sr and Zn

contents in the samples were determined by inductively coupled argon plasma optical emission spectroscopy (ICP-OES) after microwave digestion with HNO_3.

Soil procedures

Soil samples were collected from a 0–30 cm surface layer. Granulometric analysis was made by the aerometric method of Proszynski and the organic carbon by Tiurin's method [14]. Soil pH was determined by adding deionized water and 1 M KCl at a ratio 1:2 (soil:water/1 M KCl by volume). The total soil metal content was determined with ICP-OES after microwave digestion with *Aqua Regia* [15]. The extractable forms of metals were measured in 1 M HCl extractant [14] by ICP-OES. This soil extractant and procedure is currently used to estimate availability and critical levels for micronutrient cations in Poland.

Statistical procedures

Results were subjected to two or three way factors analysis of MANOWA. Means were separated by the Fisher LSD test ($p = 0.05$). Statistical analysis was performed using the Statsoft Statistica 8.0 software.

Results and discussion

Soil analyses

The content of total metals in soil (Table 2) was low and tended to be below or the lower range reported by Kabata-Pendias and Pendias [2] in non-contaminated soils in Poland. Only cadmium concentration was slightly greater than the average of Cd content in Polish non-polluted soil (0.2–1.05 mg kg dry weight) reported by Trelak [16]. A little elevated Cd concentrations (1.13–1.34 ppm) in tilled horizon of arable land with intensive agricultural system may be due to anthropogenic sources such as lime or phosphates.

Table 2

Total metals content [mg kg^{-1} dry mass] in soil in 2005–2007

Year	Cd	Cr	Cu	Fe	Mn	Ni	Sr	Zn	Pb
2005	1.34	12.6	4.67	8184	152.0	7.77	12.5	24.3	13.6
2006	1.13	9.62	4.74	6176	186.8	5.98	12.0	25.8	14.1
2007	1.23	9.70	4.75	6606	156.3	6.40	14.8	32.9	14.5

Total levels are rarely indicative of plant availability because availability depends of physical, chemical, and biological conditions in the rhisosphere [17]. In our study soil samples are extracted with 1 M HCl commonly using in Poland for estimation plant micronutrients availability and fertilization. According to criteria developed for micro-

nutrients detected by Rinkis method in Poland [18], low content of available Cu and the average of Zn and Mn were measured (Table 3). Any examined factors (fertilizer form and method of application) did not affect extractable forms of metals in soil. This technique with relatively "aggressive extractant" removes more than the soluble, exchangeable and weakly adsorbed fractions. Kashem et al [19] recommend using this procedure for first-level screening of soil contamination. Korzeniowska and Stanislawska-Glubiak [20] showed good correlation between concentration of Cu, Zn and Ni extracted from contaminated soil by 1 M HCl and the white mustard uptake of these elements. In our study plant response was not correlated with metals extracted by the 1 M HCl soil test except Cd and Sr (coefficient of correlation were r = 0.42 and r = 0.64, respectively for p = 0.05, data non published).

Table 3

Extractable fractions (1 M HCl) of metals [mg kg^{-1} dry mass] in soil after cabbage harvest in 2005–2007

Factor	Factor level	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Sr	Zn
Year	2005	0.400	0.983	2.85	982	135	2.09	10.1	9.14	11.8
	2006	0.534	0.891	2.39	993	166	2.04	13.4	10.6	15.9
	2007	0.027	0.526	3.24	1120	158	3.10	12.4	14.6	20.6
Fertilizer	$(NH_4)_2SO_4$	0.331	0.806	2.70	1044	155	2.42	12.2	11.4	16.2
	RSM	0.310	0.793	2.96	1020	151	2.40	11.7	11.4	16.0
Method of application	1	0.309	0.783	2.74	1023	150	2.46	11.6	11.6	15.8
	2	0.310	0.805	3.04	1030	149	2.38	11.8	12.0	15.7
	3	0.313	0.775	2.70	1010	151	2.44	11.8	11.7	15.6
	4	0.312	0.813	2.81	1039	152	2.33	11.8	11.1	16.8
	5	0.337	0.812	2.69	1059	157	2.46	12.4	11.1	16.4
	6	0.343	0.810	2.98	1031	157	2.38	12.4	11.2	16.2
$LSD_{0.05}$	year	0.0425	0.0439	0.426	46.8	9.6	0.07	0.26	0.55	2.33
	fertilizer	ns	ns	ns	ns	ns	ns	ns	ns	ns
	method	ns	ns	ns	ns	ns	0.09	ns	ns	ns

Presented data shows that 1 M HCl extractant removed to solution about 5–9 % of Cr total content, 12–17 % of Fe, 27–48 % of Ni, similar amounts of Zn and Cu (about 50–60 %), comparable amounts of Sr, Mn and Pb (about 70–99 %). In case of Cd obtained results did were uniform. In 2006 1 M HCl removed 47 % of total Cd content but, in 2007 only 2 %.

Plant analysis

In present work all metals concentration in the edible parts of cabbage were below the lower range of content reported for cabbage grown in non-contaminated areas. The low concentration of micronutrients such as Cu, Mn, Zn, and Fe may indicate deficiencies that would affect crop yield. In case of heavy metals, low concentration in cabbage head improves safety of plant food.

The concentration of cadmium in cabbage ranged between 0.048–0.061 mg kg^{-1} dry matter (Table 4). Cadmium content in plant varies widely and ranged between 0.05–0.20 ppm [2]. Soil pH is often inversely related to Cd uptake by plant [9]. Bolan et al [6] observed that the absorption of Cd^{2+} increases with an raise pH causes an increase in surface negative charge resulting in an increase in cations adsorption. An increase in soil pH is likely to result in the formation of hydroxy species of metal cations which are adsorbed preferently over the metal cation. In present study the significantly higher Cd concentration determined in cabbage growing in 2006 on a field characterized relatively lower pH_{H_2O} 7.18 in comparison with 2005 and 2007 (pH 8.21 and 7.90, respectively). However, total Cd concentration in soil in 2006 was at the lower level (1.13 mg kg^{-1} d.m.). The form of nitrogen fertilizers and method of fertilizers application did not affect Cd concentrations in cabbage leaves. Moreover, the small range of rhizosphere pH change (below 0.1 pH units, data non published) due to the buffering capacity of the soil was not sufficient to influence Cd bioavailability.

Table 4

Effect of nitrogen fertilization on Cd and Cr content in 'Galaxy' F_1 cabbage grown in 2005–2007

Fertilizer	Application method*		Cd [mg kg^{-1} d.m.]				Cr [mg kg^{-1} d.m.]			
			2005	2006	2007	mean	2005	2006	2007	mean
Mean for year			0.050	0.061	0.048		0.183	0.135	0.163	
Factor	$(NH_4)_2SO_4$		0.049	0.060	0.049	0.053	0.168	0.130	0.170	0.156
Fertilizer	RSM		0.050	0.061	0.046	0.052	0.199	0.141	0.155	0.165
Application method	broadcast	1	0.048	0.062	0.041	0.050	0.265	0.156	0.179	0.200
		2	0.049	0.056	0.044	0.050	0.187	0.128	0.130	0.148
		3	0.048	0.065	0.050	0.054	0.136	0.124	0.191	0.150
	placement	4	0.047	0.061	0.049	0.052	0.170	0.133	0.164	0.156
		5	0.061	0.059	0.054	0.058	0.211	0.126	0.171	0.169
		6	0.046	0.060	0.047	0.051	0.131	0.146	0.141	0.139
$LSD_{0.05}$ for	year		0.0051				0.0332			
	fertilizer		ns	ns	ns		ns	ns	ns	
	application method		ns	ns	ns		ns	ns	ns	

* 1–120 kg · ha^{-1} N broadcasted at planting of seedlings; 2–90 kg · ha^{-1} N broadcasted at planting of seedlings + 30 kg · ha^{-1} N during plant grow; 3–90 · ha^{-1} N broadcasted at planting of seedlings + foliar fertilization; 4–90 kg · ha^{-1} N placement at seedlings planting; 5–90 kg · ha^{-1} N placement at seedlings planting + 30 kg · ha^{-1} N during plant growth; 6–90 kg · ha^{-1} N placement at seedlings planting + foliar fertilization; n.s. – no significant.

Chromium is essential nutrient for human and animals. Chromium contents of grain products, fruits, and vegetables vary widely, even well-balanced diets may contain suboptimal levels of dietary chromium (50–200 μg/day). Kabata-Pendias and Pendias [2] reported that in plant tissues chromium concentrations ranged between 0.02–1 mg kg^{-1} d.m. and in cabbage heads intermediate range of Cr was 0.05 mg kg^{-1} d.m. In our study 0.135–0.183 mg Cr kg^{-1} d.m. was detected (Table 4). The highest Cr concentrations were measured in 2005 for the highest levels of pH and higher content of

chromium in soil (in total and extractable forms), and the lowest in 2006 for the smallest pH values and smaller Cr content in soil. Taylor and Olsen [5] showed that mechanism of the Cr^{3+} releases was the oxidation of some Cr^{3+} at increasing pH, producting CrO_4^{2-} which was much less sorbed at neutral to slightly alkaline condition. A probable the increasing pH escalating concentrations of soluble organic C in the soil solution and more total Cr would be solubilized.

It is well know that copper bioavailability and hence Cu toxicity is increased in acidic relative to calcareous soils [10, 21]. Increasing the pH involves an increase in the binding of Cu to soil constituents, and decrease in the mobility of copper in soil. The critical deficiency level of copper in vegetative plant parts is generally in the range of 1–5 mg Cu kg^{-1} dry matter [1, 22]. In our study copper contents in plants tended to be less than the ranges reported by Kabata-Pendias and Pendias [2] in non-contaminated sites for the cabbage heads (3–4 mg Cu kg^{-1} d.m.). The smallest value of plant Cu (1.58 mg kg^{-1}) was observed when pH of soils was highest (in 2005) while the largest (2.14 ppm) was measured for the smallest pH in 2006 (Table 5). The form of nitrogen fertilizers did not affect Cu concentrations in cabbage leaves. Similar results were presented by Smolen and Sady [23] who proved that copper content of carrot roots was not influenced by nitrogen fertilizers. The same results with tomato and rape obtained Chaignon et al [10] who concluded that Cu bioavailability was independent of N supply in the calcareous soil. Any year of presented studies the method of nitrogen application did not influence Cu concentration in cabbage (Table 5).

Table 5

Effect of nitrogen fertilization on Cu and Fe content in 'Galaxy' F_1 cabbage grown in 2005–2007

Fertilizer	Application method*		Cu [mg kg^{-1} d.m.]				Fe [mg kg^{-1} d.m.]			
			2005	2006	2007	mean	2005	2006	2007	mean
Mean for year			1.58	2.14	1.97		26.9	28.0	24.0	
Factor	$(NH_4)_2SO_4$		1.53	2.03	1.99	1.85	27.0	28.1	25.5	26.9
Fertilizer	RSM		1.63	2.24	1.95	1.94	26.8	27.8	22.6	25.7
Application method	broadcast	1	1.60	2.28	1.88	1.92	26.4	27.1	23.0	25.5
		2	1.67	2.17	1.73	1.86	27.1	28.1	22.2	25.8
		3	1.59	2.47	2.03	2.03	26.7	28.2	24.4	26.4
	placement	4	1.48	1.91	2.22	1.87	28.0	29.3	25.0	27.4
		5	1.66	2.01	1.94	1.87	28.7	27.9	25.2	27.3
		6	1.49	1.98	2.01	1.83	24.6	27.2	24.3	25.4
$LSD_{0.05}$ for:	year		0.151				1.72			
	fertilizer		ns	ns	ns		ns	ns	1.99	
	application method		ns	ns	ns		ns	ns	ns	

* See Table 3.

The iron is the fourth most abundant element in the earth's crust, yet Fe deficiency is common in crop plant. This anomaly is due to the extremely low concentration of Fe^{2+} and Fe^{3+} in soil solution especially in well aerated soil with a high pH [22]. The critical

deficiency content of iron in plant leaves is in the range of 50–150 mg Fe kg^{-1} d.m. [1]. Kabata-Pendias and Pendias [2] report that on average concentration of Fe in edible parts of cabbage there is 42 mg Fe kg^{-1} d.m. In our study iron concentrations in cabbage were low and ranged between 24.0–28.0 mg Fe kg^{-1} d.m. (Table 5). In 2007 $(NH_4)_2SO_4$ fertilization significantly increased iron concentration in cabbage heads in a comparison with RSM. Similarly tendency was also observed in case of Mn and Zn however, these differences were not always significant. Smolen and Sady [23], Gebski and Mercik [24] and Rodriguez-Otriz et al [25] reported that $(NH_4)_2SO_4$ fertilization can strongly affect the heavy metals accumulation in yield. The rhizosphere acidifications from of nitrogen supply as well as of the plant factor (enhanced net excretion of protons or of organic acid) are of particularly importance for acquisition of Fe, Zn and Mn on alkaline soils [8, 26]. The critical deficiency contents of manganese and zinc in plant vary between 10–20 mg Mn kg^{-1} and 15–30 mg Zn, respectively [1]. In our investigations Mn concentrations in cabbage leaves ranged from 13.5 mg to 15.1 mg kg^{-1} d.m. (Table 6).

Table 6

Effect of nitrogen fertilization on Mn and Ni content in 'Galaxy' F_1 cabbage grown in 2005–2007

Fertilizer	Application method*		Mn [mg kg^{-1} d.m.]				Ni [mg kg^{-1} d.m.]			
			2005	2006	2007	mean	2005	2006	2007	mean
Mean for year			14.5	15.1	13.5		0.599	0.637	0.454	0.563
Factor	$(NH_4)_2SO_4$		14.8	15.5	14.2	14.8	0.616	0.629	0.443	0.563
Fertilizer	RSM		14.1	14.7	12.7	13.8	0.582	0.644	0.466	0.564
Application method	broadcast	1	14.2	14.9	12.1	13.7	0.640	0.654	0.416	0.570
		2	15.0	15.9	12.3	14.4	0.541	0.612	0.344	0.499
		3	14.1	14.6	12.9	13.9	0.519	0.650	0.418	0.529
	placement	4	14.1	14.8	14.5	14.5	0.666	0.612	0.507	0.595
		5	15.2	15.4	15.0	15.2	0.739	0.627	0.551	0.639
		6	14.2	15.0	13.9	14.4	0.489	0.675	0.491	0.552
$LSD_{0.05}$ for:	year			0.75				0.0720		
	fertilizer		ns	ns	0.98		ns	ns	ns	
	application method		ns	ns	1.69		ns	ns	0.1369	

* See Table 3.

The Zn concentration in cabbage was below the critical values, and varied from 12.9 mg (2007) to 14.7 mg Zn kg^{-1} d.m. (2006) (Table 7). The method of application and form of nitrogen fertilization did not affect cabbage zinc concentrations any year. In 2007 slightly higher manganese concentrations for placement fertilization was noticed in comparison with N broadcasted. The same reaction was observed in case of nickel (Table 6). Beside the environmentally positive advantages, the subsurface placement of ammonium or ammonium/urea fertilizers has been proposed to supply crops under field conditions (known as the CULTAN cropping system; Controlled Uptake Long Term Ammonium Nutrition) [27]. The ammonium concentration in the deposit is toxic for

plant roots and soil microorganisms and pH in this soil area is extremely low. Probably the acidification effect of concentrated ammonium fertilizer locally on the soil improved metals uptake. Roots form a dense root net around the ammonium deposit and can take up the nitrogen as ammonium before it is nitrified and perhaps solubilized metals ions.

Table 7

Effect of nitrogen fertilization on Sr and Zn content in 'Galaxy' F_1 cabbage grown in 2005–2007

Fertilizer	Application method*		Sr [mg kg^{-1} d.m.]				Zn [mg kg^{-1} d.m.]			
			2005	2006	2007	mean	2005	2006	2007	mean
Mean for year			8.16	8.18	12.2		14.4	14.7	12.9	14.0
Factor:	$(NH_4)_2SO_4$		8.34	8.32	12.4	9.69	14.8	14.8	13.2	14.3
Fertilizer	RSM		7.98	8.04	11.9	9.31	14.0	14.5	12.6	13.7
Application method	broadcast	1	7.91	7.89	11.5	9.10	14.4	15.4	12.1	14.0
		2	7.53	7.55	12.5	9.19	15.1	15.7	12.1	14.3
		3	8.41	8.42	12.4	9.74	14.7	15.7	12.5	14.3
	placement	4	8.14	8.23	12.7	9.69	13.0	14.0	13.8	13.6
		5	8.34	8.37	11.7	9.47	15.5	14.5	13.3	14.4
		6	8.64	8.63	12.1	9.79	13.6	12.7	13.5	13.3
$LSD_{0.05}$ for:	year		0.769				0.99			
	fertilizer		ns	ns	ns		ns	ns	ns	
	application method		ns	ns	ns		ns	ns	ns	

* See Table 3.

Nickel is now generally accepted as an essential ultra-micronutrient. The only defined role of Ni is in the metabolism of urea [22]. Nickel has a significant effect on the productivity of field-grown plants, those utilizing urea as a primary nitrogen source. The Ni concentration in leaves of plants grown on uncontaminated soil ranges from 0.05–5 mg Ni kg^{-1} d.m. and is the lowest of any element [1]. In present study in cabbage edible parts nickel concentration ranged from 0.454 to 0.637 mg Ni kg^{-1} d.m. (Table 6). Plant Ni concentration were related to soil pH similar to Cu, Fe, and Mn cabbage content. The form of nitrogen application did not influence Ni concentration in cabbage.

Strontium is chemically similar to calcium, and its biogeochemical cycles are comparable. Best known is the antagonism ions action between calcium and strontium in soil solution [28, 29]. Poor Sr immobilization by soils leads to large availability for plants. Plant foods containing Sr range very low eg in corn (0.4 mg kg^{-1} dry matter) to high, eg in cabbage (45 ppm) or lettuce (74 ppm) [2]. In present study the stable elementary Sr concentration in cabbage was related to total and extractable forms of strontium in soil. We measured 8.16 to 12.2 mg Sr kg^{-1} dry matter (Table 7). Any year of presented studies the form and method of nitrogen application did not influence Sr concentration in cabbage.

Lead concentration in edible parts of cabbage was below the detective limits for using method of detection. Alkalinity as result of overlime treatment might decrease Pb uptake by plants. Lead in totally acido-labile in the soil. The pH and dissolved organic carbon (DOC) are the major factors controlling the speciation and availability of Pb in soil. The Pb^{2+} decrease as pH and DOC increase [3]. The total Pb concentration in soil was low and ranged from 13.6 to 14.5 mg Pg kg^{-1} d.m., while the extractable fraction (1 M HCl) was measured on the range 10.1–13.4 mg Pb (Tables 2, 3).

Conclusions

Field research still required to explain complex interactions of soil chemistry (eg effects of pH, soil response to liming, plant-available nutrients), soil physics, and biology, and the effects of these on crop yield. A better understand of chemistry and availability of micro/trace elements in soil, their distribution and variability in areas of crop production, their absorption by roots and translocation to edible parts of food may lead to control their accumulation by plants and improved human health.

The present study focuses on the effect of nitrogen fertilization on metals concentration in edible parts of commercially grown cabbage. The low concentration of microelements in cabbage may indicate deficiencies that would affect crop yields or human nutrition. We concluded that micronutrient deficiencies were related to soil parent material, with generally low total and extractable levels of metals. The results obtained would suggest that in commercial production on overlimed soils using nitrogen ammonium fertilizers may improve Mn, Fe and Zn uptake by plants. Consistently greater concentrations of Cd, Cu, Fe, Mn and Ni were measured in cabbage grown on the field with lower pH compared with concentrations in plants sampled at other soil sites but relatively near located. This show the importance of sampling many soil and crop combinations in a survey, or having a thorough understanding of soil metals availability. White and Zasoski [30] suggest that rational management of micronutrient fertility and metals toxicity requires understanding of how total and plant-available soil elements vary across the land. According to authors highly detailed maps of soils metals content and availability in individual fields should be developed for site-specific precision agriculture.

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WPŁYW NAWOŻENIA AZOTEM NA PRZYSWAJALNOŚĆ Cd, Cr, Cu, Fe, Mn, Ni, Pb, Sr i Zn PRZEZ KAPUSTĘ GŁOWIASTĄ BIAŁĄ (*Brassica oleracea* var. *capitata alba*) UPRAWIANĄ W WARUNKACH PRODUKCYJNYCH

Abstrakt: Doświadczenie z kapustą głowiastą białą odm. Galaxy F_1 prowadzono w latach 2005–2007 w Zagorzycach koło Miechowa. Badano wpływ rodzaju nawozu azotowego (siarczan amonu, RSM – roztwór saletrzano-mocznikowy) oraz sposobu nawożenia (rzutowo, zlokalizowanie) oraz dokarmiania pozakorzeniowego (mocznik i Supervit K) na dostępność dla roślin Cd, Cr, Cu, Fe, Mn, Ni, Sr i Zn.

Zawartość wszystkich badanych metali w główkach kapusty była poniżej poziomu przyjętego za normalny w warunkach gleb nieskażonych. Mała zawartość metali była skorelowana z małą ogólną i przyswajalną zawartością badanych metali w glebach. Rodzaj zastosowanego nawozu azotowego wpływał znacznie na zawartość Fe i Mn w kapuście. Więcej Mn i Fe zawierały rośliny nawożone siarczanem amonu w porównaniu do RSM. Jednak tendencje te były silnie uzależnione od warunków środowiskowych panujących w kolejnych latach prowadzenia badań. Podobne zależności obserwowano także dla Zn, chociaż nie były one statystycznie udowodnione.

Sposób stosowania nawozów azotowych wpływał na zawartość Mn i Ni w kapuście. Lokalizowanie depozytów azotowych w pobliżu roślin podnosiło zawartość Mn i Ni w roślinach w porównaniu do rzutowego stosowania siarczanu amonu i RSM. Zależność ta nie była jednak obserwowana we wszystkich latach.

Otrzymane wyniki mogą wskazywać, że w warunkach gleb nadmiernie wapnowanych wykorzystanie do nawożenia azotem siarczanu amonowego może poprawić zaopatrzenie roślin w Mn, Zn i Fe.

Słowa kluczowe: kapusta głowiasta biała, pierwiastki śladowe, mikroelementy, nawożenie azotem, bioprzyswajalaność

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INFLUENCE OF PESTICIDES ON MICROBIAL ACTIVITY IN SELECTED SOIL TYPES OF SLOVAKIA

WPŁYW PESTYCYDÓW NA AKTWYNOŚĆ MIKROORGANIZMÓW W WYBRANYCH TYPACH GLEBY NA SŁOWACJI

Abstract: The aim of our work was to determine influence of pesticides on the soil respiration and the numbers of microorganisms (bacteria and their spores utilizing organic and inorganic nitrogen, actinomycetes, myxobacteria, *Azotobacter chroococcum,* microscopic fungi) in the three soil types (Haplic Chernozem, Haplic Luvisol, Cambisol).

Cumulative values of basal CO_2 production for 21 days represented from 595.62 mg · kg^{-1} to 1045.79 mg · kg^{-1} d.m. soil in tested samples of Haplic Chernozems and from 424.6 mg · kg^{-1} to 540.28 mg · kg^{-1} d.m. in tested samples of Haplic Luvisols and from 1789.84 mg · kg^{-1} to 2103.81 mg · kg^{-1} d.m. in tested samples of Cambisols. Potential CO_2 production was higher (statistical significantly, $p < 0.01$) in all variants (with addition of glucose, PVAL, herbicide and fungicide) than basal one. Stimulating effect of glucose addition was more expressive in Haplic Luvisol than in Haplic Chernozem and Cambisol. Pesticides addition did not significantly affect on the decrease of numbers of bacterial vegetative forms in the soil types Haplic Chernozem and Cambisol. The insignificantly decrease was observed in the numbers of bacterial spores in the soil type Cambisol and in the numbers of microscopic fungi only in the soil type Haplic Chernozem.

Keywords: soil respiration, pesticides, physiological groups of microorganisms, soil type

Millions of tons of xenobiotic compounds are applied globally as pesticides in agricultural production in soil each year. Soil, natural water in rivers, lakes, and aquifers has been contaminated with trace amounts of pesticide residues. Microorganisms and their enzymes play an essential role in the bioconversion and total breakdown of pesticides and other xenobiotics in the environment. The principal enzymes responsible for the bioconversion are various lyases and oxydoreductases, specifically hydrolyses, oxygenases and various enzymes capable of dehalogenation [1]. Metabolic pathway diversity depends on the chemical structure of the xenobiotic compound, the organism evironmental conditions, metabolic factors, and the regulating expression of these biochemical pathways [2]. The important role played by microorganisms in the degradation of pesticide residues is well recognized. Pieces of information were

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published about microbial strains (bacteria and fungi) capable of degrading such recalcitrant pesticides as 2,4 D or Dicamba [3], Dichlobenil [4], triazine herbicide Simazine [5], Folicur or Amistar [6], Topogard [7] etc. The xenobiotics affect soil properties in many different ways. Microbial and biochemical properties of soils are evidently more sensitive to stress factors including pollutants than the chemical and physical properties of soils [8]. Mineralization of organic compounds is characteristic for growth-linked biodegradation, in which the organisms converts the substrate to CO_2, cell components, and products typical of the usual catabolic pathways [9]. Soil respiration reacts differently to variant and cultivation methods and has been used most frequently for the assessment on the side effects of chemicals such as pesticides and heavy metals, etc. [10].

The investigations were conducted to microbial respirations and microbial numbers after application of pesticides in the different soil types in Slovakia (Haplic Chernozem, Haplic Luvisol and Cambisol). The influence of pesticide on the observed microbial characteristics was evaluated by statistical method in contrast with variants without additive or with additives such as glucose (easily available source of carbon) or polyvinyl alcohol (difficultly available source of carbon).

Material and methods

Selected chemical and microbial parameters were studied during the years 2001–2007 in the different soil types (from Haplic Chernozem – arable soils, Haplic Luvisol – arable soils to Cambisol – mountain soil, meadow and pasture). The Haplic Chernozem (HCh) and Haplic Luvisol (HL) are in the most fertile south-western part of Slovakia with altitude from 121 to 190 meters above the sea level (m a.s.l.). This part of Slovakia has own abnormal, warm, climatic conditions, the average year temperature of 9.7 ^{o}C and the sum of precipitation amounting to 561 mm. Haplic Chernozems were sampled from 7 localities of Drazovce (138 m a.s.l.), Sladkovicovo (121 m a.s.l.), Voderady (137 m a.s.l.), Stefanovicova (130 m a.s.l.), Kalna nad Hronom (160 m a.s.l.), Svatoplukovo (140 m a.s.l.) and Borovce (160 m a.s.l). Haplic Luvisols were sampled from 7 localities of Golianovo (149 m a.s.l.), Kolinany (190 m a.s.l.), Plave Vozokany (185 m a.s.l.), Nove Sady (168 m a.s.l.), Malanta (180 m a.s.l.), Risnovce (161 m a.s.l.) and Velke Ripnany (174 m a.s.l.). Cambisol (C) were sampled from 4 localities of "Pod Ploskou" 1.240 m a.s.l. (National park Velka Fatra), "Strungovy prislop" 1.150 m a.s.l. (National park Mala Fatra) and "Pod Keckou" 1.100 m a.s.l. (National park Nizke Tatry) and "Diel" – 920 m a.s.l. (sierra Stolicke vrchy). The Cambisols are situated in the region with very cold and wet climate, the average year temperature of 4 ^{o}C and the sum of precipitation amounting to 1.250 mm.

Soil samples (average of 4 probes) from arable soils were taken in spring (April) period after pre-sowing preparation and mountain soils in summer (July), from the layer (0–0.3 m). The samples were mixed; stones, plant and animal residues were eliminated and sieved through the 2 mm sieve and subjected to the samply chemical analyses. Proportion of the soil samples was stored (pre-incubated) during 8 weeks at temperature 4±1 ^{o}C [11, 12], for analyzed microbial parameters and activities. The respiration

activity (basal – Variant 1 and potential soil respiration – Variant 2–5) of microorganisms was observed through production of CO_2 by titration method with HCl in four replications for 9 times during 21 days incubation at 28 °C. Potential respiration of microorganisms was measured in the presence of glucose – G (2 g · kg^{-1} of soil) – Variant 2, polyvinyl-alcohol – PVAL (2 g · kg^{-1} of soil) – Variant 3, herbicide – Gesagard 80 (0.6 g · kg^{-1} of soil) – Variant 4 and fungicide-Fundazol 50 WP (0.24 g · kg^{-1} of soil) – Variant 5.

The following parameters were determined in the fresh samples:

– dry matter (d.m.) of samples,

– actual (pH/H_2O) and exchangable (pH/KCl) soil reaction-potentiometrically in 1 mol · dm^{-3} KCl solution,

– oxidizable carbon (C_{ox}) according to Tiurin method,

– total nitrogen (N_t) by distillation method according to Kjeldahl,

– NO_3^- – N colorimetrically (Interferometer 1100 Carl Zeiss, Jena, Germany) with phenoldisulphonic acid immediately after sampling and after 14 days of incubation at 28 °C, soil moisture was adjusted to 60 % full water holding capacity,

– NH_4^+ – N colorimetrically (Interferometer 1100 Carl Zeiss, Jena, Germany) with Nessler agent immediately after sampling and after 14 days incubation at 28 °C, soil moisture was adjusted to 60 % of full water holding capacity,

– biologically releasable nitrogen (N_{biol}) was calculated as a difference of inorganic nitrogen values before and after soil samples incubation (14 days),

– inorganic nitrogen (N_{in}) was calculated as the summation of ammonium and nitrate(V) form of nitrogen content,

– nitrification, which was calculated of the values NO_3^- – N as a difference of the values determined in non-incubated and incubated samples (14 days, 28 °C, 60 % of full water holding capacity).

After pre-incubation (8 weeks) the samples were moisturised to 60 % of full water holding capacity and further characteristics were determined:

– carbon of microbial biomass (C_{mic}) by fumigation extractive method [13],

– test of respiration, basal and potential (addition of glucose per kg of soil) production of CO_2 in model trial by titrimetric absorption method [14]. Respiration rates were determined after 24, 48, 72, 96, 120, 144, 168, 336 and 504 h by trapping CO_2 in 0.1 M NaOH. The residual NaOH was titrated with 0.1 M HCl after carbonates were precipitated with 0.5 M $BaCl_2$,

– hot water extractable carbon (C_{hwl}) [15],

– dehydrogenase activity (DHA) by triphenyltetrasolium chloride (TTC) [16],

– FDA hydrolysis by fluorescein diacetate [17],

– phosphatase activity [18]

– cellulose degradation in model experiment [19],

– microorganisms counts (after 21 days finishing of experiment). The classic dilution plating method was used for evaluation of microorganism counts using different agar media for their identification: bacteria and their spores utilizing organic nitrogen on meat-peptone agar (MPA), bacteria and their spores utilizing inorganic nitrogen on Thornton agar (TA), myxobacteria on champignon agar (ChA), microscopic fungi on

Czapek-Dox agar (Cz-DA) and malt extract agar (MEA), actinomycetes on Krainsky agar (KA) and Waksmann agar (WA) and *Azotobacter chroococcum* on Ashby's agar (AA). Numbers of colony-forming units (CFU) were expressed as logarithms per 1 g d.m. soil for statistical analysis.

All results are calculated on a soil dry matter (d.m.). Statistical processing of gained data were realized in Statgraphics 5.0 programme. Two-way univariate ANOVA was used for statistical analysis of the effects of variants and localities. In case of significant F-statistics Tukey test ($p \leq 0.01$) was selected to separate the means.

Results and discussion

The biological activity of microorganisms and their numbers were observed in the three different soil types after application of pesticide in laboratory conditions, for 21 days. These soil types were different not only in cultivation of soils (arable or meadow), but in the primary chemical and biological characteristics, too (Table 1).

Table 1

Average values (± standard deviation) of biological and chemical properties of observed soil types

Parameter	Unit	Soil type		
		Haplic Chernozem (n = 7)	Haplic Luvisol (n = 7)	Cambisol (n = 4)
C_{ox}	[%]	1.78 (0.31)	1.17 (0.16)	4.69 (1.90)
N_t	[%]	0.21 (0.02)	0.14 (0.02)	0.35 (0.12)
C : N	[-]	8.0 (1.11)	8.20 (0.94)	13.22 (1.92)
pH in H_2O	[-]	7.58 (0.43)	7.02 (0.66)	5.67 (0.50)
pH in KCl	[-]	6.62 (0.60)	5.92 (0.77)	5.14 (0.45)
N_{in}	[mg · kg^{-1} d.m.]	8.20 (3.09)	6.90 (6.16)	26.44 (7.50)
N_{biol}		8.75 (3.04)	10.18 (2.71)	36.67 (18.10
Nitrification		10.23 (2.95)	11.65 (4.46)	28.67 (7.90)
C_{hwe}		0.42 (0.08)	0.33 (0.08)	0.93 (0.29)
DHA	[mg · g^{-1} · h^{-1} d.m.]	9.64 (5.70)	4.87 (1.71)	16.24 (5.47)
FDA hydrolysis	[ΔA · g^{-1} · h^{-1} d.m.]	0.15 (0.05)	0.19 (0.07)	0.73 (0.17)
Phosphatase activity	[μg PNF · g^{-1} · h^{-1} d.m.]	12.43 (4.94)	20.66 (9.12)	64.45 (16.77)
C_{mic}	[mg · g^{-1} d.m.]	369.89 (206.34)	227.36 (188.03)	1294.60 (297.44)
Cellulose degradation	[%]	44.06 (17.28)	35.15 (21.43)	34.02 (24.54)

A – absorbation, PNF – triphenyltetrasolium chloride.

The Haplic Chernozem and Haplic Luvisol were arable soil with neutral soil reaction and similar chemical and biological properties. The weak acidity and acidity soil reaction and higher values of the all measured biological parameters (except for cellulose degradation) were characteristic for the soil type Cambisol (Table 1). The Cambisol had the

high carbon (C_{ox}) content and nitrogen organic and inorganic substances from pasture of cattle in these tested localities. The incidence of bacteria in soil samples was evidently dependent on the presence of fresh organic matter rather than the total carbon content in soil [20].

Course of cumulative values of basal CO_2 production for 21 days represented from 95.40 to 739.31 mg · kg^{-1} d.m. in tested samples of Haplic Chernozem, from 63.06 to 537.77 mg · kg^{-1} d.m. in tested samples of Haplic Luvisol and 104.7 to 1905.28 mg · kg^{-1} d.m. in tested samples of Cambisol (Fig. 1). Basal respiration represented mineralization of native organic substances in soil samples. The observed positive chemical characteristics of soil type Cambisol were confirmed with values from microbial respiration. The basal respiration of Cambisol was higher than basal respiration of Haplic Luvisol and Haplic Chernozem.

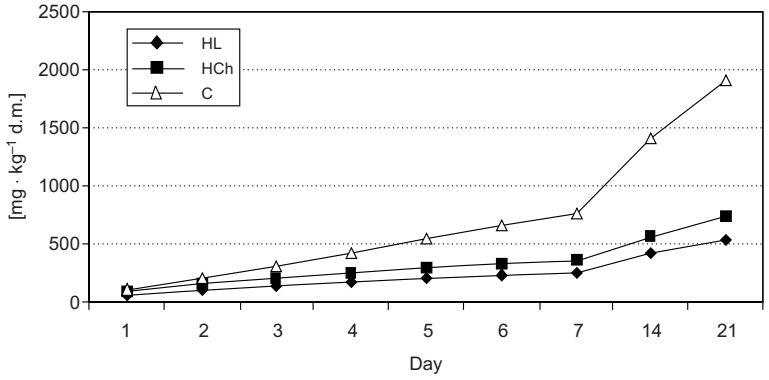

Fig. 1. Basal production (cumulative values) of CO_2 in tested soil types

Presence of active part of microflora in layer to 0.3 m of soil types was proved by determined CO_2 production values (Table 2).

Table 2

Basal and potential cumulative production of CO_2 [mg · kg^{-1} d.m.] in the observed soil types

Soil type	Variant				
	1	2	3	4	5
HCh	739.31 (218.24)	2503.99 (495.07)	1133.31 (261.21)	1053.55 (323.99)	999.28 (259.12)
HL	537.77 (115.28)	2562.58 (118.80)	877.58 (158.31)	699.08 (88.00)	781.42 (144.80)
C	1905.28 (345.32)	4435.61 (760.98)	2510.32 (406.11)	2381.61 (369.75)	2431.80 (445.42)

In brackets are values of standard deviation.

All of the additives including pesticides increased production of CO_2 in all the tested soil types during 21 days of incubation (Table 2). The negative influence (decrease) of pesticides on the microbial respiration was not confirmed as in the herbicide Topograd

application in acid soils [7]. Maximal value was measured in soil type Cambisol and the best stimulative effect (4.77 times) of glucose addition on the microbial respiration was determined in the Haplic Luvisol. It was consequence of deficiency of organic matter in this soil type. The course of respiration during 21 days respiration was similar between variants with PVAL and pesticides.

The biological activity of microorganisms in samples with pesticides in all soil types was comparable with samples which were amended with PVAL according to statistical evaluation of production of CO_2 (Table 4). Toxic influence of pesticides on the microbial respiration was not significant in the observed soil types. Fungicide was easier accessible source of carbon than herbicide for present soil microorganisms (Fig. 2). Glucose increased the production of CO_2 in comparison with values of the basal respiration. This stimulative effect was statistically significant ($p < 0.01$) in all soil types (Table 4).

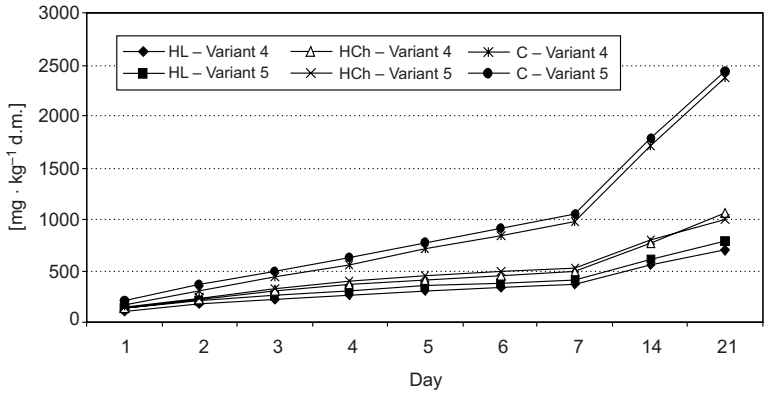

Fig. 2. Potential production (cumulative values) of CO_2 in observed soil types after addition of herbicide (Variant 4) and fungicide (Variant 5): HCh – Haplic Chernozem, HL – Haplic Luvisol, C – Cambisol

However, high CO_2 production is still not in agreement with numerous occurrences of microorganisms in samples. The very good correlation between bacterial numbers and the biodegradation of pesticides MCPP and IPU in sand soils presented Vinther et al [21]. However the high soil respiration can be a result of high activity of small soil microbial community as well as a result of low activity of large microbial community [22]. In a consequence of this it is important to know not only quantitative occurrence of microorganisms in soil, but also their specific composition and degrading capabilities.

The occurrence of microorganisms (Table 3, 4) corresponded with usual counts of microbes in arable soils [23] and soils of meadows and pastures [24]. The most numerous group from observed physiological groups in variant without additives (Variant 1) were bacterial vegetative forms and their spores utilizing organic nitrogen on meat-peptone agar (MPA) (Table 3). It was typicall for all observed physiological groups of microorganisms in comparison with soil types Haplic Chernozem and Haplic Luvisol. Preponderance of microorganisms remained multiplied after application of

pesticides in soil types Haplic Luvisol and Haplic Chernozem. The pesticides application inhibited the numbers of bacteria and their spores on the MPA and TA only in the soil type Cambisol (Table 3).

Table 3

Occurrence of bacteria and their spores in the 10^5 CFU · g^{-1} d.m. on the MPA and TA in Haplic Chernozem (n = 7), Haplic Luvisol (n = 8), Cambisol (n = 4)

Physiological group	Soil type	Variant				
		1	2	3	4	5
A	HCh	84.97 (91.52)	65.94 (68.00)	120.21 (124.32)	125.08 (119.24)	169.48 (148.74)
	HL	363.84 (716.84)	420.07 (763.12)	409.33 (757.60)	401.83 (809.69)	709.10 (1209.31)
	C	357.11 (247.05)	682.80 (664.12)	406.39 (416.93)	327.51 (361.66)	270.36 (208.48)
B	HCh	65.77 (96.80)	108.32 (155.97)	105.72 (155.46)	90.41 (141.75)	47.61 (44.32)
	HL	59.02 (63.64)	125.60 (183.92)	54.45 (56.69)	80.18 (97.23)	153.22 (204.05)
	C	704.85 (1110.76)	401.89 (367.12)	627.53 (1068.00)	150.06 (189.39)	145.81 (79.28)
C	HCh	542.33 (1186.44)	133.73 (897.63)	387.83 (736.39)	106.89 (116.48)	112.01 (113.52)
	HL	124.84 (185.11)	72.33 (64.37)	133.94 (135.35)	220.49 (343.75)	229.14 (209.95)
	C	238.79 (318.76)	401.98 (498.86)	244.52 (184.42)	209.50 (230.58)	118.41 (60.85)
D	HCh	51.24 (65.47)	72.27 (60.45)	68.67 (108.95)	54.76 (68.83)	82.72 (79.94)
	HL	80.35 (143.07)	86.62 (172.78)	96.12 (181.61)	661.96 (1457.89)	139.67 (250.95)
	C	99.14 (139.22)	16.58 (13.96)	40.24 (47.98)	63.27 (126.61)	36.14 (41.57)

A – bacteria utilizing organic nitrogen on (MPA, B – spores of bacteria utilizing organic nitrogen on MPA, C – bacteria utilizing inorganic nitrogen on TA, D – spores of bacteria utilizing inorganic nitrogen on TA, ND – non-deteremined, HCh – Haplic Chernozem, HL – Haplic Luvisol, C – Cambisol; in brackets are values of standard deviation.

Application the all of additives and pesticides had statistically insignificantly negative influence on the numbers observed physiological groups of microorganisms (Table 4). The Variant after glucose significantly stimulated the height numbers *Azotobacter chroococcum* in the Haplic Chernozem. Numbers of vegetative forms of bacteria (MPA and TA) and microscopic fungi decreased (statistically insignificantly) only in soil types: Haplic Chernozem and Cambisol. The occurrence of actinomycetes

was increased (statistically insignificantly) after application of pesticide in all the tested soil types. It is demonstration of very good adaptability of this group on the extraneously chemicals in the soil.

Table 4

Multiple range analysis for cumulative CO_2 production [mg · kg^{-1} d.m.] and total numbers of microorganism [log CFU · g^{-1} d.m.] in the soil types after 21 days of incubation (ANOVA, Tukey test, $p \leq 0.01$)

Respiration		Numbers of microorganism					
Variant	CO_2	Bacteria MPA + TA	Spore MPA + TA	Actino-mycetes	Myxo-bacteria	*Azotobacter chroococcum*	Microscopic fungi
Haplic Chernozem (n = 7)							
1	739.31^{a}	7.79^{a}	7.07^{a}	4.04^{ab}	3.24^{a}	3.22^{a}	4.31^{a}
2	2503.99^{c}	7.73^{a}	7.26^{a}	3.89^{a}	3.37^{ab}	3.77^{b}	4.28^{a}
3	1133.31^{b}	7.71^{a}	7.24^{a}	4.06^{ab}	3.33^{ab}	3.44^{a}	4.28^{a}
4	1053.55^{b}	7.37^{a}	7.16^{a}	4.23^{b}	3.42^{ab}	3.28^{a}	4.23^{a}
5	999.28^{ab}	7.45^{a}	7.12^{a}	4.14^{ab}	3.51^{b}	3.33^{a}	4.16^{a}
Haplic Luvisol (n = 7)							
1	537.77^{a}	7.72^{a}	7.18^{a}	4.18^{a}	3.48^{a}	3.40^{a}	4.07^{a}
2	2562.58^{d}	7.69^{a}	7.33^{a}	4.06^{a}	3.47^{a}	2.86^{a}	4.00^{a}
3	877.58^{c}	7.74^{ab}	7.18^{a}	4.67^{a}	3.76^{a}	3.31^{a}	4.11^{a}
4	699.079^{b}	7.79^{ab}	7.87^{a}	4.87^{a}	3.74^{a}	3.38^{a}	4.14^{a}
5	781.42^{bc}	7.97^{b}	7.47^{a}	4.87^{a}	3.88^{a}	3.45^{a}	4.10^{a}
Cambisol (n = 4)							
1	$1905,27^{a}$	7.78^{ab}	7.91^{a}	3.48^{a}	ND	ND	ND
2	4435.61^{c}	8.08^{b}	7.62^{a}	3.00^{a}	ND	ND	ND
3	2510.32^{b}	7.81^{ab}	7.82^{a}	3.78^{a}	ND	ND	ND
4	$2381,61^{b}$	7.73^{a}	7.33^{a}	3.97^{a}	ND	ND	ND
5	2431.80^{b}	7.59^{a}	7.26^{a}	3.42^{a}	ND	ND	ND

Differences between values (intra – columns) followed by the same common letter are not significant; ND – non-deteremined.

Conclusions

1. Potential CO_2 production was higher (statistically significantly, $p \leq 0.01$) in all variants (with addition of glucose, PVAL, herbicide and fungicide) than basal one.

2. Pesticides addition did not significantly affect decrease of numbers of bacterial vegetative forms in the soil types Haplic Chernozem and Cambisol. The insignificantly decrease was found only in the numbers of bacterial spores in the soil type Cambisol and in the numbers of microscopic fungi only in the soil type Haplic Chernozem.

3. Fungicide was easier accessible source of carbon than herbicide for present soil microorganisms.

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WPŁYW PESTYCYDÓW NA AKTWYNOŚĆ MIKROORGANIZMÓW W WYBRANYCH TYPACH GLEBY NA SŁOWACJI

Abstrakt: Celem naszych badań było określenie wpływu pestycydów na oddychanie gleby oraz liczebność mikroorganizmów (bakterii oraz ich spor zużywających organiczny i nieorganiczny azot, promieniowców, myxobakterii, *Azotobacter chroococcum*, mikroskopijnych grzybów) w trzech typach gleby (czarnoziemu, płowej, brunatnoziemnej). W glebie typu Haplic Chernozem kumulatywne wartości podstawowej produkcji CO_2 w ciągu 21 dni wynosiły od 595.62 do 1045.79 mg · kg^{-1} s.m. (suchej masy gleby). W glebach Haplic Luvisols wartości te wynosiły od 424.6 do 540.28 mg · kg^{-1} s.m., a w glebach typu Cambisol od 1789.84 do

2103.81 mg · kg^{-1} s.m. Wartości podstawowej produkcji CO_2 były mniejsze od potencjalnej produkcji CO_2 (różnice istotne statystycznie przy $p < 0,01$) we wszystkich wariantach eksperymentu (dodatki glukozy, PVAL, herbicydu, fungicydu). Stymulujący wpływ glukozy był bardziej wyraźny w glebie typu Haplic Luvisol niż w glebie Haplic Chernozem i Cambisol. Pestycydy nie wpłynęły w sposób istotny statystycznie na zmniejszenie liczebności wegetatywnych form bakterii w glebach typu Haplic Chernozem i Cambisol. Zaobserwowano nieistotny statystycznie spadek liczby spor bakteryjnych w glebie typu Cambisol oraz liczebności mikroskopijnych grzybów w glebie typu Haplic Chernozem.

Słowa kluczowe: oddychanie gleby, pestycydy, fizjologiczne grupy mikroorganizmów, typy gleby

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RISKS OF HEAVY METALS ENTRANCE INTO SOIL AND PLANTS AFTER CHEMICALLY AND MECHANICALLY TREATED COAL APPLICATION

KUMULACJA METALI CIĘŻKICH W ROŚLINACH ORAZ W GLEBIE MODYFIKOWANEJ ZWIĄZKAMI WĘGLA

Abstract: The effect of chemically (solid sodium humate) and mechanically (Lignofert) treated coal application as a soil remediate substance and NPK (artificial) fertilizers, on the content of heavy metals in root, straw and grain of spring barley and in the soil have been investigated on Haplic Luvisol in the pot trial realized in vegetative cage placed on the territory of Slovak Agricultural University in Nitra (48°18′ N, 18°05′ E). The achieved results did not confirm the proclaimed inhibitive effect of sodium humate (SH) and Lignofert (Lig) for entrance of heavy metals into the plants. Conversely, the content of three out of eleven metals (As, Cd, Ni) in the barley grain and one (Fe) in the straw increased after SH application in a statistically significant way in comparison with the control variant. A significant decrease was demonstrated only in the content of mercury in the grain. Lignofert statistically significantly increased the content of up to four metals (Cd, Hg, Cu and Co) in the grain and one of them (Hg) in the straw. Its application did not cause the significant decrease of any heavy metal contents in the grain. The content only of one metal (Zn) decreased in the straw significantly. The application of a rational dose of NPK fertilizers in a statistically significant way increased the content of eight out of eleven investigated heavy metals in the barley corn as a result of their moderate acidification effect on the soil. The use of SH and Lig was not more risky from the viewpoint of heavy metal cumulation in spring barley than the use of NPK fertilizers. However, their application in order to inhibit the entrance of most heavy metals into the plants is not rational. At the same time both coal substances significantly simultaneously increased the content of carbon and pH value in the soil. The use of fertilizers caused a high decrease of carbon content in the soil but their effect on heavy metal level in the soil was positive, it was lower than the effect of coal substances. The overall quantities of metals in the soil of all variants were lower than allowable limit at the end of the trial.

Keywords: coal, lignite, sodium humate, heavy metals, spring barley

In Slovakia the production of organic fertilizers has decreased in the last twenty years by 55 % and compost production by 80 %. After-harvest plant remains became the main source of organic mass in the soil. They satisfy the soil needs for organic substances at

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10–70 % in dependence on the sowing methods and technologies used. Lasting decrease of carbon entrance into the soil caused deterioration of many physical, chemical, microbiological and hygienic-toxicological parameters of the soils in Slovakia.

An alternative source of carbon, that can substantially improve present unfavourable balance of organic substances in the soil, is the application of chemically and mechanically treated coal substances. There are applied chemically treated substances such as liquid sodium, potassium, calcium and ammonium humates (the salts of isolated humic acids). A lot of scientific research work deals with the liquid humates application and their effect on seed germination [1, 2], root system development [3], plant yield parameters [4, 5], on the mechanism of their direct effect on plants [6], and nutrients mobility in the soil [7]. Less papers pay their attention to solid coal substances application. They deal with physical and chemical soil parameters [8–10] but many of their results have antagonistic character.

Agricultural practice perceives the declared by many authors inhibitive effect of chemically and mechanically treated coal applications on the sorption of heavy metals by plants [11–13] as a guarantee of lower level of undesirable metals in the plants. There have appeared some papers which do not prove their inhibitive effect [14–16], therefore there was based a trial the aim of which was to identify the effect of chemically (solid sodium humate) and mechanically (Lignofert) treated coal application as a soil remediate substance and NPK (artificial) fertilizers on the content of heavy metals in the root, straw and grain of spring barley and in the soil.

Material and methods

The pot experiment was realized in 2005 and 2006 years and conducted in a vegetation cage situated in the area of the SAU in Nitra (48°18′ N, 18°05′ E). Experimental pots were filled with the mixture of 16 kg of soil (Haplic Luvisol) and 8 kg of siliceous sand, agrochemical characteristics of which are presented in Table 1 and the methods of their determination are added under Table 1. Into each pot, 100 spring barley grain (Express c.v.) was seeded. After the germination the number of the individuals was thinned out to 75 plants per pot. Moisture of the soil in the pots was maintained on the level of 60 % of full water capacity by regular irrigation. Experiment consisted of 4 treatments (0, SH, Lig, NPK). Each treatment was in four replications.

Table 1

Agrochemical characteristics of soil used in pot trial

Year	$N\text{-}NH_4^+$	$N\text{-}NO_3^-$	N_{in}	P	K	Ca	Mg	pH_{KCl}	C_{ox}
	[mg · kg^{-1}]								[%]
2005	12.48	0.24	12.72	41.3	242	1 310	250	5.78	0.97
2006	9.80	2.99	12.80	18.9	182	1 643	303	5.57	0.99

$N\text{-}NH_4^+$ – colorimetrically (Nessler agent); $N\text{-}NO_3^-$ – colorimetrically (phenol-2,4 disulphonic acid); N_{in} – calculated as $N\text{-}NH_4^+ + N\text{-}NO_3^-$; P – colorimetrically (Mehlich II); K – flame photometry method (Mehlich II); Mg – atomic absorption spectrophotometry (Mehlich II); C_{ox} – total carbon content (Tjurin); pH_{KCl} – 1.0 M KCl.

Applied sodium humate (SH) was of Czech origin produced by an alkalic extraction (NaOH + water) from low caloric imperfectly charred subsurface coal. Its application dose was based on respecting the knowledge of Richter and Hlusek [17] and Kovacik [10] who recommend applying dose of solid sodium humate of 300 kg · ha^{-1} in pot trials. Lignofert is ground and mechanically sorted lignite of 0.1–10 mm size of particles and was produced by a Slovak company of Bana Zahorie. In the Lignofert dose calculation (900 kg · ha^{-1}) was taken into consideration the fact that the content of humic acids in sodium humate is three times higher than in Lignofert.

Table 2 shows some agrochemical and hygienic-toxicological parameters of both materials. The contents of heavy metals of mechanically and chemically treated coal substances satisfy the criteria for the soil remediate substances of peat type.

Table 2

Some agrochemical and hygienic-toxicological parameters of sodium humate and Lignofert and limit values of heavy metals for soil additives (Slovak Law No. 577/2005)

Material	pH_{KCl}	EC* [mS · cm^{-1}]	C_{ox}	HA**	Cd	As	Hg	Cr	Ni	Pb
			[%]		[mg · kg^{-1}]					
Sodium humate (SH)	9.66	13.35	44.99	61	0.100	18.4	0.385	36.6	27	5.11
Lignofert (Lig)	5.35	2.31	30.67	22	0.087	19.9	0.110	27.6	28	4.76
Limited values of heavy metals for soil additives of peat type					2	20	1	100	50	100

* Electric conductivity, ** humic acids.

The rates of NPK nutrients were calculated on the basis of the content of N_{in} and available P and K in the soil and plant requirement for these nutrients to achieve planned yield. Nitrogen was applied in the form of DAM-390 fertilizer, P in the form of single superphosphate and potassium as 60 % KCl.

The harvest of spring barley was performed at the growth stage DC 91. The content of heavy metals – Hg, As, Cd, Pb, Cr, Zn, Cu, Co, Ni, Mn and Fe in grains, straw and roots were determined by atomic absorption spectrophotometry (AAS) using the following equipments: Hg and As – AMA 254; Cd, Pb, Cr, Zn, Cu, Co, Ni, Mn and Fe – Pye Unicam.

The total forms of metals in the soil were determined after mineralization by *aqua regia*. The heavy metal contents in the grain and in the soil have been examined in all four repetitions. In the straw only in three repetitions. In consequence of getting insufficient quantity of root phytomass (for all kinds of chemical analyses performed in the trial) the root phytomass of all four repetitions were put together and thus created one average sample.

Results and discussion

The effects of tested coal applications, sodium humate and Lignofert, on the content of heavy metals in the spring barley roots, straw and grain were similar but not identical.

Sodium humate application significantly increased the content of three (Cd, As and Ni) out of eleven monitored heavy metals in the grain (Table 3) and one (Fe) in the straw. There has been the increased content of four metals (As, Cr, Mn a Fe – Table 5) in the roots. Its declared inhibitive effect on the uptake of metals by the plants has been noticed only in Hg and Cu in grain while just the decrease of Hg content was significant. There was a decrease in the content of Cd, Pb, Zn, Mn in the straw but none of them was significant (Table 4). The level of Pb, Hg, Cu and Mn in the roots has decreased moderately (Table 5).

The content of four metals (Cd, Hg, Cu and Co) has been in a statistically significant way increased in the barley grain and of one metal (Hg) in the straw after Lignofert application. In the roots there has been noticed higher increase of the same metals as after the sodium humate application. On the contrary, Lignofert application caused a slight, insignificant decrease of four metals (As, Cr, Zn and Mn). The contents of Cd, Pb, Zn, Cu, Ni, Mn and Fe (ie seven out of eleven metals) in the straw decreased, but only Zn decrease was significant.

The achieved results prove that the application of solid sodium humate and Lignofert into the soil before the sowing does not assure the lower uptake of heavy metals into the vegetative or generative organs. Moreover, their application can cause significant increases of metal contents in the plants. The same phenomenon has been noticed by Richter and Hlusek [17], Hlusek et al [14] who questioned generally found inhibitive humate uptake into the plants and consequently into the food chain.

The increase of heavy metal contents after the treatment by coal substances was not high, consequently the metal levels in the barley grain, except mercury, did not exceed the limits given by food code (Table 3). The Hg above limit content in variants 2 and 3 was the result of high mercury content in the control variant 1.

The application of rational dose of NPK fertilizers caused a significant increase of the contents of eight metals – out of eleven monitored – in the barley grain (Table 3). The increase in contents of up to ten metals (Table 5), have been monitored in the roots. Copper was an exception.

The trial has proved that the effect of both sodium humate and Lignofert on the increased content of heavy metals in the barley is lower than the effect of rational dose of NPK fertilizers. The reason of different effect on metal accumulation is their different effect on soil reaction. Coal substances alkalinized the soil and fertilizers did not change soil pH or they had moderately acidification effect (Table 6). Applied 300 $kg \cdot ha^{-1}$ of sodium humate and 900 $kg \cdot ha^{-1}$ of Lignofert significantly make the soil acidity lower. Many authors (Wisniowska-Kielian and Niemiec [18], Gondek and Filipek-Mazur [19], Vollmanova et al [20]) refer to a positive correlation dependence between the fertilizer dose or pH value and bio-accessibility of metals.

Common knowledge that the metals cumulate in the plants especially in their roots, less in vegetative mass and least in generative organs was confirmed in ten out of eleven analyzed metals. Zn was an exception. Its amount in the grain was comparable with its amount in the roots (Tables 3 and 5) but its amount in the straw was lower than in the roots.

The application of coal substances caused also the increase of heavy metal contents in the soil (Table 6). Sodium humate use in comparison with the control variant

Table 3

The effect of tested materials on the content of eleven metals in spring barley grain

Treatment		Cd	Pb	Hg	As	Cr	Zn	Cu	Co	Ni	Mn	Fe
Number	Designation	[mg · kg^{-1}]										
1	0	0.084a	0.956a	0.0057b	0.0083a	0.219a	33.35a	7.11b	0.215a	0.341a	15.91a	33.52a
2	SH	0.100b	1.047a	0.0007a	0.0173b	0.222a	37.12a	7.09b	0.293ab	0.546b	16.57a	35.65a
3	Lig	0.099b	1.042a	0.0074b	0.0074a	0.216a	32.78a	7.53c	0.323b	0.355a	15.38a	39.98a
4	NPK	0.136c	0.965a	0.0023a	0.0189b	0.299b	44.72b	5.63a	0.396b	0.630b	22.92b	52.14b
Limited values		0.1	1.0	0.05	0.2	4.0	—	10.0	—	3.0	—	—
$LSD_{0.05}$		0.0139	0.1623	0.00245	0.00433	0.0696	5.9929	0.277	0.1075	0.1523	1.325	8.172
$LSD_{0.01}$		0.0189	0.2200	0.00332	0.00587	0.0944	8.12107	0.375	0.1457	0.2064	1.796	11.075

LSD – Lowest Significant Difference at the level $\alpha = 0.05$ and $\alpha = 0.01$.

Table 4

The effect of tested materials on the content of eleven metals in spring barley straw

Treatment		Cd	Pb	Hg	As	Cr	Zn	Cu	Co	Ni	Mn	Fe
Number	Designation	[mg · kg^{-1}]										
1	0	0.184a	1.913a	0.0024a	0.0100a	3.27b	30.58b	12.31a	0.615a	0.953ab	31.04a	163.8a
2	SH	0.157a	1.481a	0.0069a	0.0196a	4.34b	27.73b	12.37a	0.730ab	1.168ab	30.89a	230.3b
3	Lig	0.166a	1.845a	0.0289b	0.0129a	3.50b	18.26a	10.22a	0.712ab	0.881a	24.11a	141.1a
4	NPK	0.339b	1.703a	0.0084a	0.0230a	1.38a	32.72b	9.13a	0.837b	1.214b	49.44b	181.0ab
$LSD_{0.05}$		0.0575	0.7214	0.01737	0.01359	1.629	7.345	3.517	0.2068	0.2924	7.882	65.312
$LSD_{0.01}$		0.0791	0.9969	0.02385	0.01867	2.237	10.090	4.831	0.2841	0.4017	10.827	89.714

LSD – Lowest Significant Difference at the level $\alpha = 0.05$ and $\alpha = 0.01$.

Table 5

The effect of tested materials on the content of eleven metals in spring barley roots

Treatment		Cd	Pb	Hg	As	Cr	Zn	Cu	Co	Ni	Mn	Fe
Number	Designation	[mg · kg^{-1}]										
1	0	0.344	3.825	0.0441	0.0458	10.25	33.67	26.81	1.325	4.885	51.32	1877
2	SH	0.365	3.785	0.0383	0.5302	13.19	29.17	21.63	1.565	5.575	68.47	2609
3	Lig	0.376	4.365	0.0382	0.4587	16.48	33.74	16.22	1.925	6.140	72.14	2949
4	NPK	0.776	4.785	0.0629	0.6793	29.18	61.33	15.87	2.565	9.510	106.66	3854

Table 6

The effect of tested materials on the content of heavy metals (total forms) and some agrochemical parameters in the soil at the end of the trial

Treatment		Cd	Pb	Hg	As	Cr	Zn	Cu	Co	Ni	Mn	Fe	pH_{KCl}	C_{ox}
Number	Destination	[mg · kg^{-1}]												[%]
1	0	0.355a	13.47a	0.031a	4.73a	16.98a	28.0a	7.68a	8.48a	14.73a	372a	11 469a	5.63a	0.954a
2	SH	0.392a	14.27a	0.030a	5.03a	18.05a	36.3b	12.97b	10.03b	16.75b	403a	12 041a	5.95b	1.107b
3	Lig	0.365a	16.25b	0.030a	4.87a	17.92a	32.4ab	12.34b	9.40ab	16.43b	419a	12 118a	5.92b	1.091b
4	NPK	0.390a	13.27a	0.028a	5.32a	17.33a	28.6a	10.05ab	8.98a	15.07a	408a	11 722a	5.60a	0.893a
BET		0.52	15.0	0.0345	5.68	19.2	34.8	11.6	9.8	18.1	412.2	14 964	5.68	0.980
Limited values		0.7	70	0.5	25	70	150	60	15	50	—	—	—	—
$LSD_{0.05}$		0.0484	1.6217	0.00683	0.767	2.614	5.733	4.325	0.980	1.3526	58.44	1 637.0	0.13	0.169
$LSD_{0.01}$		0.0665	2.2276	0.00938	1.054	3.590	7.875	5.941	1.346	1.8580	80.28	2 248.6	0.18	0.219

* Before establishing the trial, LSD – Lowest Significant Difference at the level $\alpha = 0.05$ and $\alpha = 0.01$.

significantly increased the contents of Zn, Co, Ni, Cu and Lignofert use caused the increase of Pb, Ni and Cu. On the other hand, the application of fertilizers (variant 4) did not significantly change the amounts of metals in the soil. This fact relates to the application doses of fertilizers and the contents of metals in them.

Compared amounts of metals in the soil before and at the end of the trial showed the decrease of the Cd, Hg, As, Cr, Ni and Fe contents in all variants. Application of sodium humate caused increased contents of Zn, Cu and Co and used Lignofert increased the contents of Pb, Cu and Mn. Both substances positively effected on the increase of carbon in the soil. The contents of heavy metals and C_{ox} in the variant where fertilizers were not used (variant 1) and in the variant with the application of NPK fertilizers (variant 4) were lower than before the trial. The lowest amount of carbon was in variant 4 as a result of higher mineralization of organic substances in the soil after fertilizers application.

The amounts of the metals at the end of the trial showed lower values than the allowable limit amounts are.

Conclusions

The achieved results did not confirm the inhibitive effect of sodium humate (SH) and Lignofert (Lig) for uptake of heavy metals into the plants. Conversely, the content of three out of eleven metals (As, Cd and Ni) in the barley grain and one (Fe) in the straw increased after SH application in a statistically significant way in comparison with the control variant. A significant decrease in content of mercury was demonstrated only in the grain. Lignofert statistically significantly increased the content of up to four metals (Cd, Hg, Cu and Co) in the grain and one of them (Hg) in the straw. Its application did not cause the significant decrease of any heavy metal contents in the grain. The content only of one metal (Zn) decreased in the straw significantly.

The application of a rational dose of NPK fertilizers in a statistically significant way increased the content of eight out of eleven investigated heavy metals in the barley grain as a result of their moderate acidification effect on the soil.

The use of SH and Lig was not more risky from the viewpoint of heavy metal cumulation in spring barley than the use of NPK fertilizers. However, their application in order to inhibit the entrance of most heavy metals into the plants is not rationale. Both coal substances simultaneously increased significantly the content of carbon and pH value in the soil

The use of fertilizers caused a high decrease of carbon content in the soil but their effect on heavy metal level in the soil was positive, it was lower than the effect of coal substances.

The overall quantities of metals in the soil of all variants were lower than allowable limit at the end of the trial.

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KUMULACJA METALI CIĘŻKICH W ROŚLINACH ORAZ W GLEBIE MODYFIKOWANEJ ZWIĄZKAMI WĘGLA

Abstrakt: Badano wpływ aplikacji chemicznie (humian sodu) i mechanicznie (Lignofert) modyfikowanego węgla wraz z nawozami sztucznymi na zawartość metali ciężkich w korzeniach, pędach oraz ziarnie jęczmienia. Badania prowadzono w glebie płowej z lessu w eksperymencie donicowym. Eksperyment przeprowadzono w Słowackim Uniwersytecie Rolniczym w Nitrze (48°18′ N, 18°06′ E). Uzyskane wyniki nie potwierdziły zakładanego hamującego wpływu humianu sodu (SH) i Lignofert (Lig) na pobieranie metali ciężkich z gleby przez rośliny. Przeciwnie odnotowany został statystycznie istotny wzrost zawartości trzech (As, Cd, Ni) spośród 11 badanych metali w ziarnie jęczmienia oraz jednego (Fe) w pędach tej rośliny po dodaniu SH w porównaniu z kontrolą. Lignofert spowodował statystycznie istotny wzrost zawartości czterech metali (Cd, Hg, Cu i Co) w ziarnie oraz jednego (Hg) w pędach. Zawartość cynku w pędach zmniejszyła się w sposób statystycznie istotny. Zastosowanie nawozów sztucznych spowodowało wzrost zawartości 8 spośród 11 badanych metali w ziarnie jęczmienia. Było to związane z zakwaszeniem gleby przez stosowane nawozy. Użycie SH i Lig nie stanowiło większego zagrożenia w aspekcie kumulacji metali w młodych pędach roślin niż zastosowanie nawozów sztucznych. Jednakże stosowanie SH i Lig w celu ochrony roślin przed gromadzeniem metali ciężkich nie jest racjonalne. Obydwa stosowane związki węglowe powodowały wzrost pH i zawartości węgla w glebie. Aplikacja nawozów sztucznych była przyczyną silnego zmniejszenia zawartości węgla w glebie. Zawartość metali ciężkich w glebie we wszystkich przeprowadzonych próbach była mniejsza niż ustanowione limity.

Słowa kluczowe: węgiel kamienny, węgiel brunatny (lignit), humian sodu, metale ciężkie, jęczmień

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DETERMINATION OF HEAVY METALS AND NUTRITION VALUES IN BROCCOLI

OZNACZANIE ZAWARTOŚCI METALI CIĘŻKICH I SKŁADNIKÓW ODŻYWCZYCH W BROKUŁACH

Abstract: The aim of this study was to determine the content of chosen heavy metals (mercury, lead and cadmium) and some nutritional substances (β-carotene and vitamin C) in raw broccoli samples obtained from trade network. For the assessment of heavy metals the samples were mineralized with the mixture of sulphuric acid and hydrogen peroxide and the analysis was performed using atomic absorption spectroscopy (analyzers AMA 254 and AVANTA GBC 933A, GBC, Australia). Vitamin C and β-carotene were extracted by hexane and acetone and mixture of methanol, phosphoric acid and redistilled water, respectively. Both β-carotene and vitamin C were determined by high-performance liquid chromatography with electrochemical detection (Coulochem III, ESA, USA). The broccoli samples contained 0.094 ± 0.115 μg · 100 g^{-1} of mercury and 0.0004 ± 0.0398 μg · 100 g^{-1} of lead. No cadmium was detected. It appears from this results that no heavy metals accumulate in this vegetable since all concentrations were below quality standard. The amount of β-carotene in broccoli was 1.703 ± 0.194 mg · 100 g^{-1} and the content of vitamin C was 57.974 ± 0.535 mg · 100 g^{-1}.

Keywords: broccoli, HPLC, Coulochem III, Pb, Cd, Hg, vitamin C, β-carotene, AAS

Diets rich in fruits and vegetables are protective against disease and populations that consume such diets have higher plasma antioxidant status and exhibit lower risk of cancer and cardiovascular disease [1]. Whether the health benefits of antioxidant-rich diets are due wholly or in part to their antioxidant capacity is controversial, but increased uptake of antioxidants from food is promoted globally as a simple and potentially highly effective means of health promotion [2]. There are many different kinds of antioxidants and nutrients in foods, and it is impossible to measure all [3].

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Members of the genus *Brassica* belong to the Cruciferous family and are reported to posses both antioxidant and health-promoting properties [4]. Cruciferous vegetables, such as broccoli can be eaten uncooked, but are most commonly eaten after cooking by steaming, boiling or microwaving. Broccoli is marketed as either a fresh or a processed product (eg, frozen or chopped). The United States per capita consumption of fresh broccoli has steadily increased over the last two decades [5], mainly because of its popularity in salad bars. In addition, broccoli has high vitamin C, vitamin A, fiber and mineral content, saccharides, folates, plus several cancer-preventing agents, which make broccoli a popular item among health-conscious consumers [6]. But, sometimes it is important to control heavy metals in broccoli. In addition, fresh broccoli is highly perishable, with a shelf-life of 3–4 weeks in air at 0 ^{o}C, up to 2 weeks at 4 ^{o}C, but only 2–3 days when kept at room temperature [7], mostly due to the relatively high rate of metabolism and consequent high respiration rate [8].

Materials and methods

The concentrations of three heavy metals, namely, Cd, Pb, and Hg, in the broccoli were determined by the AAS method, using the analyzers AMA 254 and AVANTA GBC 933A. Liquid sample was normally turned into an atomic gas, atomization was made up with acetylene. Samples of broccoli were mineralized with H_2SO_4 and H_2O_2. Results were evaluated on the basis of measuring calibration curves.

Broccoli has a very powerful nutritional profile, containing loads of vitamins and minerals such as vitamin A, thiamin, riboflavin, niacin, vitamin C, vitamin E, folate, vitamin K, pantothenic acid, calcium, copper, iron, magnesium, phosphorus, potassium, zinc and etc. Many authors reported that the raw green broccoli contains per 100 g edible portion (tough stems removed, 61 % of product as purchased): water 88.2 g, energy 138 kJ (33 kcal), protein 4.4 g, fat 0.9 g, carbohydrate 1.8 g, dietary fibre 2.6 g, carotene 575 μg, thiamin 0.10 mg, riboflavin 0.06 mg, niacin 0.9 mg, folate 90 μg, ascorbic acid 87 mg etc. It follows broccoli contains many antioxidants, including carotenoids, tocopherols, ascorbic acid, and flavonoids [9, 10]. Due to this we were interested in amount of β-carotene and vitamin C, especially. In our study HPLC system ESA equipped with Coulochem III detector was used. In the case of β-carotene broccoli sample was extracted by mixture of acetone:hexane (1:1, v/v) several times in water bath 27 ^{o}C, samples were protected from sun light, of course. After its at solvents were removed using rotary vacuum evaporator. Rest of sample was diluted into HPLC quality ethanol and injected into the column. Measurement conditions were following: inject volume 20 mm^3, column Supelcosil LC-8 (150 × 4.6 mm, 5 μm), temperature 30 ^{o}C, mobile phase CH_3OH : H_2O : H_3PO_4 (99 : 0.5 : 0.5, v/v/v), flow rate 1.1 cm^3 · min^{-1} in isocratic elution, detector was set to 300 and 400 mV, guard cell 750 mV. Under the same condition, calibration curve was measured. In the case of vitamin C determination sample was extracted by mobile phase directly for a period of 15 min in shaker bath at 25 ^{o}C. Sample was protected from sun light during all extracted process. After that, sample was filtered and injected into the HPLC system. Measurement conditions were following: injected volume 20 mm^3, column Supelcosil LC-8 (150 × 4.6 mm, 5 μm),

temperature 30 °C, mobile phase CH_3OH : H_2O : H_3PO_4 (99 : 0.5 : 0.5, v/v/v), flow rate 1.1 $cm^3 \cdot min^{-1}$ in isocratic elution, detector was set to 600 and 650 mV, guard cell 750 mV. Calibration curve was measured under the same condition.

Results and discussion

Lead is a poisonous metal that can damage nervous connections and cause blood and brain disorders. Long term exposure to lead or its salts can cause nephropathy and colic-like abdominal pains. Mercury is a cumulative heavy metal poison, all mercury--based toxic compounds damage the central nervous system and other organs or organ system such as the liver or gastrointestinal tract. Cadmium is one of the substances banned by the European Union's Restriction on Hazardous Substances, including proteinuria and glucosuria, cadmium-containing compounds are known carcinogens and can induce many types of cancer. We found out that our tested broccoli samples obtained 0.094 ± 0.115 μg · 100 g^{-1} of Hg, 0.0004 ± 0.0398 μg · 100 g^{-1} of Pb. We determined no cadmium. It appears from this that no heavy metals accumulate in this vegetable, all results were below the quality level. The level of significance was set at 95 %.

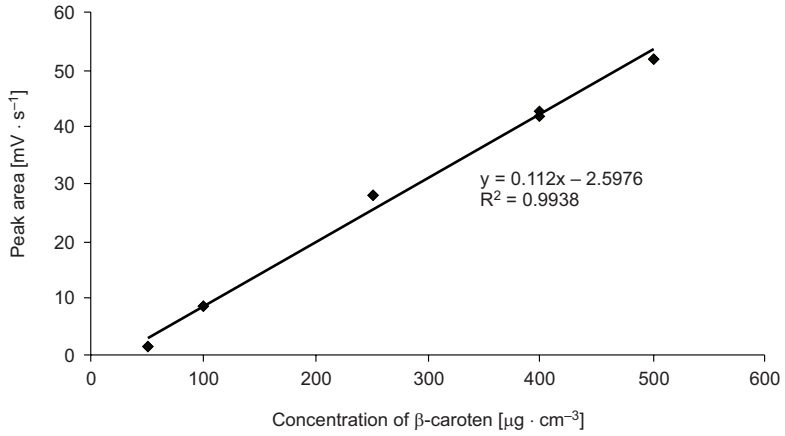

Fig. 1. Calibration curve of β-carotene

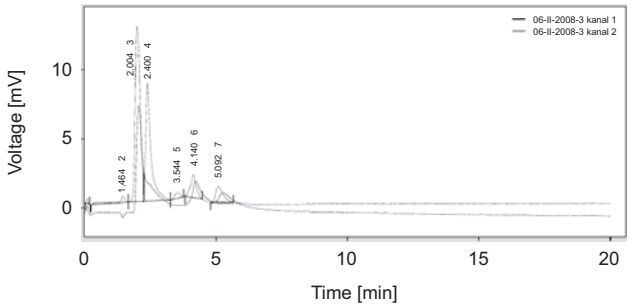

Fig. 2. Chromatogram: β-carotene in raw broccoli

β-Carotene is the most effective vitamin A precursor, and has been reported to protect humans against certain types of cancer and cardiovascular diseases [10,11]. Calibration curve was obtained analyzing five different solution of known concentration of β-carotene standard included between 50–500 μg · cm^{-3}. The curve equation y = ax + b calculated with linear regression method was utilized to determine samples concentration. Calibration curve is shown in Fig. 1. The results show that β-carotene can be determined in broccoli by HPLC with ECD, β-carotene retention time was 3.6 min. The result of our experiment showed that amount of β-carotene was 1.703 ± 0.194 mg · 100 g^{-1} in raw broccoli. The literature β-carotene content determined in leafy vegetables is 0.5–2 mg · 100 g^{-1} [5, 10, 11].

Vitamin C is considered to be the most important vitamin for human nutrition which could be best supplied by fruits (especially citrus and some tropical fruit) and vegetables. Standard solutions of L-ascorbic acid were prepared in a solution corresponding to the mobile phase (calibration curves of concentration 1–4 mm^3 · cm^{-3}) used in isocratic conditions. Triplicate injections for each standard solution were made and the peak area was plotted against the corresponding analyte concentration to obtain the calibration curves y = 72.651x – 46.433. Results are presented in Fig. 3 and 4. Retention time of vitamin C was 1.8 min.

We determined that amount of vitamin C was 57.974 ± 0.535 mg · 100 g^{-1} in raw material. There are lots of articles describing concentration of vitamin C in broccoli, average value ranges from 20 to 90 mg · 100 g^{-1}[4, 5, 7, 8]

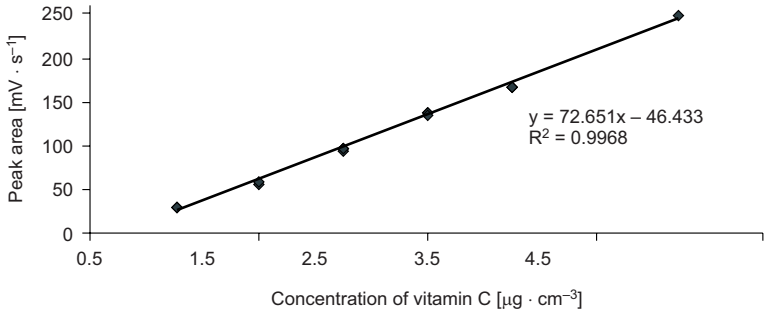

Fig. 3. Calibration curve of vitamin C

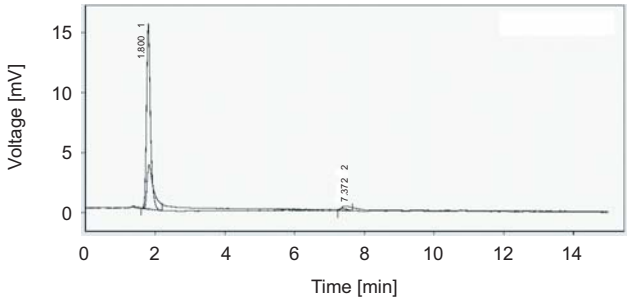

Fig. 4 Chromatogram: Vitamin C in raw broccoli

Conclusions

For determination of mercury (Hg), cadmium (Cd) and lead (Pb) analyzers AMA 254 and AVANTA GBC 933A were used. We found out that our tested broccoli samples contained 0.094 ± 0.1152 μg · 100 g^{-1} of Hg, 0.0004 ± 0.03981 μg · 100 g^{-1} of Pb. We determined no cadmium. The ECD ability to measure low levels of vitamin and flavonoids and carotenoids can provide a competitive advantage by profiling the characteristic qualities of products, to their commercial values. The result of our experiment showed that amount of β-carotene was 1.703 ± 0.1945 mg · 100 g^{-1} in raw broccoli sample and amount of vitamin C was 57.974 ± 0.535 mg · 100 g^{-1} in raw material. A high intake of broccoli has been found to reduce the risk of aggressive prostate cancer. Broccoli leaf is also edible and contains far more β-carotene than the florets.

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OZNACZANIE ZAWARTOŚCI METALI CIĘŻKICH I SKŁADNIKÓW ODŻYWCZYCH W BROKUŁACH

Abstrakt: Celem badań było zbadanie zawartości wybranych metali ciężkich (rtęć, ołów i kadm) oraz niektórych składników odżywczych (β-karoten i witamina C) w świeżych brokułach. W celu oznaczenia zawartości metali ciężkich próbki były mineralizowane w mieszaninie kwasu siarkowego i nadtlenku wodoru. Oznaczenia wykonano metodą spektrofotometrii absorpcji atomowej (analizatory AMA 254 and AVANTA

GBC 933A, GBC, Australia). Witaminę C i β-karoten izolowano przy użyciu kolejno heksanu i acetonu oraz mieszaniny metanolu, kwasu fosforowego i wody destylowanej. Witaminę C i β-karoten oznaczono przy użyciu chromatografii cieczowej oraz analizy elektrochemicznej (Coulochem III, ESA, USA). Próbki brokułów zawierały 0.094 ± 0.115 μg · 100 g^{-1} rtęci oraz 0.0004 ± 0.0398 μg · 100 g^{-1} ołowiu. Kadm nie został wykryty. Przeprowadzone badania wydają się wskazywać, że brokuły nie kumulują metali ciężkich. β-karoten występował w brokułach w ilości 1.703 ± 0.194 mg · 100 g^{-1}, a zawartość witaminy C wynosiła 57.974 ± 0.535 mg · 100 g^{-1}.

Słowa kluczowe: brokuł, HPLC, Coulochem III, Pb, Cd, Hg, witamina C, β-karoten

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BIOGENIC AMINE CONTENT IN MOULD CHEESE DURING STORAGE

ZAWARTOŚĆ AMIN BIOGENNYCH W SERZE PLEŚNIOWYM W TRAKCIE PRZECHOWYWANIA

Abstract: The aim of this research was to study the formation of seven biogenic amines (histamine, agmatine, spermine, spermidine, cadaverine, putrescine and tyramine) in three commercial mould cheeses from three different producers from the area of the Central Europe during 8-week storage in refrigerator at 6 ± 2 °C. The analysis of biogenic amines was made every week during 8-week of storage. Biogenic amines were extracted from the mould cheese by diluted HCl and determined using ion-exchange chromatography with post-column ninhydrin detection. Spermidine, spermine, putrescine and cadaverine were detected in tested mould cheeses. Spermidine was quantitatively the most important biogenic amine in all samples. While spermidine was detected immediately after purchase of samples, the rest of detected biogenic amines were developed during storage. The amount of putrescine was mostly increased during storage while the concentration of spermidine was decreased during storage. However, after 8 weeks of storage all samples contained toxicologically insignificant concentrations of detected biogenic amines in comparison with EU legislation and scientific literature and can be considered to be safe for human health.

Keywords: biogenic amine, mould cheese, ion-exchange chromatography

Biogenic amines are non-volatile low molecular nitrogen organic bases possessing biological activity. These compounds can have aliphatic, aromatic and heterocyclic structure. Biogenic amines, mainly polyamines, are indispensable parts of cells being essential for the regulation of nucleic acid function, stabilization of membranes, cell growth and proliferation, blood pressure regulation, etc. Some biogenic amines serve as free radical scavengers and antioxidants. Biogenic amines contained in food originate especially from the decarboxylation of the corresponding amino acids by micro-organisms. Decarboxylation activity is typical especially for families *Lactobacillaceae* and *Enterobacteriaceae* [1–3]. Consumption of food with high biogenic amine content (especially fish, meat and cheese products, wine, beer and other fermented food) may

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result in various health problems. They can cause eg nausea, vomiting, diarrhoea, palpitation, headache, depression, dizziness, hypo- or hypertension and, in extreme cases, anaphylactic shock, heart attack, etc. [1, 4–7]. Furthermore, some biogenic amines can participate in the formation of carcinogenic nitrosamines [5].

Camembert-type cheese is a dairy product which belongs to the semi-soft/soft mould ripened cheese. It is covered on the surface by mould *Penicillium camemberti*. The manufacture includes following main steps: milk standardization, pasteurization, starter culture and mould inoculation, renneting, whey removal, forming, salting, wrapping and ripening. The ripening period last about 2–3 weeks [8]. The ripening of cheese is characterized mainly by hydrolysis of caseins which leads to the increase of free amino acid content. Some of these amino acids can be subjected to decarboxylation to form biogenic amines. BA production in cheese can be influenced by many factors, eg by kind of starter culture strain, bacterial activity, pH of cheese, salt and fermentable saccharide concentration, storage temperature, ripening time, etc. [9, 10]. The content of BA in mould cheese could be affected also by deamination of amino acids by the enzyme activity of present moulds.

In the Czech Republic, only the law for histamine content in fish adopted from EU is valid. According to it, histamine content in 7 of 9 fish samples must not exceed 100 mg · kg^{-1}; at the same time, 2 samples of 9 can contain more than 100 mg · kg^{-1} histamine, but less then 200 mg · kg^{-1} [11]. Nevertheless, there are nowadays no other thresholds for the other biogenic amines. Previously, there were also limits for tyramine in various foods such as cheese (200 mg · kg^{-1}) or red wine (50 mg · kg^{-1}) [12].

There is numerous information about biogenic amines in cheese available in the literature [7, 13–15]. However, there was not found any paper dealing neither with the mould cheese nor with moulds producing biogenic amines. Hence, the aim of this study is to investigate the formation and the amount of biogenic amines in soft mould ripened cheeses obtained from trade network during 8-week storage in refrigerator at 6 ± 2 °C.

Materials and methods

Samples

Three different commercial brands of mould cheese (Sedlcansky hermelinek, Karel IV Camembert, Stribrnak) were assessed by biogenic amine content. All these samples were soft mould ripened cheeses covered by white mould (*Penicillium camemberti*). Sedlcansky hermelinek (H) was manufactured by Povltavske mlekarny, a.s. (Sedlcany, Czech Republic), Karel IV Camembert (C) was produced by Polser Sp. z.o.o. (Siemiatycze, Poland) and mould cheese Stribrnak (S) was made in MV Oberfranken – West eG (Wiesenfeld, Germany). All samples were stored in the refrigerator at 6 ± 2 °C until the analysis.

Chemical analysis of samples

Chemical analysis of samples was made immediately after purchase (0 day of storage) and then after 28 and 56 days of storage. Samples were characterized by dry

matter and fat content, by pH and crude protein (see Table 1). Dry matter content was determined by drying at 102 ± 2 °C to a constant weight according to ISO 5534:2004 [16]. The pH value was measured by inserting of glass electrode THETA 90 HC 113 (Gryf, Havlickuv Brod, Czech Republic) of pH-meter Gryf 208 L (Gryf, Havlickuv Brod, Czech Republic) into the water solution of sample (10 g of sample disintegrated in 30 cm^{-3} of distilled water) at 20 ± 1 °C. Fat content was determined by the acidobutyric method of van Gulik. Crude protein was assayed by analysing total nitrogen by Kjeldahl method using distillation unit Pro-Nitro A (JP SELECTA, Barcelona, Spain) and calculating crude protein content as total nitrogen multiplied by 6.38.

Biogenic amine extraction

Ten grams of sample was extracted with 25 cm^{-3} of 0.1 mol · dm^{-3} HCl in stomacher for 7 minutes. After extraction, the homogenized sample with extraction solution was centrifugated in 50 cm^3 centrifugal tubes in centrifuge Z 300 K (HERMLE Labor-technik GmbH, Wehingen, Germany) at 6500 rpm at 4 °C for 30 minutes. The supernatant was filtered and evaporated by using rotary vacuum evaporator RVO 400 A (Ingos, Prague, Czech Republic) to the syrup consistency. The rest after evaporation was dissolved in sodium-citrate buffer (pH = 2.2) in 10 cm^3 volumetric flask. The solution was filtered through the nylon membrane filter (0.45 μm) and loaded into analyzer.

Biogenic amine analysis

Isolated biogenic amines were analyzed by using ion-exchange chromatography (column 55 × 3.7 mm filled with ion exchanger OSTION Lg ANB) equipped with post-column ninhydrin derivatization and spectrophotometric detection (λ = 570 nm). The analysis was made by using Amino Acid Analyser AAA400 (Ingos, Prague, Czech Republic). The buffer system, protocols of the analysis (elution programs) and the process of ninhydrin reagent preparation were used as described in Standara et al [6]. A mixed standard solution of 7 biogenic amines (histamine, agmatine, spermine, spermidine, cadaverine, putrescine, tyramine) in sodium-citrate buffer (pH = 2.2) with the concentration of 500 nmol · cm^{-3} of each amine was prepared. Biogenic amine standards were obtained from Sigma-Aldrich (St. Louis, USA). All reagents for AAA and the ion exchanger for the column were purchased from Ingos (Prague, Czech Republic). Two samples of each brand of mould cheese were investigated every week during 8-week storage and the whole extraction procedure and biogenic amine analysis were always made at least twice for each sample.

Statistical analysis

Results obtained by chemical analysis were statistically evaluated using parametric t-test. Results were significantly different when $p < 0.05$. The dependency of BA

amount on the storage time was also evaluated using regression analysis (least squares method). Coefficient of correlation (r) for chosen model expressing changes in BA concentration depending on storage time was calculated.

Results and discussion

The formation of seven biogenic amines (histamine, agmatine, spermine, spermidine, cadaverine, putrescine, tyramine) in three commercial mould cheeses during 8-week storage in refrigerator at 6 ± 2 °C was investigated. Results obtained by basic chemical analysis of mould cheeses are presented in Table 1. The analyses showed that there were not significant differences ($p \geq 0.05$) in dry matter content during 8-week storage in refrigerator at 6 ± 2 °C while values of pH increased ($p < 0.05$) during ripening of mould cheeses. Growing pH values during storage can be explained by protein hydrolysis and ammonia creation together with the utilization of lactic acid by present microorganisms.

Table 1

Chemical analysis of mould cheeses after 0, 28 and 56 days of storage in refrigerator at 6 ± 2 °C

Sample*	Storage time [days]	DM** [% w/w]	Fat [% w/w]	pH [-]	CP* [% w/w]
H	0	46.88 ± 1.08 [a]	23.8 ± 0.4	6.67 ± 0.08 [a]	17.98 ± 0.43
	28	46.75 ± 0.32 [a]		7.85 ± 0.05 [b]	
	56	47.12 ± 0.94 [a]		8.03 ± 0.04 [c]	
C	0	54.29 ± 0.79 [a]	33.3 ± 1.8	7.06 ± 0.10 [a]	18.16 ± 0.17
	28	54.71 ± 0.59 [a]		7.64 ± 0.01 [b]	
	56	54.95 ± 1.51 [a]		7.57 ± 0.02 [c]	
S	0	40.29 ± 1.38 [a]	13.8 ± 0.4	6.76 ± 0.04 [a]	22.70 ± 0.21
	28	40.98 ± 0.34 [a]		7.74 ± 0.05 [b]	
	56	40.80 ± 0.82 [a]		7.79 ± 0.05 [b]	

* H – Sedlcansky hermelinek, C – Karel IV Camembert, S – Stribrnak, DM – Dry matter content, CP – crude protein content.

** Means in a box followed by at least one similar superscript letter are not significantly different ($p \geq 0.05$).

In this study, three different commercial brands of mould cheese were investigated in terms of their biogenic amine content. Immediately after purchase, all mould cheeses contained only spermidine but only in negligible amounts. Samples S included also small amounts of spermine and putrescine and mould cheese S contained putrescine. During storage the contents of tested biogenic amine changed. In the majority of cases, the amounts of detected biogenic amines fluctuated slightly during first 5 weeks of storage. Then, there were observed different trends in concentrations of various biogenic amines. While the amount of spermidine was mostly slightly decreased, the concentrations of putrescine substantially increased. The decline in spermidine concentration in cheese during ripening, was observed by Novella-Rodriguez et al [17]. The

rise of putrescine amount was obvious especially after 5-week storage (samples H, S), respectively after 7-week storage (sample C). This increase is evident from the Fig. 1 where the dependence of putrescine amount on the storage time is shown. Exponential model describes this dependence more accurately and at a better fitting than the other models did. There were also detected cadaverine after 5-week storage in sample S and its concentration slightly fluctuated during the further storage. In sample C cadaverine was created after 7 weeks of storage. Agmatine, tyramine and histamine were not found in any of the tested samples. These conclusions are in agreement with some authors who also did not detect histamine in cheese. Karovicova et al [18] did not determine histamine in Cottage cheese or spreadable processed cheeses.

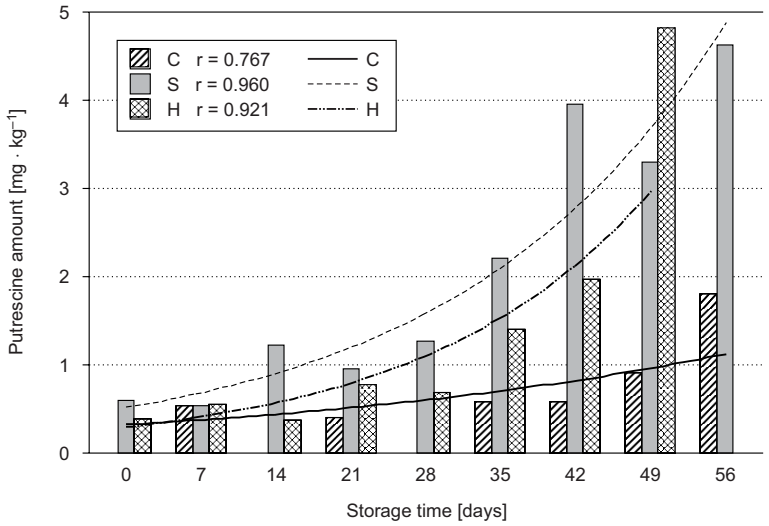

Fig. 1. The dependence of putrescine amount on storage time in 3 tested mould cheeses: H – Sedlcansky hermelinek, C – Karel IV Camembert, S – Stribrnak; r – coefficient of correlation for chosen model expressing changes in BA concentration depending on storage time

Total biogenic amine content is presented in Table 2. In samples C and S it increased mostly during storage period, while in sample H slightly fluctuated. At the end of the monitored period, concentrations of detected biogenic amines were negligible in comparison with toxic levels presented in available literature.

Results obtained in this study were significantly lower (the highest amine concentration was about 8 mg · kg^{-1}) when compared with some observations of other authors. Roig-Sagues et al [10] or Pinho et al [9], respectively, detected BA in 20 various cheese varieties in Spain or in ovine cheese Azeitao, respectively, concentrations about tens or hundreds of mg · kg^{-1}. On the other hand, Novella-Rodriguez et al [17] found similar low amounts of BA in some kinds of cheese in Spain as in this study. This wide variability of BA concentration of different cheese varieties may result from the type of cheese, the ripening time, the conditions of the

manufacturing process, the present microflora (starter culture but mainly non-starter lactic acid bacteria), etc. [17, 19].

Table 2

Total biogenic amine content of three tested mould cheeses during ripening

Storage time [days]	Total BA concentration of cheese [mg · kg^{-1}]		
	Sample H	Sample C	Sample S
0	6.0	6.4	7.8
7	8.2	8.2	8.2
14	7.4	5.9	9.0
21	6.4	7.8	6.0
28	7.5	7.4	7.3
35	6.8	10.4	9.8
42	5.0	8.7	10.0
49	5.4	8.4	9.9
56	7.6	13.0	10.0

* H – Sedlcansky hermelinek, C – Karel IV Camembert, S – Stribrnak.

Insignificant concentrations of these nitrogenous compounds in this research would be explained by the presence of microorganisms with low decarboxylation activity and by deamination of amino acids by present enzymes of moulds. Also, the conditions for their growth or BA production could be not suitable. Values of pH of all samples were always relatively high (see Table 1) and according to Halasz et al [23] the optimum pH for decarboxylase formation, and, therefore, for BA production is in more acidic environment. Higher values of pH together with low storage temperature were sufficient for keeping samples of good quality in terms of biogenic amine content. Additionally, good hygienic condition in cheese production should be maintained to avoid the outbreak of food poisoning.

Conclusions

The formation of biogenic amines in mould cheese during 8-week storage at 6 ± 2 ^{o}C was studied. Spermidine, spermine and putrescine were detected in samples immediately after purchase, while cadaverine was created during storage of samples. Concentrations of spermine, spermidine and putrescine slightly fluctuated till five week of storage and, then, the amount of putrescine substantially increased while the concentration of spermidine decreased. Histamine, agmatine and tyramine were not detected in any of the samples. Before and also after the expiration date all detected biogenic amines were present in only negligible concentrations. It can be concluded that these low amounts of biogenic amines are not toxic for healthy people and, therefore, these mould cheeses are not danger as regards biogenic amine poisoning.

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ZAWARTOŚĆ AMIN BIOGENNYCH W SERZE PLEŚNIOWYM W TRAKCIE PRZECHOWYWANIA

Abstrakt: Celem pracy było zbadanie syntezy siedmiu amin biogennych (histaminy, agmatyny, sperminy, spermidyny, kadaweryny, putrescyny i tyraminy) w trzech komercyjnie dostępnych serach pleśniowych pochodzących od różnych producentów z Europy Środkowej w czasie 8-tygodniowego przechowywania w lodówce w temperaturze 6 ± 2°C. Oznaczenia poziomu amin biogennych wykonywano raz w tygodniu. Aminy biogenne były izolowane z sera pleśniowego przez rozcieńczony HCl i oznaczane metodą chromatografii jonowymiennej i postkolumnowej reakcji ninhydrynowej. W badanych serach wykryto obecność spermidyny, sperminy, putrescyny i kadaweryny. W największych ilościach występowała spermidyna. Związek ten wykrywano w świeżo wyprodukowanym serze, natomiast pozostałe aminy pojawiały się stopniowo w czasie przechowywania. Największy wzrost stężenia w czasie przechowywania stwierdzono w przypadku putrescyny. Natomiast poziom spermidyny zmniejszał się w czasie przechowywania. Po 8 tygodniach przechowywania badane sery zawierały jednak nieznaczne ilości amin biogennych, w stężeniach dopuszczalnych przez normy UE i bezpiecznych dla ludzkiego zdrowia.

Słowa kluczowe: aminy biogenne, ser pleśniowy, chromatografia jonowymienna

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ACCUMULATION OF SELECTED HEAVY METALS IN THE FEMORA OF SMALL TERRESTRIAL MAMMALS

KUMULACJA WYBRANYCH METALI CIĘŻKICH W KOŚCI UDOWEJ DROBNYCH SSAKÓW LĄDOWYCH

Abstract: The accumulation of lead, cadmium, iron, nickel, copper and zinc in the femora of yellow-necked mouse (*Apodemus flavicollis*) and bank vole (*Clethrionomys glareolus*) living near the site of coal power station Novaky (Slovakia) was investigated. The content of heavy metals in the bones was assessed by atomic absorption spectrophotometry method. Altogether 20 femora of adult individuals were analysed. Higher concentrations of Cd, Ni, Cu and Zn were detected in the bones of bank vole. Significant differences were observed for the concentrations of Cd, Ni and Zn ($p < 0.05$). On the contrary, higher concentrations of Pb and Fe were found in the femora of yellow necked mouse. However, the differences were not significant. Our results indicate that *Clethrionomys glareolus* may be considered as more bone loaded zoomonitor in comparison with *Apodemus flavicollis*.

Keywords: heavy metals, bone, yellow necked mouse, bank vole, environment

The dynamic development of industry and motorization, as well as the continuing over-intensive use of various compounds in agriculture, cause levels of toxic heavy metals in the environment to constantly be on the increase [1]. Among the investigated elements, copper, zinc, and manganese play an important role in metabolism as components or activators of enzymes and their tissue concentrations are effectively controlled over a wide range of metal intake. Other elements, called xenobiotics such as

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cadmium and lead, are non-essential and their concentrations are physiologically more poorly regulated [2, 3].

Small terrestrial mammals have often been used as bioindicators of pollution, as residues being determined in either the carcass or in specific organs. In addition, they are small, easy to catch, have a territory of limited range, fairly short life span and they are closely adjusted to their environment [4, 5]. The yellow-necked mouse (*Apodemus flavicollis*, *Rodentia*) is slightly larger and more brightly coloured than the wood mouse (*Apodemus sylvaticus*, *Rodentia*). It is found mostly in mountainous areas of southern Europe, but extends north into parts of Scandinavia and Britain. The bank vole (*Clethrionomys glareolus*, *Rodentia*) is a small microtiny rodent that is common throughout Europe. There are only few studies focused on distribution of heavy metals in selected organs of small terrestrial mammals. They are mainly concentrated on accumulation of selected toxic elements in kidney, liver, testis, uterus and teeth [6–10]. The distribution of heavy metals in their bones is only rarely found in the literature [10, 11].

Bones have some advantages over soft tissues (such as liver and kidney) as markers of exposure to environmental pollution. It is generally known that toxic elements become incorporated into the mineral phase of bone tissue which is subject to little turnover. Anyway, an accurate historical record of exposure to various elements is retained in the bone.

In the present study, the concentrations of selected heavy metals in the femora of *Apodemus flavicollis* and *Clethrionomys glareolus* from polluted region Novaky (Slovakia) were analysed.

Material and methods

The individuals of yellow-necked mouse (*Apodemus flavicollis*) and bank vole (*Clethrionomys glareolus*) were obtained by means of the standard teriological methods and procedures from wood ecosystems [12] in the surrounding of the power station Novaky (Prievidza district, Slovakia). All animals used in the experiment were adult, in good physical condition, without pathological-anatomical changes. Our research focused on 20 femora taken from the adult mammals (12 from *Apodemus flavicollis* and 8 from *Clethrionomys glareolus*). The concentrations of selected heavy metals (Pb, Cd, Ni, Fe, Cu and Zn) were determined with the method of atomic absorption spectrophotometry (Perkin Elmer 4100 ZL) in a graphite furnace. The tissue samples were kept at –18 ^{o}C until analysis. In the laboratory the samples were dried at 105 ^{o}C until dry mass was obtained. Then, the bones were weighed (minimum 2 g) and digested in concentrated nitric(V) acid at 90 ^{o}C for 10 hours. Before the analysis, the samples were diluted to 25 cm^3 with distilled water. All metal concentrations were expressed on a dry weight basis in mg · kg^{-1}. All samples were measured on the same day. From the final data, basic statistical characteristics were calculated (mean, standard deviation, minimum, maximum, median). Since the distribution of observed levels of heavy metals was normal according to Shapiro-Wilk test, the parametric T-test were used for group comparisions with Statistica 7.0 program.

Results and discussion

Concentrations of selected heavy metals (Pb, Cd, Fe, Ni, Cu and Zn) in the femora of *Apodemus flavicollis* and *Clethrionomys glareolus* are listed in Table 1. Higher concentrations of Cd, Ni, Cu and Zn were detected in the bones of bank vole. Significant differences were observed for the concentrations of Cd, Ni and Zn ($p < 0.05$). On the other hand, higher concentrations of Pb and Fe were found in the femora of yellow necked mouse. However, the differences were not significant.

Table 1

The concentrations of selected heavy metals in the femora of small terrestrial mammals

Investigated species	Symbol	Pb	Cd	Ni	Fe	Cu	Zn
		[mg · kg^{-1}]					
Apodemus flavicollis	x	20.18	2.53	7.95	156.61	3.60	126.88
	sd	3.87	0.77	1.94	31.64	0.47	10.35
	min.	15.28	1.93	6.29	115.98	2.89	110.96
	max	26.68	3.95	11.34	204.45	4.27	141.35
	med.	18.84	2.76	7.07	168.57	3.81	129.14
Cleithrionomys glareolus	x	20.13	4.61*	9.82*	138.98	3.78	176.49*
	sd	9.51	1.13	1.89	10.15	0.74	11.20
	min.	14.08	3.71	7.93	128.19	3.20	164.21
	max	31.09	5.88	11.69	140.42	4.61	186.14
	med.	19.84	3.76	8.17	138.17	3.81	174.14

x – mean, sd – standard deviation, min – minimum, max – maximum, med – median, (*) – $p < 0.05$.

In general, there is a significant relationship between the amount of risk elements in soil, water, also in food and in the organs of mammals, first of all in liver and kidneys [8]. However, lead accumulates mainly in bone [13] and cadmium causes damage primarily to kidney, bone and lungs [14]. Cadmium alters the calcium metabolism in the bone, which leads to osteomalacia [15]. In the study by Milton et al [10] who determined lead, zinc and cadmium concentrations in a range of tissues from wild populations of bank voles trapped on an abandoned metalliferous mine site in United Kingdom, the hierarchy of Pb concentrations was bone > kidney > liver > muscle. The hierarchy of Zn concentration was bone > liver > kidney > muscle and the hierarchy of Cd in tissues was kidney > bone > liver > muscle.

It is generally known that a coal power station Novaky has a negative effect on environmental (mainly soil) pollution resulting from mine work and/or from road traffic. According to Iearadi et al [3], Roberts and Johnson [16] one of the most important sources of environmental contamination with toxic elements is the coal industry. The dust emitted contains zinc, copper, lead and cadmium, and this contamination may increase the content of the elements in the tissues of mammals inhabiting polluted areas.

We observed higher concentrations of Cd and Fe in the femora of *Apodemus flavicollis* from the area of Novaky in comparison with the data published by Damek-Poprawa and Sawicka-Kapusta [11] for the rodents caught in polluted region Bukowno (Poland). On the other hand, Pb and Zn concentrations were higher in their study. In comparison with our previous study [17], higher concentrations of Cd, Ni, Fe, Cu, and Zn were detected in the bones of yellow-necked mice from the area of Kolinany (relatively polluted region in Nitra district which is located approximately 100 km far from the town Novaky). The animals living near the site of the coal power station Novaky disposed only higher concentration of Pb. One possible reason for this phenomenon might be intensive agricultural production and the use of chemicals that is characteristic for the whole region of Nitra. There is also a possibility of fallout of dust transported in the air from big industrial regions such as Bratislava, Vienna, Budapest, or factories nearby the Nitra region. This hypothesis may also be confirmed by studies indicating the possibility of the long range transportation of toxic elements [18]. The concentration of Pb in the femora of *Clethrionomys glareolus* was lower in comparison with the one from the study by Milton et al [10]. On the contrary, Zn and Cd concentrations in the bone were higher in our study.

According to Pokarzhevskij [19] the concentration in animal organism (also in bones) of a given element is practically directly proportional to its contents in the food. The *Apodemus* food could be determined like the "purest" in comparison with all other zoomonitors. This mouse feeds mainly on seeds and fruits. The green parts of the plants which are more dusted are present in lower degree in its feeding. Really, heavy metal concentration in liver of *Apodemus* is in the last place. However, the body heavy metal loading is relatively high [6]. It may be due to lower excretion by the kidneys. It is possible that this factor results in the greater sensitivity of *Apodemus* to heavy metal pollution. On the contrary, the voles are characterized by good excretion and therefore lower contaminants remaining in the organism. But the voles feed mainly on the green parts of the plants. There are polluted in the greatest degree like more accessible to the precipitations, atmosphere dust, etc. Also, seeds and roots are present in the food of bank vole.

It is interesting to note the observations of Sawicka-Kapusta et al [20] who have investigated heavy metal content in rodents populating industrial polluted forests in southern Poland. The authors have recorded that Cd, Pb, Cu and Zn concentrations in *Apodemus flavicollis* are significantly lower than those in *Clethrionomys glareolus*. The same correlations have been established in the study by Metcheva et al [6] who studied heavy metal contentration in the liver and body of small terrestrial mammals living in different Bulgarian regions. These results are in agreement with the data obtained for Central Europe [21]. In our study, higher concentrations of Cd, Ni, Cu and Zn have been detected in the bones of bank vole as compared with yellow-necked mouse. Therefore, we presume that *Clethrionomys glareolus* may also be considered as more bone loaded zoomonitor in comparison with *Apodemus flavicollis*.

In conclusion, our contribution is a pilot study about accumulation of selected heavy metals in the bone of small terrestrial mammals living near the site of the coal power station Novaky. Further research in this direction will need to extend the number of

analysed skeletal elements and to verify the results that were obtained from our skeletal samples.

Conclusions

Accumulation of selected heavy metals in the femora of small terrestrial mammals (*Apodemus flavicollis* and *Clethrionomys glareolus*) living in polluted region in Slovakia was studied. We observed higher concentrations of Cd, Ni, Cu and Zn in the bones of *Clethrionomys glareolus*. Significant differences were observed for the concentrations of Cd, Ni and Zn ($p < 0.05$). On the contrary, higher concentrations of Pb and Fe were detected in the femora of *Apodemus flavicollis*. However, the differences were not significant. According to our results bank vole may be considered as more bone loaded zoomonitor in comparison with yellow-necked mouse.

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KUMULACJA WYBRANYCH METALI CIĘŻKICH W KOŚCI UDOWEJ DROBNYCH SSAKÓW LĄDOWYCH

Abstrakt: Zbadano kumulację ołowiu, kadmu, żelaza, niklu, miedzi i cynku w kości udowej myszy leśnej (*Apodemus flavicollis*) i nornicy rudej (*Clethrionomys glareolus*) zasiedlających tereny w pobliżu elektrowni Novaky na Słowacji. Zawartość metali ciężkich w kościach zmierzono metodą spektrofotometrii atomowej. Przebadano 20 kości udowych podchodzących od dorosłych osobników. Większe zawartości Cd, Ni, Cu i Zn stwierdzono w kościach nornicy rudej niż w kościach myszy leśnej. Istotne statystycznie różnice między badanymi ssakami dotyczyły zawartości Cd, Ni i Zn ($p < 0.05$). Z drugiej strony kości myszy leśnej zawierały więcej Pb i Fe niż kości nornicy rudej. Różnice te nie były jednak istotne statystycznie. Uzyskane wyniki wskazują, że kości nornicy rudej kumulują więcej metali ciężkich niż kości myszy leśnej, co może mieć znaczenie dla przyszłych badań monitoringowych.

Słowa kluczowe: metale ciężkie, kości, mysz leśna, nornica ruda, środowisko

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CONTAMINATION OF POTATO TUBER (*Solanum tuberosum* L.) BY NICKEL AND COPPER

ZANIECZYSZCZENIE NIKLEM I MIEDZIĄ BULW ZIEMNIAKA (*Solanum tubersosum* L.)

Abstract: The content of toxic microelements is one of the hygienic-toxicological factors of the foodstuffs quality. Lead, mercury, arsenic and cadmium belong to the most important toxic elements. Also the essential elements (minor ones – Fe, Zn or trace elements – Cr, Cu, Ni, Se) occurring in higher concentrations could have toxic effects. Copper and nickel belong to the essential elements, intake of which organisms have to take from food in certain amount, in order to provide its important biological functions. Most of foodstuffs contain less than 10 mg Cu · kg^{-1} (potatoes 0.3–0.1 mg · kg^{-1}), the nickel content in fruit, cereals and foodstuffs of animal origin (except some sea animals) is very low – hundredths to decimals mg · kg^{-1} (potatoes 0.01–0.26 mg Ni · kg^{-1}).

Keywords: potatoes, heavy metals, contaminations

Potatoes belong among staple food of global citizens. Furthermore, they have dimensional and saturating functions in human nutrition, they are also the source of mineral matters and vitamins (B_1, B_2, C, folic acid). Nowadays potatoes are cultivated in the area ca 19.5 mil. hectares, while China is the greatest producer (Table 1).

Table 1

The greatest producers of potatoes in 2006–2007 [1]

Potatoes producers	[ton]	Potatoes producers	[ton]
1. China	72,000,000	6. Germany	1,162,400
2. Russian Fed.	3,727,982	7. Poland	1,036,900
3. India	2,500,000	8. Belarus	818,501
4. Ukraine	1,946,240	9. Netherlands	677,700
5. USA	1,909,750	10. France	668,082

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At the beginning of 90ties in Slovakia there was continuous decline of cultivation acreage and the absolute minimum was recorded in 2006, with the acreage declined on 18.400 hectars and reproducing areas declined even on 760 ha. In 2007 the cultivation areas have remained on last-year values, but minimally have increased the reproducing areas (ca by 200 ha) [2].

The important factor for producers and consumers of potatoes is besides the tubers yield also external and inner quality, including its food safety, which is given also by the content of foreign substances in flesh. Heavy metals are important inorganic contaminants entering into food chain.

Regular long-termed survey of heavy metals in different international projects has showed on important increase of their concentration in soil, especially in city and industrial areas. Soil is also starting place of their enter into the plants and afterwards into food chain. Enhanced contents of heavy metals in food chain can affect significant health consequences. Obviously, hazardous ones are metals, which are accumulated in human body [3].

The occurrence of toxic elements and of chemical substances in the environment, in raw materials of plant and animal origin causes the consequence worse total hygienic quality of chosen foodstuffs, what is reflected in final point on bad health conditions of the consumer [4].

So the best solution is to prevent food chain from contamination in the beginning. If the soil contamination had originated, it is possible to monitor it or to try to eliminate its consequences [5].

The influence of heavy metals on the environment is emphasized by their persistence. The occurrence of heavy metals in plants is connected with their presence in soils [6].

Biologically essential microelements belong among heavy metals (eg Cu, Zn, Mn, Co, Cr, etc.) as well as many non-essential chemical elements (Cd, Pb, Hg, etc). They occur in soils in various concentrations, oxidation degrees and in bonds. Their risks lie in ecotoxicity and in accumulation in biotic and abiotic environmental components. Toxic ones are also biological essential microelements, when exceeding certain concentration.

The most hazardous are those elements having relative low presence in ecosystems and low border of toxicity. Metals, unlike of organic substances which have been degrading in the environment by influence of continuous activity of bacteria and fungi, by chemical degradation, are resistant to these processes and even in some cases soil microorganisms and bacteria in waters enable toxic metals to enter into complexes with organic compounds and thus change or even multiply their toxicity. It is necessary to deal with negative influence of risky elements in connections with emission situation (atmospheric gradients) [7].

Content of heavy metals in plants depends on their concentration and transfer in soil. Their transport is dependable mainly on physical and chemical soil properties [8]. The mobility or immobility of heavy metals are influenced by following parameters: soil reaction, organic matter, mineral composition, the content of oxides Fe and Mn [9].

The interaction of the amount of heavy metals in soil affect the amount of heavy metals absorbed by plants [10].

Copper belongs among elements which are essential for man, but on the other hand they are potentially toxic. Deficit of copper in human body is very rare. It is relatively frequent industrial exposure to steams of copper or dust aerosols from the standpoint of toxicity, but numerous observations of health state of workers have not detected any symptoms of chronic damages of organism.

Copper is essential element for plants. It has obvious importance in metabolic processes of the plants, because its content in plant tissues is very low and does not reach the concentrations of zinc and manganese. The copper ions form complexes with proteins and other biopolymers in plant tissue. Copper content in plants is ranging from 1 to 50 $\mu g \cdot g^{-1}$ of dry matter of tissue. Toxic effects of copper for plants can occur when the concentration is enhanced in soil after the application of fungicides.

Copper can be concentrated in mineral soil fraction, more rich are the soils containing the oxides of manganese or the mixtures rich in organic compounds. The copper content in soil is very variable. The greatest range of values is in brown soil on chalk sandstone, terraces and slopes and on non-limestone niveau deposits. Balanced set of values is gained in chernozem illimerized on loesses and in meadow soils on lime deposits.

Nickel is in line of crust composition on the 24th place, so it is not the element with abundance occurrence. Nickel is the essential element for plants and some animals.

For its low absorbing from digestive system nickel is similarly as zinc, manganese and chromium (besides Cr^{VI}) relatively less toxic. The most important consequence mostly of long-timed work-related exposure of nickel is the incidence of man's lunge cancer, nasal cavity and rarely larynx. From the standpoint of carcinogenic effects compounds of nickel sulfide and oxide are the most dangerous ones [11].

Concentration range of nickel in soils varies widely and often is in range from 1 to 300 $mg \cdot kg^{-1}$. Average values are in a range 30–80 $mg \cdot kg^{-1}$. Also extreme high contents of nickel can occur (100–7000 $mg \cdot kg^{-1}$) [12]. When nickel is present in high concentration in soil, then it is toxic for plants.

On the basis of the highest and the lowest content of four most hazardous risky elements – cadmium, mercury, lead and arsenic the scale of contamination line had been done by [13] by eight crop species, while potatoes were set on the fourth place.

Material and methods

Soil. Soil samples were taken from the site Stara zem with the acreage 62.5 ha, located in cadastre area of Imel village, between the flows of Nitra and Zitava rivers. Localisation coordinates of the site are 47°54.221′ of northern latitude and 18°10.123′ eastern longitude. Bonitation soil-ecological unit of this area is 0040001, soil type: black chernozem – carbonated, soil type: light-sandy.

Plant. The tested crop was potatoes tuber (*Solanum tuberosum* L.) in six cultivars: Volumia, Adora (very early), Vivaldi, Liva, Courage (early), Victoria (late). Potatoes were harvested in the ripeness of consuming.

The sampling sites determination (soil, plant) was done by covering of borders of the key site by raster, their distances inside the site presented sampling sites. Site

borders were gained with navigation apparatus GPS MAP 60 Cx GARMIN (GPS). After data transfer about position and above sea level into the program OziExplorer the borders were adapted and covered by raster with density of lattice of 6 seconds. Sampling places with the accuracy ±2 meters were determined with GPS. The site borders were defined by 149 points, their above sea level ranged from 105.8 to 118.0 ma.s.l. Sampling sites and varieties of potatoes are presented in Figure 1.

Fig. 1. Sampling sites and the potatoes varieties

After localization of sampling point we had done taking of the soil in this place by valid methods from two horizons (A: 0–0.2 m; B: 0.3–0.45 m) with pedological sampler GeoSampler fy. Fisher.

The content of available nutrients (P, K, Ca, Mg) in soil was determined by the Mehlich II method, the content of nitrogen by Kjeldahl method, for the agrochemical characteristics of soil we also determined: % humus, active and exchangeable form of pH (pH/H_2O; pH/KCl).

In soil samples various forms of **nickel** and **copper** were assessed in the following extracts:

– in extract of *aqua regia* – determination of pseudototal content of heavy metals – includes all their forms except of residual fraction of metals

– in soil extract HNO_3 (c = 2 mol $\cdot dm^{-3}$) – determination of so-called potential mobilizable forms of heavy metals in soil,

– in soil extract NH_4NO_3 (c = 1 mol $\cdot dm^{-3}$) – determination of mobile forms of heavy metals

Plant material was collected from the same sites as the soil.

Copper and nickel content were determined in potato tubers mineralized by dry way with AAS method on atomizer Pye Unicam SP9.

Results and discussion

The content of nutrients determined in the soil samples ranged from 1050–5250 mg N $\cdot$ kg^{-1}, 45.1–636.4 mg P $\cdot$ kg^{-1}, 146.5–647.5 mg K $\cdot$ kg^{-1}, 800–22,450 mg Ca $\cdot$ kg^{-1},

Table 2

Contents of nutrients and of humus in soil taken from 2 soil horizons (A, B)

Sampling site	Nutrients content [mg · kg^{-1}]										% Hum.	
	N		P		K		Ca		Mg			
	A	B	A	B	A	B	A	B	A	B	A	B
1	3938	6475	117.7	121.4	359.0	399.0	3790	3950	46.4	39.2	2.28	2.35
2	5250	4900	127.5	114.4	146.5	156.5	1575	1955	19.5	17.2	1.64	1.21
3	1225	1400	68.4	86.7	298.0	209.5	22450	23350	74.0	85.4	1.92	2.06
4	2275	2800	265.7	310.7	632.0	608.5	1235	1510	29.0	34.0	1.92	1.92
5	2100	2100	165.3	147.8	376.5	355.0	3655	3645	50.7	46.6	2.78	2.49
6	1575	2100	182.1	146.9	238.0	232.5	2615	2055	29.4	24.4	2.28	1.85
7	1925	2100	138.7	145.1	373.5	589.5	2180	2530	29.0	40.4	2.56	2.63
8	1925	1750	90.1	95.5	282.0	342.5	16075	15925	111.7	110.3	2.35	2.99
9	2450	4025	100.7	112.9	427.0	501.0	3630	3685	37.3	38.5	3.06	2.70
10	4025	2975	94.9	103	287.5	302.0	2005	1725	48.5	42.2	2.70	2.99
11	4025	4025	131.6	247.3	387.5	317.5	4660	5240	47.1	49.5	3.37	2.93
12	2975	4550	169.3	262.3	366.0	372.5	4295	4665	64.5	78.5	2.49	3.15
13	3500	3413	231.4	196.1	647.5	466.0	1985	2260	53.9	59.4	3.45	3.30
14	4375	5075	87.1	70.4	203.5	288.0	25400	29800	198.0	212.0	2.42	3.30
15	4375	3500	125.2	146.6	321.0	355.5	5635	5505	36.9	36.4	2.85	2.99
16	3500	3325	165.5	127.9	334.5	414.0	5525	6000	48.2	55.7	3.34	2.71
17	3850	4025	145.7	155.3	297.0	268.0	3360	3720	78.1	80.9	3.77	3.77
18	4550	4025	329.6	337.8	327.0	340.0	5625	5690	46.0	49.1	2.35	2.21
19	4725	4375	240.9	202.9	361.5	331.5	10675	13080	80.2	95.7	2.92	2.85

Table 2 contd.

Sampling site	Nutrients content [mg · kg^{-1}]										% Hum.	
	N		P		K		Ca		Mg			
	A	B	A	B	A	B	A	B	A	B	A	B
20	3500	4025	233.3	218.7	420.0	362.5	3715	3350	40.0	34.5	2.63	2.06
21	2800	3675	636.7	568.6	604.0	640.5	800	920	31.5	38.7	3.01	2.05
22	2975	3150	156.1	124.5	218.5	217.5	1540	1530	41.5	41.0	2.35	2.27
23	2100	875	167.9	173.4	384.5	395.5	6600	7475	86.7	92.8	3.45	3.45
24	1050	1050	347.8	353.8	402.5	386.5	6265	5910	53.7	51.8	2.71	2.64
25	1400	1400	248.7	210.1	360.5	449.0	1860	1795	55.6	53.4	3.08	2.86
26	2450	3850	140.9	130.9	245.5	280.0	2050	2435	43.4	48.6	3.08	2.86
27	3325	4200	45.1	42.5	189.5	136.5	20880	16500	77.5	59.6	2.71	3.67
28	5250	350	266.7	359.4	228.5	422.0	1220	1420	32.4	42.1	1.78	1.92
29	1750	3150	126.2	127.5	246.5	208.5	965	950	24.9	28.4	1.49	1.35
30	1750	3850	129.8	139.1	455.5	493.5	4780	4075	77.8	70.5	3.34	3.34

19.5–198.0 mg Mg · kg^{-1}. Exact values for individual sampling sites and A and B horizons are shown in Table 2. The gained results showed that soil had high phosphor content, low magnesium content and high content of potassium.

According [14], potatoes require soil with humus content over 2 %. All samples of soils with the exception of sampling sites 2, 3, 4, 28, 29 (Table 2) correspond to this requirement. The soil reaction should be in the range between values from pH 5.5 to 6.5, high and stabile yields of potatoes are reached when keeping the soil reaction in weakly acid area (pH 6.7) [15]. Assessed values of active form pH ranged from 6.44 to 8.70 and of exchangeable form pH ranged from 5.26 to 7.90 (Fig. 2), so it is neutral to weakly alkaline reaction, but it need not to lead to reduced production from the standpoint of potatoes cultivation.

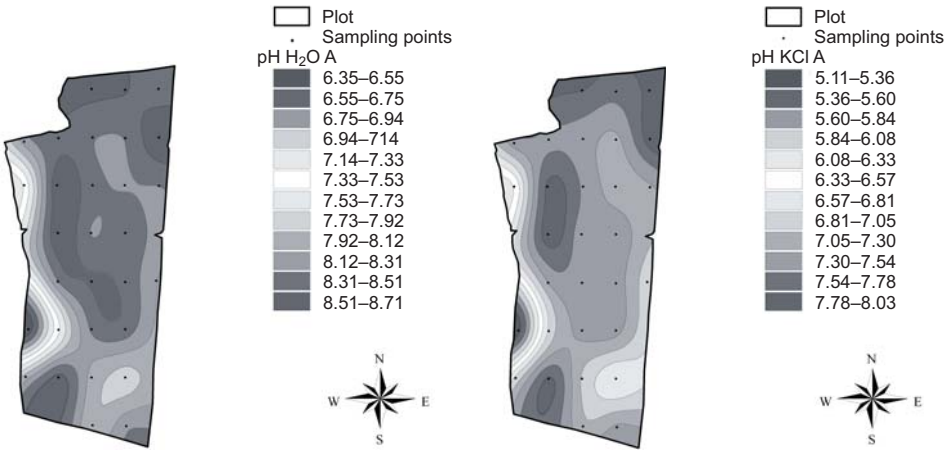

Fig. 2. Izoline maps illustrating values of pH/H_2O and pH/KCl of soil taken from A horizon

The contents of Cu and Ni in soil [mg · kg^{-1}] assessed in different extracts are presented in Table 3. The highest value determined in the extract of *aqua regia* is for Ni in A horizon 22.2 mg · kg^{-1} and B horizon 20.8 mg · kg^{-1}, for Cu 27.6 mg · kg^{-1} (in both horizons). In soil extract HNO_3 the highest content of Ni is 7.5 mg · kg^{-1} (A horizon) and 7.8 mg · kg^{-1} (B horizon), the highest content of Cu 14.6 mg · kg^{-1} (A horizon) and 12.1 mg · kg^{-1} (B horizon). In soil extract NH_4NO_3 the highest Ni content is 0.195 mg · kg^{-1} (A horizon) and 0.220 mg · kg^{-1} (B horizon), the highest content of Cu 0.175 mg · kg^{-1} (in both horizons). When the higher content of risky elements in soil occur, than their limit values, it can not mean their transfer into cultivated crops, but when the limit is exceeded this factor is significant. In contrary their content in soil below limit values will not guarance that the plants cultivated on this soil will contain tolerable amount. That is why from the hygienic standpoint it is determining, if these elements accumulate in edible parts used for consume [7].

The content of Ni assessed in potato tubers after their mineralization by dry way method is the highest one in the sample from sampling site no. 7 (0.1223 mg · kg^{-1} of

Table 3

Nickel and Copper content in soil [mg · kg^{-1}] assessed in various extracts

Sampling site	*Aqua regia*				HNO_3				NH_4NO_3			
	Ni		Cu		Ni		Cu		Ni		Cu	
	A	B	A	B	A	B	A	B	A	B	A	B
1	21.8	15.2	12.2	11.2	5.4	5.7	7.6	7.8	0.100	0.100	0.080	0.090
2	11.4	12.2	8.6	7.6	3.0	2.9	4.4	4.1	0.085	0.080	0.075	0.075
3	19.0	17.8	10.0	10.2	6.5	6.7	5.7	5.9	0.120	0.140	0.115	0.110
4	11.2	14.0	12.6	16.4	2.9	3.6	10.8	10.9	0.095	0.095	0.175	0.175
5	18.0	16.4	14.2	13.4	5.7	5.2	10.0	9.0	0.125	0.110	0.085	0.070
6	12.6	14.0	11.0	9.2	3.1	3.1	6.8	5.9	0.115	0.100	0.075	0.060
7	17.8	18.4	21.2	14.2	5.7	5.7	8.6	8.4	0.090	0.095	0.070	0.055
8	18.6	20.4	9.2	13.6	6.0	6.0	8.2	8.3	0.100	0.110	0.070	0.070
9	17.8	18.0	12.4	12.4	7.4	7.3	7.8	7.7	0.135	0.145	0.080	0.085
10	16.6	15.4	13.4	10.6	6.7	5.5	7.5	6.2	0.100	0.085	0.060	0.075
11	19.6	14.6	12.4	11.4	7.0	7.2	8.3	8.1	0.135	0.155	0.095	0.100
12	16.6	19.6	13.4	16.2	5.5	6.7	9.7	11.3	0.120	0.170	0.085	0.120
13	17.6	17.8	15.8	15.2	6.1	6.1	11.2	10.6	0.125	0.085	0.115	0.085
14	11.8	20.6	8.8	12.4	6.1	7.7	7.4	8.6	0.110	0.140	0.095	0.125
15	20.6	19.4	13.2	12.8	7.5	7.5	7.8	7.9	0.135	0.150	0.110	0.100
16	19.6	20.0	13.4	14.4	6.7	6.8	7.7	7.7	0.135	0.160	0.055	0.065
17	19.2	20.0	27.6	27.2	7.4	7.6	11.8	12.1	0.170	0.220	0.065	0.080
18	11.2	12.2	13.8	22.2	6.2	6.3	9.0	9.0	0.135	0.125	0.065	0.060
19	22.2	20.8	26.4	27.6	6.9	6.9	11.6	11.1	0.165	0.180	0.080	0.070

Table 3 contd.

Sampling site	*Aqua regia*				HNO_3				NH_4NO_3			
	Ni		Cu		Ni		Cu		Ni		Cu	
	A	B	A	B	A	B	A	B	A	B	A	B
20	19.6	14.4	18.8	20.2	5.0	3.8	8.2	6.2	0.115	0.110	0.055	0.055
21	10.6	9.6	20.8	13.8	3.4	3.5	14.6	9.9	0.130	0.110	0.055	0.075
22	6.6	11.8	5.6	10.6	4.2	4.3	6.8	7.0	0.105	0.095	0.040	0.025
23	17.0	19.2	17.4	17.0	7.5	7.8	11.9	11.7	0.195	0.170	0.110	0.075
24	19.2	18.8	13.6	24.4	7.2	7.2	10.5	10.2	0.155	0.170	0.085	0.080
25	15.8	16.8	25.6	25.6	6.6	6.5	10.1	10.0	0.125	0.135	0.050	0.050
26	14.2	12.4	19.6	19.2	4.9	5.1	7.1	7.4	0.130	0.135	0.050	0.060
27	15.8	14.4	16.4	18.2	5.8	6.1	6.5	6.6	0.155	0.155	0.075	0.070
28	7.0	11.4	12.8	22.2	3.0	3.8	7.3	9.0	0.095	0.095	0.060	0.060
29	3.4	9.0	3.8	7.6	3.0	3.1	3.9	4.1	0.105	0.070	0.035	0.030
30	15.2	12.8	13.0	11.0	7.2	7.3	9.0	9.1	0.155	0.170	0.090	0.085

Table 4

The content of Ni and Cu in tubers of potatoes [mg · kg^{-1}] fresh mater

Sampling site	Variety	Ni	Cu	Sampling site	Variety	Ni	Cu	Sampling site	Variety	Ni	Cu
1	Adora	0.029	0.088	11	Vivaldi	0.042	0.085	21	Courage	0.059	0.121
2	Vivaldi	0.016	0.065	12	Vivaldi	0.016	0.110	22	Victoria	0.020	0.105
3	Vivaldi	0.031	0.118	13	Vivaldi	0.040	0.078	23	Liva	0.073	0.178
4	Vivaldi	0.029	0.104	14	Victoria	0.019	0.142	24	Liva	0.021	0.099
5	Vivaldi	0.016	0.090	15	Vivaldi	0.018	0.084	25	Liva	0.025	0.148
6	Vivaldi	0.018	0.095	16	Vivaldi	0.025	0.118	26	Liva	0.015	0.078
7	Vivaldi	0.122	0.064	17	Liva	0.014	0.102	27	Victoria	0.041	0.063
8	Adora	0.044	0.073	18	Liva	0.015	0.117	28	Courage	0.021	0.112
9	Adora	0.018	0.085	19	Liva	0.017	0.114	29	Liva	0.032	0.095
10	Vivaldi	0.015	0.059	20	Victoria	0.016	0.095	30	Liva	0.030	0.090

fresh matter), content of Cu is the highest one in the sample from sampling site no. 23 (0.178 mg · kg^{-1} fresh matter). Results of assessment are presented in Table 4. None of the contents of Ni and Cu was higher than legislative limits. The highest acceptable amounts defined by Foodstuffs Codex of Slovak Republic are for Ni 0.5 and for Cu 3.0 mg · kg^{-1} of fresh matter [16].

Conclusions

The advantage of using of navigation system GPS by samples taking is the accuracy with which it is possible from the same sampling site to take repeatedly soil samples and plant material with certain time period and also after some years with minimal deviation and thus to observe possible changes and trends in contents of key elements, respectively possible contamination of soil.

Soil samples from the site located on cadastre area of Imeľ village do not contain enhanced contents of heavy elements Ni and Cu, the limit value for pseudototal content was not exceeded (Ni 40 mg · kg^{-1}, Cu 30 mg · kg^{-1}), for mobile forms (Ni 1.5 mg · kg^{-1}, Cu 1.0 mg · kg^{-1}) [17] and for potential mobilizable forms (Ni 10 mg · kg^{-1}, Cu 20 mg · kg^{-1}) [18]. It could be concluded that these soils are not contaminated.

Assessed contents of Ni and Cu in tubers of potatoes taken from the same sampling sites are lower than the highest acceptable amounts defined in Foodstuffs Codex SR. From the standpoint of the content of these two metals is the cultivation of the potato tuber in key locality without any risk. For the total evaluation of the safety it is very important to monitor also the contents of others risky metals which can often have not only antagonistic, but also synergic effect.

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ZANIECZYSZCZENIE NIKLEM I MIEDZIĄ BULW ZIEMNIAKA (*Solanum tubersosum* L.)

Abstrakt: Zawartość toksycznych mikroelementów jest jednym z ważnych parametrów jakości żywności. Ołów, rtęć, arsen i kadm należą do najważniejszych toksycznych pierwiastków. Mikroelementy (Fe, Zn, Cr, Cu, Ni, Se) występujące w dużych stężeniach również mogą mieć działanie toksyczne. Miedź i nikiel zaliczane są do mikroelementów, które organizm wchłania z pożywienia w ilościach niezbędnych do podtrzymania wielu procesów biologicznych. Większość pokarmów zawiera Cu w ilości nie przekraczającej 10 mg · kg^{-1} (ziemniaki 0,3–0,1 mg · kg^{-1}). Zawartość niklu w owocach, płatkach zbożowych i pokarmach pochodzenia zwierzęcego (z wyjątkiem niektórych zwierząt morskich) jest bardzo mała – od setnych do dziesiątych mg · kg^{-1} (ziemniaki 0,01–0,26 mg · kg^{-1}).

Słowa kluczowe: ziemniaki, metale ciężkie, zanieczyszczenie

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DISTRIBUTION OF DIAZINON AND SELENIUM IN VARIOUS TISSUES AFTER SINGLE AND COMMON INTRAPERITONEAL ADMINISTRATION

DYSTRYBUCJA DIAZINONU I SELENU W WYBRANYCH TKANKACH PO POJEDYNCZYM PODANIU DOOTRZEWNOWYM

Abstract: The aim of this study was to evaluate the distribution of diazinon and selenium in various tissues of laboratory rats after single and common intraperitoneal administration. Rats in the age of 135 days were randomly divided into 4 groups. Each group consisted of 10 males. Animals in the first group were administrated with diazinon 20 mg · kg^{-1} body weight intraperitoneally. Animals in the second group were administrated with selenium (Na_2SeO_3) 2 mg Se · kg^{-1} body weight intraperitoneally in physiological solution. Animals in the third group were given a mixture of diazinon 20 mg · kg^{-1} body weight and selenium 2 mg Se · kg^{-1} body weight intraperitoneally in physiological solution. The fourth group served as a control group and was administrated only with the physiological solution. 24 hours after the administration of tested substances, animals were sacrificed and samples of livers, kidneys, muscles and fat tissue were taken during the autopsy. The amount of diazinon in tissues was determined using gas chromatography with mass spectrometry. The amount of selenium was determined using atomic absorption spectrometry. We detected significant increase of selenium amount in livers, kidneys and muscles after single selenium administration and also after common administration of both substances. On the other hand we detected significant decrease of selenium amount in muscles and fat tissue after single diazinon administration. We also observed slight accumulation of diazinon amount in samples of kidneys and muscles and significant increase of diazinon in fat tissue after single diazinon administration and also after common administration of both substances.

Keywords: diazinon, selenium, liver, kidney, muscle, fat, intraperitoneal administration

Diazinon (O,O-diethyl O-2-isopropyl-6-methylpyrimidin-4-yl phosphorothioate) belongs to the group of organophosphate insecticides, used to control cockroaches, fleas and ants. It is also used to control a wide variety of sucking and leaf eating insects. It is used on rice, fruit trees, sugarcane, corn, tobacco, potatoes and on horticultural plants. Diazinon has veterinary use against fleas and ticks. Organophosphorus pesticides, including also diazinon have harmful effect on nervous system through the inhibition of

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acetylcholinesterase. Acetylcholinesterase is an enzyme, which is necessary in process for controlling of nervous signals transfer. Lack of acetylcholinesterase causes accumulation of acetylcholine. Accumulation of this neurotransmitter on the connections between nerves and muscles causes uncontrolled muscular contraction and algospasmus, between nerves and glands causes continual secretion of these glands, while acetylcholine cumulation between certain nerve cells in a brain causes sensory behavior disorders [1–3]. Most frequently described symptoms of acute diazinon toxicity are headaches, nausea, vertigo, blurred vision, feeling of pressure in chest, respiration problems, muscular weakness or convulsions, diarrhea and vomiting. Typical symptoms of irritated nervous system are confuse, anguish, melancholy and insomnia. Symptoms of chronic poisoning are always connected with depression of cholinesterase activity [2, 4].

Diazinon could be frequently found in wide range of various fruit and vegetable species, including apples, pears, cereals, soya, strawberries, tomatoes, beans [2]. During a survey that was focused on monitoring of pesticides amounts in 21 various species of fruit were analyzed 150 samples for the presence of diazinon. Results of this study show that 90 % of analyzed samples did not contain any diazinon, 1 % of samples was very close to the detection limit, 8 % of samples were positive without exceeding of valid legislation limits for maximal content of diazinon and 1 % of all analyzed fruit samples contained diazion in amount that was over the maximal legal limit for the presence of this pesticide [5].

Diazinon does not belong to the substances that are typical for its high cumulative ability in animal tissues and it is not found in the food of animal origin very frequently. However, certain amounts of this substance could be found in a fat tissue in some cases. This tissue has certain ability to cumulate diazinon during relatively short time period. This observation was found also in the cattle. Diazion was applied on the skin of tested animals in the form of a spray. Till 14 days, substance was completely excreted from the organism of tested animals. Aplication of diazinon on the skin of these animals caused also the presence of this substance in their milk during 24 hours after application [6, 7].

Residuals of this pesticide are commonly found in a sheep fat in Great Britain. In the year of 1999, food authorities in this country revealed presence of diazinon residuals in 20 samples of kidney fat. Amounts of diazinon varied from 21 $\mu g \cdot kg^{-1}$ to 150 $\mu g \cdot kg^{-1}$ [8].

Selenium is a typical biogenous element, which is necessary for all living organisms. However, selenium could act also as a toxic element, especially when it is present in organism in the surplus. Due to this fact selenium was considered to be strictly toxic and scientists did not deal with the benefits of selenium. Selenium essentiality was scientifically proven for the first time in 1957 [9]. Selenium is widely used in various industries, mostly in glass industry and electronic industry and it has been frequently used also as a part of inorganic pigments [10].

The selenium content in plants is affected by the content and availability of this element in soil in which they are grown and also varies from country to country, while the mineral composition of flesh also reflects the feeding patterns of livestock [11]. The most solid source of selenium in human nutrition is a food of animal origin, especially eggs, meat and sea food. Lower levels of selenium are usually in cereal crops and the lowest levels of this element are present in fruit and vegetable [12–14].

Elementary selenium does not have any function in living organisms and it is not absorbed by gastrointestinal tract of humans and animals. All of the known effects of selenium are accomplished through the specific selenoenzymes, or selenoproteins as are for example selenocysteine, selenomethionine, or some other selenium compounds. Selenoenzymes protect cells against oxidative damage and act also the important role in the metabolic processes of the living organism through the changing of thyroid gland hormone thyroxine to its biological active form triiodothyronine. It has been proven that various selenium compounds have also protective effect against certain kinds of a cancer. In spite of long term research, the mechanism of selenium caused protective effect against cancer diseases is still not cleared [15–17]. Humans are probably not so sensitive for effect of increased amounts of selenium in comparison with animals. Irritation of eyes, nose and throat, digestive difficulties, vomiting, increased body temperature, sleepiness, psychoneurotical symptoms, convulsion and death as the result of interrupted respiration are typical symptoms of acute selenium poisoning. Chronic toxicity of selenium compounds is usually linked with airways inflammation, pulmonary oedema, hemorrhage, dermatitis, dedentition, arthtritis, depilation, headaches, psychoneurotical symptoms and paralysis. [10, 18, 19]. A lot of studies have mentioned that effects of various toxic compounds are dependent not only on their dose but also on mutual interactions with other compounds [20–22].

Material and methods

Experimental animals

Experiment took place in the accredited breeding and experimental laboratory in the Department of Veterinary Disciplines of the Slovak Agricultural University. Laboratory rats (*Rattus norvegicus* sp.) had been chosen as the experimental animal. Animals were fed with the special feed pellets for laboratory mice and rats *ad libitum* and they were also given drinking water. Rats were housed in plastic cages and exposed to 12-h light : 12-h dark cycle, at room temperature of 18–22 ^{o}C.

Chemicals

Diazinon, purity 99 %, was obtained from Sigma-Aldrich, USA. Sodium selenate(IV), purity 98 %, was purchased from the same company.

Animal treatment schedule

Rats in the age of 135 days (weighting approximately 410 g) were randomly divided into 4 groups. Each group consisted of 10 males. Animals in the first group were administrated with diazinon (Sigma, USA) 20 mg · kg^{-1} body weight intraperiotoneally in physiological solution. Animals in the second group were administrated with selenium in the form of Na_2SeO_3 (Sigma, USA) 2 mg Se · kg^{-1} body weight intraperiotoneally in physiological solution and animals in the third group were given a mixture

of diazinon (Sigma, USA) 20 mg · kg^{-1} body weight and Na_2SeO_3 (Sigma, USA) 2 mg Se · kg^{-1} body weight intraperiotoneally in physiological solution. The fourth group served as a control group and was administrated only with the physiological solution. 24 hours after the administration of tested substances, animals were sacrificed and samples of livers, kidneys, muscles and fat tissue were taken during the autopsy.

Determination of diazinon and selenium

The amount of diazinon in tissues was determined using gas chromatography with mass spectrometry and the amount of selenium was determined with atomic absorption spectrometry. The amount of diazinon was not analyzed in the second group (selenium treated group) because dizinon was not applied to laboratory animals in this experimental group.

Statistical analysis

Basic statistical characteristics – arithmetic mean, standard deviation and variation coefficient were calculated for the amount of analyzed substance of each group. Obtained data were then processed in order to determinate statistical significance of the results. The F-test two sample for variances was used to compare the population variances. The Student's t-test (two sample assuming equal variances) was finally used for establishment of statistical significance.

Results and discussion

No deaths were observed in any of groups of experimental animals. However, animals from diazinon treated group approximately 12 hours after the administration of diazinon showed typical symptoms connected with depression of cholinesterase activity and did not react on external stimuli. The same behaviour of experimental animals was observed also after common administration of diazinon and selenium.

Table 1 presents distribution of selenium in different tissues that were obtained after single intraperitoneal administration of selenium. According to our findings, the highest level of selenium was found in the kidneys of experimental rats, lower amount of selenium was detected in livers of experimental rats and the lowest amount of selenium was in muscles and in samples of fat tissue. The amount of selenium in tissues of control animals was always lower, in comparison with tissues of experimental animals. The increasing of selenium amount in tissues of livers, kidneys and muscles in experimental animals was even statistically significant. Our observations (in control group and in experimental group of animals as well) are in accordance with a known fact that selenium amount is naturally highest in the kidney and lowest in the muscle [23]. These findings are easily observable from Fig. 1.

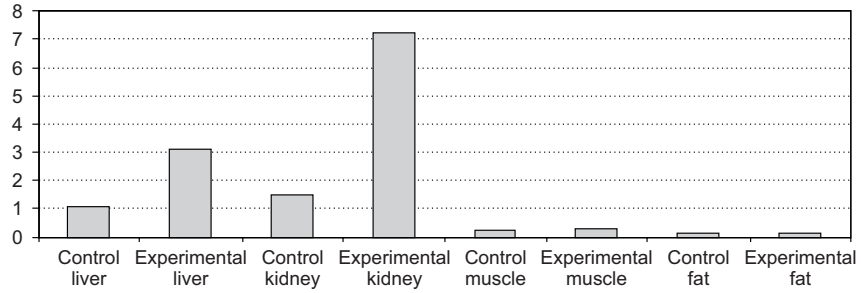

Fig. 1. Amount of selenium in different tissues after single selenium administration [mg · kg^{-1}]

Table 1

Amount of selenium in different tissues after single selenium administration

Tissue	Amount of selenium [mg · kg^{-1}]			
	Control X ± SD	Variation coefficient [%]	Experimental X ± SD	Variation coefficient [%]
Liver	1.085 ± 0.262	24.164	3.105 ± 1.09*	35.116
Kidney	1.527 ± 0.239	15.65	7.235 ± 3.995*	55.218
Muscle	0.25 ± 0.027	10.995	0.304 ± 0.061*	19.931
Fat	0.137 ± 0.048	35.267	0.122 ± 0.062	50.94

X – arithmetic mean, SD – standard deviation, * $p < 0.05$.

Table 2 together with Figure 2 presents results of selenium amount in different tissues after single intraperitoneal administration of diazinon. Results show that amount of selenium in muscles and fat tissue is lower in comparison with control group. Depression of selenium amount in tissues of muscles and fat is statistically significant. This fact could be probably connected with a response of organism on pathological condition [24]. Diazion that was administrated to the organism presumably caused a mobilization of selenium from tissues of muscle and fat. This could happen in order to protect organism of experimental animal against injury [9].

Table 2

Amount of selenium in different tissues after single diazinon administration

Tissue	Amount of selenium [mg · kg^{-1}]			
	Control X ± SD	Variation coefficient [%]	Experimental X ± SD	Variation coefficient [%]
Liver	1.085 ± 0.262	24.164	1.222 ± 0.25	20.481
Kidney	1.527 ± 0.239	15.65	1.615 ± 0.213	13.194
Muscle	0.25 ± 0.027	10.995	0.218 ± 0.03*	13.812
Fat	0.137 ± 0.048	35.267	0.062 ± 0.022*	35.5

X – arithmetic mean, SD – standard deviation, * $p < 0.05$.

On the other hand, the amount of selenium after common administration of selenium and diazinon decreased only in samples of fat tissue however, this depression was not

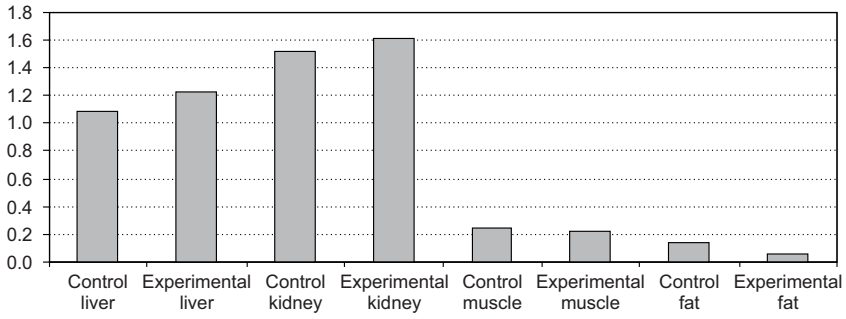

Fig. 2. Amount of selenium in different tissues after single diazinon administration [mg · kg^{-1}]

statistically significant. In this experimental group, we observed significant increase of selenium amount in samples of livers, kidneys and muscles. It means, in our opinion, that dose of selenium was high enough to protect organism against injury [9] and selenium in tissues was not mobilized to increase its status, as it is usual in pathological conditions [24]. Selenium amount in tissues was in accordance with group of animals that was administrated only with single selenium – highest amount of selenium was in kidneys, lower amount of this element was livers and the lowest amount of selenium was in the samples of muscles and fat tissue. These observations are visible in Table 3 and Fig. 3.

Table 3

Amount of selenium in different tissues after common administration

Tissue	Amount of selenium [mg · kg^{-1}]			
	Control X ± SD	Variation coefficient [%]	Experimental X ± SD	Variation coefficient [%]
Liver	1.085 ± 0.262	24.164	3.098 ± 0.779*	25.159
Kidney	1.527 ± 0.239	15.65	6.451 ± 1.966*	30.479
Muscle	0.25 ± 0.027	10.995	0.322 ± 0.032*	9.908
Fat	0.137 ± 0.048	35.267	0.098 ± 0.107	109.67

X – arithmetic mean, SD – standard deviation, * $p < 0.05$.

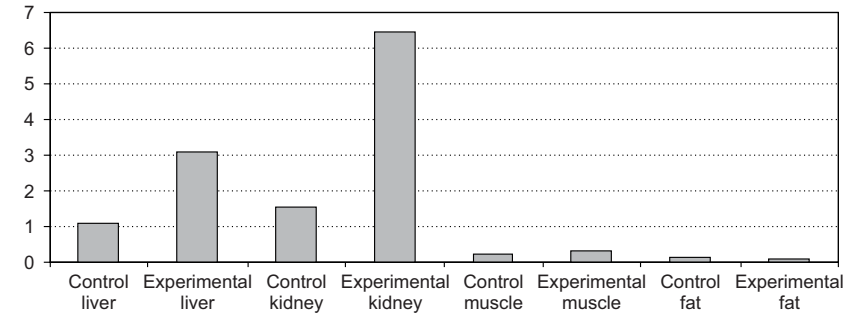

Fig. 3. Amount of selenium in different tissues after common administration [mg · kg^{-1}]

Table 4 and Figure 4 shows diazinon amount in tissues after single administration of diazinon. Amount of diazinon in samples of livers were in all samples below the detection limit. Significant increase of diazinon was detected only in samples of fat tissue. Diazinon does not belong to substances that are characteristic by cumulation in animal organism. However, it is known, that fat tissue is able to cumulate diazinon in certain cases for relatively short period of time [6]. Our finding of diazinon amount in fat (3.717 mg · kg^{-1}) is therefore equivalent to dose that were administrated to organism.

Table 4

Amount of diazinon in different tissues after single diazinon administration

Tissue	Amount of diazinon [mg · kg^{-1}]			
	Control X ± SD	Variation coefficient [%]	Experimental X ± SD	Variation coefficient [%]
Liver	UDT	—	UDT	—
Kidney	UDT	—	0.072 ± 0.036	49
Muscle	UDT	—	0.067 ± 0.041	62
Fat	UDT	—	3.717 ± 3.749*	100

UDT – under the detection limit, X – arithmetic mean, SD – standard deviation, * $p < 0.05$.

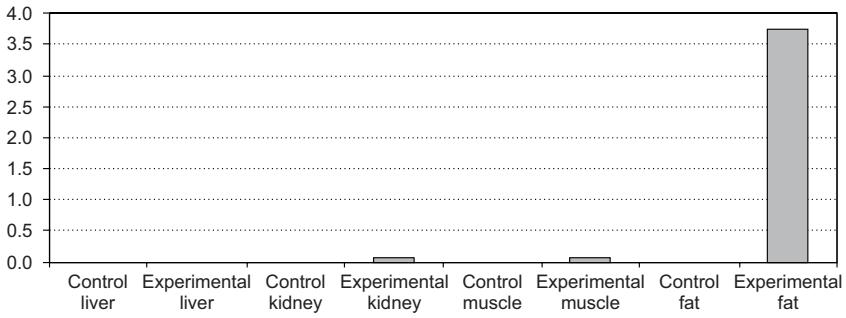

Fig. 4. Amount of diazinon in different tissues after single diazinon administration [mg · kg^{-1}]

Almost the same results were also obtained after common administration of selenium and diazinon. Results from this experimental group are presented in Table 5 and Fig. 5. The only one significant result was the increase of diazinon amount in the samples of fat tissue. However, this increase was not as high as in the previous group after single administration of diazinon. This could be caused by coadministration of selenium because selenium could act a role of protective element against diazinon. This important role of selenium was many times proven in other researches [25–27]. However, mechanisms of interaction between diazinon and selenium are still not cleared and further studies are still required

Table 5

Amount of diazinon in different tissues after common administration

Tissue	Amount of diazinon [mg · kg^{-1}]			
	Control X ± SD	Variation coefficient [%]	Experimental X ± SD	Variation coefficient [%]
Liver	UDT	—	0.0006 ± 0.002	316.228
Kidney	UDT	—	0.063 ± 0.135	212.385
Muscle	UDT	—	0.058 ± 0.119	204.424
Fat	UDT	—	1.339 ± 1.039*	77.592

UDT – under the detection limit, X – arithmetic mean, SD – standard deviation, * $p < 0.05$.

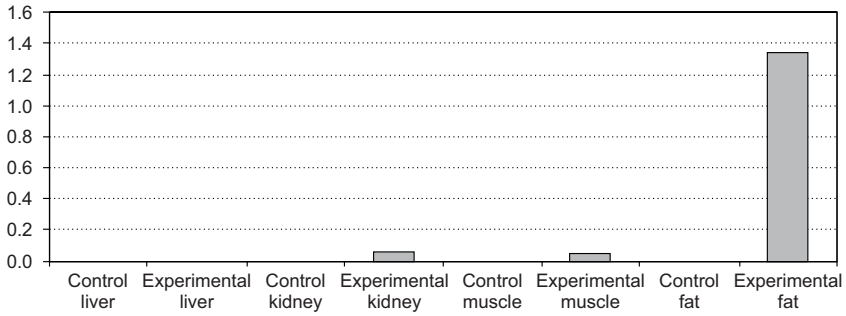

Fig. 5. Amount of diazinon in different tissues after common administration [mg · kg^{-1}]

Conclusions

We detected significant increase of selenium amount in livers, kidneys and muscles after single selenium administration and also after common administration of selenium and diazinon. On the other hand we detected significant decrease of selenium amount in muscles and fat tissue after single diazinon administration. We also observed slight accumulation of diazinon amount in samples of kidneys and muscles and significant increase of diazinon in fat tissue after single diazinon administration and also after common administration of both substances.

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DYSTRYBUCJA DIAZINONU I SELENU W WYBRANYCH TKANKACH PO POJEDYNCZYM PODANIU DOOTRZEWNOWYM

Abstrakt: Celem pracy było określenie dystrybucji dianizonu i selenu w różnych tkankach szczurów laboratoryjnych po pojedynczym dootrzewnowym podaniu. Szczury w wieku 135 dni zostały losowo przyporządkowane do 4 grup. Każda grupa składała się z 10 samców. Zwierzętom z pierwszej grupy podano dootrzewnowo dianizon w dawce 20 mg · kg^{-1} masy ciała. Zwierzętom drugiej grupy podano dootrzewnowo selen w roztworze fizjologicznym (Na_2SeO_3) w dawce 2 Se mg · kg^{-1} masy ciała. Zwierzęta trzeciej grupy otrzymały dootrzewnowo mieszaninę dianizonu (20 mg · kg^{-1}) i selenu (Na_2SeO_3 – 2 Se mg · kg^{-1}) w roztworze fizjologicznym. Czwartą grupę stanowiły zwierzęta kontrolne, którym podano wyłącznie roztwór fizjologiczny. Po 24 godzinach od podania dianizonu i selenu zwierzęta uśmiercono, po czym pobrano próbki wątroby, nerek, mięśni i tkanki tłuszczowej. Poziom dianizonu w tkankach został zbadany przy użyciu chromatografii gazowej oraz spektrofotometrii masowej. Selen oznaczono metodą spektrofotometrii absorpcji atomowej. Stwierdzono istotny statystycznie wzrost poziomu selenu w wątrobie, nerkach i mięśniach po jednorazowym podaniu selenu, a także po podaniu mieszaniny selenu i dianizonu. Z drugiej strony odnotowano również istotny statystycznie spadek zawartości selenu w mięśniach i tkance tłuszczowej po jednorazowym podaniu dianizonu. Zaobserwowano także niewielki wzrost poziomu dianizonu w nerkach i mięśniach oraz istotny statystycznie wzrost zawartości tego związku w tkance tłuszczowej zarówno po jednorazowym podaniu dianizonu, jak i po podaniu mieszaniny dianizonu i selenu.

Słowa kluczowe: dianizon, selen, wątroba, nerki, mięśnie, tłuszcz, podanie dootrzewnowe

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MOLECULAR DIAGNOSTIC OF *Streptococcus thermophilus*

DIAGNOSTYKA MOLEKULARNA *Streptococcus thermophilus*

Abstract: *Streptococcus thermophilus* is one of the most important lactic acid bacteria in the dairy industry. Despite the wide use of *Streptococcus thermophilus* in the industry, data on the phenotypic and genetic strain variations within the species are still limited. Genetic techniques are very useful for molecular discrimination of complex mixtures of starter and probiotic cultures in research laboratories. Detection and identification of various lactic acid bacteria species with rapid methods is often important for quality control of dairy products. This work deals with characterization and differentiation of strains *Streptococcus thermophilus* by PCR, RAPD and SDS-PAGE techniques. Fifteen strains of *Streptococcus thermophilus* from Czech Collection of Dairy Microorganisms (CCDM) and a strain of *Streptococcus thermophilus* from Czech Collection of Microorganisms (CCM) were used. Particular strains were confirmed with primer set THI/THII by PCR method. Consequently, their identities were examined by RAPD and SDS-PAGE techniques. Whereas, primers OPP-7 and RAPD-4, RAPD were used. It can be claimed that mentioned methods are good means for identification and characterization of streptococci.

Keywords: *Streptococcus thermophilus*, PCR, RAPD, SDS-PAGE

Streptococcus salivarius subsp. *thermophilus*, commonly named as *Streptococcus thermophilus* in food industry and throughout this paper, is one of significant species among the diverse group of lactic acid bacteria. *Streptococcus thermophilus* is widely used as a component of starters in milk fermentation processes, especially in yoghurt and cheese production and also in probiotic preparations [1–6].

Nowadays, the identification and classification of microorganisms are practised by a large variety of genotypic and phenotypic methods. The traditional time-consuming methods exploited for the identification and enumeration of bacteria are quickly replaced by methods of molecular biology, such as PCR, RAPD and SDS-PAGE. These

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genetic techniques are very useful for molecular discrimination of complex mixtures of starter and probiotic cultures in research laboratories. In addition, mentioned rapid methods are often important for quality control of dairy products [7–10]. PCR is highly sensitive technique, which is largely used as detecting and identifying tool for bacteria in different environments. Restriction analysis of genes amplified by PCR also contributes to strains distinction at the species, subspecies or other taxonomic levels [2, 11]. RAPD-PCR is one of the most popular genotypic typing techniques. It discriminates particular bacterial species of one strain and also serves as means for identification of DNA polymorphisms in the genome of selected strains. RAPD has been used for classification of a variety of food-borne microorganisms. For example, it allowed differentiation of both species and strains of lactic acid bacteria isolated from various collections and dairy products [6, 8, 12]. SDS-PAGE is widely applied method for identification of lactic acid bacteria [10].

Brigidi et al [2] tried to develop a rapid and easy-to-use PCR protocol for directly detecting and enumerating *Str. thermophilus* in their work and they affirmed that culture-independent or colony-based PCR method is available for detection of *Streptococcus thermophilus* species. Further, Langa et al [9] found that PCR is technically and economically affordable for laboratories, which do not have easy access to more sophisticated or expensive procedures. Delorme [13] used technique RAPD-PCR for investigation of new *Str. thermophilus* strains and their differentiation from *Str. salivarius* and *Enterococcus* spp. Soomro and Masud [10] analysed cell-free extracts of lactic acid bacteria by SDS-PAGE and they concluded that this method is reliable for molecular characterization of these microorganisms. Despite the wide-spread application of *Str. thermophilus* in the dairy industry, data on the phenotypic and genetic strain diversity within the species are still limited [12].

So, this work is focused on molecular diagnostic of *Streptococcus thermophilus* strains by mentioned genetic methods. Another goal of this study was to confirm applicability of given techniques for *Str. thermophilus* detection.

Materials and methods

In this study fifteen strains of *Streptococcus thermophilus* from Czech Collection of Dairy Microorganisms (CCDM) and a strain of *Streptococcus thermophilus* from Czech Collection of Microorganisms (CCM) were used. These microorganisms were grown in M17 broth for 48 hours at 37 ^{o}C.

Chromosomal DNA was isolated by modified method of Graves and Swaminathan [14]. PCR was accomplished in a DNA Engine® Peltier Thermal Cycler PTC-200 (BioRad). Amplification of *Str. thermophilus* strains was achieved using the 16S-23S rDNA primer set ThI/ThII (ThI: 5′-ACGGAATGTACTTGAGTTTC-3′; ThII: 5′-TGGCCTTTCGACCTAAC-3′) [7]. Composition of PCR reaction mixture (25 mm^3) was 15 mm^3 of distilled water, 0.5 mM dNTP mix, 0.8 μM each primer, 0.6 mM $MgCl_2$, 2.5 mm^3 of reaction buffer, 1 U of Taq polymerase and 2 mm^3 of chromosomal DNA. After optimization the thermocycle program comprise successive steps: 1 cycle of 95 ^{o}C for 5 min, followed by 40 cycles of 95 ^{o}C for 1 min, 52 ^{o}C for 30 s and 72 ^{o}C for 1 min,

then 1 cycle of 72 °C for 5 min. Amplified products were subjected to gel electrophoresis in 1.5 % gel and were visualized by ethidium bromide staining.

DNA polymorphisms were defined by RAPD-PCR using the same isolated chromosomal DNA as this for PCR. Amplification was performed on a DNA Engine® Peltier Thermal Cycler PTC-200 (BioRad) in a 25 mm^3 reaction mixture consisting of 15 mm^3 of distilled water, 0.5 mM dNTP mix, 8 μM each primer, 0.6 mM $MgCl_2$, 2.5 mm^3 of reaction buffer, 1 U of Taq polymerase and 2 mm^3 of chromosomal DNA. In this work, primers RAPD-4 (5′-AAGAGCCCGT-3′) and OPP-7 (5′-GTCCATGCCA-3′) were applied [6]. The PCR program was as follows: 1 cycle of 2 min at 94 °C, 45 cycles of 1 min at 94 °C, 1 min at 34 °C, 2 min at 72 °C and 1 cycle of 7 min at 72 °C. Amplified products from RAPD method were subjected to gel electrophoresis in 1.5 % gel and were visualized by ethidium bromide staining as well as PCR products.

Preparation of bacterial samples for SDS-PAGE was performed in this way: broth with accrued cells was centrifuged. The pellet was washed twice with distilled water and then resuspended in distilled water again. Then 3 mm^3 of lysosyme (3 mg · cm^3) were added to 100 mm^3 of resuspended cells and this mixture was incubated at 37 °C for 3 hours. After that, 25 mm^3 of 20 % SDS and sample buffer were added so that total concentration of proteins was 100 μg · cm^3 in sample and total volume of sample was 250 mm^3. Sample was immediately boiled for 10 min. In this manner prepared sample was used for analysis by SDS-PAGE. Protein Marker, Broad Range (212 to 2.3 kDa; BioLabs) was used as standard. Separating gel with concentration 12 % and 5 % resolving gel were chosen for proteins separation. Amount of 20 mm^3 of each sample was loaded on gel. After electrophoresis, gel was fixed for 20 min and then stained in silver nitrate solution according to Kirkeby et al [15].

Gels from all methods were analysed and molecular weights of DNA bands and protein fractions were calculated by using program UltraQuant (Ultra.Lum, USA). Surveyed gels from SDS-PAGE were statistically evaluated by cluster analysis using program Unistat 5.5, hence dendrograms were projected.

Results and discussion

Given strains were confirmed by technique PCR using primer set ThI/ThII. First of all, it was necessary to make gradient PCR to determine optimal annealing temperature. From obtained results temperature 52 °C was chosen. Another problem was purity of isolated DNA, so PCR reaction mixture was fortified with 0.4, 0.6, 0.8 and 1.0 mM $MgCl_2$. This study revealed that concentration 0.6 mM $MgCl_2$ is sufficient (Fig. 1). After these two optimizations, PCR with the others *Str. thermophilus* strains were performed. It was observed that applied primer set ThI/ThII is suitable for detection of the target species. Mentioned primers provided a PCR product with the expected size of 250 bp (Fig. 3). Similarly, Brigidi et al [2] described this size of PCR product in their study of primer set ThI/ThII specificity to *Streptococcus thermophilus* strains.

DNA polymorphism was examined by RAPD-PCR. After evaluation of gels by program UltraQuant (Ultra.Lum, USA), it can be claimed that obtained results of primer RAPD-4 were distinct from results of primer OPP-7. Regarding primer RAPD-4 the

Fig. 1. Optimalization of PCR for strain CCDM 128 by addition of 10 mM $MgCl_2$ to reaction mixture. M – molecular weight marker 100-bp DNA ladder, lane 1 – 0.4 mM, lane 2 – 0.6 mM, lane 3 – 0.8 mM, lane 4 – 1.0 mM $MgCl_2$

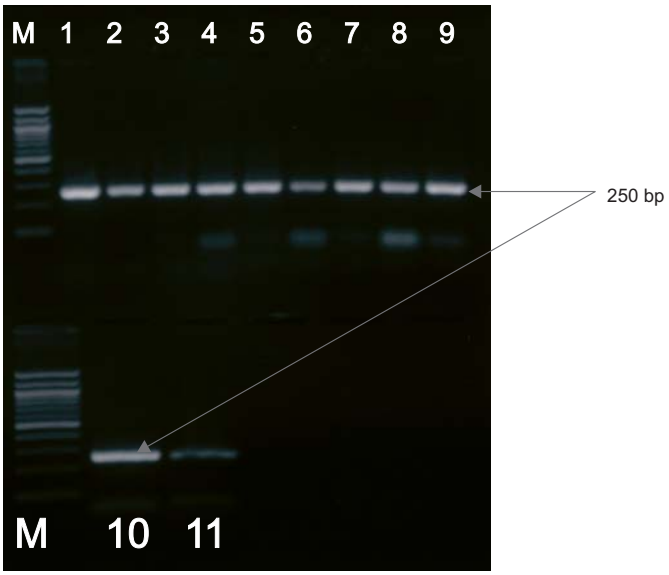

Fig. 2. PCR products of amplified chromosomal DNA of *Str. thermophilus* strains. M – molecular weight marker 100-bp DNA ladder, lane 1 – CCDM 69, lane 2 – CCDM 70, lane 3 – CCDM 126, lane 4 – CCDM 128, lane 5 – CCDM 129, lane 6 – CCDM 130, lane 7 – CCDM 131, lane 8 – CCDM 224, lane 9 – CCDM 437, lane 10 – CCDM 438, lane 11 – CCM 4757

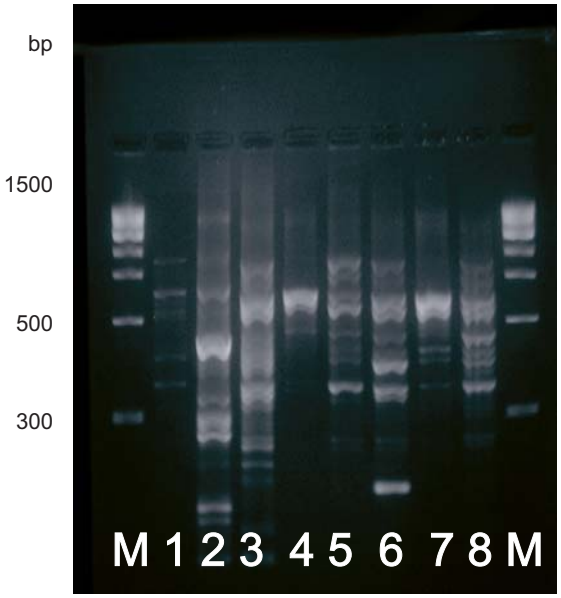

Fig. 3. RAPD products using chromosomal DNA of *Str. thermophilus* strains and RAPD primer. M – molecular weight marker 100-bp DNA ladder, lane 1 – CCDM 7, lane 2 – CCDM 33, lane 3 – CCDM 45, lane 4 – CCDM 55, lane 5 – CCDM 69, lane 6 – CCDM 70, lane 7 – CCDM 126, lane 8 – CCDM 129

DNA sizes ranged from 1.5 kb to 106 bp, biggest ones were 1.5 and 1.4 kb for strains CCDM 33 and 45, respectively. The third one was strain CCDM 131 with DNA size 0.9 kb. The lowest DNA size was observed in strain CCDM 224. The biggest DNA sizes of strains treated with primer OPP-7 were similar to that noticed in primer RAPD-4, thus 1.4 and 0.9 kb, but in contrast to primer RAPD-4 these sizes were determined in strains 128 and 70, respectively. DNA sizes for primer OPP-7 moved between 1.4 kb and 37 bp. The lowest DNA size belonged to mentioned strain 131, which is another discrepancy between these two primers. Mostly DNA sizes from 700 to 200 bp occurred in primer RAPD-4 and between 750 and 350 bp in primer OPP-7. The results are similar to that described by Urshev et al in their study [15].

Streptococcus thermophilus strains were compared by method SDS-PAGE. Figure 4 demonstrates protein profile of given strains. After normalization of gels by molecular weight standard Protein Marker, Broad range, sizes of particular proteins were obtained due to program UltraQuant (Ultra.Lum, USA). Protein sizes within the limits 10–160 kDa were determined that can be seen in Table 1, where number of proteins in mentioned molecular weight range were recorded in individual examined *Streptococcus thermophilus* strains. Salzano et al studied compute genom of *Streptococcus thermophilus* and concluded that it is possible to detect protein sizes from 10 to 210 kDa in this bacterium [16]. Mostly, proteins with molecular weights between 45–90 kDa were represented. It can be compared with results from Soomro and Masud [10] research of *Str. thermophilus*, in which they established four major proteins of about 100, 49, 47

Table 1

Amount of visualizated proteins in particular strains

Molecular mass [kDa]	CCDM 7	CCDM 33	CCDM 45	CCDM 55	CCDM 69	CCDM 70	CCDM 126	CCDM 128	CCDM 129	CCDM 130	CCDM 131	CCDM 133	CCDM 224	CCDM 437	CCDM 438	CCDM 4757
150–160	—	—	—	1	—	2	1	—	3	—	1	—	—	1	—	3
140–150	1	2	1	3	1	2	3	—	3	1	2	1	1	—	—	2
130–140	1	3	1	4	1	3	4	1	3	1	5	—	1	1	1	4
120–130	3	2	2	3	3	4	3	—	4	2	3	1	3	3	—	4
110–120	4	2	5	1	4	1	1	2	4	4	1	2	5	7	1	3
100–110	4	2	4	4	4	3	4	3	3	3	4	4	3	4	4	3
90–100	3	3	2	4	3	3	4	3	4	3	4	3	3	2	2	4
80–90	2	2	1	4	1	3	4	1	4	1	3	2	1	1	1	4
70–80	3	2	3	4	4	5	3	2	3	4	4	2	3	3	2	3
60–70	4	3	3	4	4	3	4	2	4	4	4	3	4	4	2	1
50–60	3	2	4	3	3	3	2	3	2	2	2	4	3	4	3	3
40–50	3	4	2	3	3	4	4	3	3	3	3	4	3	3	5	3
30–40	4	3	2	3	4	2	3	4	3	2	3	3	2	2	3	3
20–30	3	2	3	2	2	3	2	3	2	3	3	3	3	3	3	2
10–20	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Total number of proteins	40	34	35	45	39	43	44	29	47	35	44	34	37	40	29	44

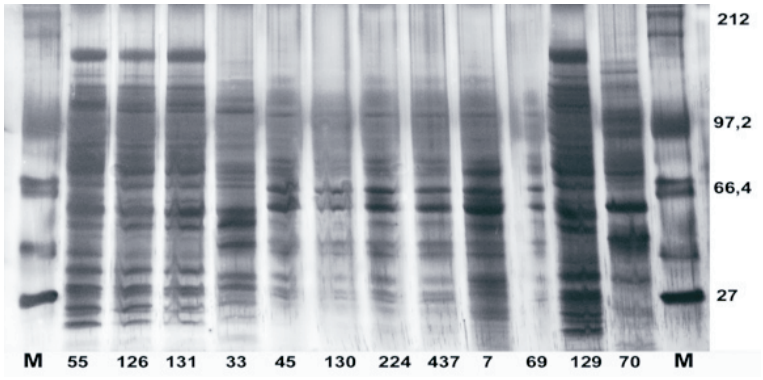

Fig. 4. Protein profile of studied *Str. thermophilus* strains obtained by SDS-PAGE method (12 % gel)

and 41 kDa. Table 1 shows number of proteins in molecular weight range of 10–160 kDa in individual examined *Streptococcus thermophilus* strains. Varcamonti et al [17] found that *Streptococcus thermophilus* protein profile also depends on temperature of cultivation, especiallly their production of heat shock proteins (Hsp proteins). With

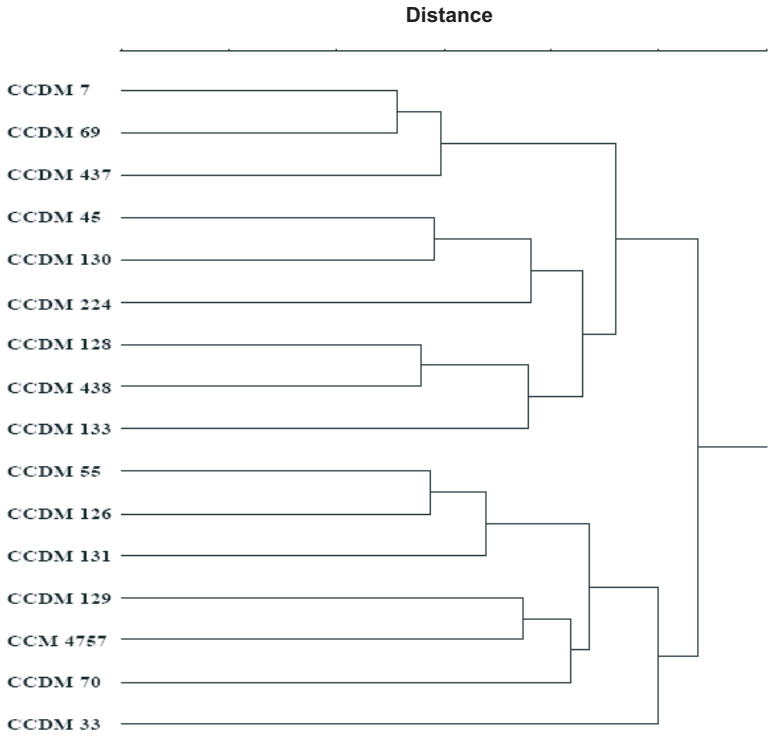

Fig. 5. Dendrogram made up of streptoccoci protein profile

respect to examined strains those were cultured at 37 ^{o}C, it could be interesting to observe influence of temperature on bacterial protein profile. Cluster analysis revealed two main groups (Fig. 5). The first group included 9 strains and it was divided into two subgroups, the first of them was consisted of 3 strains (CCDM 7, 69, 437) and the second one of 6 strains (CCDM 45, 128, 130, 133, 224, 438). The second main group was formed from two subgroups of 7 strains. The first subgroup was only one strain (CCDM 33), the second subgroup was composed of 6 strains (CCDM 55, 70, 126, 129, 131 and CCM 4757). Strain CCM 4757 resembled strain CCDM 129 the most because of their greater amount of proteins with high molecular weight.

Conclusions

In this study, expected size 250 bp of PCR products of amplified chromosomal DNA of *Streptococcus thermophilus* strains with primer set ThI/ThII were obtained. Further, technique RAPD provided similar DNA sizes in both used primers, but these sizes were observed in strange strains. Generally, DNA sizes 700 – 200 bp were detected in primer RAPD-4 and 750–350 bp in primer OPP-7. Analysis of protein profile by SDS-PAGE method revealed that proteins with middle molecular weight were mainly present. Proteins with high molecular weight were supplied only scanty and some strains lacked these proteins. From results of cluster analysis, it was established that strain CCM 4757 was mostly similar to strain CCDM 129. It could be declared that applied methods are useful means for *Streptococcus thermophilus* strains identification and detection.

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DIAGNOSTYKA MOLEKULARNA *Streptococcus thermophilus*

Abstrakt: *Streptococcus thermofilus* jest jednym z najważniejszych przedstawicieli bakterii kwasu mlekowego. Pomimo powszechnego zastosowania tego gatunku w przemyśle mleczarskim nadal nieliczne są dane na temat jego zróżnicowania fenotypowego i genetycznego *Streptococcus thermofilus*. Szybka identyfikacja różnych gatunków bakterii kwasu mlekowego ma duże znaczenie dla kontroli jakości produktów mleczarskich. Niniejsza praca dotyczy charakterystyk i różnic występujących między różnymi liniami *Streptococcus thermofilus*. Badania zostały przeprowadzone przy użyciu technik PCR, RAPD i SDS-PAGE. Do badań użyto 15 linii *Streptococcus thermofilus* z Czeskiego Zbioru Mikroorganizmów Mleczarskich oraz jednej linii *Streptococcus thermofilus* z Czeskiego Zbioru Mikroorganizmów. Dokonano porównania primerów THI/THII między poszczególnymi liniami bakterii za pomocą techniki PCR. Następnie próbki były badane i identyfikowane przy użyciu technik RAPD i SDS-PAGE. Natomiast primery OPP-7 i RAPD-4 zbadano techniką RAPD. Badania wykazały, że zastosowane techniki mogą być skutecznie wykorzystywane do identyfikacji i charakterystyki bakterii z rodzaju *Streptococcus.*

Słowa kluczowe: *Streptococcus thermophilus*, PCR, RAPD, SDS-PAGE

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Varia

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Będzie to dziewiętnasta z rzędu konferencja poświęcona badaniom podstawowym oraz działaniom praktycznym dotycząca różnych aspektów ochrony środowiska przyrodniczego. Odbędzie się ona w ośrodku „Uroczysko" na Wzgórzu Wilhelma w Piechowicach koło Szklarskiej Poręby. Doroczne konferencje ECOpole mają charakter międzynarodowy i za takie są uznane przez Ministerstwo Nauki i Szkolnictwa Wyższego.

Obrady konferencji ECOpole '10 będą zgrupowane w pięciu sekcjach

- **SI Chemiczne substancje w środowisku przyrodniczym oraz ich monitoring;**
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- **SIII Zarządzanie środowiskiem w warunkach kryzysowych;**
- **SIV Forum Młodych (FM) i Edukacja prośrodowiskowa;**
- **SV Wpływ zanieczyszczeń środowiska oraz żywności na zdrowie ludzi**.

Materiały konferencyjne będą opublikowane w postaci:

- abstraktów (0,5 strony formatu A4) na CD-ROM-ie;
- rozszerzonych streszczeń o objętości 4-6 stron w półroczniku *Proceedings of ECOpole*;
- artykułów: w abstraktowanych czasopismach: *Ecological Chemistry and Engineering/Chemia i Inżynieria Ekologiczna* (*Ecol. Chem. Eng.*) ser. A i S oraz niektórych w półroczniku *Chemia – Dydaktyka – Ekologia – Metrologia*.

Termin nadsyłania angielskiego i polskiego streszczenia o objętości 0,5–1,0 strony (wersja cyfrowa + wydruk) planowanych wystąpień upływa w dniu 15 lipca 2010 r. Lista prac zakwalifikowanych przez Radę Naukową Konferencji do prezentacji będzie sukcesywnie publikowana od 15 lipca 2010 r. na stronie webowej

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Po konferencji zostaną wydane 4-6-stronicowe rozszerzone streszczenia wystąpień w półroczniku *Proceedings of ECOpole*. Artykuły te winny być przesłane do **1 października 2010 r**. Wszystkie nadsyłane prace podlegają zwykłej procedurze recenzyjnej. Wszystkie streszczenia oraz program Konferencji zostaną wydane na CD-ROM-ie, który otrzyma każdy z uczestników podczas rejestracji. Program będzie także umieszczony na stronie webowej Konferencji.

dr hab. inż. Maria Wacławek, prof. UO
Przewodnicząca Komitetu Organizacyjnego
Konferencji ECOpole ’10

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