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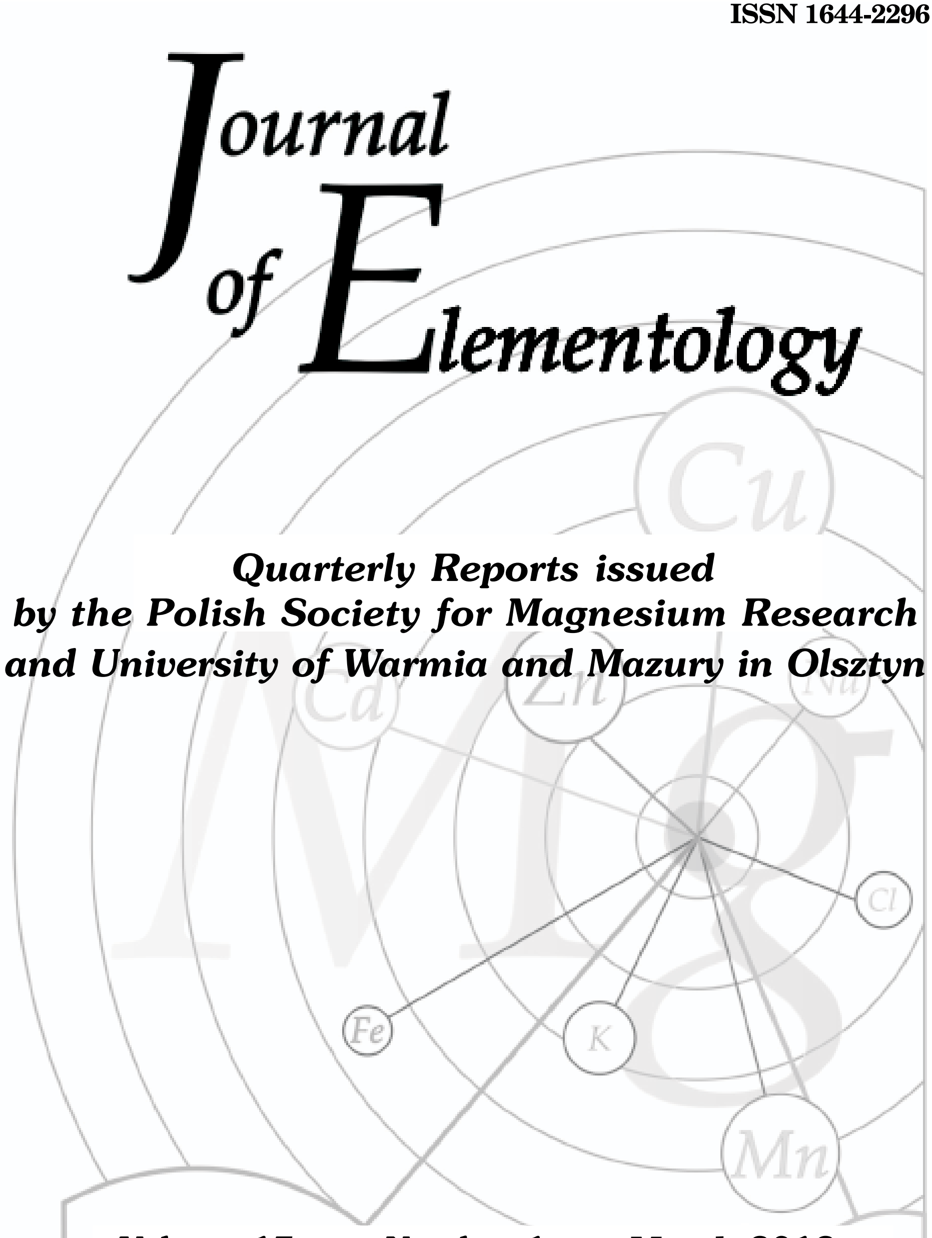

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ORIGINAL PAPERS

APPLICABILITY OF DIFFERENT KINDS OF YEAST BIOMASS TO LEAD REMOVAL FROM WATER

Aleksandra Duda-Chodak, Tomasz Tarko, Kamil Milotta

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University of Agriculture in Krakow

Abstract

The aim of the study was to assess the possibility of using different yeast biomasses for lead removal from aqueous solution. The material for the study comprised baker's yeast (BY), spent waste brewer's yeast (WBY), and fodder yeast (FY), which can be easily obtained as production waste. An amount of each yeast biomass (BY, FY, or WBY) that equals 0.1 g of dry weight was suspended in 100 cm^3 of lead solution (concentration of 200, 500, or 1000 mg dm^{-3}) and biosorption was carried out for 20, 40, 60, 90, 120, 240, and 300 minutes. The concentration of lead remaining in solution was determined using atomic absorption spectroscopy. The lead uptake by yeast biomass was calculated using the mass balance equation for the biosorbent and the results were fitted to the Langmuir isotherm model. The yeast biomasses were able to remove more than 90% of lead present in solution within 20 minutes. With BY biomass, it was possible to reduce the lead level below 1 mg dm^{-3} from the initial lead solutions of 200 and 500 mg Pb dm^{-3}. The value of q_{max} and affinity parameter b, calculated for BY after 300 minutes of biosorption, were very high (1,250 mg Pb g^{-1} d.w. and 0.363, respectively). The best efficiency was achieved for BY when the initial concentration of lead was 500 mg dm^{-3}. The final concentration of the metal (after 300 minutes of sorption) was 0.66 mg dm^{-3}, which means that 99.86% of lead was removed from the solution by the biomass of baker's yeasts.

Key words: lead, toxicity, biosorption, waste treatment, yeasts, waste yeast, Langmuir isotherm.

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MOŻLIWOŚĆ WYKORZYSTANIA RÓŻNYCH TYPÓW BIOMASY DROŻDŻY DO USUWANIA OŁOWIU Z WODY

Abstrakt

Celem pracy była ocena możliwości wykorzystania biomasy drożdżowej do usuwania ołowiu z roztworów wodnych. Materiał badawczy stanowiły: drożdże piekarskie (BY), odpadowe drożdże browarnicze (WBY) oraz drożdże paszowe (FY), stanowiące łatwo dostępny odpad produkcyjny. Ilość biomasy drożdży (BY, WBY, FY) odpowiadającą 0,1 g suchej substancji zawieszano w 100 cm^3 roztworu ołowiu (stężenie 200, 500 lub 1000 mg dm^{-3}) i przeprowadzano biosorpcję przez 20, 40, 60, 90, 120, 240 i 300 minut. Stężenie ołowiu pozostałego w roztworze mierzono metodą adsorpcyjnej spektrometrii atomowej. Wielkość ładunku ołowiu usuniętego przez biomasę drożdży wyliczano na podstawie równania równowagi masowej biosorbenta, a wyniki dopasowywano do izotermy Langmuira. Testowane drożdże usuwały z roztworu ponad 90% ołowiu w ciągu 20 min procesu. Jednakże jedynie zastosowanie BY umożliwiało obniżenie poziomu Pb poniżej 1 mg dm^{-3} z roztworów o wyjściowym stężeniu 200 i 500 mg Pb dm^{-3}. Wartość q_{max} oraz współczynnik powinowactwa *b*, wyznaczone dla drożdży piekarskich po 300 min biosorpcji, wynosiły odpowiednio 1250 mg Pb g^{-1} s.s. i 0,363. Największą wydajność usuwania Pb wykazano stosując drożdże piekarskie (BY), gdy wyjściowe stężenie ołowiu wynosiło 500 mg dm^{-3}. Stężenie równowagowe metalu po 300 min sorpcji wynosiło 0,66 mg dm^{-3}, co odpowiada usunięciu przez biomasę drożdży piekarskich 99,86% ołowiu z roztworu.

Słowa kluczowe: ołów, toksyczność, biosorpcja, oczyszczanie ścieków, drożdże, drożdże odpadowe, izoterma Langmuira.

INTRODUCTION

Heavy metals present in water, soil and air are one of the most serious ecological problems for human and animal health. Lead, toxic at very low doses, is particularly dangerous to people. It is the only heavy metal with no known beneficial effect in the human body. No case of lead deficiency has ever been noted in the medical literature. Many other elements, such as Cr, Mn, Mo, Ni, and Se, although toxic at high concentration, are actually required at lower levels. The exposure to lead can affect every organ and, on a molecular level, it interferes with fundamental biochemical processes. Lead has the ability to mimic or inhibit calcium action and to interact with proteins. The most sensitive is the nervous system, especially of children. Neuropsychological defects and IQ lowering (JUSKO et al. 2008) as well as encephalopathy and sensory deficits have been proved after exposure to lead (DAMSTRA 1977, PATRICK 2006). Lead exposure is one of the factors that contribute to the onset and development of anemia (JAIN et al. 2005), hypertension (KORRICK et al. 1999), ischemic heart disease (JAIN et al. 2007), nephritis (YU et al. 2004), diminished fertility in women (CHANG et al. 2006) and hypospermia in men (DE ROSA et al. 2003), and improper tooth and bone development (DAMSTRA 1977, PATRICK 2006). Women with blood lead level (BBL) 0.05-0.09 mg dm^{-3} were two to three times more likely to have a spontaneous abortion than were women with BLL lesser than 0.05 mg dm^{-3} (Borja-Aburto et al. 1999).

Because lead has been freely used for centuries, it is now widespread in air, soil and water. There is no safe level of lead. The US Agency of Toxic Substances and Disease Registry (ATSDR) has not developed Minimum Risk Levels for lead, "because no thresholds have been demonstrated for the most sensitive effects in humans". Therefore, any exposure to lead is of potential concern.

The WHO has estimated that long-term ambient air concentration of 0.5-1.0 μg m^{-3} would mean that 98% of the population would have blood lead levels below 0.2 mg dm^{-3}. In 1984, the WHO established the maximum lead level in drinking water at 0.05 mg dm^{-3}. In 1993, this goal was revised to 0.01 mg dm^{-3}, to be met in 15 years. In Poland, the allowed level of lead in drinking water was diminished from 0.05 to 0.025 mg dm^{-3} (*The Regulation...* 2010). According to this regulation, starting form 1 January 2013 drinking water should contain less than 0.010 mg lead dm^{-3}. This has stimulated researchers' interest in the methods of lead elimination from the environment.

The main source of lead in drinking water is lead piping and lead-combining solders. The amount of lead that may dissolve in water depends on acidity (pH), temperature, water hardness and standing time of water. Secondary pollution from industry can contaminate water through the effluents produced. Lead is released into environment with wastes from facilities that produce lead-acid batteries, lead wire or pipes, lead-based paints, etc. Industrial wastewater from production of lead-acid batteries contains 10-60 mg dm^{-3} of lead. In urban waste, the Pb level is still high and ranges from 0.05 to 1.1 mg dm^{-3} (Klimiuk, Łebkowska, 2003). Proper treatment of industrial wastewaters, which are releasing lead into the aquatic and land systems, is very important. The conventional methods of lead removal from waste include chemical precipitations, conventional adsorption, ion exchange, membrane process, oxidation and reduction, and electro-remediation methods (Lesmana et al. 2009). However, most of the methods are expensive and not environment friendly because they generate secondary effluent (sludge), whose disposal is problematic.

Biosorption is a process that utilizes low-cost biosorbents to remove toxic heavy metals (Kratochvil, Volesky 1998). Biosorption has distinct advantages over the conventional methods such as low operating cost, selectivity for a specific metal, short operation time, reusability of biomaterial and no chemical sludge. There are a few systems which have already found application in industrial or technical operations. Some examples are: AMT-BIOCLAIM, AlgaSORB, BIO-FIX, B.V. Sorbex biosorbent, bioreactors with *Alcaligenes eutrophus* designed for biosorption of Zn(II), Cd(II) and other heavy metals or radionuclides, the use of *Citrobacter* or *Methylobacillus* biomass for treatment of uranium mining drains, thorium and radium removal from mining wastewater and many others (BRIERLEY 1990, TSEZOS et al. 1997). The biomass of different microorganisms (bacteria, fungi, algae) has also been used for biosorption of different metals from aqueous solutions (SELATNIA et al. 2004, GONG et al. 2005, FARYAL et al. 2007, LESMANA et al. 2009). However, live cells need to be cultivated on special media and the cost of biosorbent grows. In the recent years, many biosorbent materials from agriculture have been utilized for heavy metal biosorption. Among easily available natural materials are different plant leaves (BENAĎSSA, ELOUCHDI 2007), coffee beans (KAIKAKE et al. 2007), fruit and vegetable pomace (KRÓL, NAWIRSKA 2003), chaff (HAN et al. 2005), oyster, almond and coconut shell, coconut fiber, and many more (LESMANA et al. 2009, QAISER et al. 2009, OPELOU et al. 2010).

The effect of different metals on *Saccharomyces cerevisiae* has already been examined by many authors (HETMAŃSKA et al. 1994, TUSZYŃSKI, MAKAREWICZ 2000, TUSZYŃSKI, PASTERNAKIEWICZ 2000). Their results have demonstrated a very rapid uptake of some metal ions by yeast cells. This has stimulated a growing interest in metal-yeast interaction and applicability of this phenomenon.

In the present research, the efficiency of lead removal from aqueous solution by three kinds of yeast biomasses has been evaluated. The selected yeasts are common in food industry and can be obtained as production waste.

MATERIAL AND METHODS

The material for the study consisted of 1) commercially available baker's yeast (BY) *Saccharomyces cerevisiae* (Lesaffre Bio-corporation S.A.); 2) spent waste brewer's yeast (WBY) *Saccharomyces cerevisiae* (waste from Tychy Brewery, after 4th passage); 3) fodder yeast (FY) *Rhodotorula* (the pure culture collection of the Department of Fermentation Technology and Technical Microbiology, University of Agriculture in Krakow, Poland).

Lead solutions

The stock solution of lead (100 g dm^{-3}) was prepared by using analytical grade lead acetate hydrate $(CH_3COO)_2Pb \cdot 3H_2O$ (POCh, Gliwice, Poland) and deionized water. An amount of 0.2, 0.5 and 1 cm^3 of stock solution was diluted in 100 cm^3 of deionized water to obtain working solutions of lead concentration 200, 500 and 1000 mg dm^{-3}, respectively.

Yeasts preparation

Baker's yeast (BY)

The yeast solution was prepared by diluting 1.000 g of baker's yeast in 10 cm^3 of physiological saline. Then, 200 cm^3 of yeast extract-peptone-dextrose medium (YEPD) was inoculated by 1 cm^3 of the yeast solution and incubated for 24 h in water bath at 30°C. The yeast biomass was obtained by centrifugation (5000 rpm, 5 min, 4°C, MPW-350R, MPW Med. Instruments, Poland) and washed with deionized water before further analysis.

Fodder yeast (FY)

Fodder yeasts of *Rhodotorula* genus were transferred on agar slants. Unhopped beer wort was diluted up to 9°Blg and sterilized (121°C, 10 min). An amount of 10 cm^3 of sterile wort was inoculated with one loop of yeast culture and incubated for 24 h at 30°C. The whole culture was then transferred into 100 cm^3 of sterile beer wort (9°Blg) and cultivation was conducted in a laboratory shaker (120 rpm, WU-4, Premed, Poland) for 24 h at 30°C. The yeast culture was then cooled (4°C, 3 h), centrifuged in aseptic condition (5000 rpm, 5 min, 4°C), and the yeasts obtained were washed with sterile deionized water.

Waste brewer's yeast (WBY)

Spent waste yeasts biomass (*Saccharomyces cerevisiae*) was obtained directly from Tychy Brewery (Poland) after 4th passage (it was treated as waste). The yeast biomass was kept at 4°C for about 72 h until analysis.

The estimation of dry weight of yeasts

In each type of yeast biomass, the dry weight content was determined with a moisture analyzer (MAC50, Radwag, Poland).

Biosorption experiments

The amount of particular yeast biomass (BY, FY, or WBY) that equals 0.1 g of dry weight was suspended in 100 cm^3 of lead solution (concentration of 200, 500, or 1000 mg dm^{-3}) in an Erlenmeyer flask and incubated with continuous shaking (200 rpm) at room temperature (RT). After 20, 40, 60, 90, 120, 240, and 300 minutes, the mixture was centrifuged (5000 rpm, 5 min, RT) and clear supernatant was transferred into disposable tubes. The concentration of lead remaining in the supernatant was determined using atomic absorption spectroscopy (AAS) (Varian AA 240 FS, Varian Inc. Agilent Technologies). Eight replicates were performed for each time-point.

Calculations

The lead uptake by yeast biomass was calculated using the following mass balance equation for the biosorbent (Volesky 2004):

$$q = [V\,(C_0 - C_f)] / S,$$

where:

q – lead uptake at equilibrium (mg Pb g^{-1} biosorbent dry weight);
V – volume of metal-bearing solution contacted (batch) with the biosorbent (dm^3);
C_0 – initial concentration of metal in solution (mg dm^{-3});
C_f – final concentration of metal in solution (mg dm^{-3});
S – dry weight of biosorbent added (g).

In the preliminary experiments (initial metal concentration ranged 50-1000 mg dm^{-3}), the Langmuir and the Freundlich models were utilized to explain the experimental data (Volesky 2004). In all the analyzed cases, the correlation coefficient (r) for the Langmuir isotherm was higher than for the Freundlich isotherm. The experimentally determined equilibrium isotherms were compared with the theoretical Langmuir and Freundlich isotherms. An example of the results of this comparison is shown in Figure 1, which demonstrates that the Langmuir model yielded better representation of the experimental data. This is in accordance with many references that have demonstrated that the Langmuir model fits better to results of heavy metal biosorption by microorganisms (Holan, Volesky 1994, Özcan et al. 2009, Qaiser et al. 2009, Velmurugan et al. 2010). This model is based on the hypothesis that the uptake occurs on a homogenous surface by monolayer sorption without interaction between adsorbed molecules. It is expressed as:

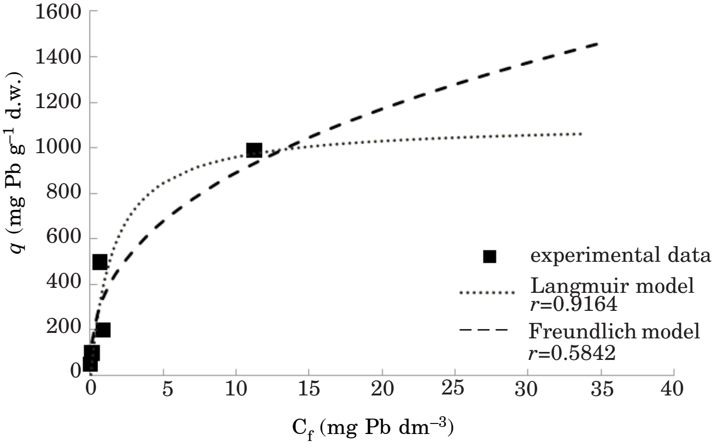

Fig. 1. Fitting of the experimental data to the theoretical (Freundlich and Langmuir model) equilibrium sorption isotherms: C_f – lead concentration at equilibrium, q – lead uptake at equilibrium, r – correlation coefficient

$$C_f/q = 1/q_{\max} \cdot C_f + 1/(q_{\max} \cdot b),$$

where:

$q_{\max}$ – represents the maximum biosorption capacity (mg Pb g^{-1} d.w.);
b – is an affinity parameter, related to the energy of biosorption.

In the experiments performed in this study, the initial concentration of lead varied from 200 to 1000 mg dm^{-3}, while the volume of lead solution and quantity of biosorbent were constant (respectively, 0.1 dm^3 and 0.1 g d.w.). Knowing these values, q was calculated and the plot C_f/q against C_f was drawn. The linear regression was used to determine the equation of best-fitted line, and the values of $q_{\max}$ and b were calculated (if possible). The results were taken into account only if the correlation coefficient was higher than r=0.7.

Statistical analysis

The results were shown as an arithmetic mean (± standard deviation) of eight replicates. A single-factor Analysis of Variance test (ANOVA) with a *post hoc* Tukey test was applied to perform statistical analysis. Kolmogorov-Smirnov test was applied to examine the normality of distribution.

RESULTS AND DISCUSSION

The level of lead was reduced significantly after biosorption with each type of yeast biomass independently from the initial lead concentration ($p<0.05$). The kinetics of the concentration changes was different but most lead was taken up within the first 20 minutes of the process. Moreover, all kinds of yeast biomass used in the study were able to remove more than 90% of lead present in the solution within 20 minutes (Table 1). These results are supported by literature references, suggesting that 95% of metal is removed by microorganisms within 30 minutes (VOLESKY et al. 1993, GONG et al. 2005).

The fodder yeasts were the most efficient biosorbent in solutions of lead concentration of 200 mg dm^{-3} when the efficiency was evaluated after 20 minutes. However, when the time of biomass contact with heavy metal was longer, the maximum uptake of lead (99.56%) was achieved by baker's yeast. Similar results were obtained for high concentration metal solution (1000 mg dm^{-3}); after 20 min the FY were more efficient than BY, but after 300 min the uptake of lead was almost the same (98.88% and 98.84%, respectively). It should be highlighted that only BY was able to reduce the lead level below 1 mg dm^{-3} from initial lead solutions of 200 and 500 mg Pb dm^{-3}.

Table 1

The efficiency of lead biosorption process by analyzed yeast at the initial lead concentration (C_0) [mg Pb dm^{-3}] and time of process (t) [min], final concentration of lead – C_f [mg Pb dm^{-3}] (arithmetic mean ± SD, n = 8) after biosorption and lead uptake at equilibrium – q [mg Pb g^{-1} d.w.]

t	C_0	BY		WBY		FY	
		C_f	q	C_f	q	C_f	q
20	200	19.62 ± 2.43 ^{a}	180.38	13.31 ± 0.43 ^{a,b}	186.69	9.16 ± 2.37 ^{a}	190.84
40		18.17 ± 1.26 ^{a}	181.83	10.29 ± 0.65 ^{c}	189.71	7.01 ± 1.33 ^{c,b}	192.99
60		8.23 ± 0.42 ^{b}	191.77	9.75 ± 2 67 ^{c}	190.25	7.40 ± 0.85 ^{a,b,d}	192.60
90		4.33 ± 0.53 ^{c}	195.68	9.54 ± 0.67 ^{c}	190.46	8.67 ± 1.48 ^{a,b}	191.33
120		1.04 ± 0.13 ^{d}	198.96	10.42 ± 3.71 ^{a}	189.58	4.42 ± 0.38 ^{e}	195.59
240		1.78 ± 0.15 ^{d}	198.22	10.55 ± 0.46 ^{b,c}	189.45	5.33 ± 0.21 ^{c,e}	194.67
300		0.88 ± 0.09 ^{d}	199.12	8.67 ± 1.31 ^{c}	191.33	5.98 ± 0.19 ^{c,d,e}	194.02
20	500	38.01 ± 1.36 ^{a}	461.99	27.54 ± 1.70 ^{a}	472.46	47.17 ± 3.68 ^{a}	452.83
40		38.47 ± 1.37 ^{a}	461.53	30.29 ± 2.27 ^{a,b}	469.72	25.77 ± 2.46 ^{b}	474.23
60		20.15 ± 1.83 ^{b}	479.85	30.04 ± 1.53 ^{a,a}	469.96	23.30 ± 1.47 ^{b,c}	476.70
90		0.94 ± 0.09 ^{c}	499.06	30.79 ± 2.14 ^{b}	469.21	21.52 ± 1.56 ^{c}	478.48
120		3.60 ± 0.41 ^{d}	496.40	30.78 ± 2.18 ^{b}	469.22	14.23 ± 2.56 ^{d}	485.77
240		1.29 ± 0.15 ^{c,d}	498.71	31.41 ± 1.68 ^{b}	468.60	12.63 ± 0.78 ^{d,e}	487.37
300		0.66 ± 0.05 ^{c}	499.35	23.06 ± 1.54 ^{c}	476.95	10.39 ± 0.50 ^{e}	489.61
20	1000	83.71 ± 5.09 ^{a}	916.29	60.27 ± 3.08 ^{a,b}	939.73	52.35 ± 1.95 ^{a}	947.65
40		79.12 ± 2.69 ^{b}	920.88	64.43 ± 3.57 ^{a}	935.57	62.73 ± 1.90 ^{b}	937.27
60		78.63 ± 2.59 ^{b}	921.37	60.80 ± 2.54 ^{a,b}	939.20	60.49 ± 2.50 ^{b}	939.51
90		38.68 ± 2.49 ^{c}	961.32	57.34 ± 2.51 ^{b,c}	942.66	55.91 ± 2.98 ^{a}	944.09
120		48.29 ± 2.17 ^{d}	951.72	56.40 ± 2.66 ^{b,c}	943.60	43.65 ± 4.64 ^{c}	956.36
240		30.39 ± 2.41 ^{e}	969.61	55.11 ± 3.57 ^{c}	944.89	24.89 ± 1.88 ^{d}	975.11
300		11.25 ± 1.43 ^{f}	988.76	53.63 ± 2.97 ^{c}	946.37	11.58 ± 1.42 ^{e}	988.42

BY – baker's yeasts, WBY – waste beer yeasts, FY – fodder yeasts *a,b,c,d…* – the same letters denote the lack of statistical significance ($p < 0.05$) between different time-points within the same kind of yeast biomass used for biosorption of particular concentration of lead

The results obtained in this study are very promising. Faryal et al. (2007) analyzed the potential of *Aspergillus niger* biomass to remove lead from aqueous solutions. In solutions containing 1000 mg Pb dm^{-3}, the maximum lead biosorption observed on day 3 was 92.04% (for *A. niger* RH 17) and 92.72 (*A. niger* RH 18). These strains removed 204.57 and 206.04 mg Pb g^{-1} of dried biomass, respectively. In our studies, the lead uptake was 980, 946, and 988 mg of Pb per gram of dried BY, WBY and FY biomass, respectively.

Table 2

The Langmuir isotherm parameters (q_{max} and b) for lead biosorption by baker's (BY), waste beer (WBY), and fodder yeast (FY) biomasses depending on the biosorption process duration (t)

T (min)	BY			WBY			FY		
	q_{max} (mg g^{-1} d.w.)	b	r	q_{max} (mg g^{-1} d.w.)	b	r	q_{max} (mg g^{-1} d.w.)	b	r
20	*NC*	*NC*	*NC*	*NC*	*NC*	*NC*	*NS*	*NS*	*NS*
40	*NC*	*NC*	*NC*	3333	0.005	0.984	2000	0.014	0.919
60	1667	0.018	0.969	*NS*	*NS*	*NS*	2000	0.014	0.987
90	1250	0.085	0.791	*NS*	*NS*	*NS*	3333	0.007	0.950
120	1000	0.250	0.999	*NS*	*NS*	*NS*	1667	0.029	0.996
240	1111	0.204	0.961	*NS*	*NS*	*NS*	*NC*	*NC*	*NC*
300	1250	0.363	0.920	3333	0.007	0.997	*NC*	*NC*	*NC*

NC – q_{max} and b were not calculated, the Langmuir isotherm did not fit
NS – the correlation coefficient (r) was below 0.7

Also, in the study of Parvathi et al. (2007) Langmuir constant q_{max} for *Saccharomyces cerevisiae* was calculated. However, the maximum concentration of lead used in that study was only 100 mg dm^{-3}, and the q_{max} was established at the level of 55.71 mg g^{-1}. In many available references, the maximum possible amount of lead ion adsorbed per unit weight of biosorbent was much lower than in our study; 137 mg g^{-1} of *Streptomyces rimosus* biomass (Selatnia et al. 2004), 85.86 mg g^{-1} and 61.35 mg g^{-1} of *Phanerochaete chrysosporium* (Say et al. 2001 and Yetis et al. 2000, respectively), 50.9 mg g^{-1} of *Enterobacter* sp. (Lu et al. 2006). Our results also exceeded the lead uptake determined in the study of Holan and Volesky (1994) for different marine algae.

The value of q_{max} and affinity parameter b (Table 2) calculated for by after 300 minutes of biosorption were very high (1250 mg Pb g^{-1} d.w. and 0.363, respectively, r = 0.920). For example, q_{max} for groundnut hull was only 31.54 mg Pb g^{-1} (Qaiser et al. 2009).

The best efficiency was achieved for BY when the initial concentration of lead was 500 mg dm^{-3}. The final concentration of the metal (after 300 minutes) was 0.66 mg dm^{-3}, which means that 99.86% of lead had been removed by the biomass of baker's yeasts. The biosorption process conducted by fodder yeasts could be presented by the Langmuir isotherm for only the first 120 minutes, and was characterized by a low affinity parameter. The longer biosorption (240 and 300 min) did not fit the above model, so q_{max} could not be calculated.

It was also demonstrated that the biosorption process conducted with baker's yeasts ran through two phases, with the first one finishing after 60 min. Further studies should be done to determine if the second phase is the bioaccumulation process. Another possibility is that cells become destroyed and new metal binding sites appear.

When q_{max} was calculated, its extremely high values (3,333 mg Pb g^{-1} d.w.) were obtained for spent waste beer yeasts after 300 minutes of biosorption. However, affinity parameter b was low (0.007) and it can be seen that in each experiment variant, the amount of lead removed from solution by WBY was the lowest. Concluding, among the analyzed kinds of yeast, WBY was the weakest biosorbent. This could have been due to the stress produced on yeast cells during fermentation in a brewery. Moreover, small gas bubbles were observed on the surface of cell in the WBY biomass. They could have significantly reduced the contact surface between the metal and cell wall. Both BY and FY were cultivated in laboratory conditions and were "non-stressed". The biphasic biosorption process can suggest that baker's yeast cells were alive and in good condition, and the bioaccumulation could take place (TUSZYŃSKI, PASTERNAKIEWICZ 2000). In spent waste beer yeasts, only physical adsorption on cell structures (mainly cell walls) probably occurred.

CONCLUSIONS

In the present research, it has been proven that yeast can be used for lead removal as a highly efficient biosorbent. Among the analyzed different yeast biomasses, the best results were achieved for baker's yeast. The lead uptake obtained in the study and the calculated values of q_{max} were higher than those demonstrated by other researchers when biosorption was performed with moulds or bacteria. The experimental data of lead biosorption by yeast biomass fit better the Langmuir than the Freundlich isotherm model.

Further research should concentrate on methods of modification of biomass in order to improve its properties. Next, the process of desorption as well as the evaluation of possible numbers of sorption/desorption cycles of the same biomass usage could be conducted.

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EFFECT OF NITROGEN FERTILIZATION ON THE YIELD AND NUTRITIVE VALUE OF *BETA VULGARIS* L.

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Abstract

Leaf beet is one of several types of *Beta vulgaris* L. The vegetable originates from the Mediterranean area but is currently grown on a large scale in the US and Western Europe, e.g. Switzerland, Germany, France or England. In Poland, it is a less known vegetable, despite its high content of many valuable nutrients and excellent taste. One of the main factors affecting the size and quality of yield is nitrogen fertilization. Nitrogen is a structural element of plants and its deficiency significantly inhibits production of new plant tissue. This element is a component of nucleic acids, nucleotides, coenzymes, chlorophyll, phytohormones and cytokinines. The aim of this study was to conduct research on the effect of varied nitrogen fertilization on yielding and chemical composition of aerial parts of leaf beet. The experiment used three doses of nitrogen (0.2, 0.4, 0.6 g N dm^{-3} of medium) in the form of ammonium nitrate or urea. Plants were cultivated in a greenhouse, in 4-litre pots filled with highmoor peat limed to pH of 5.6 with waste chalk. The following fertilizers were used in the experiment: ammonium nitrate – 34% N, urea – 46% N, monobasic potassium phosphate – 23% P, 28.2% K, magnesium sulphate – 15.6% Mg, and microelements. In the cultivation of leaf beet, application of 0.4 g N dm^{-3} NH_4NO_3 proved to be most beneficial as it led to the highest yield of fresh mass of leaves and highest content of L-ascorbic acid, whereas application of $CO(NH_2)_2$ in the amount of 0.2 g N dm^{-3} of the growth medium proved to be best, as it resulted in the lowest nitrate share in the dry mass of leaves. Regardless of the type of nitrogen fertilization, growth in the total salt concentration (EC) in the medium was shown as an effect of increasing doses of the applied fertilizers. Increasing the nitrogen concentration by 100% in the growth medium in relation to the lowest dose raised the Fe and Cu content in beet leaves and depressed that of Zn and Mn irrespective of the applied nitrogen fertilization.

Key words: *Beta vulgaris* L., doses and forms of nitrogen, nitrates, medium, macro-, micronutrients.

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ODDZIAŁYWANIE NAWOŻENIA AZOTOWEGO NA PLON I WARTOŚĆ ODŻYWCZĄ BURAKA LIŚCIOWEGO

Abstrakt

Burak liściowy jest jednym z typów uprawnych gatunku *Beta vulgaris* L. Ojczyzną buraka liściowego są kraje leżące w rejonie Morza Śródziemnego. Obecnie roślina ta jest warzywem uprawianym na szeroką skalę w USA, a także Europie Zachodniej: Szwajcarii, Niemczech, Francji, Anglii. W Polsce jest warzywem mało znanym, mimo że zawiera wiele cennych składników odżywczych i smakowych. Jednym z głównych czynników wpływających na wielkość i jakość plonu jest nawożenie azotowe. Azot jest pierwiastkiem budulcowym roślin, a jego niedobór istotnie ogranicza powstawanie nowych tkanek roślinnych. Pierwiastek ten wchodzi w skład kwasów nukleinowych, nukleotydów, koenzymów, chlorofilu, fitohormonów, cytokinin. Celem pracy było określenie wpływu zróżnicowanego nawożenia azotem na plonowanie oraz skład chemiczny części nadziemnych buraka liściowego. W doświadczeniu zastosowano trzy dawki azotu (0,2, 0,4, 0,6 g N dm^{-3} podłoża) w postaci saletry amonowej lub mocznika. Rośliny uprawiano w szklarni w doniczkach czterolitrowych wypełnionych torfem wysokim zwapnowanym kredą nawozową do pH 5,6. W doświadczeniu stosowano następujące nawozy: saletrę amonową – 34% N, mocznik – 46% N, fosforan monopotasowy – 23% P; 28,2% K, siarczan magnezu – 15,6% Mg, mikroelementy. W uprawie buraka liściowego najkorzystniejsze okazało się stosowanie 0,4 g N dm^{-3} NH_4NO_3 ze względu na największy plon świeżej masy liści i największą zawartość kwasu L-askorbinowego, natomiast ze względu na najmniejszy udział azotanów w suchej masie liści najlepsze okazało się podanie $CO(NH_2)_2$ w ilości 0,2 g N dm^{-3} podłoża. Niezależnie od rodzaju nawozu azotowego wykazano wzrost ogólnej koncentracji soli (EC) w podłożu pod wpływem wzrastających dawek stosowanych nawozów. Zwiększenie stężenia azotu o 100% w podłożu w stosunku do dawki najniższej powodowało wzrost zawartości Fe, Cu w liściach buraka, a spadek Zn i Mn niezależnie od zastosowanego nawozu azotowego.

Słowa kluczowe: *Beta vulgaris* L., dawki i formy azotu, azotany, makro-, mikroelementy.

INTRODUCTION

Leaf beet (*Beta vulgaris* L. *var. cicla* L.), a member of the family *Chenopodiaceae*, is a biennial plant (KRETSCHMER 1999). In the first year of vegetation, it produces a rosette of oval root leaves, which are sometimes crenulated, with long, slightly ribbed petioles and the colour ranging from silver white through yellow, green, pink to red. The leaf blade can be smooth or bubbly, bending downwards at the edges. Depending on a variety, the color of leaf blades is yellow and green, green or red.

Nitrogen fertilization is one of the main factors affecting the size and quality of yield of crops. With respect to species whose vegetative organs (roots, shoots, leaves) are regarded as commercial yield, higher yield can be obtained through rational increase of nitrogen fertilizer doses and supplementation with other macro- and microelements. Nitrogen is a very important structural element in plants, which contain between 1 and 6% of nitrogen in dry mass, and nitrogen deficiency significantly inhibits production of new plant tissues. An optimum supply of nitrogen ensures proper development of plants. Nitrogen is a constituent of nucleic acids, nucleotides, coenzymes, chlorophyll, phytohormones and cytokinins (Gabryś 2002).

Proper application of fertilizers has a positive effect on plant nutrition, although excessive amounts of nitrogen fertilizers diminish the quality of yield, impair resistance of plants to weather conditions (drought, wind, temperature) or pathogens, depress the consumption value and shorten storage life (Wójcik-Wojtkowiak et al. 2000). In order to keep in good health, people need an optimum diet, which must comprise fruit and vegetables of the highest possible biological value (Śmiechowska, Przybyłowski 2000).

The aim of this paper has been to investigate the effect of varied nitrogen fertilization on yield and chemical composition of edible parts of leaf beet. In the experiment, three nitrogen doses were applied (0.2, 0.4, 0.6 g N dm^{-3} of the growth medium) in the form of ammonium nitrate or urea.

MATERIAL AND METHODS

The experiment on *Beta vulgaris* L. cv. Vulcan was conducted in 2009 (20.03-19.05) and 2010 (07.04-31.05). The vegetable was grown for 62 days from sowing to harvesting (37 days from planting to the permanent site).

The experiment was set up in a complete randomization design with 6 series and 8 replications. Single plants were grown in 4-liter pots, filled with highmoor peat limed with $CaCO_3$. The content of mineral compounds in peat prior to planting to the permanent site was (in mg dm^{-3}): N-NH_4–tr., N-NO_3–15, P-PO_4–tr., K–12, Ca–58, Mg–8.

The following fertilizers were used in the experiment: ammonium nitrate – 34% N, urea – 46% N, monobasic potassium phosphate – 23% P, 28.2% K (0.5 P, 0.6 K g dm^{-3}), magnesium sulphate – 15.6% Mg (0.5 g Mg dm^{-3}), microelements.

Three doses of nitrogen: 0.2, 0.4, 0.6 g N dm^{-3} in the form of ammonium nitrate or urea were applied in the experiment. Prior to planting to the permanent site, the entire dose of phosphorus, calcium, microelements and L’ dose of nitrogen, potassium and magnesium were applied (14.04.2009; 27.04.2010). The remaining amounts of nutrients (N, K, Mg) were applied on two days during the vegetation period (24.04 and 05.05.2009; 07.05 and 17.05.2010).

Mineral compounds N-NH_4, N-NO_3, P-PO_4, K, Ca, Mg, S-SO_4, Cl in the medium were determined in an extract of 0.03 M of acetic acid at a volumetric solution to medium ratio of 10:1, with addition of active carbon (Nowosielski 1988).

Nitrate nitrogen and ammonium nitrogen were analyzed with Bremner method according to Starck modification. Phosphorus was determined with ammonium metavanadate, $S\text{-}SO_4$ – with barium chloride, Cl – with silver nitrate by colorimetry, and potassium, calcium and magnesium were determined with atomic absorption spectrometry ASA (Perkin-Elmer, Analyst 300). The pH of the medium was checked as well as the salt concentration (EC) in mS cm^{-1} in a suspension of distilled water and the examined medium in a 2 : 1 volumetric ratio (NOWOSIELSKI 1988).

Fresh mass yield was determined by measuring the height and weight of aerial parts of the plants on termination of the experiment. L-ascorbic acid was determined with Tillmans method in fresh plant material (PN-A--04019 1998) and the extract was analyzed refractometrically. The dry material was analyzed for total nitrogen with Kjeldahl method and after dry mineralization at 550°C, after dissolving ash with muriatic acid diluted in water in a 1 : 2 vol. ratio. P, K, Ca, Mg were determined with the same methods as applied to the medium analysis. $N\text{-}NH_4$, $N\text{-}NO_3$, $S\text{-}SO_4$, Cl were determined in 2% extract of acetic acid with the same methods as applied to the medium analysis. The microelements Fe, Zn, Mn and Cu were determined after dry mineralization at 550 °C with the ASA method.

The results were verified statistically using analysis of variance on mean values and the differences were evaluated with Tukey's test at the significance level α=0.05.

RESULTS AND DISCUSSION

Visually, no differences in plant pigmentation were observed under the influence of the examined factors, and all the plants were characterized by the deep green color of leaves with red ribs of leaf blades and petioles.

The experiment showed significant effect of nitrogen dose and type of nitrogen fertilizer on the yield of leaf beet and length of leaves. The highest yield of leaves (351.8 g $plant^{-1}$) was obtained after the application of 0.4 g N dm^{-3} in the form of NH_4NO_3. The lowest yield (221 g $plant^{-1}$) was obtained after the application of lowest nitrogen dose (0.2 g dm^{-3}) in the form of $CO(NH_2)_2$. When NH_4NO_3 was used for fertilization, plants were higher than from the treatments where $CO(NH_2)_2$.was applied. Analyzing the effect of a fertilizer dose on the size of plants, it was discovered that plants which had received 0.4 g N dm^{-3}) in the form of NH_4NO_3 and $CO(NH_2)_2$ were higher by approx. 3 cm than the ones from the treatments with the lowest dose of N (0.2 g dm^{-3}) – Table 1.

Many authors who have tested the effect of a nitrogen fertilizer dose on the volume of yield of vegetables confirm the positive effect of increasing nitrogen doses on yield of fresh mass (DZIDA 2004, KOŁOTA, CZERNIAK 2010).

Table 1

Plant unit weight, leaf length, L-ascorbic acid, dry matter, protein and extract content in beet leaves, depending on nitrogen fertilization

Nitrogen fertilizer	Dose N ($g\ dm^{-3}$)	Plant unit weight (g)	Leaf length (cm)	Dry matter (%)	L-ascorbic acid ($mg\ 100\ g^{-1}$ f.w.)	Protein (%)	Extract (%)
NH_4NO_3	0.2	268.8	48.25	8.91	36.60	16.75	4.87
	0.4	351.8	51.50	7.83	42.50	32.21	4.10
	0.6	327.7	49.50	7.50	39.70	37.29	3.73
Mean for NH_4NO_3		316.2	49.75	8.08	39.60	28.75	4.23
$CO(NH_2)_2$	0.2	221.0	42.63	8.45	39.70	33.56	5.17
	0.4	239.1	45.25	7.31	40.97	33.94	4.93
	0.6	224.7	41.75	8.23	32.90	37.52	5.00
Mean for $CO(NH_2)_2$		228.3	43.21	7.99	37.86	35.01	5.03
Mean for dose	0.2	244.9	45.44	8.68	38.15	25.16	5.02
	0.4	295.5	48.38	7.57	41.74	33.08	4.52
	0.6	276.2	45.63	7.86	36.30	37.41	4.37
$LSD_{0.05}$							
Fertilizer N		12.24	1.89	i.d.	i.d.	3.26	0.46
Dose N		18.03	2.79	i.d.	i.d.	4.89	i.d.
Nitrogen fertilizer x dose nitrogen		31.35	i.d.	i.d.	i.d.	8.72	i.d.

i.d. – insignificant differences

Michałojć (1994), Kozik (2006) as well as Jarosz and Dzida (2006) obtained higher yields after application of increasing nitrogen fertilization. Kalembasa and Deska (1996), however, reported different results from their research on lettuce, in which they showed that an increase of nitrogen fertilizer doses did not have a distinct effect on lettuce yield.

Our analysis of the plant material showed that as the nitrogen dose introduced to the growth medium increased, the content of nitrates in leaves likewise increased. The content of nitrates in the leaves depended on the type of nitrogen fertilizer and its dose. Less of nitrates was in leaves of plants fertilized with $CO(NH_2)_2$ than in leaves of plants fertilized with NH_4NO_3. The lowest level of nitrates (0.11% d.m.) was determined after the application of the lowest dose of $CO(NH_2)_2$, and the highest one (1% d.m.) – following the application of the highest dose of NH_4NO_3. The ammonium form of nitrogen in leaves of *Beta vulgaris* L. appeared in trace amounts (Table 2).

Increasing doses of nitrogen fertilizers can lead to the growth of nitrates in plant material (Michalik, Stępowska 1995, Wojciechowska et al. 2000). Krężel and Kołota (2007), who analyzed the content of nitrates in red beet, confirmed the above conclusion with their experimental results. Vegetables

Table 2

Macroelements in beet leave depending on nitrogen fertilizing (% d.m.)

Nitrogen fertilizer	Dose N (g dm^{-3})	N-total	N-NH$_4$	N-NO$_3$	P	K	Ca	Mg	S	Cl
NH$_4$NO$_3$	0.2	2.68	0.10	0.35	2.12	4.45	0.86	0.62	0.34	1.16
	0.4	5.15	0.06	0.70	1.87	4.45	0.61	0.67	0.29	1.22
	0.6	5.97	0.04	1.00	1.13	4.27	0.33	0.46	0.22	1.16
Mean for NH$_4$NO$_3$		4.60	0.06	0.68	1.71	4.39	0.60	0.58	0.29	1.18
CO(NH$_2$)$_2$	0.2	5.37	0.04	0.11	2.20	4.84	0.59	0.48	0.41	0.44
	0.4	5.43	0.08	0.19	2.24	4.61	0.40	0.39	0.43	0.42
	0.6	6.43	0.16	0.23	2.28	4.67	0.35	0.42	0.46	0.53
Mean for CO(NH$_2$)$_2$		5.74	0.09	0.18	2.24	4.71	0.45	0.43	0.43	0.46
Mean for dose	0.2	4.03	0.07	0.23	2.16	4.65	0.73	0.55	0.37	0.80
	0.4	5.29	0.07	0.45	2.06	4.53	0.51	0.53	0.36	0.82
	0.6	6.20	0.10	0.62	1.71	4.47	0.34	0.44	0.34	0.85
LSD$_{0.05}$										
Fertilizer N		0.320	0.019	0.026	0.116	0.221	0.07	0.04	0.033	0.063
Dose N		0.479	0.029	0.039	0.175	i.d.	0.106	0.06	i.d.	i.d.
Nitrogen fertilizer x dose nitrogen		0.854	0.052	0.071	0.311	i.d.	0.189	0.105	0.088	i.d.

i.d. – insignificant differences

with a short vegetation period (e.g. spinach) produce higher yield when more nitrogen fertilizer is applied, but simultaneously they absorb excess nitrates and the biological value of yield is lower. Therefore, fertilizing recommendations should be formulated that would consider the highest biological quality of vegetables and not just the highest yield. With the application of 200 kg N per 1 ha, the highest yield of spinach was produced, but when using 80 kg N per 1 ha the biological value of yield was much more superior (quality of protein, content of nutrients, vitamin C and carbohydrates) while the content of nitrates was low.

The content of nitrates in plant material is mostly affected by nitrogen fertilization, but also by the plant species and variety, harvest date, insolation, and the growth medium (Kozik 2006, Dzida, Jarosz 2010).

Plant species have different nitrogen demands. Czuba (1996) states that application of increasing doses of nitrogen fertilizer to a medium with a high organic matter content has a significant effect on increasing nitrogen concentrations in plants. Among several factors which can lower the nitrogen content in plants, noteworthy is the right timing of harvest.

Different parts of plants tend to accumulate different amounts of nitrogen, e.g. highest amounts of nitrates are gathered in leaf vegetables with a short vegetation period and in *Brassica* vegetables; less of nitrates will be found in root vegetables, followed by vegetable fruits, and the smallest concentrations of these compounds accumulate in generative parts of plants (Sady 2001, Wojciechowska, Rożek 2006).

The average content of total nitrogen in beet leaves was 5.17% d.m. The increasing nitrogen doses were observed to have produced an evident effect on the growth of total nitrogen accumulation in plant leaves. The highest content of N-total (6.43% d.m.) has been noted in aerial parts of beet cultivated with the highest dose of $CO(NH_2)_2$, whereas the lowest content of N-total (2.68% d.m.) has occurred in leaves of beet supplemented with the lowest dose of NH_4NO_3 (Table 2).

Interesting results were obtained from our analysis of the content L-ascorbic acid in *Beta vulgaris* L. The applied doses of nitrogen fertilizer differentiated the vitamin C concentration in the examined plants, but the effect was ambiguous. Plants fertilized with the average nitrogen dose of 0.4 g dm^{-3} in the form of NH_4NO_3 were characterized by the highest content of vitamin C (42.5 mg 100 g^{-1} f.m.), whereas the lowest content of vitamin C (32.9 mg 100 g^{-1} f.m.) was obtained in response to the fertilization with 0.6 g N dm^{-3} as $CO(NH_2)_2$. While analyzing the effect of nitrogen fertilizer type on vitamin C in leaf beet, higher vit. C concentration was found in plants fertilized with NH_4NO_3 than in plants supplemented with $CO(NH_2)_2$ – Table 1. The results of our study show that increasing doses of nitrogen in the form of $CO(NH_2)_2$ depress the L-ascorbic acid content in leaf beet plant. A similar dependence was demonstrated by Kozik (1998) in experiments conducted on lettuce. However, Dzida and Pitura (2008) as well as Venter (1983) in their experiments with leaf beet noted a higher vitamin C content in response to a growing nitrogen dose. In addition, Pokluda and Kuben (2002) claim that the vitamin C content in leaf beet plants also depends on its variety. The varieties *Beta vulgaris* which they analyzed were characterized by a wide range of vitamin C concentration, between 23.8 and 37.8 mg 100 g^{-1} f.m.

The average content of protein in beet leaves was 31.88% d.m. The increasing nitrogen doses clearly raised the protein accumulation in leaves has been noted. Raising the nitrogen dose from 0.2 to 0.4 g dm^{-3} in the growth medium led to a decrease in the dry matter and extract content in beet leaves, regardless of the applied nitrogen fertilizer (Table 1).

Significant influence of the nitrogen fertilizer type on all the determined nutrients present in aerial parts of leaf beet was noted. Leaves of plants fertilized with urea accumulated more N-total, N-NH_4, P, K, S, Fe, Zn, Mn and Cu compared to plants which were fertilized with ammonium nitrate. Also the dose of used nitrogen fertilizers had a significant effect on the mineral composition of leaf beet. When the nitrogen dose rose from 0.2 to 0.4 g dm^{-3} in the medium, the Fe and Cu content in beet leaves increased, while the concentrations of K, Ca, Zn and Mn declines, irrespective of the applied nitrogen fertilizer (Tables 2 and 3).

Table 3

Microelements in beet leave on nitrogen fertilizing (% d.m.)

Nitrogen fertilizer	Dose N (g dm^{-3})	Fe	Zn	Mn	Cu
NH_4NO_3	0.2 0.4 0.6	82.54 97.47 89.73	112.0 94.5 70.5	483.0 469.5 461.0	9.24 10.28 7.87
Mean for NH_4NO_3		89.91	92.33	471.1	9.13
$CO(NH_2)_2$	0.2 0.4 0.6	128.7 141.0 144.8	179.0 130.5 107.5	635.0 441.0 488.0	12.81 13.24 5.24
Mean for $CO(NH_2)_2$		138.2	139.0	521.3	10.43
Mean for dose	0.2 0.4 0.6	105.6 119.2 117.2	145.5 112.5 89.0	559.0 455.2 474.5	11.03 11.76 6.56
$LSD_{0.05}$					
Fertilizer N		3.758	6.422	2.854	0.488
Dose N		5.773	9.865	4.385	0.749
Nitrogen fertilizer x dose nitrogen		10.59	18.09	8.045	1.374

i.d. – insignificant differences

The results of the chemical analysis of the growth media are contained in Table 4. The mineral nitrogen content ($N\text{-}NH_4$ + $N\text{-}NO_3$) depended on the size of a dose of nitrogen fertilizers, and tended to increase at higher rates of nitrogen fertilizers. The highest concentration of ammonium nitrogen was noted in the medium enriched with the highest dose of nitrogen (0.6 g dm^{-3}) in the form of $CO(NH_2)_2$.

In the treatments with urea, the concentration of potassium was 68% higher than in the treatments fertilized with ammonium nitrate, which resulted in a higher concentration of salt in the medium when urea was used for fertilization.

The reaction of the growth media remained within the range of 5.58 and 6.15. The level of nitrogen fertilization ambiguously differentiated pH of the medium in treatments with NH_4NO_3, but when a double dose of nitrogen in the form of $CO(NH_2)_2$ had been used, a rise in the medium pH was noticed.

When the dose of 0.6 g N dm^{-3} in the form of $CO(NH_2)_2$ had been used for plant nutrition, the medium was characterized by the highest EC value (1.41 mS cm^{-1}). The total concentration of salt in the medium was in the range of 0.65 and 1.41 mS cm^{-1}. Increasing doses of nitrogen fertilization, whether as NH_4NO_3 or $CO(NH_2)_2$, were followed by increasing salt concentrations in the growth medium.

Table 4

Chemical concentration (mg dm^{-3}) and pH, EC values (mS cm^{-1}) in the media after termination of the experiment

Nitrogen fertilizer	Dose N (g dm^{-3})	N-NH_4	N-NO_3	P-PO_4	K^+	Ca^{2+}	Mg^{2+}	S-SO_4	Cl^-	EC	pH_{H_2O}
NH_4NO_3	0.2	56.33	19.33	154.3	59.0	1900	308.3	344.3	25.33	0.65	5.67
	0.4	140.6	37.33	166.0	35.33	2225	351.3	498.0	30.67	0.95	5.58
	0.6	237.3	86.67	231.3	32.67	1826	357.6	474.0	45.00	1.33	5.67
Mean for NH_4NO_3		144.7	47.78	183.8	42.33	1984	339.1	438.7	33.67	0.97	5.58-5.67
$CO(NH_2)_2$	0.2	58.67	12.67	231.0	126.3	2002	338.3	405.0	14.00	0.96	5.73
	0.4	196.6	22.33	151.3	119.3	2211	339.3	426.6	18.00	1.11	6.15
	0.6	351.0	30.33	175.6	156.3	2494	300.6	435.3	81.00	1.41	6.08
Mean for $CO(NH_2)_2$		202.1	21.78	186.00	134.0	2236	326.1	422.3	37.67	1.16	5.73-6.15
Mean for dose	0.2	57.50	16.00	192.6	92.67	1951	323.3	374.6	19.67	0.81	5.67-5.73
	0.4	168.6	29.83	158.6	77.33	2218	345.3	462.3	24.33	1.03	5.58-6.15
	0.6	294.1	58.50	203.5	94.50	2160	329.1	454.6	63.00	1.37	5.67-6.08
$LSD_{0.05}$											
Fertilizer N		24.74	3.022	i.d.	23.58	213.2	i.d.	i.d.	i.d.	0.174	
Dose N		37.09	4.531	40.32	i.d.	i.d.	i.d.	67.63	12.27	0.260	
Nitrogen fertilizer x dose nitrogen		66.09	8.073	71.85	i.d.	569.6	44.22	i.d.	21.87	i.d.	

i.d. – insignificant differences

CONCLUSIONS

1. In cultivation of leaf beet, an application of 0.4 g N dm^{-3} NH_4NO_3 proved to be the most favourable, as it led to the highest yield of fresh mass of leaves and the highest L-ascorbic acid content. In turn, owing to the lowest share of nitrates in the dry mass of leaves, an application of 0.2 g N dm^{-3} of the medium in the form of urea proved to be most superior.

2. When urea had been used for leaf beet fertilization, the aerial parts of this plant accumulated more N-total, N-NH_4, P, K, S, Fe, Zn, Mn, Cu than in plants nourished with ammonium nitrate.

3. Regardless of the type of nitrogen fertilizer, an increase of the total salt concentration (EC) in the growth medium occurred in response to the growing doses of nitrogen fertilizers.

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INFLUENCE OF WATER CONTAMINATION ON THE ACCUMULATION OF SOME METALS IN *HYDROCHARIS MORSUS-RANAE* L.

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Abstract

European frogbit (*Hydrocharis morsus-ranae)* grows mainly in stagnant, eutrophic and mesotrophic waters. It is found in various water ecosystems, but finds the optimal conditions for development in ecosystems formed with water soldier (*Stratiotes aloides*). The shortage of detailed literature data on the properties of water bodies where European frogbit and water soldier grow has stimulated this study, whose aim has been to determine the values of selected water quality parameters and concentrations of some metals in tissues of both hydrophytes.

The study was conducted in the late spring and summer of 2007 and 2008 and relied on environmental samples of water and plants (9 locations, including a field pond, a draining ditch on Pucka Isle, watercourses in Świdwie Nature Reserve, an oxbow lake of the Bug River and a flood pool near Głębokie Lake). The concentrations of $N\text{-}NO_3$, $N\text{-}NO_2$, $N\text{-}NH_4$ and PO_4^{3-} as well as the elements K, Na, Mg, Ca, Cd, Pb, Cu, Zn, Mn and Fe were measured in the water samples. Furthermore, the percentage of oxygen dissolved in water and the pH and electrolytic conductivity of water were determined. In order to assess the accumulation of metals by plants, the content of the same metals was measured in the water.

It was determined that *Hydrocharitetum morsus-ranae* L. associations grow in physiochemically very diverse aquatic environments. The range of differences in the examined water quality indicators was broader for European frogbit than for water soldier. With respect to the concentration of Zn, N and P compounds, pH and the content of dissolved oxygen in water, the ranges were the same for both species. *Hydrocharis morsus-ranae* and *Stratiotes aloides* differed in the accumulation of the metals.

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The range of the accumulation of most metals (except of K and Ca) was broader for Europen frogbit than for water soldier. In respect of Na, the range of accumulation for both plant species was the same. European frogbit spreads in water bodies in which the water is considered contaminated due to the concentrations of Cd, Pb, Cu and P.

Key words: metals, *Hydrocharis morsus-ranae* L., *Stratiotes aloides* L., water.

WPŁYW ZANIECZYSZCZENIA WÓD NA AKUMULACJĘ WYBRANYCH METALI PRZEZ *HYDROCHARIS MORSUS-RANAE* L.

Abstrakt

Żabiściek pływający (*Hydrocharis morsus – ranae* L.*)* występuje w wodach eu- i mezotroficznych. Zasiedla głównie wody stojące. Wchodzi w skład różnych zbiorowisk wodnych, lecz optymalne warunki rozwoju znajduje w obrębie zbiorowiska budowanego wraz z osoką aloesowatą (*Stratiotes aloides*). Ze względu na brak w piśmiennictwie szczegółowych danych dotyczących właściwości wód, w których występują żabiściek pływający i osoka aloesowata celem pracy było określenie wartości wybranych wskaźników jakości wód i zawartości niektórych metali w tkankach obu hydrofitów.

W latach 2007-2008, na przełomie wiosny i lata, przeprowadzono badania pobranych ze środowiska próbek wodnych i roślinnych (9 stanowisk obejmujących: oczko śródpolne, rów melioracyjny na Wyspie Puckiej, cieki na obszarze rezerwatu Świdwie, starorzecze Bugu, rozlewisko przy Jeziorze Głębokim). W próbkach wód zmierzono stężenie: $N\text{-}NO_3$, $N\text{-}NO_2$, $N\text{-}NH_4$ i PO_4^{3-} oraz K, Na, Mg, Ca, Cd, Pb, Cu, Zn, Mn i Fe. Ponadto określono procentową zawartość tlenu rozpuszczonego w wodzie oraz odczyn wody i przewodność elektrolityczną. W celu określenia wielkości akumulacji metali przez rośliny oznaczono zawartość tych samych metali w materiale roślinnym, co w wodzie.

Ustalono, że *Hydrocharitetum morsus-ranae* L. rośnie w bardzo różnorodnym fizykochemicznie środowisku wodnym, a zakres zmian wartości badanych wskaźników jakości wody był szerszy w przypadku żabiścieku pływającego niż osoki aloesowatej. W przypadku stężenia Zn, związków N i P, odczynu wody i zawartości tlenu rozpuszczonego w wodzie zakres występowania obu gatunków roślin był taki sam. Rośliny *Hydrocharis morsus-ranae* i *Stratiotes aloides* różniły się zdolnością akumulowania badanych metali. Zakres akumulacji metali, oprócz K i Ca, był szerszy w przypadku żabiścieku pływającego niż osoki aloesowatej. W przypadku zawartości Na zakres akumulacji obu gatunków roślin był taki sam. Żabiściek pływający występował m.in. w zbiornikach, gdzie ze względu na stężenie w wodzie: Cd, Pb, Cu i P, wody uznaje się za zanieczyszczone.

Słowa kluczowe: metale, *Hydrocharis morsus-ranae* L., *Stratiotes aloides* L., woda.

INTRODUCTION

Due to human pressure, degradation of aquatic ecosystems is ubiquitous (Koc et al. 2002). Pollutants entering water reservoirs affect the volume and concentration of substances dissolved in water (Grabińska et al. 2004) as well as their content in the bottom sediments (Skwierawski 2004, Szyperek 2004). The occurrence and distribution of macrophytes in aquatic ecosystems depend on a number of ecological factors, mainly: depth, type of the bottom, water movement, temperature, transparency and availability of nutrients (Bernatowicz, Wolny 1969, Gamrat, Gałczyńska 2006). The presence or absence of aquatic plants, and their quantitative ratios can define the ecological status of a given ecosystem (Sender 2007, 2008). Progressing eutrophication affects negatively the species diversity and development of macrophytes (Blindow 1992, Jeppesen et al. 2000). During the natural eutrophic and mesotrophic process of the shrinking of standing waters, plant communities of *Hydrocharitetum morsus-ranae* are the last stage in the development of aquatic vegetation. They supersede communities of plants with floating and submerged leaves, themselves being replaced by plant communities of *Equisetetum limosi, Phragmitetum, Thelypteridi-Phragmitetum, Typhetum latifoliae*. By producing huge amounts of phytomass per surface area unit, they cause rapid shallowing of occupied habitats (Podbielkowski, Tomaszewicz 1996). The content of harmful substances in an aquatic environment, such as heavy metals and organic compounds, generates various effects on the growth and development of these plant species (Maleva et al. 2004, Gałczyńska et al. 2011). The shortage of detailed literature data on the properties of water bodies in which European frogbit and water soldier occur has encouarged us to pursue this study in order to determine the values ??of selected water quality factors and concentrations of some metals in tissues of both hydrophytes. Determation of the impact of water pollution on the accumulation of selected elements by European frogbit can serve as comparative material for studies on other aquatic ecosystems settled by this species.

MATERIAL AND METHODS

Physicochemical studies of plant and water samples were carried out in the late spring and summer of 2007 and 2008. The research material was collected from nine water bodies, including a field pond, a draining ditch on Pucka Isle, watercourses in Świdwie Nature Reserve, an oxbow lake by the Bug River and a flood pool near Głębokie Lake (Table 1).

In order to determine the metal content in *Hydrocharis morsus-ranae* and *Stratiotes aloides*, all collected plant samples were dried and milled. Afterwards, the samples were mineralized in a mixture of hot acids: HNO_3 and $HClO_4$. The water samples were mineralized in HNO_3. Determinations of K, Na, Mg, Ca, Cd, Pb, Cu, Zn, Mn and Fe in mineralized plants and water samples in three replications were carried out by the atomic absorption spectrometry technique, using a Solaar S AA spectrometer.

The water samples were also submitted to determinations of nitrogen nitrate(V), ammonium cation, and orthophosphate(V). For determination of N and P in the waters, the colorimetric methods were used in accordance with the Polish Standards. The determinations were performed in a Spekol

Table 1

The occurrence of European frogbit and water soldier in the water bodies

No.	Water and plant samples location	Occurence	
		European frogbit	water soldier
1.	Draining ditch on Pucka Isle in Szczecin	*X*	-
2.	Field pond near Myślibórz	X	-
3.	Oxbow lake by the Bug River near Drohiczyn	X	X
4.	Ditch Z2, which is also a canal encompassing Świdwie Lake	X	X
5.	Gunica River flowing from Świdwie Lake	X	X
6.	Bolków-Łęgi canal, which empties into Świdwie Lake	*X*	*X*
7.	Struga Żurawia canal, which empties into Świdwie Lake	X	X
8.	Gunica River flowing from Stolsko Lake	X	-
9.	Flood pool by Głębokie Lake in Szczecin	X	-

11 spectrophotometer. The percentage of oxygen dissolved in water, water pH and electrolytic conductivity were determined in the water samples using the electroanalytical methods. The following equipment was used for the measurements of the physical parameters: an oxygen meter (HI 9145 microprocessor meter), a laboratory pH meter (CP-411 with a temperature sensor for liquids with sediments and an electrode EPS-1) and a conductometer (inoLab Coud 730 with TetraCon electrode 325).

The results of the physicochemical tests, depending on the presence of European frogbit in a given water body alone or together with water soldier, were statistically elaborated using a single factor analysis of variance. The significance of the differences between the means was determined by Tukey's test, with p=0.05. In order to determine the relationship between metal concentration in the tested water and its content in European frogbit, Pearson's linear correlation factor was calculated.

RESULTS AND THEIR DESCRIPTION

Elements leaching from agricultural soils, containing mineral fertilizers and plant protection chemicals, are an important source of water contamination in rural agricultural areas. Particularly high concentrations of PO_4^{3-} in water indicate eutrophication. However, it is worth noticing that the phosphorus concentration in water significantly decreases in summer due to the absorption of phosphorus by plant assemblages of different species of aquatic plants developing in water reservoirs. The association *Hydrocharitetum morsus-ranae* belongs to freshwater macrophyte communities which occur in very shallow water bodies. The field research comprising nine water bodies confirms the literature data (Matuszkiewicz 1984, Podbielkowski, Tomaszewicz 1996) in that this association usually inhabits eutrophic waters with an organic base (lakes or their bays, oxbow lakes, canals), in places which are sunny and sheltered from waves (Table 2). Water bodies number 1, 2 and 3 are located in an agricultural basin. The other analyzed reservoirs have not been exposed to strong human pressure over the last 20 years. In four sampling sites in water bodies nos 1, 2, 8 and 9 (Table 1), European frogbit grew alone, but in the remaining sites (nos 3-7) it occurred with water soldier (Table 1). Rather than competing, European frogbit and water perfectly complement each other in the use of water space. The water depth in which most of the plant communities grew did not exceed 1 meter, and the reaction of water was neutral or slightly alkaline (Table 2). It has been found

Table 2

Physicochemical parameters of water in the water bodies in which European frogbit occurred alone and with water soldier

Water quality indicators		Water bodies with European frogbit				Water bodies with European frogbit and water soldier				LSD$_{0.05}$
		mean	SD	min.	max.	mean	SD	min.	max.	
Physical parameters	el. conductivity (μS cm^{-1})	555	77	363	703	602	15	562	634	i.d.
	O_2 (%)	59	18	8	86	71	14	15	90	i.d.
	N-NO_3 (mg dm^{-3})	0.455	0.264	0.005	1.086	0.128	0.029	0.075	0.235	i.d.
Chemical parameters	N-NO_2^- (mg dm^{-3})	0.003	0.003	0.0	0.010	0.004	0.003	0.0	0.013	i.d.
	N-NH_4^+ (mg dm^{-3})	0.020	0.011	0.0	0.039	0.070	0.041	0.0	0.220	i.d.
	PO_4^{3-} (mg dm^{-3})	0.254	0.223	0.010	0.919	0.446	0.170	0.010	1.052	i.d.
	N_{min} (mg dm^{-3})	0.477	0.252	0.054	1.086	0.202	0.039	0.098	0.312	i.d.
	K (mg dm^{-3})	4.89	1.13	2.16	7.63	4.15	0.42	3.18	5.30	i.d.
	Mg (mg dm^{-3})	6.59	3.46	0.77	14.58	2.38	1.56	0.56	8.60	i.d.
	Ca (mg dm^{-3})	81.05	18.69	41.89	125.9	75.63	8.44	43.30	91.97	i.d.
	Na (mg dm^{-3})	19.40	5.32	10.23	33.74	11.70	1.19	8.30	15.18	i.d.
	Cd (mg dm^{-3})	0.009	0.006	0.0	0.022	0.001	0.001	0.0	0.007	i.d.
	Pb (mg dm^{-3})	0.093	0.054	0.0	0.211	0.002	0.002	0.0	0.011	i.d.
	Cu (mg dm^{-3})	0.050	0.047	0.0	0.190	0.019	0.019	0.0	0.093	i.d.
	Zn (mg dm^{-3})	0.017	0.005	0.007	0.032	0.025	0.015	0.0	0.085	i.d.
	Mn (mg dm^{-3})	0.823	0.820	0.001	3.284	0.142	0.097	0.0	0.521	i.d.
	Fe (mg dm^{-3})	1.280	0.926	0.007	4.032	0.327	0.098	0.062	0.558	i.d.

that the association *Hydrocharitetum morsus-ranae* grows in aquatic environments that possess highly different physiochemical properties (Table 2). Despite the generally higher average values of the determined water quality parameters in the water bodies with European frogbit growing alone, the performed statistical analysis indicates that there are no significant differences between the average values of these parameters depending on the classification of the examined water reservoirs (Table 2). The range of values of the analyzed examined water quality indicators was broader for the habitats occupied exclusively by European frogbit than for the ones in which it cooccurred with water soldier (Table 2). Previous studies by Pindel and Wo•niak (1998), Obolewski et al. (2009) and Gałczyńska et al. (2011) suggest that water soldier can grow in waters with higher conductivity values than European frogbit. This study has revealed that the range of occurrence of both plant species was the same with respect to the concentrations of zinc, nitrogen and phosphorus compounds, water pH and the content of dissolved oxygen in water (Table 2).

The references seem to contain no information on concentrations of nitrogen and phosphorus compounds in waters occupied by European frogbit. The data given by Tarkowska-Kukuryk (2006) on concentrations of nitrate nitrogen(V) and ammonia nitrogen in waters inhabited by water soldier are consistent with our results for the water bodies with European frogbit and water soldier (Table 2). The analysis of the results from Obolewski et al. (2009) shows that *Stratiotes aloides* inhabits waters which are characterized by even higher concentrations of orthophosphate(V) (0.63-1.31 mg dm^{-3}) than shown in this work (Table 2). In respect of pH, the literature data indicate better adaptation of water soldier to fluctuations in water pH. Pindel, Wo•niak (1998) reported that *Hydrocharis morsus-ranae* grew in waters where pH ranged from 7.0 to 7.9. On the other hand, the research carried out by Renman (1989) shows that water soldier develops in waters with pH of 5.64 to 7.50. and Obolewski et al. (2009) report that in oxbow lakes adjacent to the Łyna River water soldier colonized waters with pH of 7.6 to 8.49.

Among the analyzed water bodies (Table 2), the highest concentration of PO_4^{3-} was measured in the water of the canal surrounding Świdwie Lake (1.052 mg dm^{-3}), whereas the highest N_{min} was found in the field pond near Myślibórz (1.086 mg dm^{-3}). The lowest concentration of these elements (0.010 mg PO_4^{3-} dm^{-3} and 0.054 mg $N_{min.}$ dm^{-3}) was measured in the Gunica River flowing from Stolsko Lake. The highest concentration of the above metals was measured in three sampling points of reservoirs nos 1-3 located in the agricultural basin. The highest concentrations of potassium, calcium, magnesium, lead and copper were measured in the waters of the field pond near Myślibórz, and the highest concentrations of sodium, cadmium, manganese and iron were determined in the water of the draining ditch on Pucka Isle in Szczecin. The highest concentration of zinc in water was assayed in the oxbow lake of the Bug River (Table 2). According to the Regulation of the

Minister for Environment of 20th August 2008 on classification of surface waters, it has been concluded that European frogbit occurs in water bodies in which the water is considered to be contaminated due to the concentrations of cadmium, lead, copper and phosphorus.

Our analysis of the average levels of metals in European frogbit depending on its occurrence alone or together with water soldier showed no statistically significant differences between the means except for the concentration of zinc (Table 3). Considering the two species in the *Hydrocharitetum morsus-ranae* association, it was noticed that European frogbit and water soldier differed in the accumulation of the analysed metal ions (Tables 3 and 4). The scope of accumulation of these elements was broader for European frogbit than for water soldier, with the exception of potassium and calcium ions. In the case of sodium ions, the scope of their accumulation was the same for both plant species. With its shallow root system, European frogbit uses only the upper water layers, while water soldier's roots reach deep and take up nutrients from the lower parts of a reservoir (PODBIELKOWSKI, TOMASZEWICZ 1996).

Potassium and nitrogen dominate among the macronutrients in plants, but there are quite large amounts of calcium and much less of sulphur, phosphorus and magnesium. It was determined that the average content of potassium was 56.84 mg K g^{-1} d.w. of water soldier and 13.59 mg K g^{-1} d.w. of European frogbit. No effect of the potassium level in water on its content in dry matter of European frogbit was found. Many researchers stress that *Hydrocharitetum morsus-ranae* associations occur in waters rich in calcium

Table 3

Concentrations of metals in European frogbit either growing alone or in associations with water soldier

Metal	Water bodies with European frogbit				Water bodies with European frogbit and water soldier				$LSD_{0.05}$
	mean	SD	min.	max.	mean	SD	min.	max.	
K (mg g^{-1}d.w.)	16.34	10.53	8.59	47.80	10.84	2.26	7.79	19.79	i.d.
Mg (mg g^{-1}d.w.)	3.86	2.04	1.23	9.94	3.50	1.96	1.10	11.32	i.d.
Ca (mg g^{-1}d.w.)	12.48	6.44	1.61	27.55	16.42	4.03	7.93	30.99	i.d.
Na (mg g^{-1}d.w.)	5.83	1.84	2.90	11.14	4.28	1.57	1.46	10.38	i.d.
Cd (μg g^{-1}d.w.)	0.0	0.0	0.0	0.0	0.20	0.20	0.0	1.01	i.d.
Pb (μg g^{-1}d.w.)	16.32	7.11	0.0	32.33	21.26	11.79	0.47	67.18	i.d.
Cu (μg g^{-1}d.w.)	16.56	1.37	14.47	20.49	16.63	4.25	3.44	29.40	i.d.
Zn (μg g^{-1}d.w.)	888.4	510.9	70.96	2219	34.99	9.17	0.48	52.98	850.1
Mn (mg g^{-1}d.w.)	4.70	2.69	0.69	12.49	5.03	1.70	0.01	9.71	i.d.
Fe (mg g^{-1}d.w.)	2.60	1.77	0.67	7.89	0.66	0.24	0.09	1.43	i.d.

Table 4

Concentrations of the selected metals in dry matter of water soldier sampled from the water bodies where it co-occurred with European frogbit

Metal	Water bodies with European frogbit			
	mean	SD	min.	max.
K (mg g^{-1}d.w.)	56.84	21.53	22.20	78.65
Mg (mg g^{-1}d.w.)	6.98	2.78	3.41	10.45
Ca (mg g^{-1}d.w.)	31.46	15.82	5.40	42.46
Na (mg g^{-1}d.w.)	7.89	3.88	4.43	14.52
Cd (μg g^{-1}d.w.)	0.001	0.002	0.0	0.004
Pb (μg g^{-1}d.w.)	6.61	3.89	2.12	11.52
Cu (μg g^{-1}d.w.)	10.02	5.04	1.59	15.10
Zn (μg g^{-1}d.w.)	32.21	22.26	0.16	61.36
Mn (mg g^{-1}d.w.)	3.46	2.18	0.033	5.54
Fe (mg g^{-1}d.w.)	0.577	0.975	0.015	2.309

(ROSTAFIŃSKI 1956, RENMAN 1989). Water soldier accumulates calcium until September and its surplus precipitates in the form of carbonates on the surface of submerged leaves (KRÓLIKOWSKA 1997). In this study, water soldier accumulated an average of 31.46 mg Ca g^{-1} d.w. (Table 4) and European frogbit - just 14.45 mg Ca g^{-1} d.w. (Table 3). In all the water bodies with elevated concentrations of calcium in the water, the content of this element in the dry matter of European frogbit decreased significantly (Table 5). On the other hand, in the water bodies where European frogbit grew alone, the correlation was negative (Table 5). In the water bodies where European frogbit co-occurred with water soldier, an increase in the concentration of magnesium in water led to its significantly higher content in the dry matter of European frogbit (Table 5). Additionally, in the same water reservoirs, a statistically significant, albeit negative, linear relationship between the sodium concentration in water and its content in the dry matter of European frogbit was found (Table 5).

Heavy metals such as copper, zinc, iron and manganese are involved in many key metabolic processes. The exact role of lead and cadmium in the lifecycle of organisms is not known. The response of plants to metals, including heavy ones, depends on the sensitivity of individual plants, the severity of the stress and the form in which metal is available. The toxic effect of heavy metal absorption is related to their very high concentration in plant cells, which leads to impaired function of the membranes in photosynthetic and mitochondrial electron transport and inactivation of many en-

Table 5

Relationship between concentrations of the elements in water
and in the dry matter of European frogbit

Statistical parameters	Water bodies with European frogbit and with European frogbit and water soldier									
	metal									
	K	Mg	Ca	Na	Cd	Pb	Cu	Zn	Mn	Fe
c	0.029	0.083	i.d. **0.717**	i.d. 0.202	0.096	i.d. 0.406	i.d. 0.445	i.d. 0.202	**0.719**	**0.990**
s	0.940	0.832	**0.030**	0.603	0.806	0.278	0.230	0.602	**0.029**	**0.001**
Water bodies with European frogbit										
c	i.d. 0.107	i.d. 0.592	**0.940**	i.d. 0.323	i.d.	i.d. **0.957**	i.d. 0.534	i.d. 0.426	i.d. 0.428	**0.994**
s	0.893	0.408	**0.050**	0.671	i.d.	**0.043**	0.466	0.574	0.572	**0.006**
Water bodies with European frogbit and water soldier										
c	0.601	**0.998**	i.d. 0.259	i.d. **0.853**	i.d.	i.d. 0.164	i.d. 0.534	i.d. **0.904**	0.400	0.706
s	0.283	**0.001**	0.675	**0.046**	i.d.	0.792	0.466	**0.035**	0.505	0.136

c - correlation coefficient, *s* – significance level,
i.d. – insufficient data; statistically significant correlations in bold

zymes involved in the regulation of the basic cell metabolism. These events cause disorders of in the growth and development of plants (Gruca-Królikowska, Wacławek 2006, Gałczyńska et al. 2011).

Maleva et al. (2004) reported that when water was contaminated with cadmium or with copper at a concentration of 0.025 g dm^3, the bioaccumulation factor for European frogbit was 597 and 618, respectively, and when the contamination reached 0.25 g dm^3, it equalled 276 and 336. In the analyzed water bodies, the influence of the content of cadmium and copper in water on the concentration of these metals in the dry matter of European frogbit was not detected. With regard to lead, a statistically significant negative correlation was observed only in the water bodies where European frogbit grew alone. In turn, as the zinc concentration in water increased, the amount of this metal content in the dry matter of European frogbit significantly decreased, but this relationship was verified only in the water bodies overgrown with European frogbit and water soldier (Table 5). In respect of manganese and iron in all the water reservoirs, a significant and positive linear correlation between the concentration of these elements in water and their content in the dry matter of European frogbit was detected. In the water bodies where this species occurred independently, this relationship was identified only for iron.

CONCLUSIONS

1. European frogbit was able to live in water bodies in which the water was deemed contaminated due to excessive concentrations of cadmium, lead, copper and phosphorus.

2. The ecological range of occurrence of European frogbit, in terms of most chemical parameters of a habitat, is wider than that of water soldier. In respect of the concentrations of zinc, N and P compounds in water, the range of occurrence of both plant species is the same.

3. The range of accumulation of metals, except for potassium and calcium, was wider for European frogbit than for water soldier. By accummulating manganese and iron, European frogbit reflects concentrations of these metals in water. The occurrence of European frogbit along with water soldier affects the size of accumulation of zinc by European frogbit.

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PHYTOTOXICITY AND SPECIATION OF COPPER AND NICKEL IN COMPOSTED SEWAGE SLUDGE*

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Abstract

In recent years, sewage sludge production in Poland has increased sharply. Composting is one of the most preferable methods for sewage sludge utilization. Compost can improve the physical and chemical properties of soil. However, the quality of sewage sludge composts should be assessed before soil application by using biological and chemical methods. A germination test is used as a biological index to determine the possible phytotoxic effect of composts. Chemical methods are helpful in assessment of availability of metals to plants.

This study has been conducted to validate the effect of composting process on phytotoxicty, speciation and availability of copper and nickel in sewage sludge composted with sawdust and wheat straw. The composting process of organic wastes was carried out in chambers of a bioreactor for 28 days; afterwards each chamber was emptied and the content was stored in a closed room for 3 months to allow the maturation process to complete. Samples for analysis were collected at three characteristic phases of the composting process: mesophilic, thermophilic and mature compost. Determinations of the changes in metal speciation as well as metal bioavailability were achieved, respectively, with the technique of BCR sequential analysis and single extraction method using DTPA. Phytotoxicity of the analyzed composts was evaluated with the germination index using cress seeds.

It was found that an increasing proportion of sewage sludge in composted mixtures increased copper and nickel content in compost fractions as well as bioavailable quantities of these metals. The germination index increased only during the composting process of a mixture containing 30% of sewage sludge. The compost with 75% share of sewage sludge strongly inhibited germination of cress seeds. The process of compost maturation resulted in an increase of copper in fractions I and III. However, the smallest amounts of copper were determined in exchangeable bonds and the highest ones were determined in bonds with organic matter and sulphides. Composting sewage sludge with sawdust and wheat

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straw reduced the availability of nickel, which resulted in a decline in the content of this metal in exchangeable bonds (fraction I) but an increase in poorly- and non-extractable ones (fractions III and IV).

Key words: sewage sludge, compost, germination index, copper and nickel speciation, bioavailability.

FITOTOKSYCZNOŚĆ I SPECJACJA MIEDZI I NIKLU W KOMPOSTOWANYM OSADZIE ŚCIEKOWYM

Abstrakt

W ostatnich latach w Polsce notuje się znaczne zwiększenie masy osadów ściekowych. Kompostowanie jest uważane za jedną z najbardziej korzystnych metod wykorzystania osadów ściekowych. Kompost może przyczynić się do poprawy właściwości fizycznych i chemicznych gleby. Jednak jakość kompostów powstałych z osadów ściekowych przed ich doglebowym zastosowaniem powinna być oceniana za pomocą biologicznych i chemicznych metod. Indeks biologiczny oparty na teście kiełkowania roślin pozwala określić ewentualny fitotoksyczny efekt kompostów. Natomiast metody chemiczne pomocne są w ocenie stopnia przyswajalności metali dla roślin.

W badaniach określono fitotoksyczność, specjację i bioprzyswajalność miedzi i cynku w osadzie ściekowym kompostowanym z trocinami i słomą. Proces kompostowania odpadów organicznych przebiegał w komorach bioreaktora przez 28 dni, po czym kompostowaną biomasę zdeponowano w zamkniętym pomieszczeniu na okres 3 miesięcy celem zakończenia procesu dojrzewania. Próbki do analiz pobierano w trzech charakterystycznych fazach kompostowania: mezofilnej, termofilnej oraz kompoście dojrzałym. Zmiany specjacji oraz bioprzyswajalności metali oceniono wykorzystując odpowiednio analizę sekwencyjną BCR oraz ekstrakcję pojedynczą z roztworem DTPA. Fitotoksyczność analizowanych kompostów określono z wykorzystaniem testu kiełkowania nasion rzeżuchy.

Stwierdzono, że efektem większego udziału osadu ściekowego w kompostowanej mieszance były większe bioprzyswajalne ilości miedzi i niklu oraz ich zawartości we frakcjach kompostów. Większą wartość indeksu kiełkowania stwierdzono tylko podczas kompostowania mieszanki, gdzie udział osadu ściekowego wynosił 30%. Kompost, w którym osad ściekowy stanowił 75%, silnie hamował kiełkowanie nasion rzeżuchy. Rezultatem procesu dojrzewania kompostów była większa zawartość miedzi we frakcji I i III. Niezależnie od tego, najmniejszą zawartość tego metalu oznaczono w połączeniach wymiennych, a największą w połączeniach z materią organiczną i siarczkami. Proces kompostowania osadu ściekowego ze słomą i trocinami zredukował przyswajalność niklu, czego wyrazem było zmniejszenie jego zawartości we frakcji I, a zwiększenie w połączeniach trudno- i nieekstrahowanych (fr. III i IV).

Słowa kluczowe: osady ściekowe, kompost, indeks kiełkowania, specjacja miedzi i niklu, bioprzyswajalność.

INTRODUCTION

With continually increasing amounts of sewage sludge deposited at municipal sewage treatment plants (Central Statistical Office 2010) and the binding legal acts (Journal of Law 2010, No 28, item 145) which enforce their effective and environmentally friendly utilization, sewage sludge composts seem a possible solution. This was indicated in the Act of 27 April 2001 (Journal of Law, No 62, item 628), which stipulated that sewage sludge may be recycled by composting. The composting process based on simultaneous biochemical-microbiological transformations generates compost. It is assumed that this end-product of composting is a stable and mature substance which, when applied into soil, will evoke positive changes in physical, physicochemical and chemical soil properties, and it will stimulate soil microbiological activity (DEBOSZ et al. 2002, JORDĂO et al. 2006, SĄDEJ, NAMIOTKO 2007, HARGREAVES et. al. 2008). According to these researchers, soil fertilization with compost improves its fertility, as expressed by higher quantities of organic carbon, macro- and micronutrients as well as by better sorption and buffer capacity.

However, broad assessment of the compost fertilizing value should be performed, also including potential hazard to the environment. The risk arises mainly due to the presence of organic and inorganic compounds toxic to living organisms in composted sewage sludge (BARKER, BRYSON 2002, JAKUBUS 2005). It should be borne in mind that inorganic contaminations associated with heavy metals do not undergo biodegradation in the course of sewage sludge composting (NAIR et al. 2008), hence it is recommended to evaluate the risk factor connected with soil application of composts from sewage sludge. From this point of view, potential phytotoxicity of composts expressed by the inhibition of seed germination (WALTER et al. 2006, KO et al. 2008) or the increase in labile metal forms available to plants (HUANG et al. 2005, JORDĂO et al. 2006) is emphasized. According to MIAOMIAO et al. (2009), phytotoxicity is connected with some forms of metals, therefore metal speciation and bioavailability as well as phytotoxic effects should be studied.

In this study, changes in phytotoxicity as well as copper and nickel speciation and bioavailability during the composting process of sewage sludge with straw and sawdust were determined.

MATERIAL AND METHODS

The objective of the investigations was achieved using compost samples from an experiment carried out in controlled conditions by using a double-chamber bioreactor of the capacity of 125 dm^3 per chamber. A diagram of the bioreactor together with its description can be found in OLSZEWSKI et al. (2005). Each composting mixture was prepared in two replications, i.e. each replication of a tested composting mixture was in a separate chamber of the bioreactor. The experimental composts were prepared using sewage sludge from a mechanical-chemical sewage treatment plant, wheat straw and sawdust. All organic waste was well mixed prior to transferring to bioreactor chambers. The moisture of the mixtures was 60% and the amount of air flowing through the mixtures corresponded to the volume of 4 dm^3 min^{-1}. Some properties of the components and proportions in the composted mixtures can be found in Tables 1 and 2. Each mixture remained in the bioreactor's chambers for 28 days. Afterwards, each chamber was emptied and the content was placed in a closed room for 3 months to allow complete maturation. Analyses were performed on compost samples collected during three characteristic phases of the composting process: mesophilic, thermophilic and mature compost. Four subsamples of compost were collected in the course of each phase. The gathered samples were mixed together. The material thus prepared served as a mean sample for each compost replication and phase.

Table 1

Some properties of composted waste

Waste	Dry matter	Corg	Nog	Ni	Cu
	(g kg^{-1})	(g kg^{-1} d.m.)		(mg kg^{-1} d.m.)	
Sewage sludge	167.0	330.0	53.0	15.65	262.24
Sawdust	820.0	500.0	1.0	2.33	2.65
Wheat straw	860.0	440.0	3.0	1.29	4.35

Table 2

Composition of investigated composts

Compost	Share in compost (%)			C:N
	sewage sludge	sawdust	wheat straw	
C1	75	20	5	9.2:1
C2	60	35	5	12.1:1
C3	45	50	5	17.0 : 1
C4	30	65	5	26.4:1

Samples were dried out at 105°C for 12 hours. The dried samples was ground into fine powder and stored in plastic bags at 4°C. Copper and nickel speciation was achieved with the BCR sequential analysis. The details of the experimental protocol are given in Table 3. The bioavailable content of metals was determined with the single extraction method using DTPA complexing solution (0.005 mol dm^{-3} DTPA + 0.1 mol dm^{-3} TEA + 0.01 mol dm^{-3} $CaCl_2$, of pH 7.3) (QUEVAUVILLER et al. 1998). The compost : solution ratio was 1:2. Concentrations of copper and nickel in extracts were determined by flame atomic absorption spectrometry (FAAS) using a Varian Spectra AA 220 FS.

Table 3

BCR sequential extraction procedure (Mossop, Davidson 2003)

Fraction	Extracting agent	Extraction conditions	
		t (h)	temperature (°C)
Fr. I – exchangeable	0.11 mol dm^{-3} CH_3COOH (pH= 7.0)	16	20 - 25
Fr. II – reducible (metals bound to Fe and Mn oxides)	0.5 mol dm^{-3} NH_2OH-HCl (pH=1.5)	16	20-25
Fr. III – oxidisable (metals bound to organic matter and sulphides)	30% H_2O_2 (pH=2.0). and then 1.0 mol dm^{-3} CH_3COONH_4 (pH=2.0)	1. 2. 16	20-25. 85. 20-25
Fr. IV – residual	*aqua regia*	2.5	60-70

The analytical accuracy of the laboratory procedures was evaluated with a BCR analysis of reference material CRM 145R (trace elements in sewage sludge), and the data of three replicate analyses obtained for the total content of Cu and Ni are shown in Table 4.

Table 4

Content (mean ± SD) of copper and nickel determined for reference material BCR 145R

Metal	Found value (mg kg^{-1} d.m.)	Certified value (mg kg^{-1} d.m.)	Precision	Accuracy
Ni	294.4 ± 5.66	251.0 ± 7.71	1.94	17.3
Cu	720.7 ± 8.96	707.0 ± 8.83	1.24	1.94

The germination index (GI) has been used to determine phytotoxicty of the composts. Cress seeds (*Lepidium sativumm* L.) were germinated in GI assays. Extracts of compost were obtained by shaking 10 g of fresh matter of compost with 100 cm^3 of distilled water for 2 h at room temperature. Ten cress seeds were placed in Petri dishes (10 cm in diameter and 1.5 cm deep) filled with filter paper soaked in 5 cm^3 of the compost extract and incubated in the dark at 25°C for 48 h. The control consisted of 5 cm^3 distilled water. The percentages of relative seed germination (RSG), relative root growth (RRG) and germination index (GI) were calculated according to the following formulas (Miaomaio et al. 2009):

$$\mathrm{RSG}\ (\%) = \frac{mean\ number\ of\ seeds\ germianted\ in\ compost\ extract}{mean\ number\ of\ seeds\ germianted\ in\ control} \cdot 100,$$

$$\mathrm{RRG}\ (\%) = \frac{mean\ root\ lenght\ in\ compost\ extract}{mean\ root\ lenght\ in\ control} \cdot 100,$$

$$\mathrm{GI}\ (\%) = \frac{RSG \cdot RRG}{100}.$$

Analyses of mean samples were carried out in three replicates. The results were submitted to analysis of variance for two-factor experiments, using *F* test at the level of significance $p \leq 0.95$. Two basic experimental factors were taken into consideration: compost mixtures (A) and time of composting (B). The least significant differences were calculated using Tukey's test at the level of significance of $\alpha \leq 0.05$ and then homogenous uniform within the factor level were established.

RESULTS

As evident from the data collated in Tables 5 and 6, the experimental factors exerted significant influence on the quantitative changes of the analyzed metals in compost fractions. This impact varied depending on the metal and the type of bond it developed with the solid phase of the composts. Irrespective of the compost chemical composition and composting phase, copper quantities in fractions increased in the following order: fr. I< fr. IV < fr. II < fr. III.

The analogous sequences for nickel changed depending on the phase of the process. In samples collected during the mesophilic phase, most Ni formed bonds with Fe and Mn oxides (4.3 mg kg^{-1} d.m. for C1 and 4.1 mg kg^{-1} d.m. for C2) or exchangeable bonds (3.4 mg kg^{-1} d.m. for C3 and 2.6 mg kg^{-1} d.m. for C4). Conversely, irrespective of their chemical composition, mature composts were characterized by the following sequence of increasing quantities of this metal: fr. I< fr. II < fr. III < fr. IV.

The mutual impact of the experimental factors was least noticeable with regard to the copper content in the compost residual fraction. Following sewage sludge composting with straw and sawdust, the copper content declined but the recorded differences were non-significant (Table 5). In each consecutive phase, the content of this metal also decreased in fraction II and, consequently, in mature composts it was 40 to 60% lower than in samples from the mesophilic phase. The process of composting sewage sludge with straw and sawdust led to an increased copper content in exchangeable and organic bonds. As evidenced by the data collated in Table 5, the copper content in fraction I of samples collected during the mesophilic phase was the lowest, ranging from 1.54 mg kg^{-1} d.m. to 1.74 mg kg^{-1} d.m. (C1-C3) and 3.07 mg kg^{-1} d.m. (C4). On the other hand, mature composts were characterized by 4- to 4.5-fold (C1-C3) and two-fold (C4) higher content of the metal. It is noteworthy that during the thermophilic phase, the copper content in the discussed bonds in the composts with the highest sewage sludge proportion (C1 and C2) was the highest, ranging from 9-10 mg kg^{-1} d.m., which was 6 times more than during the mesophilic phase (Table 5). Such big differences in copper quantities in organic bonds were not record-

Table 5

Content of copper in separate fractions depending on the type of compost and composting phase (mg kg^{-1} d.m.)

Composting phase	Fractions			
	I	II	III	IV
C1				
Mesophilic	1.54*c**	31.28*a*	140.55*c*	13.64*b*
Thermophilic	9.04*a*	30.05*a*	180.00*b*	23.45*a*
Mature compost	6.49*b*	17.53*b*	212.87*a*	13.14*b*
C2				
Mesophilic	1.74*c*	36.31*a*	118.04*a*	14.58*a*
Thermophilic	10.72*a*	26.36*b*	141.30*b*	13.59*ab*
Mature compost	7.91*b*	13.28*c*	175.77*c*	10.23*b*
C3				
Mesophilic	1.61*b*	41.19*a*	72.98*b*	7.03*a*
Thermophilic	5.44*a*	31.01*b*	88.25*b*	7.89*a*
Mature compost	6.80*a*	23.41*c*	112.79*a*	6.53*a*
C4				
Mesophilic	3.07*b*	24.42*a*	47.92*a*	9.87*a*
Thermophilic	5.83*a*	25.48*a*	74.60*b*	6.81*a*
Mature compost	5.62*a*	14.5*b*	91.95*c*	5.71*a*

* values in columns followed by the same letter do not differ significantly

ed. The content of copper in fraction III of all the examined composts increased gradually from the mesophilic phase, through the thermophilic one, reaching maximum values in mature composts, in which it was 1.5- (C1-C3) and 2-fold (C4) higher than in samples collected at the beginning of the experiment (Table 5).

The experimental factors exerted the weakest impact on nickel in fraction I of the examined composts (Table 6). This effect was statistically non-significant for the compost with the highest proportion of sewage sludge in its composition. In contrast, in composts C3 and C4, the content of nickel in the analyzed bonds was found to decrease. Mature composts C3 and C4 were characterized respectively by 55 and 53% lower quantity of this metal in comparison with its level determined in samples from the mesophilic phase. The analysis of the data contained in Table 6 suggests that the lack of a mutual impact of the experimental factors also became apparent in respect to the Ni content in fraction II of compost C4. Nonetheless, in all the examined composts, an increase in Ni in bonds with Fe and Mn oxides was

Table 6

Content of nickel in separate fractions of composts depending on the type of compost and composting phase (mg kg^{-1} d.m.)

Composting phase	Fractions			
	I	II	III	IV
C1				
Mesophilic	3.32*a**	4.34*b*	3.70*c*	3.52*b*
Thermophilic	3.68*a*	5.03*b*	5.06*b*	4.63*b*
Mature compost	3.21*a*	5.98*a*	6.29*a*	6.98*a*
C2				
Mesophilic	3.50*a*	4.06*b*	3.67*c*	2.82*c*
Thermophilic	3.15*a*	4.16*b*	4.72*b*	4.18*b*
Mature compost	3.68*a*	5.14*a*	6.27*a*	6.51*a*
C3				
Mesophilic	3.42*a*	2.78*a*	2.38*c*	2.42*b*
Thermophilic	2.09*b*	3.20*a*	3.29*b*	3.46*b*
Mature compost	1.55*b*	3.71*a*	4.22*a*	5.26*a*
C4				
Mesophilic	2.56*a*	2.10*a*	1.18*b*	1.58*b*
Thermophilic	1.75*b*	2.34*a*	1.40*b*	2.57*ab*
Mature compost	1.19*b*	2.79*a*	2.50*a*	3.63*a*

* cf. Table 5

observed (in the order of 26-38%) while the process of composting sewage sludge progressed (Table 6). Changes in the nickel content in fractions III and IV of the examined composts which occurred during the composting process were identical. The lowest, i.e. 30%, sewage sludge proportion in composted matter, led to 2 times greater amount of nickel in the mature compost C4 determined in samples representing the mesophilic phase. In conditions of the remaining composts, the appropriate difference ranged from 71 to 77% (Table 6). The composition of a composted mixture did not have such a strong impact on quantitative changes of nickel in the residual fraction as in organic bonds. The data in Table 6 indicate that the content of this metal in fraction IV increased gradually from the beginning of the composting process of sewage sludge mixed with straw and sawdust and was eventually twice as high in mature composts.

The content of bioavailable copper and nickel also changed after the application in the examined sewage sludge composts. As evident from the data presented in Figure 1, the lowest Cu (17.78 mg kg^{-1} d.m.) and Ni

(1.25 mg kg^{-1} d.m.) content was determined in compost C4, while the maximum one (83.27 mg Cu kg^{-1} d.m. and 3.23 mg Ni kg^{-1} d.m.) – in compost C1. The difference between these extreme values was 6.5- and 2.5-time for copper and nickel, respectively. The mutual impact of both experimental factors was significant only in the case of copper (Figure 1). While composting sewage sludge together with straw and sawdust, the content of bioavailable copper increased gradually from the beginning of the process, reaching the highest level in mature composts. Irrespective of the composition of the composted mixture, 2.5-3.0-fold more bioavailable copper was found in the mature material. No such trends in quantitative changes were determined for nickel. The content of bioavailable nickel, irrespective of the composting phase, remained similar and statistically significant differences were determined only in the case of compost C4 (Figure 1).

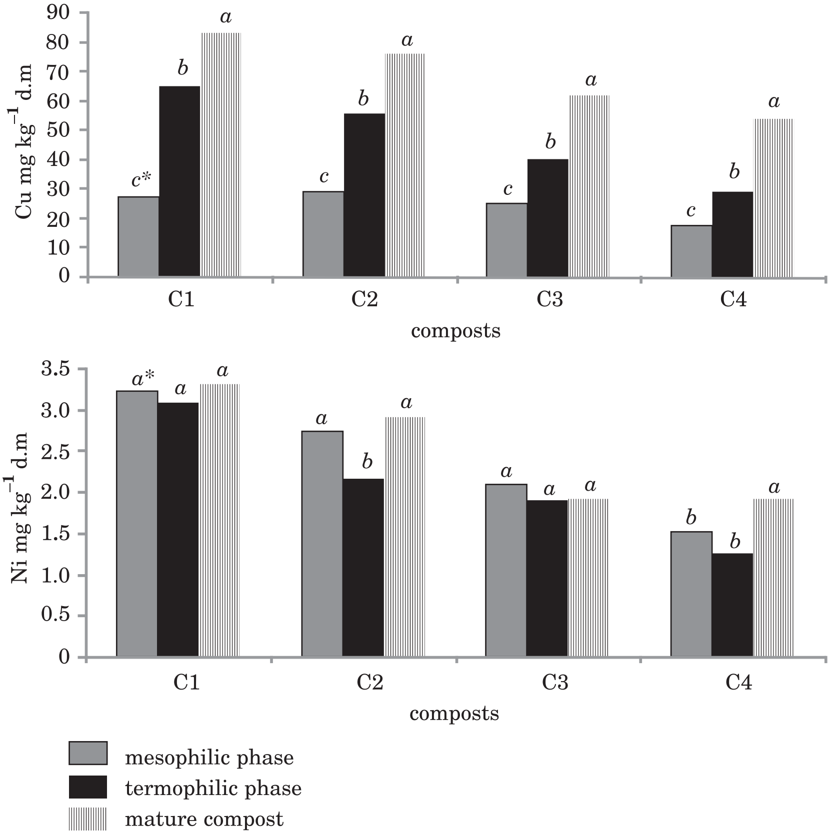

Fig. 1. Bioavailability of copper and nickel in dependence on type of compost and composting phase

*values followed by the same letter do not differ significantly

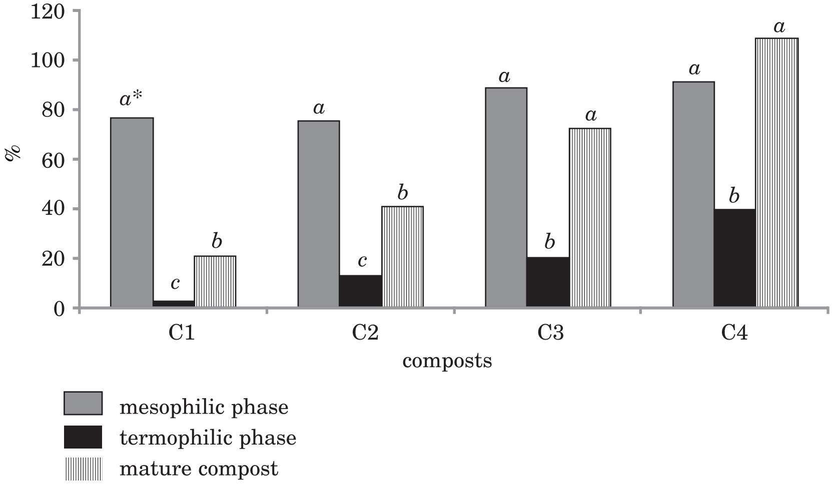

Fig. 2. Influence of composting process on index germination changes in analysed composts

*values followed by the same letter do not differ significantly

The germination index values presented in Figure 2 were evidently and statistically significantly modified by the experimental factors, ranging from 2.44% (C1) to 109.15% (C4). Mature composts in which sewage sludge constituted 45 to 75% of the total composition were characterized by 75 (C1), 46 (C2) and 18% (C3) lower GI values in comparison with the ones determined during the mesophilic phase. Mature compost containing 30% of sewage sludge (C4) was an exception, as the GI value determined in it was by 20% higher than in the initial mixture (Fig. 2). It was typical of the examined composts that the lowest GI values were determined during the thermophilic phase. These values were found to be lower when the share of sewage sludge in composted mixture was higher, i.e. 2.44% for C1 but 39.43% for C4 compost. During the mesophilic phase of the composting process, the discussed index attained values which were 32- (C1), 6- (C2), 4- (C3) and 2-fold (C4) higher than found during the thermophilic phases (Figure 2).

DISCUSSION

Unlike most of the organic compounds, metals remain in mature composts, after all the transformations of organic matter, which can be considered disadvantageous to their bioavailability. Numerous researchers (Barker, Bryson 2002, Jordão et al. 2006, Jakubus, Czekała 2010, Jakubus 2010) emphasize that during the composting process, metal availability and mobility are reduced due to their strong complexing by the forming humus. The fact that nickel mobility was reduced while composting sewage sludge with straw and sawdust was confirmed by the results of our experiments. Nickel is a mobile and water-soluble element. Besides, it tends to bind with soluble organic matter (KABATA-PENDIAS, PENDIAS 1999). Such metal bonds are so easily released that their number tends to rise in the initial phases of composting. However, further transformations which humic compounds in compost undergo make them form complexes with metals which are either poorly extractable or totally unextractable. It is therefore possible that nickel in the exchangeable fraction can undergo such quantitative transformations during the composting process of sewage sludge. Suggestions that quantitative transformations of nickel, from easily activated bonds to poorly extractable ones, occur during the sewage sludge composting process can also be found in papers published by GONDEK (2006), ZHENG et al. (2007), LIU et al. (2007), JAKUBUS and CZEKAŁA (2010). In the experiments described in this article, such transformations were particularly evident in composts C3 and C4 with a small share of sewage sludge (30% and 45%, respectively) but with a high content of sawdust (65% and 50%, respectively). According to JAKUBUS (2010), sawdust in composting mixtures can act as a sorbent of the metals released from the decomposing sewage sludge organic matter. Sawdust, as organic material from timber, undergoes slow degradation and can therefore, at least theoretically, enlarge the pool of poorly available metals.

Despite its considerable affinity with organic matter, nickel develops organic ligands characterised by poorer stability when compared with those formed by copper (NOMEDA et al. 2008). HUANG et al. (2005) came to the conclusion that sulphur compounds as well as functional groups of humic acids play a key role in this phenomenon. Also in the presented studies, this kind of preferential development of copper bonds with organic matter was demonstrated. The highest contents of the discussed metal in the organic fraction were also reported in studies by WALTER et al. (2006) and FARRELL and JONES (2009). The composting process to which sewage sludge mixed with straw and sawdust was subjected increased quantities of Cu both in the exchangeable as well as in the organic fractions. Analogous quantitative changes in copper in the same compost fractions were also presented by MIAOMIAO et al. (2009). JAKUBUS (2010) attributed this phenomenon to copper binding by soluble fulvic acid organic substances in sewage sludge and to possible precipitation of this metal in the form of carbonates. In the design of the sequential analysis applied in this study, carbonate forms and labile organic substances are described by fraction I.

Sequential analysis makes it possible to assess quantitative changes in forms and solubility of metals present in composts, which is particularly useful information when these composts are applied into soil. However, it is a time-consuming technique and therefore not routinely applied in practice.

Alternatively, application of complexing reagents, e.g. EDTA and DTPA, is recommended for determination of compost fertilizer suitability expressed by the content of bioavailable metals. According to Huang et al. (2005), bioavailability is identified with the degree of solubility and is the highest for water-soluble, exchangeable and carbonate forms of metals, but the lowest – for residual ones. In our investigations, the nickel content determined with DTPA solution was comparable with the one determined in fraction I of the examined composts. On the other hand, the content of bioavailable copper constituted the sum of the copper content in fractions I, II and partly III. In comparison with the content determined in samples from the mesophilic phase, more copper and the same amount of nickel were extracted from mature composts using DTPA solution. Similar effects of the sewage sludge composting process on changes in the content of bioavailable Ni and Cu were reported by Qiao and Ho (1997) as well as Fang and Wong (1999). However, as evident from the results presented by Huang et al. (2005) or Wong and Selvan (2006), it is by no means a rule because composting can also lead to a decrease in the content of bioavailable forms of these metals.

The decomposition of phytotoxic organic compounds occurs during the composting organic waste and can be assessed with biological methods. In practice, phytotoxicity of compost is evaluated according to the plant germination index, which is a rapid and sensitive technique for identification of substances strongly inhibiting plant germination and development (Walter et al. 2006, Ko et al. 2008, Miaomiao et al. 2009, Gao et al. 2010). These authors, after Zucconi et al. (1981), attribute inhibition of plant germination to ammonium, acetic acid, phenol or low-molecular fatty acids, that is the compounds released in the course of organic matter degradation; their presence indicates immaturity of composts.

Small values of the GI obtained in this study for the examined composts in their termophilic phase suggests the possibility of such toxic influence on cress seeds. The results indicate that the main source of these compounds was sewage sludge, a suggestion which was confirmed by the fact that higher proportions of this component in composted mixtures induced stronger germination inhibition of cress seeds. Zucconi et al. (1981, after Gao et al. 2010) maintain that the GI value above 80% is an indicator of mature, non-phytotoxic compost. In our investigations, such values were determined only for compost C4 with 30% content of sewage sludge, and they were found to increase in the course of composting. According to literature data (Bustamante et al. 2008, Ko et al. 2008, Gao et al. 2010), this is a dominant tendency in GI value changes. Nevertheless, similar GI values determined for C4 at the beginning and at the end of composting require caution in data interpretation. It is difficult to accept the fact that the examined experimental mixtures were characterized by the same degree of phytotoxicity at the beginning and at the end of the process, as suggested by the results. Ko et al. (2008) reported a similar problem but interpreted it by an extremely small threshold value of the germination index and suggested raising it 110, the value that can reliably determine compost maturity. Walter et al. (2006) attribute the ambiguity of the results to the selected test plant. Irrespectively of the results, the germination index for compost phytotoxicity assessment appears to be a prompt and important diagnostic method.

CONCLUSIONS

1. A higher proportion of sewage sludge in the composted mixture contributed to a stronger inhibition of cress seed germination and to obtaining higher quantities of bioavailable copper and nickel as well as their concentrations in the fractions.

2. Composting sewage sludge with straw and sawdust caused an increase in copper in fractions I and III and its reduction in fractions II and IV.

3. Sequential analysis of nickel in the examined composts confirmed reduced mobility of this metal during the composting process.

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INFLUENCE OF THE GŁOGÓW COPPER WORKS ON THE CONTENT OF MOBILE FORMS OF COPPER AND ZINC IN ARABLE SOILS

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Abstract

The aim of this research has been to investigate the influence of the Głogów Copper Works on profile distribution and mobility of copper and zinc in cultivated soils in the vicinity of the plant.

The following were determined in soil samples from 4 cultivated soil profiles classified as Luvisols: soil texture, pH in KCl, pH in H_2O, organic carbon, total Cu and Zn content using the ASA method after mineralization in HF and $HClO_4$ acids and the content of mobile forms of Cu and Zn using the sequential method. In terms of grain size distribution, the profiles were classified as loamy silt. The soil pH was in range of pH_{H_2O} 7.33-8.55 and pH_{KCl} 5.81-7.75. Except for P1 profile, the presence of $CaCO_3$ was observed in all soil profiles. C and Bt horizons were the richest in $CaCO_3$. The content of C-organic in humus horizons was in the range of 7.0-18.1 g kg^{-1}. The total Cu content was in the range of 4.04-57.75 mg kg^{-1}. In Ap horizons, the total Cu content was significantly higher, but in C horizons it was the lowest, which indicates that Ap horizons had been enriched with Cu by human activity. The results of the sequential analysis indicate the dominant share of fraction V, associated with organic matter, in the surface horizons. In the remaining horizons, the Cu fraction associated with iron oxides dominates. The total content of Zn was in the range of 3.34-42.65 mg kg^{-1}. The highest content of this element was observed in Ap horizons, and the lowest occurred in Eet horizons. The speciation analysis showed the highest content of Zn in the form associated with crystalline iron oxides (fraction VI), and the lowest in fractions I and II, i.e. soluble in water and exchangeable forms. Most of the investigated soils (apart from P1 profile) may be classified as unpolluted with Cu and Zn, which shows that the proximity of the Głogów Copper Works does not preclude agricultural use of the analyzed soils (with one exception).

Keywords: soils, copper, zinc.

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WPŁYW HUTY MIEDZI GŁOGÓW NA ZAWARTOŚĆ MOBILNYCH FORM MIEDZI I CYNKU W OKOLICZNYCH GLEBACH UPRAWWNYCH

Abstrakt

Celem badań była ocena wpływu Huty Miedzi Głogów na profilową dystrybucję oraz mobilność miedzi i cynku w okolicznych glebach uprawnych. Materiał badawczy stanowiły profile glebowe położone w odległości 5,7-6,8 km w kierunku południowo-wschodnim od emitora. W próbkach glebowych pochodzących z 4 profili gleb uprawnych sklasyfikowanych jako gleby płowe typowe oznaczono: uziarnienie, pH KCl (1 mol dm^{-3}) i pH w H_2O, C-organiczny oraz całkowitą zawartość Cu i Zn metodą ASA po mineralizacji w kwasach HF i $HClO_4$, a zawartość form mobilnych Cu i Zn metodą analizy sekwencyjnej. Pod względem uziarnienia badane profile zaliczono do pyłów gliniastych. W badanych glebach pH było w zakresie pH_{H_2O} 7,33-8,55 oraz pH_{KCl} 5,81-7,75, przyjmując najniższe wartości w poziomach Bt. Stwierdzono obecność węglanu wapnia, oprcz profilu (P1), którego zawartość wynosiła 0,43%-11,60%. Najzasobniejsze w $CaCO_3$ były poziomy C oraz poziomy Bt. Zawartość C-organicznego w poziomach próchnicznych wynosiła 7,0-18,1 g kg^{-1}, całkowita zawartość Cu 4,04-57,75 mg kg^{-1}. Stwierdzono wyraźnie wyższą całkowitą zawartość Cu w poziomach Ap oraz najniższą w poziomach C, co wskazuje na antropogeniczne wzbogacenie poziomów Ap w Cu. Wyniki analizy sekwencyjnej wskazują na dominujący udział w poziomach powierzchniowych frakcji V, tj. form związanych z materią organiczną, w pozostałych poziomach przeważa frakcja Cu związana z tlenkami żelaza. Całkowita zawartość Zn wynosiła 3,34-42,65 mg kg^{-1}, a najwyższą zawartość tego pierwiastka stwierdzono w poziomach Ap, najniższą w poziomach Eet. Analiza specjacyjna wykazała najwieksza zawartość Zn w formie związanej z krystalicznymi tlenkami żelaza (frakcja VI), a najniższą we frakcji I i II, tj. w formach wodnorozpuszczalnych i wymiennych. Badane gleby w większości (oprócz profilu P1) można zaklasyfikować do gleb niezanieczyszczonych Cu i Zn, co wskazuje, że bliskość Huty Głogów (oprócz jednego przypadku) nie eliminuje badanych gleb z użytkowania rolniczego.

Słowa kluczowe: gleba, miedź, cynk.

INTRODUCTION

The Głogów II Copper Works in Żukowice near Głogów has operated since 1978. It is a subsidiary plant of the Mining and Copper Industry Polska Miedź S.A. It is located in the Glogau-Baruther Urstromtal (*Pradolina Głogowska*), which is an area of agricultural and industrial use. Most of the area is occupied by cultivated soils. The Głogów Copper Works emits to the atmosphere metalliferous dusts, which permeate soil through the sedimentation process. Most of the dust emitted by non-iron works falls on soil, causing various changes. Heavy metals from dusts emitted by copper works show significantly higher solubility than lithogenic metals. They are mostly in the form of oxides, sulfides, sulfates and carbonates. Post-processing dusts emitted to the atmosphere are carriers of heavy metals, mostly Cu, Pb, Cd, Zn and As (ROSADA 2008). Emitted cement dusts affect the soil environment and the quality of crops grown on such soils.

The aim of this research has been to evaluate the influence of the Głogów Copper Works on the profile distribution and mobility of copper and zinc in arable soils in the vicinity of the plant.

MATERIAL AND METHODS

The research material consisted of 4 cultivated soil profiles, located 3.0--6.8 km away from the copper works. During the field tests, genetic horizons from which soil samples were taken were identified morphologically. The following laboratory analysis were performed on the soil samples: soil texture using Cassagrande method modified by Pruszyński, pH using the potentiometric method in H_2O and in KCl solutions (1mol dm^{-3}), organic C using Tiurin method, and content of $CaCO_3$ using Scheibler volumetric method. The total content of copper and zinc was determined after soil mineralization in a mixture of HF and $HClO_4$ acids (CROCK, SEVERSON 1980). The extraction of mobile lead was conducted by the sequential analysis according to MILLER et al. (1986) with the modification of DĄBKOWSKA-NASKRĘT (1998). The results were verified by analysis of certified material Till-3 (1995). Measurements of the content of total and mobile forms of Cu and Zn were performed using the atomic spectrometry method (ASA) in a spectrometer PU 9100X. The analyses were performed in triplicate. Statistical evaluation of the results was accomplished using Statistica 6.0 software.

RESULTS AND DISCUSSIONS

The analyzed cultivated soil profiles were classified as typical Luvisols formed from silt (PTG 1989). In agricultural categories (PTG 2008), they are medium (P1 in Wierzchosławice and P2 in Modła) and heavy soils (P3 in Kurowice and P4 in Nielubia). In the humus and eluvial horizons of the investigated profiles (apart from P2), the texture was similar to clay silt and loamy silt (Table 1), while the illuvial horizons represented clay silt (Table 1). These horizons were characterized by a low content of skeletal fraction (<8%) and sand fraction (23-28%). Three of the four examined profiles were found to contain calcium carbonate (the exception was profile P1). The richest in calcium carbonate were illuvial and bedrock horizons (Table 2), where the content of this compound was in the range of 3.46% to 11.60%. The pH of the soils was in the range of pH_{H_2O} 7.33-8.55 and pH_{KCl} 5.81--7.75, with the lowest values usually detected in the enrichment horizons of the investigated samples (Table 2). The neutral and higher values of pH were not always accompanied by a high content of $CaCO_3$, which indicates

Table 1

Soli texture

Profile	Horizon	Depth (cm)	Percentage of fraction in diameter (mm)					
			2 - 0.1	0.1 - - 0.5	0.05 - - 0.02	0.02 - - 0.002	0.002 - - 0.0002	< 0.0002
P1 Wierzchowice	Ap	0-20	19	16	35	15	5	10
	Eet	20-45	12	13	39	18	6	12
	Bt	45-90	12	15	34	16	6	17
	C	90-100	82	3	5	2	2	6
	C1	>100	22	12	39	14	4	9
P2 Modła	Ap	0-30	59	7	17	6	2	9
	Eet	30-60	37	11	27	12	3	10
	Bt	60-100	18	10	35	16	4	17
	C	>100	11	13	40	20	5	11
P3 Kurowice	Ap	0-25	15	20	33	13	5	14
	Eet	25-48	10	16	45	16	6	7
	Bt	48-90	14	11	35	17	6	17
	C	90-150	14	12	46	14	4	10
P4 Nielubia	Ap	0-20	14	6	46	19	5	10
	Eet	20-45	17	12	35	15	5	16
	Bt1	45-70	17	11	37	19	6	10
	Bt2	70-95	22	7	36	17	5	13
	C	> 95	15	12	40	21	5	7

Table 2

Physicochemical properties of soils

Profile	Horizon	pH		C- org. (g kg^{-1})	CaCo$_3$ (%)
		H$_2$O	KCl		
P1 Wierzchowice	Ap	7.33	6.07	18.1	<1
	Eet	7.54	5.91	3.2	<1
	Bt	7.95	5.81	-	<1
	C1	8.14	6.58	-	<1
	C	8.16	6.62	-	<1
P2 Modła	Ap	7.53	6.89	7.2	<0.64
	Eet	8.20	7.50	3.9	<1
	Bt	7.82	6.46	-	11.60
	C	8.84	7.65	-	<1
P3 Kurowice	Ap	8.19	7.49	7.0	1.93
	Eet	8.55	7.72	1.2	0.43
	Bt	8.13	7.30	-	6.72
	C	8.38	7.75	-	8.58
P4 Nielubia	Ap	7.55	7.22	13.0	0.93
	Eet	7.53	7.05	9.3	<1
	Bt1	7.67	6.98	-	3.46
	C	8.01	7.30	-	5.82

presence of other forms of metals alkalizing the soil environment in the soil solution and sorption complex, either in the form of salts or hydroxides (Kabata-Pendias, Pendias 2001). The content of organic carbon in the humus horizons of the analyzed soils was typical of local soils, ranging from 7.0 to 18.1 g kg^1 (Strączyński, Andruszczak 1996, Karczewska 2002, Rosada 2008) Table 2.

The total content of Cu varied from 1.98 to 57.75 mg kg^{-1} (Table 3). Significant enrichment with this element was observed in the humus horizons of the soils, which may be the result of high sorption capacity of or-

Table 3

The total content of Cu and metal fractions

Profile Horizon	Cu total (mg kg^{-1})	F I	F II	F III	F IV	F V	F VI	F VII
		(mg kg^{-1})						
P1 Wierzchowice								
Ap	24.91	0.70	0.86	1.59	1.06	7.05	9.19	1.83
Eet	9.40	0.69	0.64	0.28	1.70	2.11	2.33	1.60
Bt	11.66	0.44	0.48	0.21	0.97	1.64	2.27	2.88
C	4.85	0.60	0.71	0.15	1.18	0.86	1.27	1.03
C1	9.08	0.82	0.74	0.16	0.96	1.41	1.79	2.51
P2 Modła								
Ap	6.58	0.43	0.58	0.65	2.70	1.63	2.12	1.33
Eet	6.16	0.41	0.69	0.83	2.54	1.43	1.87	1.40
Bt	9.63	0.37	0.76	0.72	2.43	1.35	2.83	2.17
C	7.08	0.40	0.62	1.10	2.25	1.42	1.75	1.53
P3 Kurowice								
Ap	30.62	0.70	0.71	3.50	2.47	11.19	3.52	2.52
Eet	4.04	0.41	0.43	0.72	0.88	0.70	1.37	1.64
Bt	4.85	0.43	0.43	0.80	1.32	1.21	1.03	1.76
C	1.98	0.30	0.32	0.49	0.68	0.55	0.62	0.83
P4 Nielubia								
Ap	57.75	0.51	0.91	4.45	2.70	25.71	22.11	3.11
Eet	10.40	0.60	0.71	0.56	1.10	3.86	3.72	1.11
Bt	4.98	0.34	0.59	0.21	0.93	1.42	2.08	0.86
C	4.48	0.36	0.62	0.24	1.07	1.55	0.94	1.59

FI – exchangeable and soluble in water forms, FII – forms soluble in acids, FIII – forms occluded on manganese oxides, FIV – forms related to organic matter, FV – forms related to amorphous iron oxides, FVI – forms related to crystal iron oxides, FVII – residual form ganic substances towards Cu, forming associations of different mobility (Migaszewski et. al. 2004). It may have also been caused by man-made enrichment due to the proximity of the copper works. The indicators of Cu arrangements were calculated from the ratio of this element in a given horizon to its average content in the bedrock, assuming value >1, which may prove its anthropogenic origin (Table 3). The highest content of Cu in surface horizons was reported by other authors (Weber 1995, Strączyński, Andruszczak 1996, Karczewska 2002). This finding indicates the dependence on the distance to the copper works (P4 profile) and the content of organic substances. The factors which influence solubility, migration and availability of Cu are organic substances, clay minerals and pH (Dąbkowska-Naskręt et al. 2002). In the analyzed soils, correlation between the total Cu content and pH in 1M KCl was found.

Sequential extraction helps to evaluate behavior of metals in soil environment and their possible migration in the biochemical circulation (Gworek 1985). The sequential analysis performed in this study extracted 7 fractions of metals (Table 3). The results of copper sequential extraction were varied within and between the profiles (Table 3) and the share of each copper fraction in the total copper content in each genetic horizons of the investigated profiles can be ordered as follow:

Ap- FV> FVI> FIII> FIV> FVII> FII> FI
Eet- FVI> FV> FIV> FVII > FII> FIII> FI
Bt- FVI> FVII> FIV> FV> FII> FIII> FI
C- FVII> FVI> FIV> FV> FII> FIII> FI

Fraction V, composed of forms associated with organic matter, dominates in the humus horizons, and especially in Ap horizon of P4 profile, the closest to the emitter, where it equalled 44.52% of the average total Cu content. However, P2 profile was an exception in that its Ap horizon *did* contain a high amount of fraction V but the dominant form of copper was fraction IV, i.e. forms related to manganese oxides. The lowest content of high mobility fractions (FI and FII) and the highest share of hardly soluble forms (FV and FVI) were observed in the humus horizons of the examined soils, which proves the low mobility of Cu in the surface horizons – a desirable characteristic when soils are used for agricultural purposes. Similar results for soils from the environs of a copper smelter were obtained by Rosada (2008), who confirms the predominant role of organic matter in association of Cu in surface samples, mostly in soils with silt texture. This author demonstrated a 5-fold higher content of Cu in fraction III (forms related to carbonates) in Ap horizons of soil profiles than in deeper horizons, a finding attributable to routine liming of fields, which was recommended as the simplest method of soil reclamation to be carried out during peak emission of dust from copper works (Rosada 2008). In the deeper genetic horizons of the investigated soils, the highest content of Cu forms associated to iron oxides was observed. In Eet and Bt horizons, the Cu forms related to amorphous iron oxides (FV) and crystalline iron oxides (FIV) make 3.97% and 26.38% of the average total Cu content, respectively, and in the parent horizon, where Cu forms related to crystal iron oxides (FVI) were dominant, it equalled 28.53% of the average total Cu content. In the bedrock horizons of the analyzed soils, the average percentage of Cu related to fractions of the highest mobility (FI – 8.30% and FII – 10.16%) is 4-fold higher than in the average total Cu content in the humus horizons (FI – 1.95% and FII – 2.55%), which may indicate copper release from the bedrock to higher horizons, where it is bound by clay minerals (Bt) and organic substance (Ap).

The total Zn content in the investigated soil profiles ranged from 3.34 to 42.65 mg kg^{-1} (Table 4). The highest total Zn content was observed in Ap horizons of P1 and P4 profiles, which may be related to the fact that organic matter forms fairy stable associations with zinc (Kabata-Pendias, Pendias 2001), and in Bt horizons of P2 and P3 profiles, which may be attributed to the well-developed illuvial horizon and progressing illuvial process. Highly significant statistical correlation was found between the total content of Zn and C-org. content in the soils (0.96 p=0.0500). Simultaneously, the eluvial horizons were the poorest in total Zn. The role of pH is very significant for Zn sorption by organic substance, optimal pH is 5.8 (Kabata-Pendias et. al. 1993). Acidification usually favours the weathering of minerals and accumulation of their products in a soil profile (Allen, Fanning 1983, Dudka 1992, Gondek 2010). In the analyzed soil profiles, such a low value of pH was observed only in Bt horizon of P1 profile and it does not confirm the above dependences. The indicator of Zn distribution is >1 for surface profiles P1, P2 and P4, which indicates its anthropogenic accumulation. It was only in P3 profile that this value was <1 (0.76), proving its lithogenic character. In the conducted sequential analysis, the following order of the shares of Zn fractions in relation to the average total Zn content was observed (Table 4):

Ap- FVII> FVI> FIII> FIV> FV> FII> FI
Eet- FVII> FVI> FIII> FV > FIV> FII> FI
Bt- FVII> FVI> FIII> FV> FIV> FII> FI
C- FVII> FV> FIII> FVI> FIV> FII> FI

In all the genetic horizons of the profiles, forms of zinc related to crystalline iron oxides (FVI) and to amorphous iron oxides (FV) were clearly dominant. Such high levels of Zn in the least mobile forms indicate a significant tendency to Zn occlusion by soil mineral oxides (Rosada 2008). Moreover, in soils with low pH, a particularly significant content of mobile and easily absorbed forms of heavy metals was observed on iron oxides (Karczewska 2002). The relatively high pH of the investigated soils (pH_{KCl} 5.81--7.75) led to a very low content of labile Zn fractions (soluble in water and exchangeable) in all the genetic horizons of the soils. The content of Zn in these forms did not exceed 5% of its total content. The fractions related to organic matter (FIV) varied in the range of 0.56-2.26 mg kg^{-1} and the highest values were observed in Ap horizons (except P2 profile). The content of

Table 4

The total content of Zn and metal fractions

Profile Horizon	Zn total (mg kg^{-1})	F I	F II	F III	F IV	F V	F VI	F VII
		(mg kg^{-1})						
P1 Wierzchowice								
Ap	42.65	0.31	1.48	5.86	2.63	2.18	7.02	9.61
Eet	22.60	0.66	0.64	1.11	1.39	1.88	2.68	5.88
Bt	25.49	0.43	0.66	0.73	1.17	1.69	1.93	5.60
C	3.73	0.16	0.32	0.53	0.75	0.83	0.64	1.04
C1	28.26	0.48	0.57	1.32	1.14	1.48	1.00	4.38
P2 Modła								
Ap	15.91	0.67	0.30	2.45	1.05	1.43	2.63	5.32
Eet	13.43	0.69	0.41	1.65	0.96	1.30	2.11	4.44
Bt	22.63	0.62	0.36	2.04	0.84	1.90	1.02	4.68
C	15.18	0.13	0.49	1.52	0.81	2.28	0.73	4.53
P3 Kurowice								
Ap	15.75	0.31	0.33	3.34	2.10	2.04	4.12	6.41
Eet	6.21	0.18	0.39	1.29	0.76	1.24	1.36	3.05
Bt	22.39	0.33	0.46	2.12	1.09	1.47	4.93	6.72
C	20.85	0.11	0.33	1.64	0.68	1.29	1.91	4.75
P4 Nielubia								
Ap	41.71	0.41	0.70	7.69	2.75	2.66	6.63	9.04
Eet	41.56	0.30	0.48	4.45	2.12	2.07	4.63	7.75
Bt	3.34	0.27	0.41	0.72	0.79	0.56	0.68	0.96
C	24.96	0.49	0.41	1.60	1.07	1.46	1.93	4.21

Key: cf. Table 3. p.d - below detection limit

Zn in the organically bound fraction (IV) was significantly lower than the content of Cu related to fraction IV. The results suggest lower affinity of Zn than Cu to soil organic substances (Kabata-Pendias, Pendias 2001). Furthermore, similarly to Cu, the Zn content in fraction II (forms related to carbonates) was three-fold higher in the humus horizons of the soils. This fraction makes 16.67% of the total Zn content, and its share is comparable to the forms related to amorphous iron oxides in these horizons. Similar results on Zn fractions in soils from the surroundings of a copper smelter are given by Rosada (2008) and Karczewska (2002), which indicates the predominance of Zn in most stable fractions, related to crystalline iron oxides.

CONCLUSIONS

1. The total content of Cu in Ap horizons of the analyzed soil profiles in the vicinity of the copper works was significantly higher than its total content in the bedrock horizon, which was confirmed by the values of the profile arrangement of Cu indicator (>1) and indicates the anthropogenic origin of copper in the soil.

2. In the sequential analysis, Cu forms related to organic matter were dominant among all forms of Cu in Ap horizons, which confirms high affinity of this metal to organic matter. In other soil horizons, the forms of Cu associated iron oxides dominate, which – along with the small share of Cu lower mobility fractions, may indicate low mobility of Cu in the investigated soils. This finding is ecologically advantageous.

3. The highest total Zn content in the investigated soils was observed in the humus horizons. However, it was only a little higher than the content of this metal in the bedrock, which indicates small enrichment of the investigated soils with this element due to human activity.

4. The dominant fraction of Zn in the investigated soils contained forms related to crystalline iron oxides, while smaller amounts of hardly soluble and exchangeable forms, which suggests low mobility of Zn in the soils.

5. Statistical analysis of the results showed significant correlation between the total content of zinc and organic carbon in the surface horizons of the soils.

6. Generally, no negative effect was observed of the Głogów Copper Works on the Cu and Zn content in arable soils in the vicinity of the plant. Thus, the analyzed soils can be used as farmland. However, a slightly higher total Cu content in P4 profile, in the closest proximity of the copper smelter, indicates that these soils should be monitored.

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EFFECT OF TRAFFIC POLLUTION ON CHEMICAL COMPOSITION OF RAW ELDERBERRY (S*AMBUCUS NIGR*A L.)

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Abstract

The aim of the study was to determine the effect of road traffic on the chemical composition of elderberry flowers and fruits. The raw material for the study was collected from sixteen different wild stands in south-east Poland. Each stand was located at a different distance from roads, carrying different loads of traffic. The raw material was collected from June (flower) to August (fruit) 2009 and dried at 30°C. Determination of the content of flavonoids (flavonols converted into quercetin) was performed by spectrophotometry according to the Polish Pharmacopoeia VIII (2008) procedure. Some minerals, including heavy metals (Cr, Zn, Pb, Cu, Cd, Ni, Fe and Mn), were determined with the ICP-AES method after dry digestion of the plant material.

A closer distance to transportation routes and heavier traffic had a significant impact on the chemical composition of raw elderberry, which had a lower total content of flavonoids than the material collected from sites further away from roads. Significant correlation was found between the content of Cr, Fe, Cd and Cu in elderberry fruit or the content of Fe, Zn, Cu, Cr, Cd, Pb and Mg in elderberry flowers and the influence of traffic, where closer proximity and heavier road traffic contributed significantly to an increase in the concentration of these elements in the analyzed raw material. The chemical composition of elderberry flowers and fruits was significantly affected by the traffic and depended on a harvest site.

Key words: elderberry, *Sambucus nigra*, traffic pollution, content of flavonoids, macro- and micronutrients.

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WPŁYW ZANIECZYSZCZEŃ KOMUNIKACYJNYCH NA SKŁAD CHEMICZNY SUROWCA BZU CZARNEGO (*SAMBUCUS NIGRA* L.)

Abstrakt

Celem badań było określenie wpływu zanieczyszczeń komunikacyjnych na skład chemiczny kwiatów i owoców bzu czarnego. Surowce pozyskano z 16. stanowisk naturalnych w południowo-wschodniej części Polski. Stanowiska znajdowały się w różnym oddaleniu od ciągów komunikacyjnych o zróżnicowanym natężeniu ruchu drogowego. Surowiec zbierano od czerwca (kwiat) do sierpnia (owoc) 2009 r. i natychmiast po zbiorze suszono w temp. 30°C. Zawartość flawonoidów (w przeliczeniu na kwercetynę) oznaczono spektrofotometrycznie wg Farmakopei Polskiej VIII (2008), a wybranych składników mineralnych – włącznie z metalami ciężkimi (Cr, Zn, Pb, Cu, Cd, Ni, Fe i Mn) – po mineralizacji materiału roślinnego na sucho metodą IPC.

Bliska odległość od ciągów komunikacyjnych oraz większa intensywność ruchu drogowego wpłynęły w istotny sposób na skład chemiczny surowca bzu czarnego, który zawierał mniej flawonoidów w stosunku do surowców pozyskiwanych z terenów bardziej odległych. W doświadczeniu stwierdzono istotną zależność między zawartością Cr, Fe, Cd i Cu w owocu bzu czarnego oraz Fe, Zn, Cu, Cr, Cd, Pb i Mg w kwiecie bzu czarnego a miejscem ich pozyskiwania. Bliskość ciągów komunikacyjnych i większa intensywność ruchu drogowego znacznie przyczyniły się do zwiększenia zawartości tych pierwiastków w analizowanym surowcu. Na skład chemiczny obydwu przebadanych rodzajów surowca bzu czarnego istotny wpływ ma miejsce ich pozyskiwania ze stanu naturalnego.

Słowa kluczowe: bez czarny, *Sambucus nigra*, zanieczyszczenia komunikacyjne, zawartość flawonoidów, zawartość makro- i mikroelementów.

INTRODUCTION

Common elderberry (*Sambucus nigra* L.) has many medicinal properties. Elder flowers contain mainly flavonoids (min. 0.8% acc. to Polish Pharmacopoeia VIII (2008)), organic acids and essential oils. Elderberry fruits have anthocyanins, flavonoids and phenolic acids. Elderberry extract and extract-containing preparations demonstrate antiviral properties, reducing the duration of flu symptoms to 3-4 days. They stimulate the immune system by increasing production of inflammatory cytokines, and are probably immunoprotective when administered to cancer and AIDS patients. Elderberry extract acts as an antioxidant, neutralizing free radicals and inhibiting the cooxidation reactions of linolenic acid and b-carotene(BARAK et al. 2001, DAWIDOWICZ et al. 2006, SIMONOVIK et al. 2007). Elderberry fruits have been used for making preserves, wines, winter cordials, and for adding flavor and color to other products (ice cream, beverages, yoghurt). Dietary supplements containing elderberry extracts, juices or syrups have become popular as remedies for treating cold and flu symptoms (*The ABC…* 2003). Elderberry plants are also grown as ornamentals. Unfortunately, wild elderberry shrubs often grow in areas polluted by vehicles. Elderberry grows mostly in ruderal habitats (landfill, rubble), in thickets, on roadsides and along busy streets (ATKINSON, ATKINSON 2002). Harvesting flowers and fruits from such sites raises concern about the quality of elderberry extracts and efficacy of treatment. Increasing pollution leads to a steady rise in levels of toxic heavy metals in plants, which precludes their optimal use as medicinal raw produce. Using contaminated herbs, apart from the desired pharmaceutical effect, causes intoxication of a patient with metals (MIROSŁAWSKI et al. 1995, OGNIK et al. 2006). Thus, it seems necessary to examine medicinal plants for their content of heavy metals. This is dictated by the fact that our current knowledge does not reflect the negative effects of toxic heavy metals and their possible interactions (MIROSŁAWSKI et al. 1995).

The purpose of this study is to determine the effect of road traffic on the chemical composition of elderberry raw material. The scope of work includes the determination of heavy metals as well as active substances (flavonoids) and their relation to the distance from the nearest roads.

MATERIAL AND METHODS

The raw material for the study consisted of flowers and fruits of common elderberry harvested in 2009 from sixteen different wild stands in the south-eastern part of Poland. The stands were located at a different distance from roads (Table 1). The raw material was collected from June (flower) to August (fruit) 2009 and then dried at 30°C. Voucher specimens have been deposited at the Department of Industrial and Medicinal Plants, University of Life Sciences, Lublin.

Determination of flavonoid content (flavonoles converted into quercetine) was performed by spectrophotometry according to the Polish Pharmacopoeia VIII (2008) procedure. In addition, the content of selected minerals, including heavy metals (Cr, Zn, Pb, Cu, Cd, Ni, Fe and Mn), after digestion of plant material to dryness with the ICP-AES method was determined at the Department of Agricultural and Environmental Chemistry, the University of Agriculture in Krakow. For chemical analysis, elderberry samples (5 g dry weight) of plant material from each replication were collected. The plant samples, after drying at 70°C in an oven with forced air flow, were dry-mineralized in a muffle furnace at 450°C. The ash was dissolved in HNO_3 (1:2) and evaporated to dryness in sand bath. After evaporation, the ash was re-mineralized (in order of total ash) in a muffle furnace. The burned ash was dissolved first in 20% HCl, followed by evaporation to dryness, dissolution in HNO_3 (1:2) and boiling. Thus prepared, the plant material samples were transferred to volumetric flasks. During the study, the content of heavy metals (Cr, Zn, Pb, Cu, Cd, Ni and Mn) and macroelements (K, Ca, Mg) was determined with the inductively coupled plasma atomic emission spectroscopy (ICP-AES) method. Chemical analyses were performed in three replications.

Table 1

Location of the stands of obtaining elderberry raw material along with an indication of their distance from the route and traffic intensity

No	Stand	Location	Distance from the oad (m)	Traffic intensity near the stand	Possible impact of road traffic on raw material on a conventional scale (1-6)
1	Lublin Konopnicka estate	51°14'22.34"N 22°31'09.29"N	150	+/-	2
2	Lublin Nałęczowska street	51°15'01.29"N 22°30'41.65"E	0.5	+++	6
3	Lublin Skromna street	51°14'57.40"N 22°30'33.01"E	1.5	+	4
4	Lublin Głęboka street	51°14'50.92"N 22°31'14.70"E	5	+++	5
5	Lublin Słowacki estate	51°14'44.23"N 22°31'11.02"N	21	+	3
6	Goraj Lubelski	50°43'14"N 22°40'9"E	60	-	1
7	Kocudza	50°40'9"N 22°36'52"E	4	-	1
8	Lublin Morwowa street	51°14'57.11"N 22°30'32.68"E	15	++	5
9	Lublin Kolorowa street	51°15'02.08"N 22°30'59.04"E	10.5	+	3
10	Lublin Kraśnicka street	51°14'47.35"N 22°30'56.44"E	1.5	+++	6
11	Świdnik	51°13'42"N 22°39'56" E	10	+	3
12	Żyrzyn	51°30'24" N 22°04'28" E	2	+/-	2
13	Syczyn	51°16'36" N 23°14'34" E	3	+/-	2
14	Bekiesza	51°17'44" N 23°12'05" E	50	-	1
15	Pacanów	50°24'19" N 21°03'06" E	5	+	3
16	Ławy	50°12'18" N 20°38'49" E	150	++	2

Key:

+++ very large, ++ large, + medium,
+/- small, - very small

6 – maximum, 5 – very big, 4 – big,
3 – average, 2 – small, 1 – minimum

Statistical analysis

The data were analyzed with the SAS general linear model procedure (version 8.2 SAS Institute, Cary, N.C.). Testing for significance of mean effects and interactions on all variables was calculated using ANOVA analysis of variance. Statistical significance was set at P=0.05. The strength of the linear relationship between two variables was expressed using Pearson's linear correlation coefficient (Rxy), and taking values from –1.0 to 1.0 (where -1.0 is a perfect negative correlation, 0.0 is no correlation, and 1.0 is a perfect positive correlation). The test of significance is based on the assumption that the distribution of residual values (i.e. deviations from the regression line) for dependent variable y follows normal distribution, and that the variability of residual values is the same for all values of independent variable x. In the interpretation of Pearson's correlation coefficient, the following scale was used: 0 <Rxy <0.25 low degree of correlation; 0.25 <Rxy <0.75 moderate correlation; 0.75 = <Rxy <1 high degree of correlation (Stanisz 2000).

RESULTS

Mineral composition analysis of elderberry flowers and fruits showed significant differences in the concentration of individual elements (including heavy metals) in examined samples of plant material (Tables 2, 3, 4).

The content of macroelements in elderberry flowers ranged from 32.4 (stand number 16) to 83.1g K kg^{-1} d.m. (stand number 12); from 4.15 (stand number 7) to 8.83 g Mg kg^{-1} d.m. (stand number 3), and from 1.99 (stand N^{o} 6) to 5.15 g C kg^{-1} d.m. (stand number 15) – Table 2. It is worth noticing that the content of each analyzed element was significantly related to the site of raw material collection (the least significant difference P=0.05), which implies that it was most likely influenced by the composition of soil on which elderberry plants grew.

A similar relationship between the mineral content and the from the place from which the raw material originated was observed for heavy metals (Table 2). The analyzed flower samples contained the highest levels of iron, followed by zinc and manganese. Elderberry flowers accumulated also copper: from 5.28 (stand 16) to 16.10 mg kg^{-1} d.m. (stand 10), chromium: from 0.63 (stand 3) to 2.31 mg kg^{-1} d.m. (stand 2), nickel: from 0.252 (stand 7) to 2,264 mg kg^{-1}d.m. (stand 12) and cadmium: from 0 (stands 6, 12, 13, 14 and 15) to 0.04 mg kg^{-1} d.m. (stand 11). The lead content was the highest in the raw material gathered closer to roads. Generally, the raw material gathered near the busiest routes (stands 2 and 10) had the highest total content of most heavy metals (Tables 3, 4). The lowest total content of heavy metals was observed in elderberry flowers from rural areas far from roads. In general, elderberry flowers contained almost twice as much of the analyzed

Table 2

The content of selected macronutrients in elderberry flowers and fruits (g kg^{-1} d.m.)

Stand number	Flower			Fruit		
	K	Mg	Ca	K	Mg	Ca
1*	53.4	6.87	3.50	30.6	4.05	2.28
2	66.8	6.41	3.43	41.4	5.46	2.55
3	48.5	8.83	3.60	26.7	5.75	2.83
4	59.2	6.05	4.04	29.4	4.08	2.44
5	62.1	6.11	2.23	37.4	4.50	3.02
6	53.3	4.30	1.99	34.6	3.26	1.53
7	60.2	4.15	2.42	35.3	4.57	1.97
8	59.5	5.97	3.08	40.4	3.29	1.37
9	56.2	7.07	2.91	32.7	4.53	2.29
10	66.2	5.84	3.55	38.0	2.98	1.59
11	61.5	6.92	4.76	35.1	4.58	2.72
12	83.1	5.62	2.60	49.6	2.95	1.66
13	56.6	4.50	3.14	37.0	5.04	4.22
14	45.4	6.31	3.75	27.2	3.67	1.93
15	71.3	6.85	5.15	49.0	5.87	3.86
16	32.4	6.33	3.54	33.0	3.82	2.53
LSD**$_{0.05}$	7.99	0.777	0.781	7.75	1.091	1.573

*key under Table 1, **LSD$_{0.05}$ – least significant difference at P=0.05

elements as fruits harvested from the same plants growing in the wild (Tables 2, 3, 4).

Differences in the mineral composition of elderberry fruit as well as a significant effect of the location from which the raw material had been harvested were evidenced during the experiment (Tables 2, 4). The content of macroelements in elderberry fruits ranged from 26.7 (stand 3) to 49.6 g K kg^{-1} d.m. (stand 12), from 2.95 (stand 12) to 5.87 g Mg kg^{-1} d.m. (stand 15) and from 1.37 (stand 8) to 4.22 g Ca kg^{-1} d.m. (stand 13). It is important that the content of each macronutrient in elderberry fruits, like in the flowers, significantly depended on the place where the raw material had been gathered.

A relationship was also found between the content of heavy metals in elderberry fruits and the site where the raw materials had been collected (Table 4). As in the flowers, elderberry fruit samples contained the highest amounts of iron, zinc and manganese of all the heavy metals analyzed. Elderberry fruits also accumulated copper: from 3.93 (stand 6) to 8.54 mg

Table 3

The content of heavy metals in elderberry flowers (mg kg^{-1} d.m.)

Stand number	Cu	Zn	Mn	Fe	Cr	Ni	Cd	Pb
1*	11.26	35.63	30.82	60.23	0.68	0.410	0.007	0
2	9.94	38.35	25.21	214.58	2.31	1.227	0.018	0.232
3	9.23	24.34	26.46	65.61	0.63	1.396	0.034	0
4	9.10	37.22	27.38	75.70	0.72	0.421	0.025	0.369
5	7.71	23.92	26.57	55.16	1.08	1.531	0.014	0
6	5.47	26.97	13.85	48.67	0.83	0.396	0	0
7	7.11	25.22	14.93	35.77	0.77	0.252	0.002	0.009
8	8.93	38.58	22.38	76.36	0.86	0.582	0.017	0.013
9	13.21	36.13	23.61	50.84	0.84	1.474	0.015	0
10	16.10	39.29	27.80	192.47	1.48	0.634	0.008	0.202
11	9.11	37.01	31.714	111.21	0.78	0.652	0.040	0.310
12	11.45	36.36	38.266	53.84	1.19	2.264	0	0.063
13	6.81	30.15	20.562	52.80	0.68	0.843	0	0.004
14	6.88	31.78	33.39	54.75	0.70	0.909	0	0.212
15	6.58	26.33	30.08	78.93	0.91	0.855	0	0.202
16	5.28	32.04	30.17	68.18	0.67	0.838	0.002	0.328
LSD**$_{0.05}$	1.009	8.333	4.5089	15.5334	0.229	0.7749	0.0042	0.1321

*key under Table 1, **LSD$_{0.05}$ – least significant difference at P=0.05

kg^{-1} d.m. (stand 4), chromium: from 0.388 (stand 5) to 0.714 mg kg^{-1} d.m. (stand 2), nickel: from 0.100 (stand 7) to 0.803 mg kg^{-1} d.m. (stand 3) and cadmium: from 0 (stands 3, 9, 11, 15) to 0.076 mg kg^{-1} d.m. (stand 10) The raw material from stands 15, 2 and 3, gathered in close proximity to busy roads, possessed the highest total content of all the heavy metals (121.03, 99.59 and 97.91 mg kg^{-1} d.m., respectively). Generally, elderberry fruits contained half the concentrations of the analyzed elements determined in flowers but accumulated them in a similar way. Cadmium was exceptional in that it did not tend to rise in the raw material (Table 2).

The content of flavonoids was higher in elderberry flowers than in fruit (Figure 1). The percentage of these active substances in elderberry flowers of ranged from 0.55% to 1.56% of air dry matter, while in fruits it varied from 0.028% to 0.116%. The highest content of flavonoids in flowers appeared in samples nos 6 and 15 (1.45 and 1.56% respectively). These samples originated from stands in a rural area, far from any roads. The lowest content of flavonoids was observed in the raw material from the most con-

Table 4

The content of heavy metals in elderberry fruit (mg kg^{-1} d.m.)

Stand number	Cu	Zn	Mn	Fe	Cr	Ni	Cd
1*	7.28	15.63	13.60	32.08	0.440	0.242	0.013
2	5.73	17.65	15.78	58.96	0.714	0.248	0.014
3	8.51	19.65	14.01	54.05	0.542	0.803	0
4	8.54	25.79	11.30	50.42	0.504	0.186	0.029
5	7.99	16.39	30.66	38.02	0.388	0.160	0.009
6	3.93	12.71	7.71	37.37	0.443	0.176	0.018
7	5.53	11.86	11.50	29.52	0.431	0.100	0.034
8	5.68	13.14	9.99	31.43	0.465	0.168	0.015
9	6.16	13.99	11.79	27.63	0.482	0.231	0
10	5.99	8.76	9.81	54.47	0.629	0.261	0.076
11	4.70	18.59	9.96	58.46	0.517	0.407	0
12	7.31	14.29	27.72	34.03	0.407	0.523	0.001
13	4.94	17.28	19.63	51.89	0.642	0.462	0.011
14	5.55	13.37	30.03	39.98	0.476	0.458	0.017
15	6.27	16.08	18.41	79.12	0.630	0.523	0
16	4.68	15.35	18.86	45.37	0.608	0.500	0.011
$LSD^{**}_{0.05}$	1.110	4.774	1.709	8.112	0.1087	0.0768	0.0049

*key under Table 1, $^{**}LSD_{0.05}$ – least significant difference at P=0.05

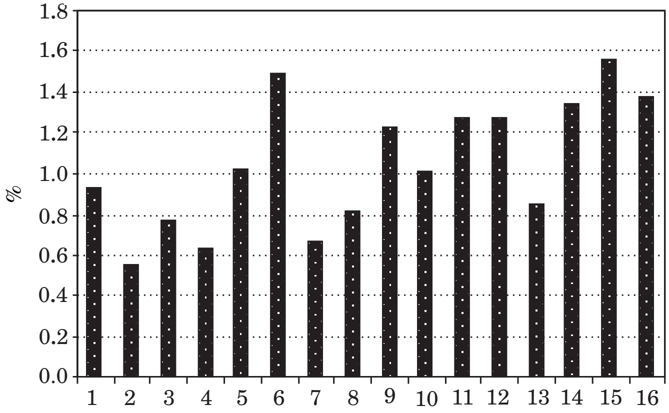

Fig. 1. Total content of flavonoids in elderberry flower (in % of dry weight) depending on a stand from which raw material has been obtained (N° 1-16)

taminated stands (nos 2, 4), where it was lower than required by Polish Pharmacopoeia VIII (2008)). The largest content of flavonoids in elderberry fruits was determined in the raw material harvested from stands 5, 9, 14 and 15 (0.101 and 0.115%, respectively). The lowest content of flavonoids was found in the raw material from stands 10 (0.028%) and 2 (approx. 0.029%) (sites most severely contaminated with heavy metals, near to roads) (Figure 2).

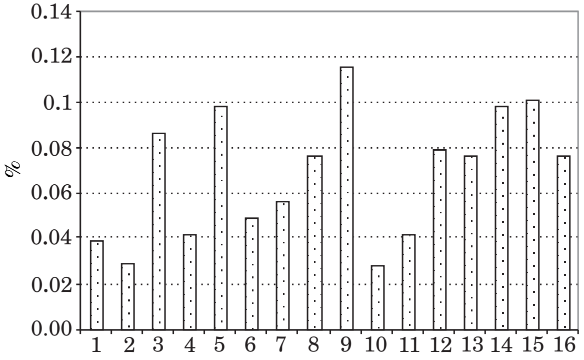

Fig. 2. Total content of flavonoids in elderberry fruit (in % of dry weight) depending on a stand from which raw material has been obtained (N° 1-16)

Table 5

Pearson's correlation coefficient r_{xy} between the effect of traffic routes and content of minerals in elderberry raw material

Component	Effect of traffic routes on a conventional (1-6) scale (see Table 1)	
	flowers	fruits
K	0.03	0.14*
Mg	0.37*	0.11
Ca	0.28*	-0.05
Cu	0.53**	0.33**
Zn	0.64**	0.23*
Mn	0.14	-0.33**
Fe	0.75**	0.38*
Cr	0.50*	0.48**
Ni	- 0.05	- 0.10
Cd	0.48**	0.35**
Pb	0.41**	-
Flavonoids in total	- 0.47**	- 0.36*

**significant at $P \leq 0.01$, * significant at $P \leq 0.05$

A high positive correlation was verified in the experiment between the impact of road traffic (the distance from the nearest road and the load of traffic) and the content of Fe, Zn, Cu and Cr in elderberry flowers (Pearson's correlation coefficient reached 0.75, 0.64, 0.53 and 0.50; Table 5). A weaker correlation was obtained between the impact of transportation routes and the content of Cd, Pb and Mg in elderberry flowers (Pearson's correlation coefficient was 0.48, 0.41, 0.37, respectively) or Cr, Fe, Cd and Cu in elderberry fruit (r=0.48, 0.38, 0.35, 0.33; Table 5). Positive correlation was also noted between the negative impact of transportation routes and the total content of flavonoids in elderberry flowers and fruits or the manganese content in elderberry fruits.

DISCUSSION

Increasing environmental pollution causes continuous rise in the content of toxic heavy metals in plants, which may precludes their optimal use as medicinal raw material (SROGI 2005). There are many factors influencing the content of heavy metals in the plant. The most important one is the degree of contamination and the distance from its source. Possible sources of environmental contamination with heavy metals are industry, transportation, public facilities, landfill waste and fertilizers (GAMBUŚ, GORLACH 2001). The study has shown that the distance from transport routes and traffic load intensity affects the mineral composition of the analyzed material. Elderberry fruit and flowers collected far from roads in rural areas were characterized by the smallest total content of heavy metals, a finding which coincides with the results reported by OGNIK et al. (2006). However, TRĘTOWSKA et al. (1998) suggested that the place where elderberry plants grow has no effect on the content of Cd, Zn, Fe, Mg and Ca in its fruit. According to these authors, an increasing traffic flow correlated positively with the content of Pb and Cu, but negatively with Mn. Our experiment showed a clear relationship between the content of Fe, Zn, Cu and Cr in elderberry raw material and road traffic. Analogously to the study by KUŹNIEWSKI et al. (1993), we found out that the amount of accumulated heavy metals depends on the anatomical parts of plants (flowers of *S. nigra* contained almost twice as much of the analyzed elements as fruits).

Sambuci flos is characterized by a relatively high content of flavonoids (up to 1.8%). In our experiment, the content of flavonoids ranged from 0.55 to 1.56% in flowers and from 0.03 to 0.12% in elderberry fruits. Similar amount of flavonoids was found in elderberry flowers by DAWIDOWICZ et al. (2005) and WACH et al. (2007) and in fruits by RIEGER et al. (2008). Moreover, the lowest content of flavonoids was observed in the raw material from the most contaminated stands (2, 3, 4), where it was below the amount required by Polish Pharmacopoeia VIII (2008).

CONCLUSIONS

1. The chemical composition of both types of elderberry raw material was significantly affected by the place of harvest.

2. Close proximity to transportation routes and more intensive traffic had a significant impact on the chemical composition of raw elderberry, which was characterized by a lower total content of flavonoids in comparison to the material collected from uncontaminated areas.

3. A significant correlation was found between the content of Cr, Fe, Cd and Cu in elderberry fruit, and Fe, Zn, Cu, Cr, Cd, Pb and Mg in elderberry flowers versus the influence of traffic routes, where proximity and higher intensity of road traffic contributed significantly to an increase in the content of these elements in the analyzed raw material.

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EFFECT OF *SACCHAROMYCES CEREVISIAE* YEAST ON MILK PROTEIN CONTENT AND COMPOSITION AND SERUM MINERAL CONCENTRATIONS IN SHEEP

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Abstract

The experiment was conducted on 26 nursing Kamieniec ewes from a breeding herd. The animals were divided into two equal groups characterized by the same litter type, age and sex of suckling lambs: I – control and II – experimental. Throughout the 70-day lactation period, both groups were fed identical diets comprising haylage of grasses and legumes, meadow hay and CJ concentrate in daily rations of: 2.80 kg haylage of grasses and legumes, 0.6 kg meadow hay and 0.6 kg CJ concentrate per ewe. Group II animals were fed the CJ concentrate with the addition of Inter Yeast S® *Saccharomyces cerevisiae* dried yeast in the amount of 50 g kg^{-1} of the concentrate. Milk was sampled during control milking runs on lactation day 28 and 70. The collected samples were analyzed to determine the content of protein, whey protein, casein, α_s, β and κ casein fractions. Blood was sampled twice from the jugular vein on lactation day 28 and 70. After separation of serum, the following nutrient concentrations were determined: inorganic phosphorus (P_{in}), calcium (Ca) and magnesium (Mg). No significant variations were reported in the percentage share of whey proteins in ewe's milk during the peak and towards the end of lactation. The yeast preparation had no effect on the casein content of milk. Yeast supplementation si-

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gnificantly influenced the proportions of casein fractions on lactation day 70. A significant drop in β-casein levels (by 2.32%) with a simultaneous increase in κ-casein concentrations (by 1.86%) were noted in the milk of group II animals. Similar calcium, phosphorus and magnesium concentrations were determined in the blood serum of both animal groups throughout the experiment (on lactation day 28 and 70) and these results were within the reference values.

The results of this study indicate that the inclusion of *Saccharomyces cerevisiae* brewer's yeast into the diet of lactating ewes could enhance the quality of milk proteins.

Key words: ewes, *Saccharomyces cerevisiae,* milk proteins, blood minerals.

WPŁYW PREPARATU DROŻDŻY *SACCHAROMYCES CEREVISIAE* NA ZAWARTOŚĆ BIAŁKA I JEGO FRAKCJI W MLEKU ORAZ WSKAŹNIKI MINERALNE W SUROWICY OWIEC

Abstrakt

Doświadczenie przeprowadzono na 26 maciorkach karmiących owcy kamienieckiej, pochodzących ze stada hodowlanego, podzielonych na 2 równe grupy, analogiczne pod względem wieku oraz typu miotu i płci odchowywanych jagniąt: I – kontrolną, II – doświadczalną. Zwierzęta obu grup otrzymywały podczas 70-dniowej laktacji taki sam zestaw pasz objętościowych: sianokiszonkę z traw i roślin motylkowych, siano łąkowe oraz mieszankę CJ. Dzienna dawka pokarmowa, w przeliczeniu na 1 matkę, obejmowała: 2,80 kg sianokiszonki z traw i roślin motylkowych, siano łąkowe i 0,6 kg mieszanki treściwej CJ. Maciorki z grupy doświadczalnej otrzymywały mieszankę CJ z dodatkiem suszonych drożdży piwowarskich *Saccharomyces cerevisiae* Inter Yeast S®, w proporcji 50 g kg^{-1} mieszanki. Próby mleka do analiz pobierano podczas doju kontrolnego dwukrotnie, w 28. i 70. dniu laktacji, i określono w nich procentową zawartość: białka, białka serwatkowego i kazein, a także udział frakcji α_s-, β- i κ-kazeiny w kazeinie całkowitej. Krew do badań pobierano dwukrotnie z żyły jarzmowej, a w otrzymanej surowicy określono koncentrację składników mineralnych: Ca, P_{in} i Mg. Dodatek drożdży nie spowodował istotnych zmian w zawartości białka oraz białka serwatki w mleku, zarówno w szczytowej, jak i końcowej fazie laktacji, natomiast wpłynął na kształtowanie się udziału frakcji kazein. Stwierdzono, że w mleku owiec z grupy II nastąpiło istotne obniżenie poziomu β-kazeiny (o 2,32%), a równocześnie wzrost ($p \leq 0{,}01$) zawartości κ-kazeiny (o 1,86%). Stężenie wskaźników mineralnych surowicy (Ca, P i Mg) pozostawało na zbliżonym poziomie w obydwu grupach owiec zarówno w 28., jak i 70. dniu laktacji i mieściło się w granicach wartości referencyjnych.

Wyniki badań wskazują, że dodatek suszonych drożdży piwowarskich *Saccharomyces cerevisiae* do diety owiec karmiących może mieć wpływ na jakość białka mleka.

Słowa kluczowe: owce, *Saccharomyces cerevisiae*, białka mleka, wskaźniki mineralne krwi.

INTRODUCTION

Dry extracts of *Saccharomyces cerevisiae* brewer's yeast are a rich source of easily digestible protein (around 43%), B vitamins, minerals, enzymes as well as immunomodulators: β-1,3/1,6-D-glucan (up to 15%) and mannan-oligosaccharides (MOS) (up to 11%). The properties of yeast extracts are determined by the processing technology. Preparations containing only dead cells demonstrate probiotic properties, whereas extracts that contain live cells show prosymbiotic and symbiotic qualities (Grela, Semeniuk 2006). The supplementation of animal diets with yeast improves the feed conversion ratio, body gains, fattening performance, carcass quality, milk yield and lowers disease incidence (Dobicki et al. 2004, Dobrzyński et al. 2006, Fuchs et al. 2007, Milewski, Sobiech 2009). Glucan and mannan, structural components of yeast cell walls, strongly inhibit the growth of pathogenic bacteria, such as *Escherichia coli* and *Klebsiella pneumoniae,* in the gastrointestinal tract. *Saccharomyces cerevisiae* yeast can, therefore, be applied as a natural stimulator in animal nutrition (Dobicki et al. 2007, Grela, Semeniuk 2006, Wójcik et al. 2007). The stimulating effect of yeast extracts on lambs was demonstrated by Milewski (2009) and Milewski and Sobiech (2009). Lambs fed dried yeast were characterized by higher growth rates, improved muscle development and higher humoral immunity parameters (Milewski 2009). A study investigating the milk yield of ewes has demonstrated that yeast significantly improves milk production and increases the dry matter and fat content of milk (Milewski, Sobiech 2009). To date, no experiments have been conducted to investigate the effect of *Saccharomyces cerevisiae* dried brewer's yeast on the protein composition of ewe's milk.

The objective of this study was to determine the effect of *Saccharomyces cerevisiae* dried yeast on milk protein content and composition and the blood serum concentrations of selected minerals in sheep.

MATERIAL AND METHODS

The experiment was conducted on 26 nursing Kamieniec ewes from a breeding herd. The animals were divided into two equal groups characterized by the same litter type, age and sex of suckling lambs: I – control and II – experimental. Throughout the 70-day lactation period, both groups were fed identical diets comprising haylage of grasses and legumes, meadow hay and CJ concentrate in daily rations of: 2.80 kg haylage of grasses and legumes, 0.6 kg meadow hay and 0.6 kg CJ concentrate per ewe. Group II animals were fed the CJ concentrate with the addition of Inter Yeast S® *Saccharomyces cerevisiae* dried yeast in the amount of 50 g kg^{-1} of the concentrate. The quantity of administered feed and leftovers was monitored throughout the experiment. Milk was sampled during control milking runs on lactation day 28 and 70. The animals were milked manually following the administration of oxytocin in accordance with the method described by Milewski and Ząbek (2008). The collected samples were analyzed to determine the content of protein, whey protein, casein, α_s, β and κ casein fractions. The percentage content of protein was determined using a Combi

Foss 6000 analyzer. Casein nitrogen and whey protein nitrogen were analyzed by Kiejdahl method. Casein fractions were identified by protein electrophoresis. Milk samples for electrophoretic separation were diluted in the 1:10 ratio. Proteins were separated on 14.5% polyacrylamide gel in the presence of SDS using a Mini-PROTEAN 3-cell system in line with the manufacturer's recommendations (Bio-Rad). The percentage of casein fractions in milk was determined with the application of Bio-Rad Quantity One 4.6 software. Blood was sampled from the jugular vein on lactation day 28 and 70. The following nutrient concentrations were determined in the blood serum: inorganic phosphorus (P_{in}) by the phosphomolybdenum reduction assay without deproteinization, calcium (Ca) by the Moorehead and Briggs method involving o-cresolphthalein complexone (CCP), magnesium (Mg) using the Calmagite dye method and the approach proposed by Gindler and Heth and Khayem-Bashi et al. The analyses were performed in an Epoll 200 spectrophotometer using Alpha Diagnostics kits.

The results were verified statistically by the Student's *t*-test with the use of Statistica 10.0 software.

RESULTS AND DISCUSSION

During the experiment, no differences in feed consumption were reported. Only small quantities of haylage leftovers were observed, suggesting that nutrient uptake levels were similar in both groups. The data presented in Table 1 show comparable protein levels in the milk of both ewe groups, which reached 4.98% in group I and 4.96% in group II on lactation day 28. A higher milk protein content was noted in the experimental group on lactation day 70 (a non-significant difference of 0.45%). No significant variations were reported in the percentage share of whey proteins in ewe's milk during the peak and towards the end of lactation. The yeast preparation

Table 1

Percentage of protein, whey protein and casein in sheep milk

Paremeter	Day of lactation	Group I		Group II	
		$\overline{x}$	SD	$\overline{x}$	SD
Protein	28	4.98	0.43	4.96	0.44
	70	5.55	0.88	6.00	0.35
Whey protein	28	0.77	0.04	0.74	0.08
	70	0.96	0.10	0.88	0.12
Casein	28	4.00	0.29	4.07	0.40
	70	4.43	0.64	4.85	0.39

had no effect on the casein content of milk. Yeast supplementation significantly influenced the proportions of casein fractions on lactation day 70 (Figure 1). A significant drop (P=0.01) in β-casein levels (by 2.32%) with a simultaneous increase (P=0.01) in k-casein concentrations (by 1.86%) were noted in the milk of group II animals. The protein content of milk noted in this study is consistent with the findings of Dobicki et al. (2006), Piva et al. (1993), Robinson and Garrett (1999), and Swartz et al. (1994). In the above experiments, the addition of *Saccharomyces cerevisiae* dry brewer's yeast to diets for high-yielding cows had no effect on the protein content of milk. The percentage content of casein fractions in ewe's milk differed from that reported by Ono et al. (1989) – higher β-casein levels and lower β_s-casein concentrations were observed in our study. Caseins are bioactive milk components that affect gastric functions, demonstrate anti-thrombotic and anti-diabetic properties, prevent tooth demineralization and decay (Abd-El-Salam et al. 1996). Caseins deliver many health benefits. κ-casein prevents the adhesion of pathogenic bacteria, such as *Strepcococcus pneumoniae, Haemophilus influenzae* and *Helicobacter pylori*, to gastric mucosal cells. The protective properties of β-casein have been demonstrated in cancer research (Nakajima et al. 1996) and during experimental contaminations with bacteria and endotoxins (Noursadeghi et al. 2002). A drop in β-casein levels and an increase in the κ-casein content of milk noted in this study towards the end of the lactation period suggest that the protein composition of ewe's milk can be modified through supplementation with *Saccharomyces cerevisiae* dry brewer's yeast. The results of this study indicate that the use of brewer's yeast enhances the quality of ewe's milk, delivering significant health benefits for suckling lambs. When administered to dairy sheep, brewer's yeast improves the processing suitability of milk. These findings offer a valuable incentive for further research into the use of yeast in animal nutrition.

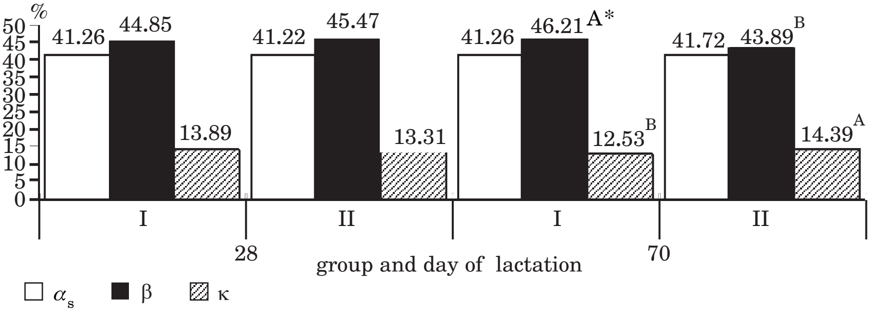

Fig. 1. Percentage content of fractions αs-, β- i κ-casein in total casein of sheep milk

*A, B – difference statistically significant at $p \leq 0.01$

Table 2

Concentration of minerals in sheep serum

Paremeter	Day of lactation	Group I		Group II	
		$\overline{x}$	SD	$\overline{x}$	SD
Calcium (mmol l^{-1})	28	2.53	0.27	2.76	0.56
	70	2.41	0.29	2.66	0.34
Phopsphorus inorganic (mmol l^{-1})	28	1.61	0.41	1.71	0.34
	70	1.68	0.33	1.62	0.46
Magnesium (mmol l^{-1})	28	1.08	0.06	1.11	0.07
	70	1.12	0.11	1.19	0.23

Concentrations of calcium and magnesium in serum of sheep from the experimental group were higher than in the control group throughout the experiment, but these changes were not statistically important. Concentrations of phosphorus in serum of sheep from the experimental group were higher on 28 day of lactation, but decreased on 70 day of lactation and these changes were not statistically important as well (Table 2). These results indicate that yeast has no effect on the content of the bioactive elements studied, as previously demonstrated by GALIP (2006), PAYANDEH and KAFILZADEH (2007) and MILEWSKI and SOBIECH (2009). A decrease in Mg concentrations in the blood serum of cows fed yeast was reported by NIKKHAH et al. (2004), but in the described experiment, feed was supplemented with live yeast cultures. The underlying mechanism has not been fully explained, and it could be attributed to the ability of selected cations to bind across the yeast cell wall, which limits cation absorption from feed (GARCIA et al. 2000).

CONCLUSIONS

1. The addition of yeast to diets for nursing ewes did not significantly change milk protein content and mineral concentrations in the serum.
2. The supplement improved the proportions of casein fractions in total milk casein.
3. A drop in β-casein levels and a simultaneous increase in the k-casein content of milk was noted towards the end of the lactation period, suggesting that dry brewer's yeast improves the nutritional value of ewe's milk.

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CONTENT OF MACRO- AND MICROELEMENTS IN MEAT OF MALE KIDS AND RAM LAMBS IN RELATION TO THEIR SLAUGHTER AGE

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Abstract

The aim of the experiment was to determine the content of some chemical elements in meat tissue in kids of the White Improved breed and in rams of the Polish Lowland breed. After weaning (60 days of life), the animals were fed all-mash CJ and meadow hay. The mixtures were produced from components originating from the Polish region called Podlasie. At the age of 1 month, all kids and rams were castrated using the blood method. They were slaughtered at the age of 90, 120, 150 and 180 days of life. Samples to analyse the chemical elements were taken from *longissimus dorsi* muscles. Concentrations of Pb, Cd, Zn, Mn, Fe and Mg were determined.

Generally, more Pb in meat tissue of kids than rams was found, and a significant difference (p=0.01) for the slaughter on the 120th day of life was proven. However, the content of Cd was larger (p=0.05 for 90, 120 and 150 days) in ram tissues (0.003-0.014 mg kg^{-1}) than in kids. It should be noticed that both in kid and ram meat tissues, the norms for Pb and Cd content, established by the Minister of Health and the European Committee, were not exceeded, which makes the meat suitable for consumers. The meat tissue of kids was richer in iron and poorer in zinc and copper than that of lambs, and the level of these elements decreased with the slaughter age. It was also observed that the iron content in meat tissue on the 180-day slaughter of animals was less than 17 mg kg^{-1}. More copper in meat tissue of rams and zinc in kids slaughtered at the age of 150 and 180 days were found. To sum up, intensive feeding applied to fatten kids and lambs did not affect the accumulation of chemical elements in meat, and especially that of heavy metals.

Key words: meat tissue, male kids, ram lambs, macroelements, heavy metals.

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ZAWARTOŚĆ MAKRO- I MIKROELEMENTÓW W MIĘSIE KOZIOŁKÓW I TRYCZKÓW W ZALEŻNOŚCI OD ICH WIEKU UBOJU

Abstrakt

Celem badań było określenie zawartości wybranych pierwiastków w tkance mięśniowej koziołków rasy białej uszlachetnionej i tryczków polskiej owcy nizinnej. Zwierzęta po odsadzeniu od matek (60 dni życia) żywiono mieszanką pełnoporcjową CJ oraz sianem łąkowym. Pasze wyprodukowano z komponentów pochodzących z regionu Podlasia. W wieku ok. 1 miesiąca wszystkie koziołki tryczki wykastrowano metodą krwawą. Zwierzęta ubijano w wieku 90, 120, 150 i 180 dni życia. Próby do oznaczenia pierwiastków pobierano z *m. longissimus dorsi*. Oznaczono zawartość: Pb, Cd, Zn, Cu, Mn, Fe i Mg.

W tkance mięśniowej koziołków stwierdzono wyższą zawartość Pb niż u tryczków, a istotną ($p \leq 0.01$) różnicę stwierdzono w przypadku uboju w 120. dniu życia, natomiast zawartość Cd była wyższa ($p \leq 0.05$) w przypadku 90., 120. i 150. dnia w tkankach tryczków (0.003-0.014 mg kg^{-1}) niż koziołków. Należy podkreślić, że w tkance mięśniowej koziołków i tryczków nie zostały przekroczone normy zawartości Pb i Cd ustalone przez Ministra Zdrowia oraz Komisję Europejską, co czyni to mięso w pełni przydatne dla konsumenta. Analizowana tkanka mięsna koźląt okazała się bogatsza w żelazo i uboższa w cynk i miedź niż u jagniąt, a poziom tych pierwiastków obniżał się wraz z wiekiem ubijanych zwierząt. Zaobserwowano, że u zwierząt ubijanych w 180. dniu życia zawartość Fe w tkance mięsnej u niektórych sztuk wynosiła poniżej 17 mg kg^{-1}. Natomiast wyższy poziom miedzi stwierdzono w tkance mięśniowej tryczków, a cynku u koziołków ubijanych w wieku 150 i 180 dni życia. Zaobserwowano, że zastosowane intensywne żywienie w tuczu koźląt i jagniąt nie wpłynęło na kumulację pierwiastków, a szczególnie metali ciężkich w mięsie.

Słowa kluczowe: tkanka mięsna, koziołek, tryczek, makroelementy, metale ciężkie.

INTRODUCTION

Meat obtained from young kids has excellent quality and delicate taste. It is also rich in phosphorus, sulphur, copper, iron and calcium. This type of meat competes with veal and lamb as regards its nutritive value (LIDWIN-KA•MIERKIEWICZ 2006, NIEDZIÓŁKA et al. 2007). The world production of meat from lambs and goats is approximately 14 million tons (i.e. 4.9% of total meat quantity). Despite the fact that production of these two kinds of meat has increased by about 2.6 million tons, in some regions of the world, e.g. the EU, meat from lambs and kids remains in short supply (FAOSTAT 2008). Issues connected with contamination of the environment, feed or water pollution as well as accumulation of chemical elements in food products (meat) can reduce food export from countries of large soil, water or food pollution (WĘGLARZY 2007).

Studies conducted so far have often shown some differentiation of levels of elements in tissues and edible organs, from trace amounts to values exceeding physiological ones. The levels of chemical elements in animal tissues depend on species, gender, age and kind of tissue (ABOU DONIA 2008, Hoffman et al. 2003, Johnson et al. 1995, Krupa and Kogut 2000, Lipecka et al. 2003, Pieniak-Lendzion et al. 2006).

The aim of the study was to define the content of some chemical elements in meat tissue of male kids and ram lambs fed all-mash and slaughtered at different age.

MATERIAL AND METHODS

Castrated male kids (*n*=24) of the White Improved breed and castrated ram lambs (*n*=24) of the Polish Lowland sheep breed fattened up to 90, 120, 150 and 180 days of life were used as the experimental material. Male kids and ram lambs were kept together with their mothers and drank mothers' milk until the 60th day of life. At the age of 1 month, all the kids and rams were castrated using the blood method. The experimental animals were fed *ad libitum* with dry mash CJ and meadow hay as a structural supplement. The mixtures were produced from components grown in Podlasie. Samples were taken from *longissimus dorsi* muscles. The content of chemical elements such as Zn, Cu, Mn, Fe was tested with an atomic absorption spectrophotometer AAS. The content of Pb and Cd was analysed using the extraction method. An atomic absorption spectrophotometer AAS-30 manufactured by Carl Zeiss Jena was used to conduct the analyses. Sample consisting of 10 g of material each were dried at the temperature 150°C for 24 hours. Then, each sample was combusted in a muffle stove (temp. 420°C). The ash was moistened with nitric acid and, after distilling with nitrogen oxide, it was placed back in the muffle stove at the temperature 420°C for 30 minutes. The white rest was dissolved in muriatic acid (1 mol dm^{-3}) and analysed directly from the aqueous solution by aspirating to the flame of an atomic absorption spectrophotometer. The content of heavy metals in meat was tested in a laboratory at the Institute of Chemistry of the University of Podlasie.

The results were statistically described in tables showing mean values ($\overline{x}$) as well as the minimum and maximum for each trait (min-max). Significance of differences between means for the species were worked out according to Tukey's test. Statistical calculations were done using Statistica 6.0 PL.

RESULTS AND DISCUSSION

Heavy metals such as Pb, Cd, Cu, Hg and Zn are the most common food contaminants and the most dangerous ones for health. According to the Ordinance of the Minister of Health (2003), the maximum content of Cd in meat tissue is 0.05, and Pb 0.10 mg kg^{-1}. However, according to the European Committee Recommendation (2006), the maximum level of Pb and Cd in food products are 0.10 and 0.05 mg kg^{-1}, respectively. Both Pb and Cd concentrations in the analysed meat tissue of kids and rams did not exceed the maximum permissible amounts. Generally, more Pb was found in meat tissue of kids than that of rams, and significant ($p \leq 0.01$) difference (by 0.012 mg kg^{-1}) for the slaughter on the 120th day of life was demonstrated. On the other hand, the Cd content was larger ($p \leq 0.05$ for 90, 120 and 150 day) in tissues of ram lambs (0.003-0.014 mg kg^{-1}) than in male kids (Table 1). Lower Cd and Pb concentrations in female and male kids which were slaughtered at 150 days of age were found in some previous research (PIENIAK-LENDZION et al. 2006). However, a higher content in goats and sheep in the Rzeszow region, especially that of cadmium (above norms) was found by KRUPA and KOGUT (2000). MOREOVER, KREŁOWSKA-KUŁAS (1998) paid attention to differences in the lead and cadmium content in meat of some animal species with regard to the level of environmental contamination. The author compared the cadmium and lead content in meat tissue of lambs from the Kraków region with the content of these metals in lamb meat from the Maków Podhalanski region. She noticed that the cadmium accumulation amounted to 0.01 and 0.002 mg kg^{-1}, and lead accumulation reached 0.089 and 0.007 mg kg^{-1}, respectively. In another study completed by ABOU DONIA (2008), the lead content in buffalo, cattle, sheep, goat and elk meat was studied with reference to some industrial, urban and communication areas. It was found that sheep and goats accumulated the least Pb in meat tissue and internal organs compared with the other species. The lowest content of

Table 1

Content of lead and cadmium in muscle tissue of male kids and ram lambs (mg kg^{-1} fresh tissue)

Elements	Age of slaughter	Animals' species			
		male kids		ram lambs	
	(days)	$\overline{x}$	min. - max.	$\overline{x}$	min. - max.
Pb	90	0.031	0.021 - 0.041	0.028	0.018 - 0.028
	120	0.036**	0.026 - 0.046	0.024**	0.004 - 0.041
	150	0.040	0.010 - 0.071	0.041	0.021 - 0.061
	180	0.041	0.021 - 0.061	0.047	0.007 - 0.087
Cd	90	0.001*	0.000 - 0.004	0.003*	0.002 - 0.004
	120	0.002*	0.001 - 0.006	0.005*	0.002 - 0.008
	150	0.004*	0.002 - 0.006	0.006*	0.004 - 0.008
	180	0.006	0.001 - 0.011	0.014	0.008 - 0.020

* values with different letters differ significantly ($p<0.05$) for the animal species

** as above for $p<0.01$

Pb in meat tissue of animals living close to transportation routes was found for sheep and goats (0.006 mg kg^{-1} for sheep and 0.009 mg kg^{-1} for goats). The highest level of Pb was determined in both meat tissue and giblets of animals inhabiting an industrial area. The content of chemical elements varied in relation to a lamb breed and sex, which was confirmed by HOFFMAN et al. (2003). The authors found that lambs of the Suffolk pure and crossbred race, which were slaughtered at 40 kg of body weight, were characterized by the largest level of Pb (0.048-0.064 mg kg^{-1}). LITWINCZUK et al. (2001) confirmed the results with regard to the metal content in cows and bulls. These authors concluded that that the Pb and Cd content in *longissimus dorsi* muscles was higher in cows than in bulls, being equal to 0.39 mg kg^{-1} of Pb and 0.083 mg kg^{-1} of Cd, with significant differences at $p \leq 0.05$.

Meat tissue of male kids was characterized by a bigger iron content ($p \leq 0.05$ at 90 and 120 days) than that of ram lambs. The content of this chemical element decreased together with the slaughter age from 41.67 to 23.73 mg kg^{-1} (Table 2). It was also observed that the element content in meat tissue of older animals fell below 17 mg kg^{-1}. A similar content (16.29--18.83 mg kg^{-1}) of iron in meat tissue of lambs slaughtered at 40 kg of body weight was found by HOFFMAN et al. (2003). Cadmium and copper could affect the iron accumulation and decrease its content in tissues (MORAWIEC 1991, WĘGLARZY 2007). Lower amounts of zinc and copper in the analysed meat tissue of male kids and ram lambs, regardless of the slaughter age, were found. Significantly ($p \leq 0.01$) more Cu and Zn in meat tissue of rams (150 days) was determined, i.e. 1.42 and 39.56 mg kg^{-1}, respectively (Table 2). Similar amounts of Cu and Zn in *m. semimembranosus* of lambs were discovered by HOFFMAN et al. (2003). The content of Zn ranged from 27.86 to 54.66 mg kg^{-1}, and the Cu content varied from 0.87 to 1.40 mg kg^{-1}. The level of the chemical elements strongly depends on a breed and genotype. In the research presented by PIENIAK-LENDZION et al. (2006), gender had an effect on the Zn and Cu content. Female kids slaughtered at the age of 150 days were characterized by a 0.29 mg kg^{-1} higher Cu content and male kids had 0.52 mg kg^{-1} more Zn by ($p \leq 0.05$). JOHNSON et al. (1995) obtained on average 28.1 mg 100 g^{-1} of calcium, 92.3 mg 100 g^{-1} of zinc and 4.4 mg 100 g^{-1} of iron in meat tissue of kids. In an experiment reported by LIPECKA et al. (2003), the effect of a feeding system on the content of chemical elements in meat tissue of lambs slaughtered at 25-30 kg of body weight was tested. Non-significant differences for the content of Pb, Cd, Cu and Zn in meat tissue of lambs fattened intensively and on pasture were revealed. The differences between the groups were only noticed with regard to magnesium, which appeared in higher quantities in lambs fattened with concentrates (317.30 mg kg^{-1}) than in those pastured (281.44 mg kg^{-1}). In the present study, a lower average content of Mg was found, which in meat tissue of lambs ranged from 262.54 to 295.95 mg kg^{-1} (Table 2). Moreover, a significantly larger content of this chemical element was found in meat tissue of male kids than in that of ram lambs aged 90 and 150 days.

Table 2

Content of copper, zinc, manganese, iron and magnesium in muscle tissue of male kids and ram lambs (mg kg^{-1} fresh tissue)

Elements	Age of slaughter	Animals' species			
		male kids		ram lambs	
	(days)	$\overline{x}$	min. - max.	$\overline{x}$	min. - max.
Cu	90	0.94	0.74 - 1.18	1.08	0.87 - 1.29
	120	1.12	0.85 - 1.39	1.17	0.96 - 1.40
	150	1.32**	0.98 - 1.66	1.42**	0.86 - 1.98
	180	2.28	1.61 - 2.95	2.41	2.02 - 2.80
Zn	90	29.12	24.58 - 33.66	29.56	26.14 - 32.98
	120	29.43	26.19 - 32.67	31.87	28.66 - 35.08
	150	33.26**	29.37 - 37.15	39.56**	36.90 - 42.22
	180	51.71	49.15 - 54.27	49.41	45.94 - 52.88
Mn	90	0.46**	0.40 - 0.52	0.38**	0.31 - 0.45
	120	0.34	0.25 - 0.43	0.33	0.24 - 0.42
	150	0.30	0.23 - 0.37	0.32	0.28 - 0.36
	180	0.30**	0.22 - 0.38	0.40**	0.35 - 0.45
Fe	90	41.67*	34.80 - 48.54	24.89*	18.11 - 31.67
	120	38.22*	32.90 - 43.54	27.62*	21.62 - 33.62
	150	29.14	25.69 - 32.59	27.15	21.48 - 32.82
	180	23.73	16.84 - 30.62	23.89	16.68 - 31.11
Mg	90	295.32*	228.62-362.02	262.54*	219.33-307.75
	120	314.89	259.22-370.56	303.78	269.24-338.32
	150	289.23*	243.56-334.90	269.73*	215.08-324.38
	180	292.56	228.56-356.56	295.95	230.86-361.04

* values with different letters differ significantly ($p<0.05$) for the animal species

** as above for $p<0.01$

CONCLUSIONS

1. Low content of lead and cadmium in meat tissue was found in the course of intensive fattening of kids and rams. Meat tissue in kids compared with rams contained more lead and less cadmium. The levels of the heavy metals in meat did not exceed the maximum permissible amounts, i.e. 0.10 mg kg^{-1} of Pb and 0.05 mg kg^{-1} of Cd.

2. Kid meat was characterized by a larger content of Fe (41.67-23.73 mg kg^{-1}) than ram meat (24.89-23.89 mg kg^{-1}). The content of chemical elements decreased with the slaughter age, which was caused by a larger content of Cu in mixtures, which in turn could have affected the iron level in animal bodies.

3. The fattening period ended in a larger accumulation of Cu and Zn especially. More Cu in meat tissue of rams and Zn in kids slaughtered at the age of 150 and 180 days were found.

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THE EFFECT OF MULTI-COMPONENT FERTILIZERS ON SPRING TRITICALE YIELD, THE CONTENT AND UPTAKE OF MACRONUTRIENTS*

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Abstract

Fertilization is the main factor and an indicator of the effectiveness of agricultural production. A wide variety and range of mineral fertilizers are currently available on the Polish market. They differ with respect to quality and price, while their names are often similar, which makes it difficult for the farmer to select the best one. There has been a steady increase in the share of multi-component fertilizers in total mineral fertilizer consumption in Poland, which seems very positive. Multi-component fertilizers supply a combination of nutrients at a time, in adequate amounts and proportions, in view of the content of available phosphorus, potassium and magnesium in the soil. The objective of this study was to determine the effect of mixed multi-component fertilizers on spring triticale yield, the content and uptake of macronutrients. A three-year field experiment (2005-2007) was carried out in a randomized block design at the Research and Experimental Station in Tomaszkowo, at the University of Warmia and Mazury in Olsztyn. The experiment comprised three fertilization treatments: control treatment (simple fertilizers) and two treatments with mixed multi-component fertilizers, Amofosmag 4 (NPKMg 4:15:15:2) and Amofosmag 3 (NPKMg 3:14:20:2). The tested crop was spring triticale (*Triticosecale Wittm*) cv. Wanad. Amofosmag 4 had the most beneficial influence on the yield of spring triticale grain, which increased by 11% on average, compared with the control treatment. The effect of Amofosmag 3 was similar to that of simple fertilizers. Simple and multi-component fertilizers exerted a comparable effect on the mineral composition of triticale grain and straw, which remained within normal limits. More pronounced differences were observed in this respect between successive years of the study. The highest total uptake of nitrogen, phosphorus, potassium, calcium and magnesium by spring triticale was noted in plots fertilized with

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Amofosmag 4, which indicates that the nutrients contained in this fertilizer are more readily available to plants compared with simple fertilizers and Amofosmag 3.

Key words: spring triticale, yield, macronutrients, multi-component fertilizers.

DZIAŁANIE NAWOZÓW WIELOSKŁADNIKOWYCH NA PLON, ZAWARTOŚĆ I POBRANIE MAKROELEMENTÓW PRZEZ PSZENŻYTO JARE

Abstrakt

Nawożenie jest podstawowym czynnikiem i zarazem wskaźnikiem efektywności produkcji rolniczej. Dostepny obecnie na polskim rynku bardzo szeroki i zróżnicowany pod względem jakościowym i cenowym asortyment nawozów mineralnych, o podobnych nazwach, często utrudnia rolnikowi wybór odpowiedniego nawozu. Pozytywnym zjawiskiem jest powolny, ale systematyczny wzrost udziału nawozów wieloskładnikowych w ogólnym zużyciu nawozów mineralnych przez polskiego rolnika. Nawozy wieloskładnikowe umożliwiają dostarczanie roślinom jednocześnie kilku składników pokarmowych w odpowiednich ilościach i proporcjach, najczęściej z uwzględnieniem zasobności gleby w przyswajalny fosfor, potas i magnez. Celem pracy była ocena wpływu zastosowanych nawozów wieloskładnikowych mieszanych na wielkość plonu, zawartość i pobranie makroelementów przez pszenżyto jare. Trzyletnie doświadczenie polowe (2005-2007) przeprowadzono w Zakładzie Dydaktyczno-Doświadczalnym w Tomaszkowie należącym do Uniwersytetu Warmińsko-Mazurskiego w Olsztynie. Doświadczenie, założone metodą losowanych bloków, obejmowało trzy obiekty nawozowe: obiekt kontrolny (nawozy jednoskładnikowe) oraz dwa nawozy wieloskładnikowe mieszane – Amofosmag 4 (NPKMg 4:15:15:2) i Amofosmag 3 (NPKMg 3:14:20:2). Rośliną testowaną było pszenżyto jare (*Triticosecale Wittm*) odmiany Wanad. Z badań wynika, że najkorzystniej na plon ziarna pszenżyta jarego wpłynął Amofosmag 4, który zwiększył go średnio o ok. 11% w porównaniu z obiektem kontrolnym. Natomiast Amofosmag 3 działał na poziomie nawozów jednoskładnikowych. Nawozy jednoskładnikowe i wieloskładnikowe podobnie kształtowały skład mineralny ziarna i słomy pszenżyta, który mieścił się w ogólnie przyjętych normach. Większe zróżnicowanie wystąpiło między poszczególnymi latami badań. Największe łączne pobranie azotu, fosforu, potasu, wapnia i magnezu przez pszenżyto jare stwierdzono po zastosowaniu Amofosmagu 4, co świadczy o lepszej przyswajalności składników pokarmowych z tego nawozu niż z nawozów jednoskładnikowych oraz z Amofosmagu 3.

Słowa kluczowe: pszenżyto jare, plon, makroelementy, nawozy wieloskładnikowe.

INTRODUCTION

A wide variety and range of multi-component fertilizers (solid, liquid and suspension) are currently available on the Polish market. They often have a similar composition, but are sold under different brand names, which makes it difficult for the farmer to select the best one. Fertilization rates should be adjusted to the requirements of the plant species, and should be determined in view of crop yield and quality, fertilizer efficiency and environmental issues. Compound fertilizers provide crops with essential nutrients in adequate amounts and proportions, and they help prevent or reduce nutrient leaching (Czuba 1998, Zawartka, Skwierawska 2004a). Multi-component fertilizers, which provide three primary macronutrients, N, P and K, and secondary nutrients, Mg, Ca, S and Na, in varying proportions, are applied to counterbalance the progress of soil acidification, to correct magnesium deficiency in the soil and to reduce sulfur emissions, thus preventing nutrient deficiency in plants (Filipek 2001, Nowak, Draszawka-Bołzan 2001).

The objective of this study was to determine the effect of mixed multi-component fertilizers Amofosmag 3 and Amofosmag 4 on spring triticale yield, the content and uptake of macronutrients.

MATERIALS AND METHODS

In 2005-2007, a field experiment was carried out in a randomized block design at the Research and Experimental Station in Tomaszkowo, at the University of Warmia and Mazury in Olsztyn. The experiment, which comprised three fertilization treatments in four replications: control treatment (simple fertilizers), Amofosmag 3 and Amofosmag 4, was established on proper brown soil developed from sandy loam, of quality class III b and very good rye complex (IV). The physicochemical properties of soil in each year of the study are presented in Table 1. The tested crop was spring triticale (*Tritico-secale Wittm*) cv. Wanad. The preceding plants were winter triticale in the first year, and winter rapeseed in the following two years. The plot surface area was 10 m˛.

Table 1

Selected physicochemical and chemical properties of the soil used in the experiment

Year	pH w 1 M KCl	Available forms (mg kg^{-1})		
		P	K	Mg
2005	6.15	56.70	112.0	31.0
2006	7.20	112.9	145.3	25.0
2007	5.60	116.0	224.0	87.0

Based on the average levels of available phosphorus in the soil, 300 kg ha^{-1} Amofosmag 3 (NPKMg 3:14:20:2+22% CaO+9% SO_3; 9 kg N, 18 kg P and 50 kg K on pure ingredient basis) and Amofosmag 4 (NPKMg 4:15:15:2+24% CaO+9% SO_3; 12 kg N, 20 P and 37 kg K on pure ingredient basis) were applied pre-sowing. In the control treatment, the following fertilizers were applied pre-sowing: 12 kg N in the form of urea, 45 kg P_2O_5 (20 kg P) in the form of triple superphosphate and 45 kg K_2O (37 kg K) per ha in the form of potash salt. The nitrogen rate of 90 kg ha^{-1} was supplemented with two doses of ammonium nitrate applied by top-dressing, as follows: control treatment and Amofosmag 4 treatment – 50 and 28 kg N, Amofosmag 3 treatment – 50 and 31 kg N ha^{-1}.

Samples of spring triticale were collected at the stage of full maturity. The grain and straw harvested in each plot were dried and weighed individually. Wet mineralized samples were assayed for the content of: total nitrogen – by the hypochlorite method, phosphorus – by the vanadium-molybdenum method, calcium and potassium – by atomic emission spectrometry (AES), and magnesium – by atomic absorption spectrometry (AAS). The results of chemical analyses were verified statistically by a two-factorial analysis of variance for a randomized block design. The experimental factors were as follows: *a* – fertilization, *b* – duration of the experiment. The least significant difference was assumed at p=0.05.

RESULTS AND DISCUSSION

The distribution of air temperatures in 2005 differed insignificantly from the long-term average (Table 2). Total precipitation in April was substantially lower than the long-term average, which could have contributed to uneven emergence, whereas July was too wet. In 2006, the mean monthly temperatures were similar to the long-term average. The highest temperature was recorded in July. Precipitation levels differed considerably from the average values in July and August. Precipitation total in July and August was over 2.5-fold lower and nearly 2.5-fold higher, respectively, than the long period average, which made harvest difficult. In 2007, air temperatures during the growing season were slightly above the long-term average. July was

Table 2

Weather conditions in 2005-2007 – data provided by the Meteorological Station in Tomaszkowo

Month	Mean daily temperature (°C)				Total precipitation (mm)			
	2005	2006	2007	1970-2000	2005	2006	2007	1970-2000
April	8.2	7.3	7.5	6.9	22.0	25.6	24.7	36.1
May	11.6	12.5	13.8	12.7	68.2	89.2	93.5	51.9
June	14.2	16.0	17.7	15.9	35.4	79.2	88.1	79.3
July	19.7	20.9	17.7	17.7	83.9	29.3	173.7	73.8
August	16.9	17.2	18.3	17.2	39.6	165.0	68.0	67.1
Mean	14.1	14.8	15.0	14.1	Σ249.1	Σ388.4	Σ448.0	Σ308.2

wet, with a difference of 99.9 mm between the mean monthly rainfall and the long period average. The weather conditions affected the yield of spring triticale.

The highest average yield of spring triticale grain (5.82 t ha^{-1}) was obtained in the first year of the study (Table 3). Amofosmag 4 contributed to a significant increment in grain yield, which was by 27% and 25% higher than in the control treatment (simple fertilizers) and in the Amofosmag 3 treatment, respectively. In another experiment, spring wheat also responded by yield increase to the application of Amofosmag 4 (NOGALSKA et al. 2010).

Table 3

Spring triticale yield after the application of Amofosmag 4 and Amofosmag 3 (t ha^{-1})

Treatment	Grain				Straw			
	2005	2006	2007	mean (*a*)	2005	2006	2007	mean (a)
NPK	5.31	4.25	3.81	4.46	5.70	7.05	5.15	5.97
Amofosmag 4	6.75	4.39	3.78	4.97	7.41	6.40	5.60	6.47
Amofosmag	5.39	4.02	3.73	4.38	6.29	6.33	5.14	5.92
Mean (*b*)	5.82	4.22	3.77		6.47	6.59	5.30	
LSD$_{p=0.05}$ for:								
a	0.29				n.s.			
b	0.33				0.54			
*a*x*b*	0.50				0.94			

Legend: *a* – fertilization, *b* – duration of the experiment

In the second year of the study (2006), the yield of spring triticale grain ranged from 4.02 to 4.39 t ha^{-1}, depending on the applied fertilizer, and it was significantly lower (by 38% on average) than in the first year. Amofosmag 4 contributed to an approximately 10% increase in triticale grain yield, in comparison with Amofosmag 3. The lowest triticale grain yield was attained in 2007 – it was 35% and 11% lower than in 2005 and 2006, respectively. The above could have been due to the less favorable weather conditions. Triticale straw yield was less affected by the applied fertilizers. The highest straw yield, similarly as the highest grain yield, was obtained in the Amofosmag 4 treatment. Triticale straw yield was significantly lower (by over 1.2 tons) in the last year of the experiment than in the first and second year.

The means from the three years show that Amofosmag 4 caused an approximately 11% and 8% increase in the yield of triticale grain and straw, respectively, compared with the control treatment. The effect of Amofosmag 3 was similar to that of simple fertilizers. Other authors (MAZUR et al. 2001, STĘPIEŃ, MERCIK 2001, ZAWARTKA, SKWIERAWSKA 2004b) also observed a yield-forming effect of compound fertilizers in many plant species. On the other hand, KRZYWY et al. (2000) demonstrated that multi-component fertilizers had an insignificant effect on the yield of winter triticale and spring barley.

The results of chemical analyses of spring triticale grain and straw, presented in Table 4, suggest that the concentrations of the analyzed macronutrients varied insignificantly between fertilization treatments, and in most cases they remained within normal limits (*Norms for nutritions...* 2009). The grain of spring triticale fertilized with Amofosmag 3 had a significantly lower average nitrogen content, compared with the Amofosmag 4 and control treatments. The concentrations of phosphorus, potassium, calcium and magnesium were not significantly modified by the applied fertilizers. More pronounced differences were observed in this respect between successive years of the study. In the first year of the study (2005), the grain of spring triticale contained significantly less nitrogen and calcium and more magnesium, compared with the values noted in the two consecutive years. In the second year of the experiment, triticale grain contained larger amounts of nitrogen than in the first year, and significantly higher quantities of phosphorus and calcium and less potassium than in the first and third year. The highest average nitrogen content (23.0 g kg^{-1} d.m.) of triticale grain was observed in 2007, and it was significantly higher than in 2005 and 2006 (by 60% and 18%, respectively). The concentrations of the analyzed macronutrients in triticale straw varied between the years. The highest content of nitrogen was noted in the first year, of phosphorus and magnesium in the second year, and of potassium – in the third year. The findings of many other authors (KRZYWY et al. 2000, FILIPEK 2001, KRZYWY et al. 2001, MAZUR et al. 2001, NOGALSKA et al. 2010) suggest that multi-component fertilizers have an insignificant effect on the macronutrient content of tested plants.

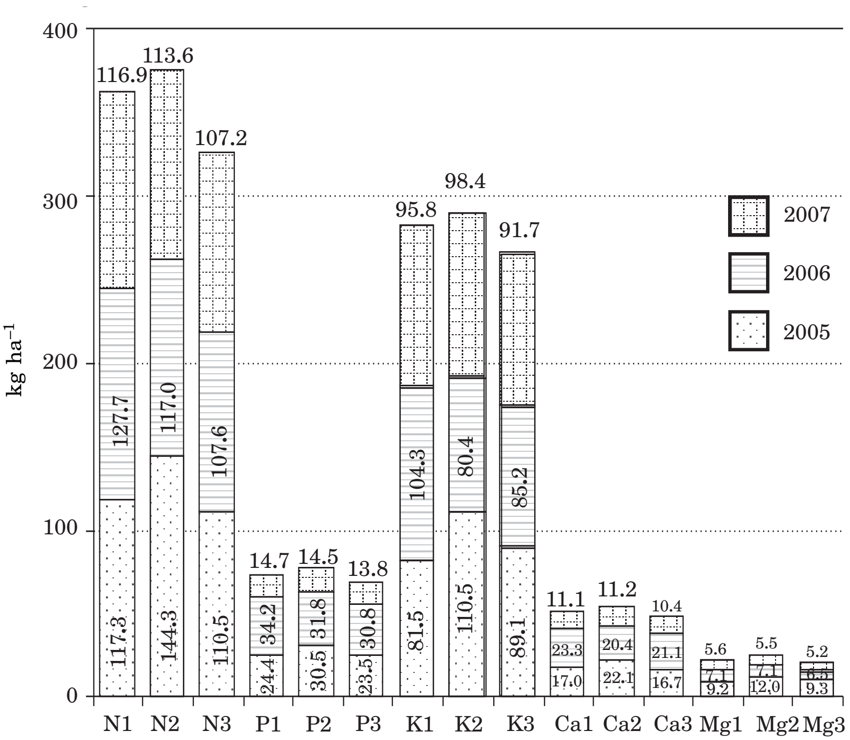
Macronutrient uptake was estimated based on the yield and macronutrient content of spring triticale grain and straw (Figure 1). The highest nitrogen uptake by spring triticale plants (144.3 kg N ha^{-1}) was noted in the first year of the experiment, following the application of Amofosmag 4, which resulted from the highest yield of triticale grain and straw in this treatment. Phosphorus uptake levels were comparable in all treatments. Phosphorus uptake varied between years – it was the highest in 2006 (32.3 kg P ha^{-1} on average) when triticale grain and straw were abundant in phosphorus, and nearly two-fold lower in the third year of the experiment. A similar trend was observed with regard to calcium uptake. Magnesium uptake decreased over time. The highest total (mean values of three years) uptake of the analyzed macronutrients (N, P, K, Ca and Mg) was noted in plots fertilized with Amofosmag 4. In the Amofosmag 3 treatment, macronutrient uptake was 7% lower than in the control treatment. This indicates that the nutrients contained in Amofosmag 4 are more readily available to spring triticale, in comparison with simple fertilizers and Amofosmag 3. STĘPIEŃ, MERCIK (2001), KRUCZEK, SULEWSKA (2005) and NOGALSKA et al. (2010) reported that multi-component fertilizers, compared with simple fertilizers, supported higher nutrient uptake by various plant species.

Table 4

Macronutrient content of spring triticale after the application of Amofosmag 4 and Amofosmag 3 (g kg^{-1} d.m.)

Macro-nutrient	Treatment	Grain				Straw			
		2005	2006	2007	mean (*a*)	2005	2006	2007	mean (*a*)
Nitrogen	NPK	14.9	20.1	23.8	19.6	6.65	6.04	5.07	5.92
	Amofosmag4	14.9	19.5	23.1	19.2	5.86	4.89	4.71	5.15
	Amofosmag3	13.5	18.9	22.0	18.1	5.97	4.99	4.90	5.29
Mean (*b*)		14.4	19.5	23.0	-	6.16	5.31	4.89	-
$LSD_{p=0.05}$ for:	*a* *b* *a*xb	0.89 0.90 n.s.				n.s. 0.62 n.s.			
Phosphorus	NPK	3.72	5.40	2.37	3.83	0.84	1.60	1.09	1.18
	Amofosmag4	3.62	5.25	2.37	3.75	0.84	1.40	0.97	1.07
	Amofosmag3	3.64	5.33	2.30	3.76	0.66	1.50	1.02	1.06
Mean (*b*)		3.66	5.32	2.35	-	0.78	1.50	1.03	-
$LSD_{p=0.05}$ for:	*a* *b* *a*xb	n.s. 0.15 n.s.				n.s. 0.14 n.s.			
Potassium	NPK	4.50	3.30	4.61	4.14	10.1	12.8	15.2	12.7
	Amofosmag4	4.30	3.05	4.37	3.91	11.0	10.5	14.6	12.0
	Amofosmag3	4.45	3.07	4.25	3.92	10.4	11.5	14.8	12.2
Mean (*b*)		4.42	3.14	4.41		10.5	11.6	14.9	-
$LSD_{p=0.05}$ for:	*a* *b* *a*xb	n.s. 0.21 n.s.				n.s. 1.49 n.s.			
Calcium	NPK	0.31	1.00	0.47	0.59	2.75	2.68	1.80	2.41
	Amofosmag4	0.31	1.01	0.44	0.59	2.75	2.54	1.75	2.35
	Amofosmag3	0.30	0.99	0.45	0.58	2.40	2.67	1.75	2.27
Mean (*b*)		0.31	1.00	0.45		2.63	2.63	1.77	-
$LSD_{p=0.05}$ for:	*a* *b* *a*xb	n.s. 0.061 n.s.				n.s. 0.28 n.s.			
Magnesium	NPK	1.43	0.99	1.02	1.15	0.29	0.41	0.34	0.35
	Amofosmag4	1.40	0.98	1.01	1.13	0.34	0.44	0.30	0.36
	Amofosmag3	1.42	0.99	0.95	1.12	0.26	0.40	0.32	0.33
Mean (*b*)		1.42	0.99	0.99	-	0.30	0.42	0.32	-
$LSD_{p=0.05}$ for:	*a* *b* *a*xb	n.s. 0.048 n.s.				n.s. 0.037 n.s.			

Explanations as in Table 3



1 – NPK, 2 – Amofosmag 4, 3 – Amofosmag 3

Fig. 1. Nutrient uptake by spring triticale grain and straw in 2005-2007 (kg ha^{-1})

CONCLUSIONS

1. Amofosmag 4 contributed to an approximately 11% and 8% increase in the yield of spring triticale grain and straw, respectively, compared with the control treatment. The effect of Amofosmag 3 was similar to that of simple fertilizers. Triticale grain yield was significantly higher in the first year of the study than in the second and third year.

2. The concentrations of the analyzed macronutrients in spring triticale grain and straw varied insignificantly between fertilization treatments. In most cases, simple and multi-component fertilizers exerted a comparable effect on the chemical composition of the tested crop. Significant differences were observed in this respect between successive years of the study.

3. The highest total uptake of N, P, K, Ca and Mg by spring triticale was noted in plots fertilized with Amofosmag 4. Macronutrient uptake from Amofosmag 3 was lower than from simple fertilizers.

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COMPARISON OF THE CHEMICAL COMPOSITION OF SPRING DURUM WHEAT GRAIN (*TRITICUM DURUM*) AND COMMON WHEAT GRAIN (*TRITICUM AESTIVUM* SSP. *VULGARE*)

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Abstract

The research was conducted at the Experimental Farm in Felin, property of the University of Life Sciences in Lublin, in 2007–2009. The experiment was located on soil of good wheat complex. The research included the spring form of durum wheat (*Triticum durum* Desf.) – breeding line LGR 896/23 (selected at ULS, Lublin) and cultivars Lloyd (American), Chado and Kharkivska 27 (Ukrainian), which were compared with spring common wheat (*Triticum aestivum ssp. vulgare*) cv. Torka.

In the study, the chemical composition of durum wheat grain and common wheat grain was analysed. The content of total protein, fibre, fat, ash, nitrogen–free extracts, macronutrients (phosphorus, potassium, calcium, magnesium) and micronutrients (copper, iron, manganese, zinc) was determined. Variation coefficients and coefficients of correlation were also calculated.

All the lines and cultivars of spring durum wheat were characterised by a higher content of total protein and zinc compared to common wheat. Common wheat showed a higher content of copper and manganese in grain compared to durum wheat. Among the quality traits of wheat grain, regardless of the species, the content of nitrogen–free extracts was the least variable (cv=1.5%), whereas the highest variability (cv=37.2%) characterised the content of manganese. Significant correlations were shown in wheat grain for the following pairs of quality traits: protein–nitrogen–free extracts, protein–ash, and ash–protein–free extracts. The results of the qualitative evaluation of the wheat species show that the line and cultivars of durum wheat as well as the cultivars of common wheat fulfil the protein content norms set for raw material for pasta production. Higher ash levels in flour may cause a risk of obtaining darker colour pasta.

Key words: durum wheat, common wheat, pasta, grain quality, chemical composition, macroelements, microelements, coefficient of variation, correlation coefficient.

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PORÓWNANIE SKŁADU CHEMICZNEGO ZIARNA PSZENICY JAREJ TWARDEJ (*TRITICUM DURUM*) Z PSZENICĄ ZWYCZAJNĄ (*TRITICUM AESTIVUM* SSP. *VULGARE)*

Abstrakt

Badania przeprowadzono w latach 2007–2009 na terenie Gospodarstwa Doświadczalnego w Felinie, należącego do Uniwersytetu Przyrodniczego w Lublinie. Doświadczenie zlokalizowano na glebie zaliczanej do kompleksu pszennego dobrego. Badaniami objęto formę jarą pszenicy twardej (*Triticum durum* Desf.): linia hodowlana LGR 896/23 (wyselekcjonowana w UP w Lublinie) i odmiany Lloyd (amerykańska), Chado i Kharkivska 27 (ukraińskie), którą porównano z pszenicą jarą zwyczajną (*Triticum aestivum* ssp. *vulgare*) odmiany Torka.

W pracy analizowano skład chemiczny ziarna pszenicy twardej i pszenicy zwyczajnej. Określono zawartość białka ogólnego, włókna, tłuszczu, popiołu, bezazotowych substancji wyciągowych, makroelementów (fosforu, potasu, wapnia, magnezu) i mikroelementów (miedzi, żelaza, manganu, cynku). Obliczono także współczynniki zmienności oraz współczynniki korelacji.

Wszystkie badane linie i odmiany pszenicy jarej twardej zawierały więcej białka ogólnego oraz cynku w porównaniu z pszenicą zwyczajną. Pszenica zwyczajna miała wyższą zawartość miedzi i manganu w ziarnie w porównaniu z pszenicą twardą. Spośród cech jakościowych ziarna badanych pszenic, niezależnie od gatunku, zawartość substancji bezazotowych wyciągowych okazała się cechą najmniej zmienną (cv=1,5%), największą zaś zmienność (cv=37,2%) stwierdzono w przypadku zawartości manganu. W ziarnie pszenicy wykazano istotne korelacje dla następujących par cech jakościowych: białko–substancje bezazotowe wyciągowe, białko–popiół, oraz popiół–substancje bezazotowe wyciagowe. Wykazano, że zarówno linia i odmiany pszenicy twardej, jak też odmiana pszenicy zwyczajnej spełniają normy surowca do produkcji makaronu pod względem zawartości białka. Podwyższona popiołowość mąki może stwarzać niebezpieczeństwo uzyskania makaronu o ciemniejszej barwie.

Słowa kluczowe: pszenica twarda, pszenica zwyczajna, makaron, jakość ziarna, skład chemiczny, makroelementy, mikroelementy, współczynnik zmienności, współczynnik korelacji.

INTRODUCTION

Because of its high content of carotenoid pigments and high kernel glassiness and hardness, durum wheat is mainly used in the production of special coarse flour, known as semolina, from which pasta is made (SZWED-URBAŚ et al. 1997). However, high quality semolina can be obtained only from high quality grain, and then pasta has appropriate viscoelasticity, colour, and does not form clusters while drying or overcooking, as may happen when flour from common wheat is used (OBUCHOWSKI 1998). The chemical and mineral composition of wheat grain has essential influence on its quality. Minerals are a group of compounds necessary in human nutrition. The human organism is incapable of producing them, hence they must be supplied in appropriate amounts with food. *Triticum durum* grain should not contain large amounts of ash because ash gives a dark colour to pasta, which should be yellow owing to the natural concentration of carotenoid pigments (ZALEWSKI, BOJARCZUK 2004). The colour of semolina and flour obtained from *T. durum* grain is very important, because synthetic dyes are not used in the production of pasta (SZWED-URBAŚ et al. 1997). At the same time, grain of this species should be rich in proteins for the semolina will contain a minimum 12-13% of protein and no less than 30% of gluten. With lower levels of these components, the pasta will be brittle and fragile (OBUCHOWSKI 2000, RACHOŃ 2004), prone to cracking during drying and transport (RACHOŃ et al. 2002), and thus of lower quality.

At present, the demand of the Polish the food industry for durum wheat grain is covered entirely by import (SZWED-URBAŚ 1997, RACHOŃ 2004). However, high prices of grain of this species on the international market and the industry's increasing demand, dictated by the increased consumption of products made from durum wheat (e.g. pastas, grits), have renewed the interest in cultivation of specific varieties of this wheat in our country (SZWED-URBAŚ et al. 2000, RACHOŃ, SZUMIŁO 2006). Since 1976, research has been conducted in the Lublin region with the goal of studying the world resources of *Triticum durum* and choosing appropriate initial material for the national program of cultivating this wheat (SZWED-URBAŚ 1992).

The aim of this study has been to determine the chemical composition of grain of spring varieties and lines of durum wheat in comparison to common wheat and to assess their technological suitability for the production of pasta.

MATERIAL AND METHODS

A field experiment was conducted in 2007-2009 at the Experimental Farm in Felin, property of the University of Life Sciences in Lublin. The experimental field was located on soil of good wheat complex, rich in nutrients: P –76, K –119 and Mg –55 (in mg kg^{-1} of soil), with slightly acid reaction (pH in KCl solution – 6.3) (RACHOŃ 2001).

The experiment was set up on soil after winter rape in random blocks with 4 replications. The research covered the spring form of durum wheat: breeding line LGR 896/23 (selected in the Institute of Plant Genetics, Breeding and Biotechnology, ULS in Lublin) and cultivars Lloyd (American), Chado and Kharkivska 27 (Ukrainian), and spring common wheat of cv. Torka, which belongs to the grain technology value group E. The harvest area of the plots was 10 m^2. Soil tillage was a typical plough system. The following were applied pre-sowing: phosphorus fertilisation at a dose of 26.2 kg P ha^{-1}, potassium at a dose of 66.6 kg K ha^{-1} and nitrogen at a dose of 40.0 kg N ha^{-1}. Another dose of nitrogen (40.0 kg N ha^{-1}) was applied as top dressing. Sowing density of both species was 5 million germinating kernels per 1 ha. Treatments (herbicides, fungicides, insecticides, retardant) were performed according to current recommendations.

Chemical analyses were performed annually in grain samples taken from the plots. After wet mineralisation (concentrated sulphuric acid + hydrogen peroxide), the amounts of the following were determined: fibre (gravimetric method), fat (Soxhlet gravimetric method) and ash (gravimetric method at 580°C) – PN-ISO 2171. Protein content was determined using Kjeldahl method (N%·5.75) – PN-75/A-04018. Nitrogen-free extract content was obtained by subtracting the sum of the other ingredients of dry matter from 100. The concentration of the following macro- and micronutrients was also determined: P (flow spectrophotometry), K (flame spectrometry emission), Ca, Mg, Cu, Fe, Mn and Zn (atomic absorption spectrometry).

The experimental results were statistically processed with the analysis of variance, determining the significance of differences by means of Tukey's test at the significance level 0.05. The results were given as average values from three years of the research with standard deviation (SD). The variability of the examined grain quality parameters was determined based on the variation coefficient. Next, the relationships between grain quality traits were determined by calculating the coefficients of correlation for the significance level 0.05.

The weather conditions during the experiment were highly variable (Table 1). In 2007, April was dry, which had an unfavourable effect on the germination of wheat. Later, from May to July of that year, high temperatures were noted and the rainfall was above the long-term average (1951-2000), which was favourable for intensive growth and development of the plants. In 2008, April was warm, with the rainfall above the long-term norm,

Table 1

Rainfalls and air temperatures according to the Meteorological Observatory at Felin

Year	Month						Period March-August
	March	April	May	June	July	August	
Rainfalls (mm)							sum
2007	30.2	17.4	81.5	87.8	87.0	37.6	341.5
2008	64.8	55.8	101.6	25.9	77.1	45.0	370.2
2009	69.6	2.9	71.1	125.5	57.1	54.7	380.9
Mean for years 1951-2000	25.8	40.6	58.3	65.8	78.0	69.7	338.2
Temperature (°C)							mean
2007	6.2	8.7	15.0	18.1	19.2	18.4	14.3
2008	3.4	9.3	12.8	17.7	18.3	19.3	13.5
2009	1.4	11.4	13.6	16.4	19.9	19.0	13.6
Mean for years 1951-2000	1.0	7.5	13.0	16.5	17.9	17.3	12.2

which favoured the sprouting of cereals. May was characterised by moderate temperatures and a significant surplus of rainfall, a deficiency of which occurred in June, whereas in July the temperature and precipitation were close to the long-term average. In the last year of the research (2009), severe precipitation deficit in April and at the beginning of May inhibited the germination, growth and development of spring wheat, and frequent rainfall in June encouraged re-infestation of the plantation with weeds. In 2009, the lowest quality of durum and common wheat grain was obtained.

RESULTS AND DISCUSSION

Chemical composition analysis of the grain of the examined species of wheat and their lines and varieties showed significant variations of most of the examined characteristics (Table 2). One of the more important distin-

Table 2

The chemical composition of spring wheat (g kg^{-1} d.m.)

Cultivars and line		Total protein	Crude fibre	Crude fat	Crude ash	N-free extract
Common wheat						
Torka	M	136	22.0	20.0	22.0	801
	SD	12.0	1.2	0.9	2.1	32.7
Durum wheat						
LGR 896/23	M	154	23.0	19.0	23.0	781
	SD	13.5	1.2	1.6	1.9	32.5
Lloyd	M	148	20.0	19.0	22.0	791
	SD	10.5	1.7	1.2	1.5	31.6
Chado	M	148	24.0	19.0	21.0	788
	SD	5.6	2.1	1.1	0.12	29.8
Kharkivska 27	M	147	22.0	18.0	21.0	792
	SD	9.9	1.1	2.1	1.5	31.1
Mean		149	22.3	18.7	21.8	788
LSD		11.3	2.10	1.50	1.40	13.6
CV (%)		7.4	7.4	6.9	7.3	1.5

M – mean for the years 2007-2009
SD – standard deviation
LSD(p=0.05) for cultivars and line
CV – coefficient of variation

guishing qualities of wheat is the grain protein content. The average protein content in durum wheat grain equalled 149 g kg^{-1} and was significantly higher compared to common wheat of Torka variety (136 g kg^{-1}). The examined lines and varieties of durum wheat were characterised by high total protein content in the grain. The highest protein content (154 g kg^{-1}) was noted in the grain of durum wheat of line LGR 896/23, and the lowest (147 g kg^{-1}) was determined in the grain of cv. Kharkivska 27. Many authors point out a higher protein content in durum wheat grain compared to common wheat. In the studies by RACHOŃ and KULPA (2004) and RACHOŃ and SZUMIŁO (2006, 2009), durum wheat contained on average 2.3-3.1% more protein, whereas in the investigations by SZWED-URBAŚ (1993) and SEGIT and SZWED-URBAŚ (2006), the differences were even bigger and reached up to 4.6%. In the study by RACHOŃ and SZUMIŁO (2002), the protein content in grain of cv. Torka equalled 135 g kg^{-1} and was close to the content of this component in another variety of common wheat – Sigma (135 g kg^{-1}), but significantly lower (by 2.9%) compared to lines and varieties of durum wheat.

The average fibre content in durum wheat grain equalled 22.3 g kg^{-1}, and fat – 18.7 g kg$^{-1,}$ in which they did not differ significantly from the common wheat variety (22.0 g kg^{-1} and 20.0 g kg^{-1}, respectively). Among the compared lines and varieties of durum wheat, significant differences in fibre content were noted between the cultivars Chado (24.0 g kg^{-1}) and Lloyd (20.0 g kg^{-1}). The nitrogen-free extract content was similar in both compared wheat species. A significant difference was noted only between line LGR 896/23 of durum wheat (781.0 g kg^{-1}) and common wheat (801.0 g kg^{-1}).

Determination of the ash content and level of minerals is significant for a comprehensive analysis of wheat grain quality traits, as these components determine whether it is technologically possible to process given wheat into final products i.e. bread or pasta. Cereal products, which form 40-50% of the human diet, are one of the main sources of minerals. High content of minerals is nutritionally beneficial, but an excessive ash content may impede technological processes and production high quality food (e.g. darkening of pasta). In our study, the average ash content in durum wheat grain equalled 21.8 g kg^{-1} and did not significantly differ compared to common wheat (22.0 g kg^{-1}). A significant difference was noted only between line LGR 896/23 (23.0 g kg^{-1}) and cv. Chado v (21.0 g kg^{-1}). Other authors, e.g. GĄSIOROWSKI and OBUCHOWSKI (1978), MAKARSKA et al. (2001), RACHOŃ (2001), RACHOŃ and SZUMIŁO (2009), demonstrated a higher content of this component in durum wheat grain compared to common wheat.

For an organism to function properly, the daily supply of basic nutrients is just as important as the availability, often even in trace amounts, of elements participating in the metabolism: macro- and micronutrients. One of their sources is wheat grain.

Among the analysed macroelements, phosphorus, calcium and magnesium were demonstrated to vary in their content (Table 3). The average phosphorus content of durum wheat grain was 4.73 g kg^{-1} and did not differ

Table 3

The content of macronutrients in spring wheat (g kg $^{-1}$ d.m.)

Cultivars and line		Phosphorus	Potassium	Calcium	Magnesium
Common wheat					
Torka	M	4.60	5.00	0.77	1.40
	SD	0.35	0.65	0.11	0.18
Durum wheat					
LGR 896/23	M	5.00	5.50	0.64	1.40
	SD	0.45	0.94	0.12	0.10
Lloyd	M	4.70	5.20	0.86	1.20
	SD	0.43	0.88	0.06	0.10
Chado	M	4.60	5.30	0.76	1.30
	SD	0.35	0.64	0.11	0.11
Kharkivska 27	M	4.60	4.90	0.74	1.20
	SD	0.45	0.65	0.06	0.10
Mean		4.73	5.23	0.75	1.28
LSD		0.250	n.s.	0.120	1.800
CV (%)		8.4	14.5	15.0	11.1

* key under Table 2

significantly compared to common wheat – 4.60 g kg^{-1} The highest phosphorus content among the examined durum wheat lines and varieties was determined in line LGR 896/23 – 5.00 g kg^{-1}. RACHOŃ and SZUMIŁO (2009) found a higher phosphorus content in durum wheat grain compared to common wheat. The highest content of calcium was observed in durum wheat grain of cv. Lloyd – 0.86 g kg^{-1}, and of magnesium in durum wheat grain of line LGR 896/23 and in the common wheat variety – 1.40 g kg^{-1}. At the same time, no significant differences were shown between the average content of both elements in durum wheat grain compared to common wheat, which in the case of magnesium is confirmed by MAKARSKA et al. (2001). The calculated variance coefficients (cv) for the respective macroelements, regardless of the examined species, were varied. The highest variance was noted for calcium (cv=15.0%) and potassium (cv=14.5%), and the lowest one for phosphorus (cv=8.4%).

The examined species varied significantly in terms of copper, manganese and zinc content (Table 4). Common wheat grain was characterised by the highest copper and manganese content: 3.23 mg kg^{-1} and 37.7 mg kg^{-1}, respectively. Significantly lower values were obtained in durum wheat grain (2.54 mg kg^{-1} and 30.5 mg kg^{-1} on average). Of the examined durum wheat

Table 4

The content micronutrients in spring wheat (mg kg^{-1} d.m.)

Cultivars and line		Copper	Iron	Manganese	Zinc
Common wheat					
Torka	M	3.23	37.7	37.7	32.5
	SD	0.34	6.72	8.02	5.94
Durum wheat					
LGR 896/23	M	2.83	37.6	30.3	39.4
	SD	0.25	3.55	10.66	5.90
Lloyd	M	2.64	37.5	26.7	38.7
	SD	0.25	8.25	11.47	7.47
Chado	M	2.33	36.3	35.1	46.4
	SD	0.63	4.76	13.90	8.16
Kharkivska 27	M	2.35	34.6	29.8	40.8
	SD	0.47	1.92	12.59	9.21
Mean		2.54	36.5	30.5	41.3
LSD		0.680	n.s.	7.74	5.42
CV (%)		19.6	14.5	37.2	21.4

* key under Table 2

varieties, the lowest content of copper was noted in the grain of the cultivars Chado – 2.33 mg kg^{-1} and Kharkivska 27-2.35 mg kg^{-1}; in respect of manganese, the following varieties contained the smallest concentrations of this element: Lloyd – 26.7 mg kg^{-1} and Kharkivska 27-29.8 mg kg^{-1}. Significantly higher zinc content was shown in durum wheat grain (on average, 41.3 mg kg^{-1}) compared to common wheat (32.5 mg kg^{-1}). VILLEGAS et al. (1970), MAKARSKA et al. (2001) and RACHOŃ (2001) also indicate higher zinc content in durum wheat compared to common wheat. The lowest variance among the analysed micronutrients was demonstrated for iron (cv = 14.5%) and copper (cv=19.6%), and the highest one for manganese (cv = 37.2%).

In the above research, regardless of the wheat species, significant relations were observed between the protein content and ash or nitrogen-free extract content in grain (Table 5). The protein content in grain was positively correlated with ash (r=0.658) and negatively with nitrogen-free extract content (r=–0.985). RACHOŃ and SZUMIŁO (2009) also demonstrated a negative correlation between the protein content and nitrogen-free extracts in wheat grain. A negative, significant correlation was evidenced also between the ash content and nitrogen-free extracts in wheat grain (r=–0.696).

Table 5

Values of correlation coefficients

Correlation coefficients	Total protein	Crude fibre	Crude fat	Crude ash
Crude fibre	0.288	*x*		
Crude fat	-0.332	-0.149	*x*	
Crude ash	0.658*	0.169	-0.433	*x*
N-free extract	-0.985*	-0.400	0.266	-0.696*

* values of significant correlation coefficients

CONCLUSIONS

1. Durum wheat grain was characterised by a higher content of total protein and zinc compared to common wheat.
2. Common wheat showed a higher copper and manganese content in grain compared to durum wheat.
3. High protein content in the grain of the examined varieties and lines of durum wheat indicates its usefulness in the production of pasta, and the relatively high ash content in the grain indicates worse colouring of pasta.
4. In wheat grain, regardless of its species, significant correlations were shown for the following pairs of quality traits: protein-nitrogen-free extracts, protein-ash, and ash-protein-free extracts.

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THE INFLUENCE OF NITROGEN FERTILIZATION WITH ENTEC-26 AND AMMONIUM NITRATE ON THE CONCENTRATION OF THIRTY-ONE ELEMENTS IN CARROT (*DAUCUS CAROTA* L.) STORAGE ROOTS

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Abstract

Simultaneous application of nitrification inhibitors and nitrogen fertilizers (containing reduced forms of nitrogen (ammonium and urea) can increase soil acidification caused by these fertilizers. As a result, it can lower soil reaction (pH) and influence the content of available forms of heavy metals and trace elements. The aim of the study was to evaluate the effect of the application of: ENTEC-26 (containing 3.4-dimethylpyrazol phosphate /DMPP/ – nitrification inhibitor) and ammonium nitrate on the mineral composition of carrot storage roots. A two-year research was conducted with field cultivation of the carrot cultivar Kazan F_1. The experiment was arranged in a split-plot design with four replicates. The following combinations with different N fertilization were distinguished: 1 – control (without N fertilization), 2 – ENTEC-26 35+35, 3 – ENTEC-26 70+70, 4 – ENTEC-26 105+105, 5 – ammonium nitrate 35+35, 6 – ammonium nitrate 70+70, 7 – ammonium nitrate 105+105, where: 35+35, 70+70 and 105+105 denote nitrogen doses (kg N ha^{-1}) used for pre-plant fertilization and top-dressing, respectively. In carrot storage roots as well as in soil after carrot cultivation, concentrations of Ag, Al, B, Ba, Ca, Ce, Co, Cr, Dy, Fe, Ga, In, K, La, Li, Lu, Mg, Mn, Na, Ni, P, Pb, S, Sc, Sn, Sr, Ti, Tm, Y, Yb and V were determined using the ICP-OES technique. Nutrition with nitrogen had significantly influenced the content of Co, Fe, In, Li, Mn, Ni, S, Sc, Sr, Y, Yb and V in carrot roots and this effect varied depending on the type of fertilization regime used in the experiment. No significant

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impact of N fertilization was found in reference to the accumulation of Ag, Al, B, Ba, Ca, Ce, Cr, Dy, Ga, K, La, Lu, Mg, Na, P, Pb, Sn and Ti in carrot roots. In relation to the control, application of all doses of both ENTEC-26 and ammonium nitrate resulted in decreased concentration of Mg, Al, B, Ba, Ce, Fe, Ga, La, Ni, Pb, Ti, Y, V, Cr, Dy, In, Li, Lu, Sc and Yb as well as enhanced accumulation of Ca, Sr and Ag in soil after carrot cultivation. Nonetheless, the above changes in the concentration of elements in soil due to N fertilization were not reflected in their levels detected in carrot storage roots.

Keywords: nitrogen fertilization, nitrification inhibitor, DMPP, heavy metals, trace elements, rare elements.

WPŁYW NAWOŻENIA AZOTEM Z ENTEC-26 I SALETRĄ AMONOWĄ NA ZAWARTOŚĆ TRZYDZIESTU JEDEN PIERWIASTKÓW W KORZENIACH SPICHRZOWYCH MARCHWI (*DAUCUS CAROTA* L.)

Abstrakt

Aplikowanie inhibitorów nitryfikacji wraz z nawozami azotowymi (zawierającymi zredukowane formy azotu: amonową i amidową) może wzmacniać zakwaszające działanie tych nawozów na glebę. W efekcie może to prowadzić do obniżenia pH oraz zmian zawartości dostępnych dla roślin form metali ciężkich i pierwiastków śladowych w glebie. Celem badań była ocena wpływu stosowania nawozów azotowych z ENTEC-26 (zawierającego inhibitor nitryfikacji 3,4-dimetylopyrazolofosfat /DMPP/) i saletrą amonową na skład mineralny korzeni spichrzowych marchwi. Przeprowadzono dwuletnie badania z polową uprawą marchwi Kazan F$_1$. Doświadczenie założono metodą split-plot w czterech powtórzeniach. Obiektami badań były kombinacje ze zróżnicowanym nawożeniem azotem: 1 – kontrola (bez nawożenia azotem), 2 – ENTEC-26 35+35, 3 – ENTEC-26 70+70, 4 – ENTEC-26 105+105, 5 – saletra amonowa 35+35, 6 – saletra amonowa 70+70, 7 – saletra amonowa 105+105, gdzie: 35+35, 70+70 i 105+105 oznacza dawkę azotu w kg N ha^{-1} stosowaną w nawożeniu przedsiewnym i w nawożeniu pogłównym. W korzeniach spichrzowych oraz w glebie po uprawie marchwi zawartość: Ag, Al, B, Ba, Ca, Ce, Co, Cr, Dy, Fe, Ga, In, K, La, Li, Lu, Mg, Mn, Na, Ni, P, Pb, S, Sc, Sn, Sr, Ti, Tm, Y, Yb i V była oznaczana techniką ICP-OES. Nawożenie azotem miało istotny wpływ na zawartość Co, Fe, In, Li, Mn, Ni, S, Sc, Sr, Y, Yb i V w marchwi. Jednakże wpływ ten był zróżnicowany w zależności od zastosowanego sposobu nawożenia azotem w badanych kombinacjach. Nie stwierdzono natomiast istotnego oddziaływania nawożenia azotem na zawartość Ag, Al, B, Ba, Ca, Ce, Cr, Dy, Ga, K, La, Lu, Mg, Na, P, Pb, Ti i Sn w marchwi. W porównaniu z kontrolą, zastosowane nawożenie azotem we wszystkich dawkach zarówno w formie ENTEC-26, jak i saletry amonowej powodowało zmniejszenie zawartości Mg, Al, B, Ba, Ce, Fe, Ga, La, Ni, Pb, Ti, Y, V, Cr, Dy, In, Li, Lu, Sc i Yb oraz zwiększenie zawartości Ca, Sr i Ag w glebie po uprawie marchwi. Jednakże wykazane zmiany zawartości tych pierwiastków w glebie pod wpływem nawożenia azotem nie miały odzwierciedlenia w ich zawartości w korzeniach spichrzowych marchwi.

Słowa kluczowe: azot, nawożenie, inhibitor nitryfikacji, DMPP, metale ciężkie, pierwiastki śladowe, pierwiastki rzadkie.

INTRODUCTION

Nitrogen fertilizers containing reduced forms of this element (ammonium, urea) belong to physiologically acid fertilizers as their application decreases soil pH (Gębski 1998, Jentschke et al. 1998, Maier et al. 2002). Consequently, altered solubility of mineral nutrients may occur, manifested as an increased content of easily available forms of microelements (excluding molybdenum), heavy metals and trace elements in soil. As a result, enhanced uptake (and therefore accumulation) of these elements in plant tissues can be observed (Marschner 1995, Rodríguez-Ortíz et al. 2006). However, it should be underlined that the actual influence exerted by N fertilizers depends on physicochemical properties of soil, including its buffer capacity (Diatta, Grzebisz 2006).

Application of nitrification inhibitors together with nitrogen fertilizers is aimed at limiting the negative effect of NO_3^- on the environment, mainly through its reduced leaching from soil. Activity of nitrification inhibitors is generally based on processes of $N\text{-}NH_4$ stabilization in soil by inactivation of nitrifying microorganisms (Li et al. 2008).

Increased concentration of $N\text{-}NH_4$ resulting from the introduction of nitrification inhibitors can significantly enhance the acidification effect exerted by this nitrogen form on soil. Depending on physicochemical properties of soil, various changes in soil content of available forms of heavy metals and trace elements may occur. As a result, plant concentration of these elements is seriously affected, which contributes to large differences in the quality of crop.

In research on $N\text{-}NH_4$ stabilization in soil, numerous natural nitrification inhibitors have been tested, including *Chenopodium album* L. extract (Jafari, Kholdebarin 2002), natural essential oils (Kiran, Patra 2002) or *Mentha spicata* L. oil (Patra et al. 2002). Additionally, synthetic inhibitors of this process were applied, such as nitrapyrin (Abbasi et al. 2003), dicyandiamide /DCD/ (Davies, Williams 1995, Blankenau et al. 2002, Cookson, Cornforth 2002) or 3.4-dimethylpyrazol phosphate /DMPP/ (Zerulla et al. 2001, Hähndel, Wissemeier 2004, Gioacchini et al. 2006). The above compounds differ in their power to depress the nitrification rate as well as in the spectrum of activities towards soil microflora.

The negative impact of high concentrations of heavy metals manifested as a limited nitrification rate is relatively well documented (Rother et al. 1982, Cela, Sumer 2002). In contrast, acknowledging the influence of nitrification inhibitors on the solubility of available forms of mineral elements in soil requires thorough research. The recognition of the effect exerted by N fertilizers containing nitrification inhibitors on the mineral composition of plants is particularly important for obtaining high quality of crops. The mineral composition of consumed plant food has direct influence on human health. For this reason, an objective assessment of the activity of nitrification inhibitors is essential for vegetable production, including carrots processed into baby food, as carrot is one of the main components used in this kind of products.

The influence of nitrogen fertilization on soil environment and mineral composition of plants is also determined by many other factors related with physicochemical properties of soil, e.g. soil type, texture and redox potential, the content of organic matter (GĘBSKI 1998), cation exchange capacity, base saturation ratio as well as soil content of Ca, Mg and heavy metals (SADY, ROŻEK 2002). There are some interesting results of long-term studies on carrot cultivation on soils with differentiated physicochemical properties (SADY et al. 1999, 2000, SADY, ROŻEK 2002, SMOLEŃ, SADY 2006). The lowest rate of heavy metal (cadmium) accumulation in carrot storage roots was noted in the case of plants grown on soils characterized by: $pH_{KCl} > 7.0$, base saturation ratio $V > 98\%$ as well as Ca content exceeding 2,900 mg dm^{-3}. Carrot cultivation on soils with similar physicochemical properties provides storage roots with a relatively low level of heavy metal (Cd and Pb) accumulation, which means they can be used for production of baby food. Such food products must contain significantly less cadmium and lead than those dedicated to adult consumers. Baby food manufacturing plants located in south-eastern Poland use carrots cultivated only on this type of soil, as demonstrated by the unpublished data obtained from direct interviews and long-term field studies conducted in this part of the country (SADY et al. 1999, 2000, SADY, ROŻEK 2002, SMOLEŃ, SADY 2006). The above finding encouraged us to undertake the present study on carrot cultivation on alkaline soil with high calcium content.

The problem of nitrogen fertilization affecting soil environment and mineral composition of plants (defined as plant nutrition and heavy metal accumulation) has been relatively well documented. Nevertheless, previous studies rarely included the effect of N application on changes in soil level of trace elements and their uptake by plants. The importance of numerous trace elements to living organisms is yet to be revealed. Recognition of their levels in plants tissues as well as factors affecting their accumulation can significantly broaden our current scope of knowledge.

The aim of this work has been to evaluate the influence of the application of nitrogen fertilizer ENTEC-26 (containing nitrification inhibitor DMPP), in association with ammonium nitrate, on the mineral composition of carrot storage roots. An attempt has been made to comprehensively assess the effect of the above fertilizers on the content of macro- and microelements, heavy metals and trace elements (including rare earth elements) in carrot roots.

MATERIAL AND METHODS

Plant material and treatments

In 2004-2005, a field experiment involving cv. Kazan F_1 carrot was conducted in Trzciana (50°06’ N, 21°85’ E in south-eastern Poland). Each year carrot plants grew on a different site within the same soil complex. Carrot was cultivated in a three-year crop rotation including sugar beets (1st year), winter wheat (2nd year) and carrot (3rd year). Carrot plants were grown on raised beds, 140 cm wide and 30 cm high, on which seeds were sown in three rows, spaced 30 cm apart, at a rate of 45 seeds m^{-1} (1 million of seeds per hectare). The seeds were sown on 24 April 2004 and 30 April 2005.

The experiment was arranged in a split-plot design. The following combinations with different nitrogen fertilization were distinguished: 1 – control (without nitrogen fertilization), 2 – ENTEC-26 35+35 kg N ha^{-1}, 3 – ENTEC-26 70+70 kg N ha^{-1}, 4 – ENTEC-26 105+105 kg N ha^{-1}, 5 – ammonium nitrate (NH_4NO_3 35+35 kg N ha^{-1}), 6 – ammonium nitrate (NH_4NO_3 70+70 kg N ha^{-1}), 7 – ammonium nitrate (NH_4NO_3 105+105 kg N ha^{-1}), where 35+35, 70+70 and 105+105 denote nitrogen doses applied for pre-plant fertilization and top-dressing, respectively. Solid nitrogen fertilizer was added to soil in the form of ammonium nitrate (Zakłady Azotowe in Puławy, Poland) and ENTEC-26 as COMPO (GmbH & Co. KG, Germany). ENTEC-26 fertilizer contains 26% N (7.5% N-NO_3, 18.5% N-NH_4), 13% S and 0.8% nitrification inhibitor DMPP. Pre-sowing nitrogen fertilization was conducted directly before bed formation, whereas top dressing was carried out at canopy closure. Each experimental treatment was randomized in four replicates on 2.4 m×6 m (14.4 m^2) plots. The total area used for experiment was 403 m^2.

Carrots were harvested on 24 September 2004 and 8 September 2005. At harvest, samples of about 5 kg of carrot storage roots were collected in four replications (i.e. from each plot) for laboratory analyses.

A detailed description of the course of carrot cultivation (together with the climatic conditions) as well as yield and the results of chemical analysis of storage roots and soil (prior to and after carrot cultivation) has been presented in previous works (Smoleń, Sady 2007, 2008 a, 2009a, b, 2011).

Plant analysis

Each year, shredded plant material (carrot storage roots) was dried at 70°C, ground and mineralized in 65% super pure HNO_3 (Merck no. 100443.2500) in a CEM MARS-5 Xpress microwave oven (Pasławski, Migaszewski 2006). Certified material, i.e. peach leaves (CRM 3 1547), was mineralized in the same way. Concentrations of Ag, Al, B, Ba, Ca, Ce, Co, Cr, Dy, Fe, Ga, In, K, La, Li, Lu, Mg, Mn, Na, Ni, P, Pb, S, Sc, Sn, Sr, Ti, Tm, Y, Yb and V in mineralized plant samples were determined using the ICP-OES technique.

Soil analysis

Soils samples from two layers (0-30 cm and 30-60 cm) were collected on two dates – in spring prior to the start of the experiment as well as during carrot harvesting. In spring, eight samples were collected at random from the total area of the experiment. During carrot harvest, soil samples were collected individually for each nitrogen fertilization treatment. Samples were collected from 0-30 cm and 30-60 cm soil layers because the uptake of mineral nutrients by carrot plants occurs in both arable layer (0-30 cm, from which raised beds are formed) and lower soil layers (30-60 cm), a finding reported by Westerveld et al. (2006 a, b).

Before the experiment, the following tests were made to characterize the type of soil: soil texture was analysed using Casagrande method, modified by Pruszyński (Komornicki et al. 1991), organic matter content was assessed by Tiurin method, modified by Oleksynowa (Komornicki et al. 1991), and pH was determined with a potentiometer (1:2 v/v soil in 1 M KCl and in H_2O). The content of macronutrients (N-NH_4, N-NO_3, P, K, Mg, Ca) was determined after soil extraction with 0.03 M acetic acid (Nowosielski 1988). Soil concentration of N-NO_3 and N-NH_4 was assayed using the micro-distilling method (Nowosielski 1988), while K, Mg, Ca were determined by the AAS technique (Ostrowska et al. 1991). The phosphorus level was determined using the vanadium-molybdenum method (Nowosielski 1988). Soil sorption properties were characterized by hydrolytic acidity (Ha) in 1 M $(CH_3COOH)_2Ca$ with pH = 8.2 using Kappen method (Ostrowska et al. 1991), sum of exchangeable basic cations (abbreviated as S, $S = S_{Na}+S_K+S_{Ca}+S_{Mg}$) after soil extraction using 1 M NH_4Cl (Kociałkowski et al. 1984) as well as cation exchange capacity (abbreviated as CEC, CEC = Ha + S) and base saturation ratio V% ($V\% = S \cdot 100 : T$).

Soil samples collected after carrot cultivation were dried in open air, ground and passed through a 1 mm mesh sieve. Subsequently, soil extraction with 1 M HCl was carried out according to Rinkis method (Gorlach et al. 1999). The extracts were assayed for their content of the same elements as carrot roots with the ICP-OES technique. Soil extraction with 1 M HCl is commonly applied for analysis of micronutrients in soil.

Soil samples collected before carrot cultivation were analyzed to determine both physicochemical properties of soil and fertilization requirements of plants for mineral nutrients (N, P, K, Mg and Ca analysis after soil extraction with 0.03 M acetic acid) according to methods commonly applied in vegetable production in Poland, that is mineral analysis based on extraction with 0.03 M acetic acid (Nowosielski 1988).

Mineral composition of soil after carrot cultivation was assayed using the universal extraction solution of 1 M HCl. In the diagnostic practice of soil chemical properties, the preferred methods are the ones which enable researchers to determine available forms of numerous mineral nutrients in a single soil extract (Smoleń et al. 2010a, b). Soil extraction with 1 M HCl was applied in order to evaluate the effect of N fertilization on the content of easily assimilable forms of the tested elements in soil. The aim of this procedure was to determine the geochemical background (concentration of elements in soil) for the influence of nitrogen application on the content of the tested elements in carrot roots.

Previous studies (Smoleń et al. 2010a) revealed the applicability of this method to the assessment of the relation between the soil content of Co, Fe, Mn, Mo and Pb in 0-30 cm, 30-60 cm and 60-90 cm layers and accumulation of these elements in carrot storage roots (on the basis of correlation coefficient values).

Determination of elements in carrot and soil samples

The content of thirty-one elements (Ag, Al, B, Ba, Ca, Ce, Co, Cr, Dy, Fe, Ga, In, K, La, Li, Lu, Mg, Mn, Na, Ni, P, Pb, S, Sc, Sn, Sr, Ti, Tm, Y,

Table 1

Results of determination of elements in certified reference material – peach leaves (CRM 3 1547)

Concentration of elements	(% d.w.)				
	Ca	K	Mg	P	S
Certified value	1.56	2.43	0.432	-	0.2*
Obtained value	1.64	2.27	0.46	0.15	0.18
	(mg kg^{-1} d.w.)				
	Ag	Al	B		
Certified value	-	249	29		
Obtained value	0.39	192.43	27.45		
	Ba	Ce	Co	Cr	
Certified value	124	10*	0.07*	1*	
Obtained value	113.71	11.21	0.08	0.99	
	Dy	Fe	Ga	In	
Certified value	-	220*	-	-	
Obtained value	1.56	191.38	3.66	0.18	
	La	Li	Lu	Mn	Na
Certified value	9*	-	-	98	24
Obtained value	8.99	0.06	0.016	91.26	24.68
	Ni	Pb	Sc	Sr	Sn
Certified value	0.69	0.87	0.04*	53	<0.2*
Obtained value	0.98	0.91	0.022	45.99	0.0035
	Ti	Tm	Y	Yb	V
Certified value	-	-	-	-	0.37
Obtained value	11.00	0.0021	2.68	0.103	0.20

*approximate value (not certified)

Yb and V) in carrot and soil samples was determined with the use of a ICP-OES spectrometer (Prodigy Teledyne Leeman Labs USA). The ICP-OES instrument was calibrated using Merck's ICP multi-element standards nos VI and XVI, Inorganic Ventures ICP single element standards of Ca, K, Mg, Na, P and S as well as standard no. 69 for a group of rare elements. Analysis of the certified material (CRM 3 1547 – peach leaves) was conducted using the same emission and calibration lines as for carrot samples. The results of determination of elements in the certified material are presented in Table 1.

Soil characteristics

In both years, the 0-30 cm and the 30-60 cm soil layers were classified as heavy soils (Table 2). In 2004-2005, all the soil sites had a similar organic matter content in both layers. Soil reaction pH_{KCl} on all sites ranged from 7.15 to 7.35, while pH_{H_2O} was 7.55-7.80. The average content of mineral nitrogen ($N\text{-}NH_4$+$N\text{-}NO_3$) in the 0-60 cm layers in spring (before the experiment) was 273 and 246 kg N ha^{-1} in 2004 and 2005, respectively. In both years, the soil contained comparable levels of phosphorus. The level of easily soluble forms of K, Mg, and Ca in the tested soil layers differed between the two years. In 2005, the soil had a slightly higher potassium content than in 2004. A relatively high concentration of magnesium in soil was observed in 2004, whereas in 2005, the soil had a high calcium content. Cation exchange capacity of the soil remained similar in both years and base saturation ratio (V %) surpassed 90%.

Table 2

Physical and chemical properties of 0-30 and 30-60 cm soil layers prior to the start of experiments in 2004-2005

Characteristics	2004		2005	
	0-30 cm	30-60 cm	0-30 cm	30-60 cm
Soil texture class	silty clay	silty clay	silty clay	silty clay
Organic matter (%)	2.20	1.33	2.26	1.50
pH_{KCl}	7.16	7.15	7.35	7.32
pH_{H_2O}	7.80	7.75	7.55	7.70
$N\text{-}NH_4$ (mg dm^{-3})	12.3	10.5	21.0	7.0
$N\text{-}NO_3$ (mg dm^{-3})	33.3	35.0	33.3	21.0
P (mg dm^{-3})	50.6	50.5	53.1	43.2
K (mg dm^{-3})	35.8	14.7	128.7	64.6
Mg (mg dm^{-3})	81.0	83.2	61.5	63.3
Ca (mg dm^{-3})	6 433.3	6 613.3	7 258.0	7 352.5
Cation exchange capacity (CEC cmol kg^{-1})	14.7	18.0	16.6	18.0
Saturation of the sorption complex with alkaline elements (%)	94.9	93.6	96.9	96.7

Statistical analysis

The results were statistically verified with the ANOVA module of Statistica 8.0 PL at $p<0.05$. The significance of differences was calculated using Duncan's test.

RESULTS

Soil fertilization with nitrogen had a significant influence on the content of S, Fe, Mn, Ni and Sr (Table 3) as well as Co, In, Li, Sc, Y, Yb and V (Table 4) in carrot storage roots. No effect of N nutrition was observed in respect of the concentration of Ca, K, Mg, Na, P, Al, B, Ba, Ga, Ti, Ag, Ce, Cr, Dy, La, Lu, Pb and Sn in carrot (Tables 3 and 4).

In comparison to the control, nitrogen fertilization, both in the form of ENTEC-26 and ammonium nitrate and in all the applied doses, contributed to a comparable increase in the accumulation of S, Mn and Sr in carrot roots. A similar tendency was found for Fe but the highest content of this element was noted in roots of carrot plants fertilized with ENTEC-26 in the dose of 35+35 kg N ha^{-1}.

A significant increase in the accumulation of Co (versus the control) was noted only in carrot fertilized with ENTEC-26 in the dose of 35+35 kg N ha^{-1} (Table 4). It is worth mentioning that fertilization with higher amounts of ENTEC-26 (70+70 as well as 105+105 kg N ha^{-1}) significantly reduced the Co concentration in carrot in comparison to the application of 35+35 kg N ha^{-1}. In the case of ammonium nitrate, storage roots of carrot fertilized with 105+105 kg N ha^{-1} contained significantly more cobalt than plants fertilized with lower doses of this nitrogen source (35+35 as well as 70+70 kg N ha^{-1}). Roots of carrot fertilized with 105+105 kg N ha^{-1} dose of ammonium nitrate accumulated the highest amount of indium while plants grown in the presence of 35+35 kg N ha^{-1} were characterized by the highest concentration of lithium. In comparison to the control, a significant decrease in the content of scandium was noted in plants fertilized with the highest dose of ENTEC-26 (105+105 kg N ha^{-1}). In the case of Sc and Yb, decreased accumulation of the former and increased one of the latter element were observed along with an increasing dose of ENTEC-26. The highest concentrations of Sc, Y and Yb were determined in storage roots of carrot grown in the presence of the lowest level of ammonium nitrate (35+35 kg N ha^{-1}). In comparison to the control, reduced accumulation of yttrium was noted in carrot storage roots fertilized with ENTEC-26 in the dose of 70+70 or 105+105 kg N ha^{-1}. The applied N fertilization, both in the form of ENTEC-26 and ammonium nitrate, contributed to a general decrease in the vanadium content in roots. A significant change in the accumulation of this element was observed after the application of 70+70 and 105+105 kg N ha^{-1}

Table 3

Concentration of Ca, K, Mg, Na, P, S, Al, B, Ba, Fe, Ga, Mn, Ni, Sr and Ti in carrot storage roots depending on nitrogen fertilization – means from 2004-2005

Combinations	(% d.w.)					
	Ca	K	Mg	Na	P	S
Control	0.45	2.61	0.13	0.56	0.42	0.14 *a*
ENTEC-26 35+35 kg N ha^{-1}	0.48	3.14	0.14	0.57	0.47	0.17 *b*
ENTEC-26 70+70 kg N ha^{-1}	0.53	3.23	0.16	0.63	0.51	0.19 *b*
ENTEC-26 105+105 kg N ha^{-1}	0.49	3.06	0.14	0.56	0.47	0.18 *b*
NH_4NO_3 35+35 kg N ha^{-1}	0.51	3.40	0.15	0.69	0.51	0.18 *b*
NH_4NO_3 70+70 kg N ha^{-1}	0.50	3.10	0.15	0.58	0.48	0.16 *ab*
NH_4NO_3 105+105 kg N ha^{-1}	0.52	3.34	0.15	0.60	0.51	0.17 *b*
Test *F* for fertilization	n.s.	n.s.	n.s.	n.s.	n.s.	*
	(mg kg^{-1} d.w.)					
Control	43.74	31.43	19.62	44.59 *a*	1.77	5.88 *a*
ENTEC-26 35+35 kg N ha^{-1}	36.89	34.38	18.41	50.74 *ab*	1.57	7.44 *b*
ENTEC-26 70+70 kg N ha^{-1}	30.45	36.60	17.85	51.97 *ab*	1.59	8.20 *b*
ENTEC-26 105+105 kg N ha^{-1}	31.88	35.24	15.98	48.12 *ab*	1.42	7.32 *b*
NH_4NO_3 35+35 kg N ha^{-1}	47.50	36.04	19.30	63.24 *c*	1.66	7.86 *b*
NH_4NO_3 70+70 kg N ha^{-1}	36.02	34.31	16.36	56.53 *bc*	1.50	7.71 *b*
NH_4NO_3 105+105 kg N ha^{-1}	40.28	36.94	17.58	57.08 *bc*	1.52	8.11 *b*
Test *F* for fertilization	n.s.	n.s.	n.s.	*	n.s.	*
			Ni	Sr	Ti	
Control			2.87 *b*	16.71 *a*	3.06	
ENTEC-26 35+35 kg N ha^{-1}			3.31 *b*	43.12 *b*	2.55	
ENTEC-26 70+70 kg N ha^{-1}			2.31 *ab*	41.89 *b*	3.03	
ENTEC-26 105+105 kg N ha^{-1}			1.06 *a*	45.24 *b*	2.20	
NH_4NO_3 35+35 kg N ha^{-1}			1.11 *a*	46.91 *b*	3.27	
NH_4NO_3 70+70 kg N ha^{-1}			1.11 *a*	45.99 *b*	2.98	
NH_4NO_3 105+105 kg N ha^{-1}			1.18 *a*	43.03 *b*	3.47	
Test *F* for fertilization			*	*	n.s.	

Test *F*: * means are significantly different, n.s. – not significant.
Means followed by the same letters are not significantly different at $p<0.05$.

Table 4

Concentration of Ag, Ce, Co, Cr, Dy, In, La, Li, Lu, Pb, Sc, Sn, Tm, Y, Yb and V in carrot storage roots depending on nitrogen fertilizatio – means from 2004-20055

Combinations	(µg kg-1 d.w.)					
	Ag	Ce	Co	Cr	Dy	In
Control	99.02	991.05	19.35 *ab*	208.39	43.49	185.82 *abc*
ENTEC-26 35+35 kg N ha^{-1}	98.36	1029.99	49.10 *c*	267.70	51.15	137.61 *ab*
ENTEC-26 70+70 kg N ha^{-1}	137.73	1065.73	13.03 *ab*	210.44	45.64	199.89 *ab*
ENTEC-26 105+105 kg N ha^{-1}	104.01	1026.78	13.03 *ab*	191.57	50.62	121.80 *a*
NH_4NO_3 35+35 kg N ha^{-1}	104.02	976.28	5.40 *a*	224.98	56.92	168.68 *abc*
NH_4NO_3 70+70 kg N ha^{-1}	94.54	1002.30	9.71 *a*	189.29	51.13	120.48 *a*
NH_4NO_3 105+105 kg N ha^{-1}	92.81	990.65	23.98 *b*	310.73	50.25	214.85 *c*
Test *F* for fertilization	n.s.	n.s.	*	n.s.	n.s.	*
	La	Li	Lu	Pb	Sc	Sn
Control	109.32	49.45 *a*	1.33	77.64	2.81 *bc*	1.22
ENTEC-26 35+35 kg N ha^{-1}	114.69	58.64 *a*	1.41	76.52	2.66 *bc*	0.89
ENTEC-26 70+70 kg N ha^{-1}	93.32	50.81 *a*	0.65	19.03	1.17 *ab*	0.80
ENTEC-26 105+105 kg N ha^{-1}	105.01	44.79 *a*	1.57	59.10	0.88 *a*	1.43
NH_4NO_3 35+35 kg N ha^{-1}	113.43	75.66 *b*	1.54	70.84	4.16 *c*	1.25
NH_4NO_3 70+70 kg N ha^{-1}	107.71	56.44 *a*	1.26	68.60	1.50 *ab*	0.94
NH_4NO_3 105+105 kg N ha^{-1}	117.25	56.59 *a*	1.60	72.82	2.25 *ab*	0.79
Test *F* for fertilization	n.s.	*	n.s.	n.s.	*	n.s.
		Tm	Y	Yb	V	
Control		0.85	11.75 *c*	2.90 *bc*	93.32 *c*	
ENTEC-26 35+35 kg N ha^{-1}		1.38	9.74 *bc*	1.22 *a*	73.66 *abc*	
ENTEC-26 70+70 kg N ha^{-1}		0.33	5.45 *a*	1.97 *ab*	52.82 *a*	
ENTEC-26 105+105 kg N ha^{-1}		3.77	7.79 *ab*	3.85 *cd*	62.60 *ab*	
NH_4NO_3 35+35 kg N ha^{-1}		7.64	15.27 *d*	5.32 *d*	83.54 *bc*	
NH_4NO_3 70+70 kg N ha^{-1}		<l.d.	10.33 *bc*	2.59 *abc*	58.30 *ab*	
NH_4NO_3 105+105 kg N ha^{-1}		<l.d.	11.99 *c*	3.99 *cd*	77.85 *abc*	
Test *F* for fertilization		-	*	*	*	

Test *F*: * means are significantly different, n.s. – not significant.
Means followed by the same letters are not significantly different at $p<0.05$.

of ENTEC-26 as well as 70+70 kg N ha^{-1} of ammonium nitrate. Nitrogen fertilization had a variable effect on thulium accumulation in carrot. The highest concentration of Tm was found in roots fertilized with ammonium nitrate in the dose of 35+35 kg N ha^{-1}. In carrot roots treated with higher doses of this nitrogen compound (70+70 and 105+105 kg N ha^{-1}), the content of thulium was below its detectability on a ICP-OES spectrometer.

It should be mentioned that nitrogen application resulted in increased accumulation of Cu by carrot storage roots (when compared to the control plants) except the combination with ENTEC-26 35+35 kg N ha^{-1}. More detailed results were presented previously (SMOLEŃ, SADY 2007). This level of nitrogen supply did not affect Cd and Zn concentrations in carrot storage roots (SMOLEŃ, SADY 2007).

In the soil after carrot cultivation, significant variation was observed in concentrations of Ca, K, Mg, S, Al, B, Ba, Ce, Co, Fe, Ga, La, Mn, Ni, Pb, Sr, Ti, Y, V, Ag, Cr, Dy, In, Li, Lu, Sc and Yb, which was related to the applied nitrogen nutrition (in soil layers 0-30 cm and 30-60 cm as well as for means 0-60 cm – Tables 5, 6 and 7). No significant changes were noticed in the soil content of Na, P, Tm (in soil layers 0-30 cm and 30-60 cm as well as for means 0-60 cm) and Sn (in the 0-30 cm layer as well as for means 0-60 cm) in all the tested combinations. In comparison to the control, nitrogen fertilization (regardless of its dose and used compound) induced lower levels of Mg, Al, B, Ba, Ce, Fe, Ga, La, Ni, Pb, Ti, Y, V, Cr, Dy, In, Li, Lu, Sc and Yb as well as higher accumulation of Ca, Sr and Ag in soil (in both soil layers and means 0-60 cm – Tables 5, 6 and 7) after carrot cultivation. It should be underlined that the Ni content in the 30-60 cm layer from the fertilization variant ENTEC-26 105+105 kg N ha^{-1} was comparable to the control. In the case of Sn, a significant influence of N application was noted only in the 30-60 cm soil layer. Nonetheless, the level of Sn in soil fertilized with nitrogen was similar to the control value. The highest content of tin was found in the 30-60 cm layer of soil fertilized with NH_4NO_3 70+70 kg N ha^{-1}.

Along with an increasing dose of ENTEC-26, a significant rise in the soil content of sulphur was found. In the soil supplied with ammonium nitrate (in all the tested doses), the S concentration remained comparable to the control.

In respect of potassium, application of higher N doses in both tested form (ENTEC-26 and ammonium nitrate) resulted in reduced soil accumulation of this element when compared to fertilization with 35+35 kg N ha^{-1}. The lowest level of cobalt in soil was noted due to fertilization with ENTEC-26 and ammonium nitrate in the concentration of 70+70 kg N ha^{-1}. In comparison to the control, a significant decrease in the soil content of manganese was observed for the 35+35 kg N ha^{-1} dose of ENTEC-26 as well as ammonium nitrate in the doses of 70+70 and 105+105 kg N ha^{-1}.

Table 5

Concentrations of Ca, K, Mg, Na, P, S, Al, B, Ba and Ce in soil after carrot cultivation depending on pre-plant and top dressing nitrogen fertilization - means from years 2004-2005

Soil layer	Combinations	(mg kg^{-1} soil)									
		Ca	K	Mg	Na	P	S	Al	B	Ba	Ce
0-30 cm	control	14185.7 *a*	132.2 *d*	866.4 *b*	31.6	390.5	37.2 *a*	964.1 *c*	2.6 *d*	29.5 *c*	9.5 *c*
	ENTEC-26 35+35 kg N ha^{-1}	15 728.8 *bc*	143.9 *e*	673.9 *a*	32.6	374.1	37.2 *a*	697.8 *ab*	2.0 *c*	26.2 *b*	8.3 *b*
	ENTEC-26 70+70 kg N ha^{-1}	16 614.8 *d*	122.1 *b*	674.9 *a*	33.2	344.1	38.1 *a*	676.2 *a*	1.8 *ab*	25.4 *a*	8.2 *ab*
	ENTEC-26 105+105 kg N ha^{-1}	16 262.0 *cd*	105.7 *a*	668.5 *a*	31.6	339.2	70.8 *b*	708.9 *bc*	1.8 *b*	26.1 *b*	8.1 *ab*
	NH_4NO_3 35+35 kg N ha^{-1}	16 693.0 *d*	127.0 *c*	678.6 *a*	32.0	357.4	35.7 *a*	677.1 *a*	1.8 *ab*	25.9 *ab*	8.2 *ab*
	NH_4NO_3 70+70 kg N ha^{-1}	15 636.2 *b*	105.2 *a*	666.9 *a*	33.2	371.9	39.4 *a*	694.5 *ab*	1.7 *a*	25.8 *ab*	8.1 *a*
	NH_4NO_3 105+105 kg N ha^{-1}	16 127.6 *bcd*	124.0 *bc*	678.9 *a*	33.8	349.8	36.8 *a*	690.3 *ab*	1.8 *b*	26.4 *b*	8.3 *b*
	test *F* for fertilization	*	*	*	n.s.	n.s.	*	*	*	*	*
30-60 cm	control	15 312.0 *a*	84.2 *c*	1002.3 *d*	32.8	226.1	29.3 a	1001.6 *c*	1.3 *e*	27.1 *e*	9.0 *e*
	ENTEC-26 35+35 kg N ha^{-1}	18 699.0 *b*	99.3 *d*	756.6 *c*	34.7	232.0	37.0 *c*	705.9 *b*	1.1 *cd*	22.8 *b*	7.8 *bc*
	ENTEC-26 70+70 kg N ha^{-1}	19 780.9 *d*	73.7 *b*	713.0 *a*	35.8	243.7	50.4 *d*	665.9 *a*	0.8 *a*	22.4 *ab*	7.7 *bc*
	ENTEC-26 105+105 kg N ha^{-1}	19 064.2 *bc*	76.6 *b*	733.1 *b*	36.7	254.3	52.1 *d*	713.6 *b*	1.1 *d*	23.8 *c*	7.9 *cd*
	NH_4NO_3 35+35 kg N ha^{-1}	19 314.7 *cd*	75.6 *b*	735.6 *b*	34.4	261.4	31.1 *ab*	726.9 *b*	1.0 *b*	24.5 *d*	8.0 *d*
	NH_4NO_3 70+70 kg N ha^{-1}	18 702.4 *b*	63.4 *a*	710.4 *a*	34.9	263.3	31.6 *ab*	657.8 *a*	0.8 *a*	22.0 *a*	7.5 a
	NH_4NO_3 105+105 kg N ha^{-1}	18 622.6 *b*	72.1 *b*	735.5 *b*	36.8	254.1	27.6 *a*	703.7 *b*	1.0 *bc*	23.6 *c*	7.7 *b*
	test *F* for fertilization	*	*	*	n.s.	*	*	*	*	*	*

Test *F*: * means are significantly different, n.s. – not significant.
Means followed by the same letters are not significantly different at $p<0.05$.

Table 6

Concentrations of Co, Fe, Ga, La, Mn, Ni, Pb, Sr, Ti, Y and V in soil after carrot cultivation depending on pre-plant and top dressing nitrogen fertilization – means from years 2004-2005

Soil layer	Combinations	(mg kg^{-1} soil)										
		Co	Fe	Ga	La	Mn	Ni	Pb	Sr	Ti	Y	V
0-30 cm	control	2.29 *c*	2365.0 c	2.15 *b*	3.91 *c*	276.4 *b*	4.13 c	10.02 c	32.2 *a*	5.79 *d*	4.57 *d*	5.0 *d*
	ENTEC-26 35+35 kg N ha^{-1}	2.15 *b*	1854.0 *a*	1.86 *a*	3.35 *b*	266.8 *a*	3.70 *b*	8.16 *b*	61.1 *c*	4.62 *ab*	3.89 *c*	3.8 *bc*
	ENTEC-26 70+70 kg N ha^{-1}	2.09 *ab*	1922.7 *ab*	1.93 *a*	3.27 *ab*	266.7 *a*	3.57 *a*	7.68 *a*	59.6 *c*	4.76 *bc*	3.81 *ab*	3.7 *a*
	ENTEC-26 105+105 kg N ha^{-1}	2.15 *b*	1934.2 *b*	1.92 *a*	3.27 *ab*	268.5 *ab*	3.71 *b*	8.21 *b*	63.2 *d*	4.80 *c*	3.81 *ab*	3.9 *c*
	NH_4NO_3 35+35 kg N ha^{-1}	2.14 *b*	1891.8 *ab*	1.91 *a*	3.31 *ab*	268.0 *a*	3.64 *ab*	8.02 *ab*	63.0 *d*	4.61 *a*	3.81 *ab*	3.7 *a*
	NH_4NO_3 70+70 kg N ha^{-1}	2.11 *ab*	1890.2 *ab*	1.87 *a*	3.23 *a*	262.2 *a*	3.65 *ab*	8.46 *b*	57.7 *b*	4.66 *abc*	3.76 *a*	3.8 *ab*
	NH_4NO_3 105+105 kg N ha^{-1}	2.15 *b*	1873.3 *ab*	1.91 *a*	3.32 *b*	268.1 *a*	3.73 *b*	8.17 *b*	62.9 *d*	4.61 *a*	3.86 *ab*	3.8 *ab*
	test *F* for fertilization	*	*	*	*	*	*	*	*	*	*	*
30-60 cm	control	2.11 *ab*	2503.7 *e*	2.47 *d*	3.63 *d*	251.6 *ab*	3.86 *d*	6.77 *e*	34.8 *a*	8.69 *d*	4.32 *e*	4.2 *e*
	ENTEC-26 35+35 kg N ha^{-1}	2.11 *ab*	2073.6 *b*	2.09 *ab*	3.09 *b*	248.7 *ab*	3.52 *bc*	5.60 *ab*	64.1 *cd*	6.26 *b*	3.60 *bc*	3.2 *bc*
	ENTEC-26 70+70 kg N ha^{-1}	2.07 *a*	2160.2 *c*	2.12 *ab*	3.03 *b*	253.0 *ab*	3.38 *a*	5.49 *a*	63.2 *c*	6.28 *b*	3.51 *b*	3.1 *ab*
	ENTEC-26 105+105 kg N ha^{-1}	2.23 *c*	2150.4 *c*	2.15 *b*	3.10 *b*	269.3 *c*	3.76 *d*	6.40 *de*	66.9 *e*	6.30 *b*	3.62 *c*	3.4 *d*
	NH_4NO_3 35+35 kg N ha^{-1}	2.14 *b*	2382.5 *d*	2.31 *c*	3.18 *c*	256.9 *bc*	3.56 *c*	5.94 *bc*	71.1 *f*	7.05 *c*	3.72 *d*	3.4 *d*
	NH_4NO_3 70+70 kg N ha^{-1}	2.06 *a*	2080.4 *b*	2.05 *a*	2.94 a	254.0 *ab*	3.44 *ab*	5.63 *ab*	60.5 *b*	6.01 *a*	3.41 *a*	3.0 *a*
	NH_4NO_3 105+105 kg N ha^{-1}	2.07 *a*	1999.2 *a*	2.08 *ab*	3.07 *b*	247.0 a	3.54 *bc*	6.19 *cd*	65.6 *de*	6.30 *b*	3.57 *bc*	3.2 *c*
	test *F* for fertilization	*	*	*	*	*	*	*	*	*	*	*

Test *F*: * means are significantly different, n.s. – not significant.
Means followed by the same letters are not significantly different at $p<0.05$.

Table 7

Concentrations of Ag, Cr, Dy, In, Li, Lu, Sc, Sn, Tm and Yb in soil after carrot cultivation depending on pre-plant and top dressing nitrogen fertilization – means from years 2004-2005

Soil layer	Combinations	(µg kg^{-1} soil)									
		Ag	Cr	Dy	In	Li	Lu	Sc	Sn	Tm	Yb
0-30 cm	control	164.8 *a*	1100.3 *c*	487.5 *d*	379.8 *c*	858.0 *c*	93.5 *c*	39.0 *c*	153.8	39.3	372.3 *b*
	ENTEC-26 35+35 kg N ha^{-1}	188.8 *bc*	925.0 *b*	360.8 *c*	294.3 *abc*	641.0 *a*	77.0 *abc*	29.8 *a*	166.8	35.5	313.0 *a*
	ENTEC-26 70+70 kg N ha^{-1}	190.5 *bc*	897.8 *ab*	324.3 *a*	242.0 *a*	637.3 *a*	76.8 *ab*	32.3 *b*	155.5	37.8	307.3 *a*
	ENTEC-26 105+105 kg N ha^{-1}	189.0 *bc*	907.3 *b*	332.0 *a*	280.3 *ab*	680.0 *b*	77.0 *abc*	30.5 *ab*	177.8	34.8	309.8 *a*
	NH_4NO_3 35+35 kg N ha^{-1}	188.5 *bc*	904.5 *b*	325.0 *a*	299.5 *abc*	638.0 *a*	76.5 *a*	31.5 *ab*	196.0	36.0	307.8 *a*
	NH_4NO_3 70+70 kg N ha^{-1}	180.5 *b*	913.0 *b*	334.8 *a*	313.0 *abc*	658.5 *bc*	76.0 *a*	30.0 *a*	149.3	40.3	305.0 *a*
	NH_4NO_3 105+105 kg N ha^{-1}	194.0 *c*	907.3 *b*	349.0 *b*	291.3 *abc*	642.3 *a*	76.0 *a*	30.8 *ab*	148.0	40.8	312.3 *a*
	test *F* for fertilization	*	*	*	*	*	*	*	n.s.	n.s.	*
30-60 cm	control	176.5 *a*	1100.5 *e*	412.8 *d*	387.5 *c*	1036.8 *e*	94.8 *d*	65.5 *e*	193.5 *ab*	41.8	358.8 *e*
	ENTEC-26 35+35 kg N ha^{-1}	210.0 *bc*	840.5 *ab*	244.8 *c*	293.8 *ab*	768.5 *bcd*	79.3 *ab*	52.0 *b*	96.3 *a*	36.5	296.5 *bc*
	ENTEC-26 70+70 kg N ha^{-1}	218.5 *c*	844.5 *ab*	199.8 *a*	307.5 *ab*	746.0 *ab*	79.5 *bc*	55.3 *c*	129.8 *ab*	38.8	290.0 *b*
	ENTEC-26 105+105 kg N ha^{-1}	214.3 *c*	869.8 *bc*	222.8 *b*	323.3 *ab*	775.8 *cd*	79.5 *b*	48.3 *a*	152.3 *ab*	44.8	298.3 *cd*
	NH_4NO_3 35+35 kg N ha^{-1}	216.3 *c*	908.8 *d*	226.3 *b*	362.0 *abc*	794.0 *d*	86.5 *c*	58.3 *d*	140.8 *ab*	39.0	308.8 *d*
	NH_4NO_3 70+70 kg N ha^{-1}	217.5 *c*	829.8 *a*	196.0 *a*	326.5 *ab*	734.5 *a*	77.3 *ab*	48.5 *a*	373.8 *b*	38.5	282.5 *a*
	NH_4NO_3 105+105 kg N ha^{-1}	201.0 *b*	848.0 *ab*	237.5 *c*	281.0 *a*	761.8 *bc*	76.8 *a*	47.3 *a*	105.8 *a*	39.5	292.3 *bc*
	test *F* for fertilization	*	*	*	*	*	*	*	*	n.s.	*

Test *F*: * means are significantly different, n.s. – not significant.
Means followed by the same letters are not significantly different at $p<0.05$.

DISCUSSION

One of the major factors influencing the mineral uptake by plants is soil pH. Fluctuations in this parameter stimulated by the use of mineral fertilizers can affect the level of macronutrient uptake by plants (Marschner 1995). In this context, it should be underlined that the application of physiologically acid nitrogen fertilizers tested in this experiment did not significantly affect soil pH (data published previously, Smoleń, Sady 2007, 2011). Soil pH after carrot cultivation was above of 7.5 (means from 2004 and 2005 for the 0-30 cm and 30-60 cm of soil layer, respectively) and equalled 1) 7.37-7.78, 2) 7.65-7.85, 3) 7.74-7.79, 4) 7.62-7.67, 5) 7.58-7.70, 6) 7.61-7.73, 7) 7.60-7.69 (Smoleń, Sady 2007). The lack of influence on soil pH by either of the tested, physiologically acid N fertilizer could have been caused by the favorable physicochemical properties of soil, particularly a relatively high soil buffer index and sorption capacity. Therefore, these characteristics of the cultivated soil could have considerably influenced the results of our quantitative analysis of elements in carrot storage roots.

Although the applied N fertilization had no significant influence on soil pH, diverse effects of its dose as well as the applied nitrogen compounds were found in relation to the content of available forms of Ca, K, Mg, S, Al, B, Ba, Ce, Co, Fe, Ga, La, Mn, Ni, Pb, Sr, Ti, Y, V, Ag, Cr, Dy, In, Li, Lu, Sc and Yb in both tested soil layers. The observed variation could have been caused by the interaction between the introduced mineral N forms (($N\text{-}NO_3$, $N\text{-}NH_4$) as well as the nitrification inhibitor and the analysed elements (macro- and microelements, heavy metals and trace elements). In the present study, the changes in the soil content (in both soil layers 0-30 cm and 30-60 cm) of Ca, K, Mg, S, Al, B, Ba, Fe, Ga, Mn, Ni, Ti, Ag, Ce, Co, Cr, Dy, In, La, Li, Lu, Pb, Tm, Y and Yb induced by nitrogen fertilization were not reflected by the level of accumulation of these elements in carrot storage roots. However, there was an exception, namely the differentiated soil level of Sr, Sc and V (related to the application of ENTEC-26 and ammonium nitrate) corresponded with the concentration of these elements in carrot roots. It can be concluded that the content of the tested elements in carrot was more strongly related to the application of N fertilizers affecting the mineral uptake from soil than its interaction with the availability of the tested elements for plants. The fact that there was no direct relationship between the soil content and carrot root accumulation of the above elements could have resulted from the application of a relatively strong soil extractant, i.e. 1 M HCl. The mineral content of soil determined after extraction with strong extractants is usually weakly correlated with the concentration of respective elements in plant material (Westerman 1990). Our previous studies (Smoleń et al. 2010 a) indicated better applicability of 0.03 M acetic acid in comparison to 1 M HCl for the estimation of the relationship (expressed by correlation coefficients) between soil level of Al, B, Ba, Cd, Cr, Cu, Li, Ni, Sr, Ti as well as Zn and its accumulation in carrot roots. The weak effect of N nutrition on the content of available forms of micronutrients and heavy metals in soil has been previously reported by Czekała and Jakubus (2006) (26- year field-trial study carried on medium sands) as well as Łukowski (2006) [three-year experiment conducted on typical brown soil].

Nitrogen fertilization often strengthens the dilution effect on other nutrient elements i.e. reduced plant concentration of macro- and micronutrients as well as trace elements and heavy metals due to increased crop yield (Gębski 1998, Sorensen 1999). Nevertheless, it was also shown that more intense plant growth occurring as a result of N fertilization may enhance the uptake of heavy metals by crops (Giordano, Mortvedt 1976). In the present study, the highest total yield of carrot storage roots was obtained after the application of ENTEC-26 35+35 kg N ha^{-1} – the data shown in the earlier publication by Smoleń, Sady (2008 a). The yield of carrot fertilized with ammonium nitrate in a dose of 70+70 or 105+105 kg N ha^{-1} was lower than noted for the control plants. Thus, it can be stated that the interaction of nitrogen applied in the form of NO_3^- or NH_4^+ as well as SO_4^{2-} (from ENTEC-26) with mineral elements in soil environment (Ag, Al, B, Ba, Ca, Ce, Co, Cr, Dy, Fe, Ga, In, K, La, Li, Lu, Mg, Mn, Na, Ni, P, Pb, Sc, Sn, Sr, Ti, Tm, Y, Yb and V) could have remarkably modified the results obtained during our study. This statement encompasses both potentially antagonistic or synergistic effects between particular elements (during the uptake stage) as well as chemical interactions occurring in soil solution. As a result, different forms (speciations) of tested elements, characterized by various solubility and availability for plants, may have occurred in soil (Uygur, Rimmer 2000, Marcinkonis 2008). In the present study, the aforementioned factors could have significantly influenced the uptake of particular elements by plants and thus affected the level of their accumulation in carrot storage roots.

It is interesting to notice that fertilization with ENTEC-26 (containing sulphur in the form of $S\text{-}SO_4$) had no effect on S content in carrot storage roots in comparison to ammonium nitrate nutrition. Very little sulphur could have been leached by precipitation water to soil layers deeper than 60 cm, a conclusion which is confirmed by the results of soil analysis after carrot cultivation. The results can be explained by a relatively low demand of carrot for sulphur.

The fertilizers used in our experiment, i.e. ENTEC-26 and ammonium nitrate, had variable effect (depending on a nitrogen dose) on the content of Co, Fe, In, Li, Ni, Sc, Y, Yb and V in carrot roots. This variation could have been caused by adverse chemical properties, different solubility in soil (i.e. forming speciations of differential availability for plants) as well as different mobility of particular elements in the soil-plant system. Mineral transport in this system is affected, for example, by physicochemical properties of soil (Tyler, Olsson 2001). At this point, let us cite the results of the study conducted by Tills, Alloway (1981), which suggested that nitrogen applied in the ammonium form lowered the copper concentration in wheat grown on copper-deficient soil when compared with the nitrate source of nitrogen. Increasing concentrations of ammonium ions in solution culture reduced both plant concentration of copper as well as vegetative yield. MARSCHNER, RÖMHELD (1993) stated that accumulation of Fe, Mn, Zn, and Cu in bean plants was higher after fertilization with $N\text{-}NH_4$ (especially when combined with the nitrification inhibitor N-Serve) than application of $N\text{-}NO_3$. Total-N, P, K and Mg concentrations in flue-cured tobacco leaves were non-significantly affected by the form of nitrogen fertilizer forms: 100% $N\text{-}NO_3$, 100% $N\text{-}NH_4$, 50%+50% $N\text{-}NH_4$+$N\text{-}NO_3$ (KARAIVAZOGLOU et al. 2007). In the research conducted by ZACCHEO et al. (2006), 15-day incubation of soil with $(NH_4)_2SO_4$+ +nitrification inhibitor DMPP as well as $Ca(NO_3)_2$ did not significantly affect soil pH when compared to strongly acidic activity of $(NH_4)_2SO_4$ and $(NH_4)_2S_2O_3$. On the other hand, application of $(NH_4)_2SO_4$+DMPP contributed to a significant increase in the content of Cd, Cu, Ni, Pb and Zn in sunflower shoots in comparison to fertilization with $Ca(NO_3)_2$. XIE et al. (2009) revealed that the acidification occurring due to treatments with $N\text{-}NH_4$ and $N\text{-}NH_4$ with the nitrification inhibitor DCD increased the concentrations of extractable Cd and Zn, both of which were largely depleted in the rhizosphere. However, the total uptake of Cd and Zn was the highest in the $N\text{-}NO_3$ treatment, despite the fact that concentrations of extractable Cd and Zn in the rhizosphere were the lowest in this combination.

RODRÍGUEZ-ORTÍZ et al. (2006) showed that application of NH_4NO_3 in doses of 50, 100 and 150 mg N kg^{-1} increased Cd and Pb accumulation in tobacco plants higher than soil fertilization with $CO(NH_2)_2$ in 50 and 100 mg N kg^{-1} doses. In the studies conducted by JURKOWSKA et al. (1981) as well as JURKOWSKA and ROGÓŻ (1981), N fertilization (as ammonium nitrate, calcium nitrate, urea and ammonium sulphate) resulted in increased levels of macronutrients (N, P, S, Cl, K, Na, Ca and Mg) and micronutrients (Fe, Mn, Zn, Cu, Mo and B) taken up by barley and sorrel plants cultivated in a pot experiment. In the case of microelements, JURKOWSKA and ROGÓŻ (1981) observed differences in the influence of the tested fertilizers on the uptake of micronutrients, which were related to the influence of these compounds on soil pH. Application of various nitrogen treatments (including ammonium sulphate, ammonium nitrate and urea) in red cabbage grown on heavy soil (pH_{H_2O} 6.89) significantly affected the levels of Al, Cu, Fe, Mn, Sr, Zn, Cd, Co, Li, Mo Ti and V in cabbage heads (SMOLEŃ, SADY 2008b). Nevertheless, the tested fertilizers did not have a statistically verifiable effect on the concentration of B, Cr, Ni, Pb and Ti in cabbage. In the research conducted by DOMAGAŁA et al. (2009) on ammonium sulphate and RSM (solution of ammonium nitrate + urea), interaction between an N dose with the method of its introduction to soil (placement and broadcast technique application) and concentration of Cd, Cr, Cu, Fe, Mn, Ni, Sr and Zn in white cabbage was dependent on the physicochemical parameters of soil, especially soil reaction

(pH_{H_2O} 7.18-8.21), in each year of the study. In this context, the results of our previous study (Smoleń, Sady 2009c) on pot cultivation of carrot cv. Kazan F_1 fertilized with different types of N compounds: calcium and ammonium nitrate, ammonium sulphate as well as urea, seem interesting. In that experiment, among all the analyzed elements (Al, As, B, Ba, Be, Ca, Co, Cr, Fe, Ga, In, K, Li, Mg, Mn, Mo, Na, Ni, Pb, Sb, Se, Sr, Ti and V), nitrogen fertilization (irrespective of the applied compound) contributed to increased uptake of Mg and Se by carrot plants when compared to the control (cultivated without N addition). Treatment with ammonium sulphate led to reduced concentration of Ba and Mo as well as increased Mn content in carrot roots. As a result of calcium nitrate application, higher accumulation of Sr and Be in carrot was observed. In the present study, different results were found than those obtained in the previously mentioned pot experiment (Smoleń, Sady 2009c) in respect of N fertilization affecting the mineral composition of storage roots of carrot cv. Kazan F_1, including the content of macro- and micronutrients, heavy metals and trace elements. Conditions of plant cultivation (field experiment), with a particular emphasis on different physicochemical properties of soil as well as application of lower nitrogen doses introduced with other fertilizers, can possibly explain such discrepancy.

Other authors (Czekała, Jakubus 2006, Jentschke et al. 1998, Karaivazoglou et al. 2007, Łukowski 2006, Maier et al. 2002, Marschner, Römheld 1993, Rodríguez-Ortíz et al. 2006, Sady, Rożek 2002, Sady et al. 1999, 2000) have rarely included in their work on agronomic factors affecting mineral nutrition the heavy metals and trace elements presented in our study. Each of these elements (Ag, Al, Ba, Ce, Co, Cr, Dy, Ga, In, La, Li, Lu, Ni, Pb, Sc, Sn, Sr, Ti, Tm, Y, Yb and V) is characterized by specific physicochemical properties and mobility in the soil-plant system (Tyler, Olsson 2001). The deleterious effect on human and livestock health of the above elements is not very well recognized, particularly in the context of their intake (introduction to the food chain) due to excessive accumulation in consumed plants. What is better understood is the negative impact of some speciations of these elements on organisms resulting from environmental contamination (Chmielnicka 2002). Thus, it is exceptionally difficult to discuss our results on bioaccumulation of heavy metals and trace elements in carrot in comparison with the data provided by other authors. Those elements were relatively rarely analyzed due to limited access to high-performance analytical devices, which enable determination of chemicals appearing in very small quantities. Out of all the elements classified as heavy metals, the European regulations provide maximum permissible levels in vegetables of just two elements: Cd and Pb (*Commission Regulation*...2001). In the case of lead, the threshold is set at 100 μg Pb kg^{-1} f.w. In the present study, the Pb content in carrot did not exceed the maximum level and remained in the range of 19.03-77.64 μg Pb kg^{-1} d.w. (Table 4), i.e. 2.12-8.66 μg Pb kg^{-1} f.w., which means it was tens of fold lower than the permissible level.

In conclusion, the results presented in this paper provide some basic knowledge on the content of heavy metals and trace elements in carrot storage roots as well as their availability in soil resulting from applied nitrogen fertilization. Because we lack norms on maximum permissible levels of heavy metals and trace elements in vegetables (except Cd and Pb), it is difficult to evaluate the effect of the tested nitrogen application on the biological quality of carrot yield. Our research has been carried out on soil characterized by such physicochemical properties that ensured carrot yield with a relatively low content of cadmium (SADY et al. 1999, 2000, SADY, ROŻEK 2002, SMOLEŃ, SADY 2006), which is particularly important for production of baby food. Thus, it is an interesting finding that the nitrogen fertilization tested in this experiment had no significant influence on the content of numerous, potentially toxic elements in carrot roots, including Ag, Al, Ba, Ce, Cr, Dy, Ga, La, Lu, Pb, Sn and Ti.

CONCLUSIONS

1. Nutrition with nitrogen had significantly affected the content of Co, Fe, In, Li, Mn, Ni, S, Sc, Sr, Y, Yb and V in carrot roots and this effect varied depending on the type of fertilization regime used in the experiment.
2. No significant impact of N fertilization was found in reference to the accumulation of Ag, Al, B, Ba, Ca, Ce, Cr, Dy, Ga, K, La, Lu, Mg, Na, P, Pb, Sn and Ti in carrot roots.
3. In relation to the control, application of all doses of either ENTEC-26 or ammonium nitrate resulted in decreased concentration of Mg, Al, B, Ba, Ce, Fe, Ga, La, Ni, Pb, Ti, Y, V, Cr, Dy, In, Li, Lu, Sc and Yb as well as enhanced accumulation of Ca, Sr and Ag in soil after carrot cultivation. Nonetheless, the revealed changes in the concentration of elements in soil induced by N fertilization were not reflected in the levels of elements detected in carrot storage roots.
4. Comparable doses of nitrogen introduced with ENTEC-26 and ammonium nitrate did not significantly influence soil pH or the relationship between soil and plant concentration of tested elements.
5. The results of the present experiment confirmed relatively low sulphur requirements of carrot plants.

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CHANGES IN THE CONTENT OF ORGANIC CARBON AND AVAILABLE FORMS OF MACRONUTRIENTS IN SOIL UNDER THE INFLUENCE OF SOIL CONTAMINATION WITH FUEL OIL AND APPLICATION OF DIFFERENT SUBSTANCES*

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Abstract

The aim of the study has been to determine the effect of soil contamination with fuel oil (0-20 g kg^{-1} of soil) on the content of organic carbon and available forms of some macronutrients in soil under the influence of different neutralizing substances and crop cultivation. The content of organic carbon as well as available potassium and magnesium in soil was positively correlated with the increasing rates of fuel oil, whereas the fluctuations in available phosphorus did not show any regularity. Among the analyzed elements, the highest increase in the concentration was found for potassium. The content of available macronutrients in soil was correlated with the plants' yield and uptake of elements. The substances introduced to soil in order to alleviate the effect of fuel oil on soil properties caused the weakest changes in the content of organic carbon but affected most profoundly the content of available potassium and magnesium. Compared to the series without soil amending substances, bentonite caused an increase in the average content of available forms of phosphorus, potassium and magnesium, whereas calcium oxide modified the content of available potassium and magnesium. CaO decreased the content of organic carbon and available phosphorus in soil. The effect of the other tested soil amending substances on the analyzed soil properties was weaker than that produced by bentonite or calcium oxide, and the direction of changes in the levels of the elements depended on the type of a substance.

Key words: fuel oil contamination, nitrogen, compost, bentonite, zeolite, calcium oxide, soil, organic carbon, macroelements.

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ZMIANY ZAWARTOŚCI WĘGLA ORGANICZNEGO I PRZYSWAJALNYCH FORM MAKROPIERWIASTKÓW W GLEBIE POD WPŁYWEM ZANIECZYSZCZENIA OLEJEM OPAŁOWYM I APLIKACJI RÓŻNYCH SUBSTANCJI

Abstrakt

Celem badań było określenie wpływu zanieczyszczenia gleby olejem opałowym (0-20 g kg^{-1} gleby) na zawartość węgla organicznego i przyswajalnych form wybranych makropierwiastków w glebie w warunkach aplikacji różnych substancji i uprawy roślin. Zawartości węgla organicznego oraz przyswajalnego potasu i magnezu w glebie były dodatnio skorelowane ze zwiększającymi się dawkami oleju opałowego, zaś w zawartości fosforu przyswajalnego nie wykazano ukierunkowanych zmian.

Największy wzrost zawartości odnotowano w przypadku potasu. Zawartość przyswajalnych makroskładników w glebie była skorelowana z plonem i pobraniem pierwiastków przez rośliny. Substancje zastosowane w celu ograniczenia wpływu oleju opałowego na właściwości gleby wywołały najmniejsze zmiany w zawartości węgla organicznego, a największe w zawartości przyswajalnego potasu i magnezu w glebie. Bentonit spowodował, w porównaniu z serią bez dodatków, zwiększenie średniej zawartości przyswajalnych form fosforu, potasu i magnezu, a tlenek wapnia – zawartości przyswajalnego potasu i magnezu. Tlenek wapnia wpłynął także na zmniejszenie zawartości węgla organicznego i przyswajalnego fosforu w glebie. Wpływ pozostałych substancji na badane właściwości gleby był mniejszy niż bentonitu i tlenku wapnia, a kierunek zmian w zawartości pierwiastków uzależniony od rodzaju zastosowanej substancji.

Słowa kluczowe: zanieczyszczenie olejem opałowym, azot, kompost, bentonit, zeolit, tlenek wapnia, gleba, węgiel organiczny, makroskładniki.

INTRODUCTION

Contamination of soil with petroleum derivatives, including fuel oil, is a growing problem. Globally, over 45 million of tons of crude oil and petroleum end products, i.e. 2% of the annual production, contaminate the natural environment. The loss of petroleum during its production and storage on land reaches 5 million tons a year, while another 8 million is lost during its processing and transport – of that amount, 3 million tons pollute soil. Each year, about 27 million tons of petroleum derivatives are lost, of which 9 million permeate soil (Vinnik, Ovcharov 2004). Once in soil, petroleum derivatives trigger a series of negative changes, for example the morphological and physicochemical soil properties have been observed to undergo very intensive transformations (Zaharov, Zhitin 2008). The content of such macronutrients as carbon, phosphorus, potassium and magnesium in soil depends on a number of factors, including soil contamination with petroleum-based substances. When soil becomes polluted with such substances, the content and availability of the above elements change, which affects their concentrations in plants and the crop weight (Wyszkowski et al. 2004, Wyszkowski, Ziółkowska 2009a,b). The adverse influence of petroleum end products on the quality of crops growing on contaminated land (Ziółkowska, Wyszkowski

2010, Wyszkowska, Wyszkowski 2010) as well as on other live organisms (Ziółkowska, Wyszkowski 2010, Wyszkowska, Wyszkowski 2010) is associated with the fact that such pollutants can be transmitted to subsequent links in trophic chains. Therefore, it is essential to have these contaminants neutralized in the environment in which they occur. There are many methods for the stimulation of degradation of petroleum contaminants, e.g. soil amendment with biogenic substances, aeration, inoculation with active microorganisms or regulation of soil reaction (Rusek 2006).

The above considerations have encouraged the authors to undertake a study whose aim has been to determine the effect of soil contamination with fuel oil on the content of organic carbon and available forms of some macronutrients in soil treated with different neutralizing substances.

MATERIAL AND METHODS

A pot experiment has been conducted in a greenhouse at the University of Warmia and Mazury in Olsztyn (north-eastern Poland). The tested soil had the following characteristics: pH in 1 mol KCl dm^{-3} 4.52, hydrolytic acidity (Hh) 25.4 $mmol(H^+)$ kg^{-1}, total exchange bases (TEB) 29.1 mmol(+) kg^{-1}, cation exchange capacity (CEC) 54.5 mmol(+) kg^{-1}, base saturation (BS) 53%, C_{org} content 11.28 g kg^{-1}, concentrations of available phosphorus 71.88 mg P kg^{-1}, potassium 118.6 mg K kg^{-1} and magnesium 104.18 mg Mg kg^{-1}. Increasing rates of fuel oil were tested, that is 0, 5, 10, 15 and 20 g kg^{-1} d.m. of soil. In order to reduce the effect of fuel oil on soil, the following neutralizing substances were applied: compost 3% (270 g per pot), bentonite and zeolite 2% (180 g per pot), urea (200 mg N kg^{-1} of soil) and 50% calcium oxide applied in a rate equivalent to one full hydrolytic acidity (10.93 g Ca kg^{-1} of soil). The experiment was run in three replications. Additionally, each pot was supplemented with macro- and micronutrients in the following amounts (in mg kg^{-1} of soil): N – 100 [$CO(NH_2)_2$], P – 30 [KH_2PO_4]; K – 100 [KH_2PO_4 + KCl]; Mg – 50 [$MgSO_4 \cdot 7H_2O$]; Mn – 5 [$MnCl_2 \cdot 4H_2O$]; Mo – 5 [$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$]; B – 0.33 [$H_3BO_3$]. To set up the experiment, fuel oil, compost, bentonite and lime as well as the macro- and micronutrients in the form of aqueous solutions were mixed with 9 kg of soil, which was then placed in polyethylene pots. Afterwards, maize (*Zea mays* L.) cv. Reduta was sown. During the experiment, the soil moisture was maintained at the level of 60% of water capillary capacity. Soil samples for analyses were taken while harvesting maize in the intensive stem elongation phase.

The soil samples were dried and passed through a sieve (mesh 1 mm). The following determinations were made: content of organic carbon (C_{org}) with Tiurin method in potassium dichromate with diluted sulphuric acid

(Lityński et al. 1976), the content of available phosphorus and potassium with Egner-Riehm method (Lityński et al. 1976), the content of available magnesium with Schachtschabel method (Lityński et al. 1976). The results were processed statistically by a two-factorial analysis of variance ANOVA using Statistica software package (StatSoft, Inc. 2010).

RESULTS AND DISCUSSION

At present, with the natural environment being exposed to strong man-made pressure, we lack sufficient knowledge about the behaviour of elements, including potassium and magnesium, in soil. Any disturbance in the balance and ratios between particular elements may cause their transfer and modify concentrations in subsequent links of the trophic chain (Sapek 2007).

In the series with no neutralizing substances added to soil, the content of organic carbon in soil rose by 86% (r=0.970) under the influence of increasingly higher rates of fuel oil (Table 1). In all the treatment tested in the present experiment, with or without soil amending substances, the content of organic carbon increased as the rates of fuel oil rose, which can be explained by the structure of petroleum derivatives, which consist of aliphatic (paraffin) hydrocarbons, cycloalkanes (naphthalene oils), arenes and olefins, while light fuel oil in turn contains up to 86% of carbon atoms (Solecki 2005). Similar results on the effect of petroleum compounds (diesel oil and petrol) have been reported by Wyszkowski and Ziółkowska (2009c). In their study, the content of organic carbon in soil rose by 82%. Soil contamination with petroleum end products most strongly affects the content of organic carbon (Obire, Nwaubeta 2002, Caravaca, Roldán 2003, Riffaldi et al. 2006). Vinnik and Ovcharov (2004) found out a rise in soil pH caused by an increasing concentration of petroleum contaminants, while the content of organic carbon was two- to ten-fold higher. In the authors' own experiment, increased contamination of soil with fuel oil caused a considerable increase in the content of organic carbon in soil. Kucharski and Jastrzębska (2005) also noticed that soil contamination with fuel oil most profoundly influences the content of organic carbon, which was positively correlated with increasing rates of the contaminant.

The content of available phosphorus in soil, compared to the other analyzed elements, did not show any regular changes in the treatments with increasing rates of fuel oil (Table 1). The content of available potassium in soil rose as the rates of fuel oil added to soil increased. In the series without neutralizing substances, the content of potassium in the treatments with the highest rate of fuel oil (20 g kg^{-1}) was twice as high as in the control soil, without fuel oil (r=0.975) – Table 1. Magnesium behaved similarly to

Table 1

Content of organic-C and available form of phosphorus (P), potassium (K) and magnesium (Mg) in soil after plants harvest

Dose of fuel oil (g kg^{-1} of soil)	Kind of substance neutralizing effect of fuel oil						
	without additions	nitrogen	compost	bentonite	zeolite	CaO	average
Organic (g kg^{-1})							
0	9.2	9.4	9.7	9.8	8.5	8.3	9.1
5	12.4	11.3	12.6	12.6	12.3	12.4	12.2
10	13.1	12.9	14.3	14.5	14.2	11.8	13.5
15	14.2	14.0	15.0	14.2	15.5	12.4	14.2
20	17.1	15.6	18.5	16.0	16.6	14.2	16.3
Average	13.2	12.6	14.0	13.4	13.4	11.8	13.1
r	0.970	0.996	0.978	0.939	0.966	0.861	0.973
LSD	a – 0.8**, b – 0.9**, a· b – 2.1**						
Phosphorus (mg kg^{-1})							
0	100.0	108.7	108.0	135.7	107.4	83.3	107.2
5	96.0	121.4	94.1	135.6	107.5	70.6	104.2
10	97.2	105.0	97.8	136.5	98.9	78.0	102.2
15	105.3	95.4	100.1	129.3	73.1	79.1	97.1
20	99.9	89.9	96.2	117.9	76.3	81.2	93.6
Average	99.7	104.1	99.2	131.0	92.6	78.4	100.8
r	0.401	-0.822	-0.519	-0.841	-0.910	0.141	-0.990
LSD	a – 3.7 **, b – 4.0 **, a· b – 9.0**						
Potassium (mg kg^{-1})							
0	89.3	131.5	128.2	186.6	103.9	131.5	128.5
5	100.7	176.9	129.9	144.5	131.5	147.7	138.5
10	139.6	173.7	147.7	214.2	136.3	157.4	161.5
15	180.2	172.0	178.5	230.4	157.4	199.6	186.4
20	186.6	160.7	183.4	222.3	154.2	186.6	182.3
Average	139.3	163.0	153.5	199.6	136.7	164.6	159.4
r	0.975	0.454	0.959	0.712	0.933	0.914	0.956
LSD	a – 5.3**, b – 5.8**, a· b – 13.0**						
Magnesium (mg kg^{-1})							
0	118.1	119.1	118.3	164.5	145.4	153.8	136.5
5	121.7	116.9	115.7	174.1	157.2	153.8	139.9
10	129.2	118.9	132.6	178.3	159.2	161.3	146.6
15	132.4	116.9	132.6	184.5	158.8	171.5	149.5
20	135.0	126.3	132.6	186.7	173.3	163.5	152.9
Average	127.3	119.6	126.4	177.6	158.8	160.8	145.1
r	0.983	0.587	0.837	0.977	0.916	0.791	0.990
LSD	a – 4.4**, b – 4.8**, a· b – 10.8**						

LSD for: *a* – fuel oil dose, *b* – kind of neutralizing substance;
significant for: **p=0.01; *r* – correlation coefficient

organic carbon and potassium in that its concentration in soil increased directly proportionally to the increase in the degree of soil contamination with fuel oil. The maximum increase was 14% (r=0.938) in the pot polluted with 20 g of fuel oil per kg of soil, compared to the control soil. The content of available macroelements in soil was correlated with the yield and uptake of elements by tested plants (Wyszkowski, Sivitskaya 2011). The yield and uptake of the analyzed macroelements by plants in uncontaminated pots were higher than in the ones contaminated with fuel oil. A reverse relationship was observed in the content of available macroelements in soil.

Wyszkowski and Ziółkowska (2008) noticed that the content of phosphorus decreased as the rates of diesel oil added to soil rose. A depressed content of phosphorus and an increased content of magnesium in soil exposed to petroleum hydrocarbons have also been reported by Obire and Nwaubeta (2002). In the experiments conducted by Dimitrow and Markow (2000), or Wyszkowski and Ziółkowska (2009c), a lower content of phosphorus and elevated levels of magnesium and potassium appeared in soil contaminated with diesel oil, which - in terms of its properties – is similar to fuel oil. Such dependences have been confirmed in the present experiment.

Our comparison of the effects produced by all the tested neutralizing substances have demonstrated certain differences (Figures 1-2), which correlated with yields (Wyszkowski, Sivitskaya 2011). The strongest effect was produced by bentonite and calcium oxide. In soil polluted with fuel oil (5 to 20 g kg^{-1}), bentonite contributed to a large increase in the average content of available forms of phosphorus, potassium and magnesium, by 30, 34 and 40% respectively, in comparison to the series without soil amending substances. Calcium oxide raised the average content of available potassium by 14% and magnesium by 25%, but lowered the content of organic carbon by an average 11% and that of available phosphorus by 23%. Zeolite favoured the accumulation of magnesium (+25%) in soil contaminated with fuel oil, in contrast to potassium (-5%) and phosphorus (-11%). Compost and nitrogen

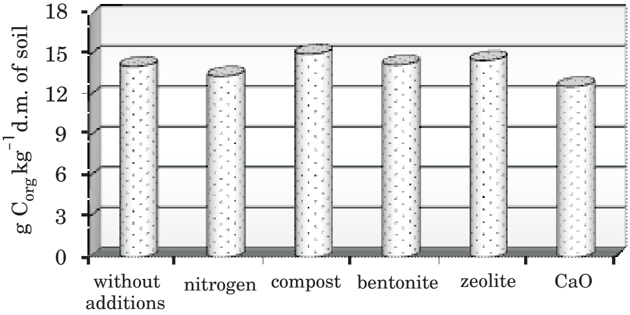

Fig. 1. Content of organic-C in soil after plant harvest
(average from series contaminated with 5-20 g fuel oil per kg of soil)

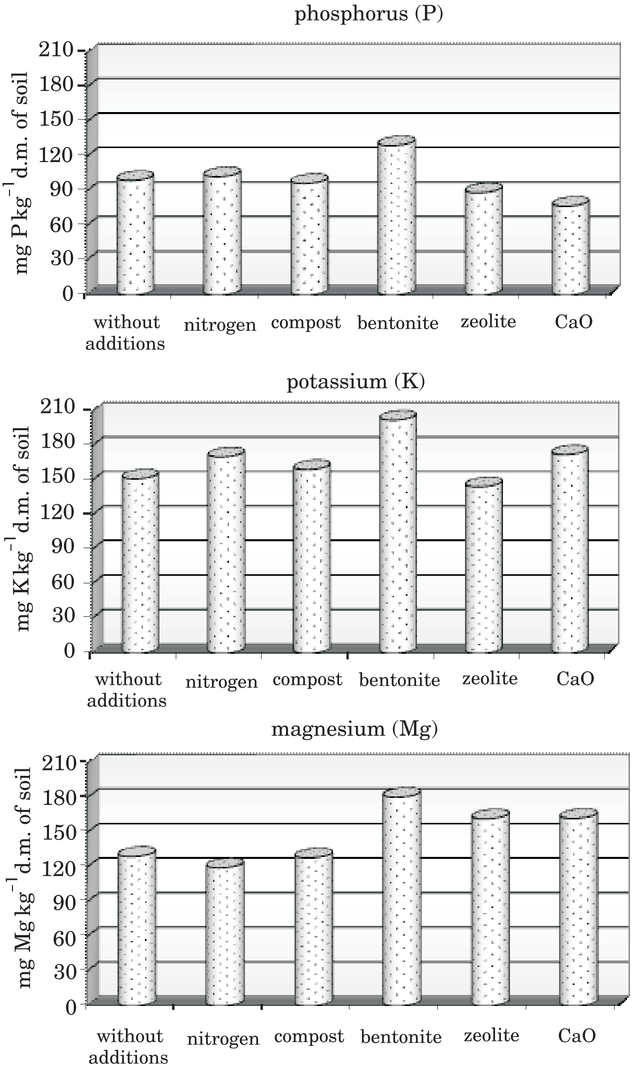

Fig. 2. Content of available forms of phosphorus, potassium and magnesium in soil after plant harvest (average from series contaminated with 5-20 g fuel oil per kg of soil)

produced much weaker effects. In the compost amended soil, a slight increase in the mean content of organic carbon and available potassium was noticed when compared to the series without any neutralizing substances. In turn, nitrogen caused a small decrease in the content of organic carbon and available magnesium but an increase in the content of potassium in soil.

The results obtained in the present experiment have been confirmed by studies reported by other authors (Kwiatkowska, Maciejewska 2008, Wyszkowski, Ziółkowska 2008, 2009c). Organic substance present in soil plays a very important role as it determines the quality of soil environment. The content of organic substance in soil can be raised by application of various substances, e.g. composts (Riffaldi et al. 2006, Quintern et al. 2006). In another experiment run by Wyszkowski and Ziółkowska (2008, 2009c), the biggest changes in soil properties were caused by bentonite and compost, while in the present study such modifications were attributed to bentonite and calcium oxide. In the study by Wyszkowski and Ziółkowska (2009c), application of compost to soil had a positive effect on the content of organic carbon and available potassium in soil, causing a two-fold and 24% increase in their concentrations, respectively. In another trial conducted by the same authors (Wyszkowski, Ziółkowska 2008), application of compost to soil did not have any significant effect on the content of magnesium but raised the content of phosphorus in soil contaminated with diesel oil. Kwiatkowska and Maciejewska (2008) also demonstrated that enrichment of soil with organic substance caused an increase in the content of macronutrients in soil, and lignite was a better source of organic substance in soil than peat or manure.

Wyszkowski and Ziółkowska (2008, 2009c) did not find evidence suggesting that bentonite had any significant effect on the content of organic carbon but proved that it significantly raised the content of potassium and magnesium, analogously to the results provided by the present experiment. Depending on a tested plant, bentonite either had no effect on the content of phosphorus (Wyszkowski, Ziółkowska 2009c) or caused an increase in the content of this element in soil (Wyszkowski, Ziółkowska 2008).

Soil liming improves soil porosity, stability and other physicochemical properties; it also creates a more suitable environment for soil microorganisms (Kucharski, Jastrzębska 2006), which may have a favourable influence on the content of available elements in soil. In the study conducted by Gruba (2009), increased solubility of organic matter was found out in soils of pH less than 4-4.5, and two elements, i.e. aluminium and iron, which are capable of saturating function groups (mainly COOH), played the most important role in dissolving and precipitating organic matter. In the experiment conducted by Wyszkowski and Ziółkowska (2008, 2009c), calcium oxide had a favourable effect on the content of phosphorus, potassium (Wyszkowski, Ziółkowska 2008) and magnesium in soil (Wyszkowski, Ziółkowska 2009c).

Mercik et al. (1995) observed that an increase in the content of organic carbon in soil was the smallest when mineral nitrogen had been applied, bigger after application of farmyard manure and the biggest – when both fertilizers had been introduced to soil simultaneously and leguminous plants had been sown.

CONCLUSIONS

1. The content of organic carbon and available potassium and magnesium in soil was positively correlated with the increasing rates of fuel oil, whereas changes in the content of available phosphorus did not show any regularity. The highest increase in the concentration of the analyzed soil components was found for potassium.

2. The substances introduced to soil in order to neutralize the effect of fuel oil on soil properties caused the weakest changes in the content of organic carbon but affected most profoundly the content of available potassium and magnesium.

3. Compared to the series without soil amending substances, bentonite caused an increase in the average content of available forms of phorphorus, potassium and magnesium, whereas calcium oxide modified the content of available potassium and magnesium. CaO decreased the content of organic carbon and available phosphorus in soil.

4. The effect of the other tested soil amending substances on the analyzed soil properties was weaker than that produced by bentonite or calcium oxide, and the direction of changes in the levels of the elements depended on the type of a substance.

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REVIEW PAPER

HEALTH-SUPPORTING PROPERTIES OF BEEF

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Abstract

Contemporary human diet contains excessive quantities of *n*-6 saturated fatty acids (SFA) and polyunsaturated fatty acids (PUFA), but it is deficient in *n*-3 fatty acids. This imbalance could be the cause of respiratory diseases, obesity and cancer. Until recently, animal fat, including meat fat, was regarded solely as a source of saturated fatty acids, which are a risk factor for atherosclerosis, obesity and certain types of cancer. Recent studies have shown, however, that animal fats contain biologically active substances beneficial for health, and that only some saturated fatty acids have adverse consequences. The latter group includes lauric acid (C12), myristic acid (C14) and palmitic acid (C16), i.e. mainly the ones with an unfavorable *n*-6/*n*-3 fatty acid ratio. Apart from fat, beef also contains high amounts of minerals, mainly phosphorus, potassium, magnesium and zinc.

Conjugated linoleic acid (CLA), in particular its cis-9 trans-11 and trans-9 cis-11 isomers, is one of the substances with health-supporting properties. Synthesized solely in the gastrointestinal tract of ruminants, CLA reaches the blood stream and is used by the mammary gland to synthesize milk fat. CLA is embedded in the animal's adipose tissue. There is a large body of research confirming CLA's ability to reduce the risk of atherosclerosis and obesity, and to lower cholesterol levels. When incorporated into the human diet in the amount of 1.5 to 3.5 g, CLA exerts anticarcinogenic effects (it inhibits the development of breast cancer, malignant melanoma, colorectal cancer and lung cancer). CLA prevents

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and alleviates the symptoms of type 2 diabetes; it is a powerful antioxidant and it boosts immunity. The highest quantities of CLA have been noted in the meat of ruminants. The CLA content of ruminant meat has been determined in the range of 10 to 33 mg 100 g^{-1} of fat, being much higher than in pork (2-19 mg) and poultry (3.4 mg). Beef also has the most desirable *n*-6/*n*-3 fatty acid ratio at 6.3:1 in comparison with pork (12.7:1) and poultry (8.3:1). The highest levels of *n*-3 fatty acids can also be found in beef (5-6 g in 100 g fat).

The objective of this study was to present and discuss the most recent findings concerning the fatty acid content of beef and its implications for human health. Source data were gathered and grouped thematically, and an attempt was made to characterize beef and its fatty acid profile.

Key words: fatty acids, CLA, minerals, health-promoting properties of beef.

PROZDROWOTNE WŁAŚCIWOŚCI WOŁOWINY

Abstrakt

Dieta współczesnego człowieka zawiera zbyt dużo kwasów nasyconych (SFA–saturated fatty acid) i wielonienasyconych (PUFA–polly unsaturated fatty acid) typu *n*-6, a zbyt mało typu *n*-3, co jest przyczyną m.in. chorób związanych z układem krążenia, otyłością, a także chorobą nowotworową. Do niedawna tłuszcz zwierzęcy, w tym tłuszcz mięsa, był postrzegany jedynie jako źródło niekorzystnych dla człowieka nasyconych kwasów tłuszczowych powodujących miażdżycę, otyłość i niektóre nowotwory. Tymczasem, jak wykazują badania ostatnich lat, tłuszcze zwierzęce zawierają substancje biologicznie czynne o charakterze prozdrowotnym, a niebezpieczne dla zdrowia człowieka są jedynie niektóre kwasy nasycone (laurynowy C12), mirystynowy (C14) oraz palmitynowy C16), a głównie te, które występują w niewłaściwej proporcji, rodziny kwasów *n*-6 do *n*-3. Mięso wołowe zawiera także znaczne ilości składników mineralnych, zwłaszcza fosforu, potasu, magnezu oraz cynku.

Celem pracy było zebranie najnowszej literatury dotyczącej wpływu kwasów tłuszczowych znajdujących się w mięsie wołowym na zdrowie ludzi w aspekcie jej prozdrowotnych właściwości. W tym celu zgromadzono i pogrupowano tematycznie dostępną na ten temat literaturę oraz dokonano próby opisu charakteryzującego mięso wołowe i zawartych w nim kwasów tłuszczowych.

Jedną z substancji o działaniu prozdrowotnym jest sprzężony kwas linolowy (CLA–conjugated linoleic acid), powstający wyłącznie w przewodzie pokarmowym przeżuwaczy (stąd trafia do krwi, a dalej wykorzystywany jest przez gruczoł mleczny do syntezy tłuszczu mleka oraz wbudowany jest w tkankę tłuszczową zwierzęcia), a szczególnie jego izomery cis-9 trans-11 i trans-9 cis-11. Wiele badań potwierdza jego działanie antymiażdżycowe i przeciwdziałające otyłości, a także ograniczające zawartość cholesterolu. Zawartość CLA od 1,5 do 3,5 g w diecie człowieka ma działanie antynowotworowe (hamuje rozwój raka sutka, czerniaka złośliwego, raka jelita grubego oraz raka płuc). Ponadto CLA jest czynnikiem zapobiegającym i łagodzącym objawy cukrzycy insulinozależnej II typu, ma działanie antyoksydacyjne jako silny przeciwutleniacz, a także wykazuje dodatni wpływ na funkcje układu odpornościowego.

Najwięcej kwasu CLA zawiera mięso zwierząt przeżuwających – od 10 do 33 mg CLA, mięso wieprzowe 2-19 mg CLA, a drobiowe 3,4 mg CLA w 100 g tłuszczu. Mięso wołowe ma również najkorzystniejszy stosunek kwasów *n*-6 do *n*-3, który wynosi 6,3:1, podczas gdy w mięsie wieprzowym 12,7:1, a drobiowym 8,3:1. W mięsie wołowym stwierdzono również najwyższą zawartość kwasów z grupy *n*-3 (od 5 do 6 g w 100 g tłuszczu).

Słowa kluczowe: kwasy tłuszczowe, CLA, składniki mineralne, prozdrowotne właściwości wołowiny.

INTRODUCTION

Beef is a highly valued type of meat around the world, and it ranks second to pork in the global meat consumption structure. The highest beef consumption is noted in developed countries whose inhabitants show a clear preference for beef owing to its high nutritive value and sensory properties. Beef is tender, juicy and characterized by a bright red color and delicate flavor. Beef protein constitutes muscle building material of high biological value, and its flavor attributes are determined by fat content (Barowicz 2007). The availability of beef protein for consumers varies from 70 to 100%, subject to the content of connective tissue. Beef has moderate calorific value and is leaner than mutton and pork. It is a rich source of minerals, in particular phosphorus, potassium, magnesium and zinc (Figure 1). Phosphorus is the key bone-building material, potassium regulates water use in the body, magnesium enhances heart function and zinc improves immunity and contributes to healthy skin (Kowalski et al. 2010). Beef has the highest zinc content among all types of meat. Determined at 3.8 mg/100, its zinc content is two-fold higher than in pork and veal, and 5- to 7-fold higher than in poultry (Barowicz, Brejta 2000, Barowicz 2007).

Beef is also the main source of vitamins B1, B2, B6 (50%) and iron (35%) in human nutrition. The highest levels of vitamin B2 are observed in veal at 0.3 mg 100 g^{-1}, whereas beef contains only insignificantly smaller amounts of this nutrient at 0.2 mg 100 g^{-1}. Beef contains 1.4 mg 100 g^{-1} of vitamin B12, 0.1 mg 100 g^{-1} of vitamin B1 (essential for carbohydrate metabolism and normal function of the nervous system). The highest iron concentrations are noted in beef and veal (around 2.5 mg 100 g^{-1}), and iron levels are 2.5-fold lower in pork and 5-fold lower in poultry. Iron plays a vital role in the human body. Iron deficiency leads to anemia, fatigue, poor concentration, impaired physical and mental activity, and higher susceptibility to infection (Kowalski et al. 2010, Zwierzchowski et al. 2011). Animal products contain easily available heme iron, whereas plants are a source of non-heme iron, which is less readily absorbed by the body. Figure 1 indicates the relevant quantities of other food products that deliver the same amounts of zinc, iron and B vitamins as 100 g of beef (Department of Agriculture 2002).

Unlike pork, beef is not intended solely for processing, and it often consumed in raw (steak tartare) or semi-raw form (rare steak). For this reason, beef cattle have to be healthy, free of parasites and adequately fed during fattening. Nutritional regime is an environmental factor that affects the quality of beef. Fattening intensity could influence beef carcass fatness. Daily gains should fall in the range of 900-1000 g to guarantee a high content of lean meat in the carcass. The meat of young bulls weighing 475--500 kg, fed silage or grass haylage, is lighter in color than the meat of animals whose diets are composed mainly of concentrate. Age at slaughter is also an important factor that affects the quality and cooking suitability

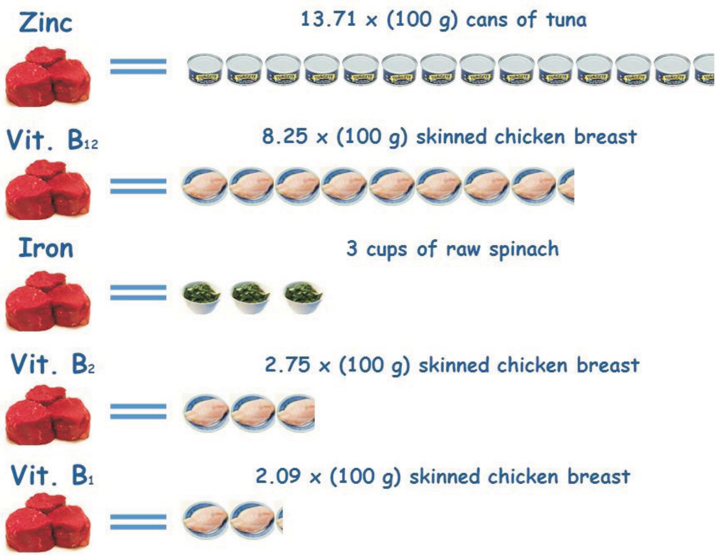

Fig. 1. Zinc, iron and B vitamin concentrations in other food products. (*Agricultural Research Service* 2002).

of beef. Aging leads to higher muscle mass and a decline in meat tenderness (due to an increased diameter of muscle fibers), decreased marbling and lower water-holding capacity. For this reason, attempts are made to lower slaughter age and intensify the fattening regime to increase the final body weights of animals.

OBJECTIVE OF THE STUDY

The objective of this study was to discuss the health benefits of beef for consumers, in particular the role of beef fatty acids in the prevention of atherosclerosis, cancer, heart disease and diabetes.

MATERIALS AND METHODS

This paper overviews the most recent research findings concerning the health-promoting properties of beef. Source materials were gathered and grouped thematically to describe the characteristics of beef and its fatty acid profile.

DISCUSSION

Until recently, animal fat, including meat fat, was regarded solely as a source of saturated fatty acids which are a risk factor for atherosclerosis, obesity and certain types of cancer. Recent studies indicate, however, that animal fats contain biologically active substances that deliver health benefits (STRZETELSKI, STASINIEWICZ 1999, STRZETELSKI et al. 2003), and that adverse health consequences are noted only in respect of some saturated fatty acids (KHOSLA, SUNDRUM 1996) as well as polyunsaturated fatty acids (PUFA) with an unfavorable *n*-6/*n*-3 fatty acid ratio. Beef has a relatively high content of saturated fatty acids and lower levels of unsaturated fatty acids. Recent research indicates, however, that in the group of saturated fatty acids, only lauric acid (C12), myristic acid (C14) and, to a smaller extent, palmitic acid (C16) contribute to the risk of respiratory disease.

Contemporary human diet contains excessive quantities of *n*-6 saturated fatty acids (SFA) and polyunsaturated fatty acids (PUFA), but is deficient in *n*-3 fatty acids. This imbalance could be a cause of respiratory diseases, obesity and cancer (SKRZYPEK 1999). According to LABORDE et al. (2001), every 1000 kcal consumed should deliver a maximum of 11 g SFA, a minimum of 3300 mg *n*-6 PUFA and a minimum of 500 mg *n*-3 PUFA. According to contemporary nutritional standards, a healthy *n*-6/*n*-3 PUFA ratio is 2-4 to 1, whereas in developed countries, the actual ratio is 10-14 to 1 (SIMOPOULOS et al. 1994a, SIMOPOULOS et al. 1994b, SIMOPOULOS 2001).

Table 1

Average fat and cholesterol content of beef cuts
(HONIKEL ARNETH 1996)

Cut	Fat content g)	Cholesterol content (mg)
Loin end	2.6	49.0
Sirloin	2.7	51.0
Loin	6.3	48.5
Best ribs	11.3	48.0
Ribs	13.9	66.5

Conjugated linoleic acid (CLA), in particular its cis-9 trans-11 and trans-9 cis-11 isomers, is one of the substances with health-supporting properties (STANTON et al. 1997). Synthesized solely in the gastrointestinal tract of ruminants, CLA reaches the blood stream and is used by the mammary gland to synthesize milk fat. CLA is embedded in the animal's adipose tissue. There is a large body of research confirming CLA's ability to reduce the risk of atherosclerosis and obesity and to lower cholesterol levels (STANTON et al. 1997, PARODI 1999, FRITSCHE, STEINHARD 1997). As demonstrated by LABORDE et al. (2001), when incorporated into the human diet in the amount of 1.5 to

3.5 g, CLA exerts anticarcinogenic effects (it inhibits the development of breast cancer, malignant melanoma, colorectal cancer and lung cancer). According to Ip et al. (2001). CLA has the above properties only when consumed in quantities higher than 3.5 g. CLA prevents and alleviates the symptoms of type 2 diabetes, is a powerful antioxidant and boosts immunity.

The highest quantities of CLA have been noted in milk and fat of ruminants. The CLA content of ruminant meat has been determined in the range of 10 to 33 mg 100 g^{-1} of fat, and it is much higher than in pork (2-19 mg) and poultry (3.4 mg) (Fogerty 1988). Beef also has the most desirable *n*-6/*n*-3 fatty acid ratio at 6.3:1 in comparison with pork (12.7:1) and poultry (8.3:1).

Table 2

Composition of the fatty acid pool (%) in the fat of slaughter animals
(Bartnikowska et al. 2002)

Fatty acid	Pork	Beef	Poultry
Saturated (SFA)	37.0-50.6	42.6-57.6	25.2-33.9
Monounsaturated (MUFA)	41.1-51.6	39.5-51.8	43.7-52.
Polyunsaturated (PUFA)	6.6-14.2	2.6-5.5	17.5-26.0
C12:0 + C14 :0	1.2-14.2	2.0-3.7	1.1-2.1
C16:0	23.0-27.0	22.5-26.0	18.0-24.0

Table 3

The effect of selected factors on SFA, PUFA and CLA concentrations in beef

Specification	Fatty acid (% total acids)						Authors
	SFA		PUFA			CLA	
	$C_{16:0}$	$C_{18:0}$	*n*-3	*n*-6	*n*-6/*n*-3		
Breed							
Hereford	20.1	-	3.27	12.67	3.96	0.32	Dymnicka et al. 2004
Charolaise	20.9	-	3.96	16.03	4.06	0.26	
Limousine	20.7	-	2.90	15.23	5.45	0.26	
Feed type							
Maize silage + concentrate	20.7	-	2.96	13.81	4.77	0.30	Dymnicka et al. 2004
Grass haylage + concentrate	20.3	-	3.50	15.81	4.66	0.24	
Green forage	24.3	15.8	-	-	1.32	-	Enser et al. 1998
Concentrate (ground barley, soybean meal + ground barley)	23.2	13.7	-	-	9.20	-	
Meadow grass	22.8	14.7	1.36	3.14	2.33	1.08	French et al. 2000
Concentrate 8 kg + hay 1 kg)	27.4	15.9	0.84	3.21	4.15	0.37	
Grass haylage + concentrate 4 kg	26.6	16.0	0.91	2.96	3.61	0.47	
Green forage	21.7	17.1	2.37	3.46	1.46	0.78	
Grass haylage + concentrate 3 kg	24.1	16.9	1.59	3.50	2.21	0.53	Noci et al. 2005

The highest levels of n-3 fatty acids can also be found in beef (5-6 g in 100 g fat) – SKRZYPEK 1999.

The fatty acid content of meat fat is affected by breed (SIEBERT et al. 1998, ZEMBYASHI et al. 1999, KAZALA et al. 1999, LABORDE et al. 2001), nutrition (DI LUCCIA et al. 2003) and, as demonstrated by RULE (1994), fat type (subcutaneous, kidney and intramuscular fat). DI LUCIA et al. (2003) have observed differences in the concentrations of SFA and UFA subject to the analyzed muscles.

CONCLUSIONS

In comparison with other types of meat, beef is a source of highly digestible proteins with a desirable amino acid composition. Beef contains more B vitamins, minerals with antioxidant properties (Fe, Zn, Cu, Mg, Se) and biologically active substances that deliver health benefits. Beef fat is a source of unsaturated fatty acids and conjugated linoleic acid that prevent and inhibit the development of various diseases. The use of green forage in the dietary regime of ruminants increases the share of *n*-3 PUFA in beef fat, guarantees a healthier *n*-6/*n*-3 PUFA ratio and increases the concentrations of non-enzymatic antioxidants: vitamin E, ascorbic acid and beta-carotene. The incorporation of oilseed plants, plant fats and green forage in cattle's diets is believed to be the most effective method of increasing the concentrations of desirable fatty acids in beef.

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