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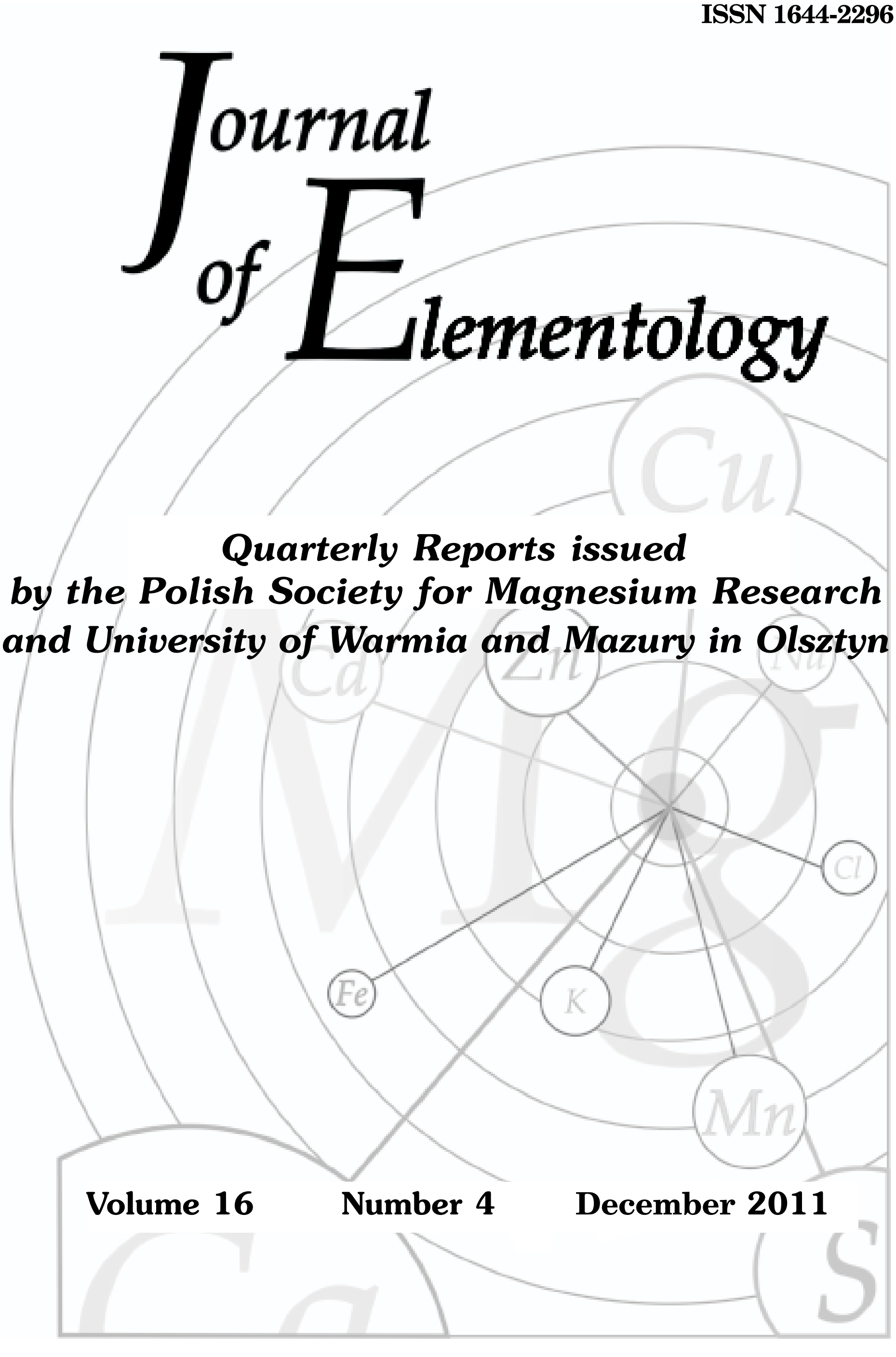

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Professor Kazimierz Pasternak – Remembering a Friend

On 5th September 2011, we lost a dear friend. Professor Kazimierz Pasternak, PhD, who for many years had been the Deputy Chairman of the Polish Society for Magnesium Research, Chairman of the Lublin Branch of the Society, and Head of the Chair and Institute of Medical Chemistry at the Medical University of Lublin, passed away. He died after barely sixty years of a hard-working life. Death of any person evokes sorrow, pain and nostalgia. But when a close friend dies, the sorrow intensifies, the pain grows and the feeling of nostalgia becomes so overwhelming that we begin to rebel against such a premature and unexpected death, which has deprived us of someone so close, beloved and respectful.

To many, Professor Kazimierz Pasternak was, and will remain, a true friend, someone who endeared himself to us with his good nature, friendly manner and constant willingness to help others. His assistance not only led to numerous research projects and publications, but also - and perhaps more importantly – inspired others to preserve and continue work All Professor Pasternak's assistants and students as well as everyone who has had the honour of collaborating with him can testify to this.

Professor Pasternak was not only an outstanding chemist but also an eminent physician, who understood our human needs. While reviewing his impressive scientific attainments, we notice than many of his articles and conference reports were produced in collaboration with clinical doctors representing various branches of medicine. Internists, paediatricians, surgeons and gynaecologists owe him a debt of gratitude, but the professor was also familiar with issues related to toxicology and immunology. Being fully aware of the importance of chemistry in medicine, Professor Pasternak introduced elements of this science into clinical medicine. He devoted most of his attention to both beneficial and adverse effects of various elements on the human organism. Magnesium was certainly his favourite element. He also investigated the role of free radicals and biogenic amines, metabolism of amino acids and behaviour of enzymes in healthy and ill patients.

Professor Kazimierz Pasternak will remain in our memory as an excellent leader, full of creative passion. He proved his leadership skills as the Head of the Chair of Medical Chemistry, Deputy Dean of the Faulty of Nursing and Health Science, Chairman of the Society of Friends of the University of Medicine in Lublin but - above all - as the main founder, deputy chairman and chairman of the Lublin Branch of the Polish Society of Magnesium Research. He was truly the moving spirit behind all initiatives of the society.

Professor Pasternak understood young researchers and created the best possible environment for their development. He supervised nearly twenty doctoral dissertations and many more master theses. And he gave his students an up-to-date and comprehensible chemistry textbook.

Professor Pasternak worked rationally, passionately and cheerfully. When Kazio – as his close friends called him – smiled, he made others smile, and then any obstacle proved surmountable. Always active and full of energy, he wanted to work and knew how to do so. He continued to be busy working and making plans for the future until the very last days. Suddenly, prematurely and unjustly, his beautiful and fruitful life came to an end.

With the death of Professor Kazimierz Pasternak, the Polish Society of Magnesium Research lost a distinguished Deputy Chairman and a devoted researcher, for whom the Society and its affairs were the aim and essence of a professional career. Professor Pasternak organised and participated in many conventions, conferences and symposia, where he earned the respect and friendship of other participants.

On 15 September this year, we paid our last respects to Professor Kazimierz Pasternak, at the cemetery in Lipowa Street, in Lublin. His grieving family were consoled by the Professor's friends, colleagues, students and acquaintances, many with tears in their eyes ...

We shall remember his as a good and warm person, and we shall never forget him. One must never forget a true friend!

Andrzej Papierkowski

Profesor Kazimierz Pasternak – wspomnienie o Przyjacielu

W dniu 5 września 2011 roku zmarł Profesor dr hab. Kazimierz Pasternak, wieloletni wiceprezes Polskiego Towarzystwa Magnezologicznego, prezes Oddziału Lubelskiego, kierownik Katedry i Zakładu Chemii Medycznej Uniwersytetu Medycznego w Lublinie. Odszedł w szóstej dekadzie pracowitego życia. Śmierć człowieka przynosi z sobą smutek, cierpienie i tęsknotę. Gdy umiera przyjaciel, potęguje się smutek, narasta cierpienie i pogłębia tęsknota, a wraz z nimi pojawia się uczucie protestu przeciwko przedwczesnej, niespodziewanej śmierci, zabierającej spośród nas kogoś bardzo nam bliskiego, kochanego i szanowanego.

Profesor Kazimierz Pasternak był i pozostanie przyjacielem wielu ludzi, których zjednywał sobie dobrocią, życzliwością i stałą gotowością do niesienia pomocy, owocującej nie tylko licznymi prowadzonymi wraz z Nim badaniami i ich publikacją, lecz także – a może przede wszystkim – inspirowaną przez Niego zachętą do dalszej twórczej pracy.

Wiedzą o tym Jego asystenci i uczniowie, wiedzą wszyscy ci, którzy mieli satysfakcję i przyjemność z Nim współpracować. Był Profesor nie tylko znakomitym chemikiem, lecz również – światłym, rozumiejącym ludzkie potrzeby lekarzem.

Przeglądając Jego imponujący dorobek naukowy, spostrzegamy, iż znaczna liczba publikacji i doniesień zjazdowych jest efektem współdziałania z klinicystami różnych specjalności. Zawdzięczają Mu niemało interniści, pediatrzy, chirurdzy, ginekolodzy; nieobce były Mu zagadnienia toksykologii i immunologii. Rozumiejąc doskonale znaczenie chemii w medycynie, wprowadzał jej elementy do nauk klinicznych. Najwięcej uwagi poświęcił korzystnemu i niekorzystnemu wpływowi różnych pierwiastków na organizm ludzki i zwierzęcy, a ulubionym Jego pierwiastkiem był bez wątpienia magnez. Badał znaczenie wolnych rodników i amin biogennych, przemiany aminokwasów i zachowanie się enzymów w stanach zdrowia i choroby.

Profesor Kazimierz Pasternak pozostanie w naszej pamięci doskonałym, pełnym twórczej inwencji organizatorem pracy. Dowiódł tego jako kierownik Katedry, prodziekan Wydziału Pielęgniarstwa i Nauk o Zdrowiu, prezes Towarzystwa Przyjaciół Akademii Medycznej w Lublinie, a przede wszystkim jako główny kreator, wiceprezes i prezes Lubelskiego Oddziału Polskiego Towarzystwa Magnezologicznego. Był niczym *spiritus movens* wszelkich jego poczynań.

Rozumiał potrzeby młodych i chętnie stwarzał im optymalne możliwości rozwoju. Był promotorem blisko 20 rozpraw doktorskich i wielu prac magisterskich a studentom pozostawił nowoczesny i zarazem przystępny podręcznik biochemii.

Pracował z rozumną pasją i zawsze z uśmiechem a uśmiech Kazia rozjaśniał twarze i niweczył przeszkody. Wypełniała Go energia i aktywność, chciał i potrafił pracować. Pracował i planował dalszą działalność do ostatnich dni. Niespodziewanie, przedwcześnie i niesprawiedliwie nadszedł jednak kres Jego pięknego, pożytecznego życia...

Z odejściem Profesora Kazimierza Pasternaka Polskie Towarzystwo Magnezologiczne traci nie tylko zasłużonego wiceprezesa, traci też oddanego mu badacza, dla którego Towarzystwo i jego sprawy były celem i esencją działalności. Organizował wiele zjazdów, konferencji i sympozjów, aktywnie w nich uczestniczył i zawsze zdobywał sympatię i szacunek ich uczestników.

W dniu 15 września br. żegnaliśmy Profesora na cmentarzu przy ulicy Lipowej w Lublinie. Pogrążoną w bólu Rodzinę otoczyli Jego przyjaciele, koledzy, uczniowie i znajomi, a w wielu oczach pojawiły się łzy...

Zapamiętamy Go jako dobrego, ciepłego człowieka, i nie zapomnimy, bo nie wolno zapominać o Przyjacielu!

Andrzej Papierkowski

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ORIGINAL PAPERS

CONCENTRATION AND POOLS OF HEAVY METALS IN ORGANIC SOILS IN POST-FIRE AREAS USED AS FORESTS AND MEADOWS

Adam Bogacz, Przemysław Woźniczka, Beata Łabaz

Institute of Soil Science and Environmental Protection
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Abstract

High concentration of heavy metals in organic soils may be the result of intensive, deep-seated fires causing high temperatures. This research has been carried in four post-fire areas, located in forests or on meadows in Lower Silesia. The aim has been to determine the impact of some parameters on the content of heavy metals in soils under post-fire meadows and forests. The concentration and pool of the analyzed heavy metals were determined in soils against the background of such parameters as the depth in a horizon, organic matter content, soil colour and soil reaction. Twenty Histosol soil profiles (85 soil samples) were analyzed, representing peat-muck, muck (MtIIc1, MtIIIc1) and mineral-mucky (Me11) and on-muck soils (according to the Polish taxonomy of soils). The soils were strongly desiccated. Some physicochemical and chemical properties of pyrogenic soils were analyzed in dry samples collected into plastic bags with an Instorfu auger. Heavy metals (Zn, Cu, Pb, Ni, Cr) were determined in HCl + HNO_3. Pools of heavy metals were recalculated into g m^{-2} in 0-20 cm layers of soils and compared among forest and meadow soils. Due to intensive fires, the reaction of pyrogenic soils was slightly acid, neutral or alkaline. As a consequence of the high temperatures, a broad spectrum of soil colours was noticed. The results showed increased concentrations of heavy metals in the topmost and muddy soil horizons. The alkaline soil reaction favoured accumulation of heavy metals. Pools of heavy metals in soils were significantly lower in forest than in meadow areas, which could be attributed to different time periods which elapsed since the fires. The Hue tint of the colour of soil horizons containing ashes indicated the presence of oxidized iron forms and

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a low content of organic carbon. The accumulation of heavy metals in post-fire sites in Lower Silesia was negatively correlated with the soil's strongly acid reaction. The determined concentrations of heavy metals did not exceed the threshold values set for unpolluted soils.

Keywords: soil, peat, fire, heavy metals, valley areas, dewatering process.

KONCENTRACJA I ZASOBNOŚĆ METALI CIĘŻKICH W GLEBACH ORGANICZNYCH NA OBSZARACH POPOŻAROWYCH UŻYTKOWANYCH JAKO LASY I ŁĄKI

Abstrakt

Wysoka koncentracja metali ciężkich w glebach organicznych może być wynikiem intensywnych, wysokotemperaturowych pożarów wgłębnych. Badania prowadzono na leśnych i łąkowych obszarach popożarowych Dolnego Śląska. W glebach określono koncentrację oraz zasoby wybranych metali ciężkich na tle: głębokości w profilu, zawartości materii organicznej, barwy gleby oraz odczynu środowiska glebowego. Łącznie przeanalizowano 20 profilów płytkich gleb: torfowo-murszowych, murszowych (MtIIc1, MtIIIc1), murszowatych (Me11) i namurszowych (85 próbek glebowych). Właściwości fizykochemiczne i chemiczne gleb popożarowych oznaczono w próbkach suchych pobranych świdrem Instorf do plastikowych woreczków. Zawartość metali ciężkich (Zn, Cu, Pb, Ni, Cr) w glebie oznaczano po mineralizacji w mieszaninie $HCl+HNO_3$. Zasoby metali ciężkich w glebie przeliczano na g m^{-2} w 0-20 cm warstwie gleby i porównywano z glebami leśnymi i łąkowymi. Badania pokazały wzrost koncentracji metali ciężkich oraz ich zasoby w poziomach powierzchniowych 0-20 cm. Poziomy popożarowe gleb wykazywały odczyn lekko kwaśny, obojętny lub zasadowy. W konsekwencji temperatury pożarów można obserwować zróżnicowane spektrum kolorów gleb. Pożary na glebach leśnych i łąkowych wzbogaciły wiele poziomów w Zn, Cu, Pb, Ni, Cr. Koncentrację metali w glebach obserwowano głównie w powierzchniowych i zamulonych poziomach. Odczyn gleb sprzyjał koncentracji metali ciężkich. Zasoby metali ciężkich w glebach były wyraźnie niższe na obszarach leśnych niż łąkowych. Było to m.in. związane z czasem, jaki upłynął od zakończenia pożarów. Odcień barwy Hue w poziomach z popiołem był warunkowany intensywnym utlenianiem żelaza i niską zawartością węgla ogólnego. Gromadzenie się metali ciężkich na obszarach popożarowych Dolnego Śląska było negatywnie skorelowane z silnie kwaśnym odczynem środowiska. Nie obserwowano przekroczenia wartości granicznych metali ciężkich w przypadku gleb niezanieczyszczonych.

Słowa kluczowe: gleba, torf, pożar, metale ciężkie, obszary dolinowe, przesuszenie.

INTRODUCTION

Significant increase in the content of heavy metals is observed in ashes generated by peat fires, especially the ones causing high temperatures (Zaidelman et al. 1999). The main reasons are the loss of organic matter and elevated quantities of mineral particles in ash (Bannikov et al. 2008). The concentration of trace elements is several-fold higher in ash than in peat, depending on analyzed trace elements (Zaidelman et al. 2003, Dikici, Ylmaz 2006).

This research has been carried out to determine the impact of some physical and chemical parameters on concentration of heavy metals (Pb, Zn, Cu, Cr and Ni) in soils of post-fire meadow and forest areas and to compare distribution of metals in the described soil horizons.

METHODS

Soil samples have been collected from two post-fire forest sites: Gromadka (GR) in Chocianów Forest Division and Mikorzyce-Górowo (MG) in Wołów Forest Division, and from two meadow sites: Lubsko (LU) and Sobin-Jędrzychów (SJ). The forest fires occurred in 1986 and 1992 and the meadow fires broke out in 2006 and 2008. In total, 20 soil profiles were analyzed and 85 soil samples were collected. Forest soil samples were collected from profiles No 9-12MG (12 years after a fire) and profiles No 1-8GR (21 years after a fire). Meadow soil samples were collected from profiles No 12-15LU (immediately after a fire) and profiles No 16-20SJ (2 years after a fire). The soil samples represented the following soil subtypes: muck, peat-muck, mineral-mucky and on-muck soils. The horizons represented strongly dried peat-muck soils and medium deep (MtIIc1 and MtIIIc1) and mineral-mucky (Me11) and on-muck soil (Okruszko 1974). The peat found in post-fire sites has a distinctly high degree of organic matter decomposition (Bogacz 2009, Bogacz at al. 2010). In the collected soil samples, the following properties were determined: soil colour according to Munsell notation, ash content by peat combustion in a muffle furnace at 550°C for 4 hours, bulk density with 100 cm^{-3} Kopecky's metal rings, organic carbon content in a Bushi analyzer, pH in H_2O and 1 mol dm^{-3} KCl $_{(pH\ KCl)}$ potentiometrically at a 1:2.5 soil to solution ratio, total content of the heavy metals such as zinc (Zn_t), copper (Cu_t), lead (Pb_t), nickel (Ni_t) and chromium (Cr_t) with the flame atomic absorption spectroscopy (FAAS). The samples had been digested in mixed $HCl+HNO_{3.}$ Concentrations of trace elements were analyzed in this mixture, in mg kg^{-1}. Afterwards, their pools were converted into g m^{-2} in the 0-20 cm layer of soil and compared between forest and meadow soils. For each of the investigated sites, a correlation coefficient was determined between concentration of heavy metals, pH and depth. Differences between pools of heavy metals in soil used as forests and meadows were calculated using the mean standard deviation (SD) and basic analyses of variance. Statistical analyses were made using logarithmic data with Stat-Soft Statistica software, version 8.0.

RESULTS AND DISCUSSION

The organic post-fire soils under forests and meadows were characterized by strongly acid and acid reaction in a deeper horizon, but neutral and alkaline pH in the horizons with ashes (Table 1). A study performed by ZAIDELMAN et al. (2003) on pyrogenic peat soil in Moscow region confirms this regularity. The lowest carbon content was observed in muddy horizons and those with ashes, whereas the highest one, exceeding 500 g kg^{-1} of soil, appeared in bog peat and intermediate peat horizons. Following an intensive fire, the reaction of pyrogenic soils is neutral or alkaline. As a consequences of the high temperature, a very broad soil colour spectrum, between N, 10YR and 5YR, can be observed (Table 1).

According to the Polish norms prepared for arable soils by the *Threshold quantities...* (1985), an elevated Pb content in the surface and subsurface layers of post-fire soils (ashes and horizons with ashes) occurred in some samples. Excessive metal concentrations were determined versus the Polish soil quality standards and Polish ground quality standards (*Ordinance*…2002). Generally, the highest levels of metals appeared in the topmost horizons, just a few centimetres thick, which with time might be dispersed by wind or leached by water. Fires enriched many horizons of meadow and forest soils with Zn, Cu, Cr and Ni. The highest Pb concentration was observed in the Olfh layers of litter, zinc in the thin layers of peat and litter, copper in muck and also in a thin layer of peat. The chromium content was observed mainly in ash horizons A and organic muddy horizons Om. No such regular tendencies were observed for nickel (Table 1).

Pools of lead in the analyzed forest soils layer ranged from 0.76 to 9.09 g m^{-2}. The Zn content was less varied and did not exceed 3.0 g m^{-2}. Copper, chromium and nickel were found to appear in even less diverse quantities, for example below 1.0 g m^{-2} in the 0-20 cm forest soil layer (Table 2). In post-fire meadow soils, which had been ravaged by a fire just a couple of years before, concentrations of the metals were different. Significantly higher pools of microelements, such as lead, zinc and copper as well as chromium and nickel, were observed in surface layers (Table 2).

Large quantities of organic carbon have been observed in soils under the two analyzed meadows and under forests, in layers unaffected by high- or low-temperature fires (HOGG et al. 1992). These layers were characterized by black colour 10YR 2/1. The research conducted by AFREMOV and AFREMOVA (2000) on deep peat soils implied a high carbon content, over 600 g kg^{-1} of soil, in layers affected by a low-temperature fire. An increase in the carbon content in surface layers may also be influenced by the initially low microbial activity (JASTRZĘBSKA, KUCHARSKI 2006), resulting in organic matter decomposition (TSIBART, GENNADIEV 2008). In horizons where ash suggested occurrence of fires generating temperatures over 200°C (ULERY, GRAHAM 1995),

Table 1

Heavy metal concentration and some properties in pyrogenic soil genetic horizons

Genetic horizon	Depth (cm)	Soil colors	pH_{KCl}	C-total (g kg^{-1})	Pb	Zn	Cu	Cr	Ni
					(mg kg^{-1})				
Olfh	2-7* 4**	10YR 3/4*** 2.5YR 2.5/0	3.1-4.3 3.4	139-475 336	20.2-246 87.4	8.50-136 70.5	7.70-501 23.7	3.82-18.4 8.85	3.15-37.0 10.8
A	4-34 11	N 3/0 5YR 5/5	3.3-7.9 5.5	10.7-112 62.5	14.8-81.7 38.2	9.20-91.7 31.7	2.50-41.2 16.7	8.10-42.7 17.6	3.95-37.2 16.6
Mt	5-17 9	10YR 2/1 5Y 3/2	2.4-6.8 4.6	253-497 411	4.10-145 55.6	8.50-77.5 29.1	6.39-203 33.3	2.01-22.1 9.64	2.50-60.5 9.86
Otni	5-30 13	10YR 2/1 5YR 3/3	2.3-5.8 3.6	137-519 319	1.40-333 42.0	5.6-46.2 16.6	3.10-32.3 11.7	1.99-67.1 27.0	0.90-74.2 22.7
Otpr	13-20 18	10YR 2/1 5YR 3/1	1.7-3.5 2.6	225-415 350	1.4-8.00 6.85	7.70-32.1 19.9	4.10-28.6 14.8	5.60-8.00 7.25	21.3-76.7 51.6
O	2-16 10	N 3/0 5YR 2.5/1	4.0-6.3 5.4	171-389 268	4.97-79.4 49.8	3.51-865 297	7.91-130 50.0	2.94-46.6 21.0	1.38-33.6 15.8
O/D	8-15 11	10YR 2/1 7.5YR 4/3	3.9-5.9 4.5	55.5-109 83.9	5.23-14.8 9.54	4.25-15.0 7.66	1.50-13.0 5.72	8.40-34.2 16.1	0.40-9.00 3.48

Key: Olfh – forest litter, A – acumulation, Mt – moorsh, t – peat, tni – low peat, tpr – mediate peat, O – organic, O/D – organic/mineral, *range (minimum – maximum, **arithmetic mean, ***soil color range

Table 2

Heavy metals pools in upper horizons of pyrogenic soils in forests areas

Site No.	Layer (cm)	Heavy metals calculated (g m^{-2})									
		Pb		Zn		Cu		Cr		Ni	
1/GR	0-10	1.24	1.70	0.78	1.87	0.65	1.08	0.16	0.31	0.14	0.28
	10-20	0.46		1.09		0.43		0.15		0.14	
2/GR	0-10	0.41	10.76	0.61	0.90	0.26	0.80	0.09	0.36	0.08	0.21
	10-20	0.35		0.29		0.54		0.27		0.13	
3/GR	0-10	1.20	11.77	1.23	1.66	0.43	0.74	0.17	0.31	0.17	0.35
	10-20	0.57		0.43		0.31		0.14		0.18	
4/GR	0-10	4.82	14.97	2.77	3.02	1.06	1.25	0.38	0.43	0.23	0.29
	10-20	0.15		0.25		0.19		0.05		0.06	
5/GR	0-10	2.37	2.58	1.07	1.40	0.57	0.70	0.28	0.32	0.16	0.23
	10-20	0.21		0.33		0.13		0.04		0.07	
6/GR	0-10	1.49	13.44	1.50	2.51	0.38	0.88	0.40	0.99	0.30	0.94
	10-20	1.95		1.01		0.50		0.59		0.64	
7/GR	0-10	1.15	12.55	1.12	1.89	0.30	0.56	0.18	0.56	0.16	0.42
	10-20	1.40		0.77		0.26		0.38		0.26	
8/MG	0-10	1.65	3.58	0.82	2.75	0.30	0.61	0.28	0.75	1.40	3.92
	10-20	1.93		1.93		0.31		0.47		2.52	
9/MG	0-10	2.95	9.56	0.91	2.16	0.79	1.10	0.24	0.43	0.15	0.34
	10-20	6.61		1.25		0.31		0.19		0.19	
10/MG	0-10	1.30	1.39	0.52	1.07	0.40	1.14	0,23	0.24	0.18	0.59
	10-20	0.09		0.55		0.74		0.01		0.41	
11/MG	0-10	1.27	3.31	0.20	0.51	0.24	0.55	0.13	0.39	0.10	0.26
	10-20	2.04		0.31		0.31		0.26		0.16	

Key: GR – Gromadka, MG – Mikorzyce-Górowo

a decrease in the organic matter content was observed (Table 1). This phenomenon has been pointed out by many scientists studying post-fire soils (ZAIDELMAN et. al. 2003, KANIA et. al. 2006). It has also been confirmed by the colour of a horizon containing ashes, such as red-yellow Hue 5YR, indicating the presence of oxidized iron forms and a low content of organic carbon (Table 1).

The accumulation of organic matter determined in the analyzed post-fire sites in Lower Silesia was negatively correlated with strongly acid reaction of the soil environment (r=-0.60**, p<0.01, n=85) – Table 3. After a fire, these soils were enriched with different heavy metals in post-ash or ash horizons. This is also clearly confirmed by the correlation coefficients for the depth and concentration of Pb (r=-0.39*), Cu (r=-0.26*) and Zn (r=-0.26*) $p<0.05$, n=85). The Cr and Ni content in soils depends not only on their concentration in ashes but also on the intensity of mud formation in soils, a process which is often observed in deeper horizons (Tables 2 and 4). The negative correlation coefficients computed between chromium and nickel depth and concentration were not significant (Table 3). A study completed by Arkhipova and Karataieva (2002) on vast peat areas in Siberia confirms the above findings. Ashes generated by fires neutralize organic acids occurring in peat, mainly in surface layers, and change base saturation by adding easily soluble, alkaline substances (Gynikova, Sympilova 1999, Krasnoshchekov 1994).

Table 3

Coefficients of correlation between concentration of heavy meta and properties of pyrogenic soils in post-fire areas

Value	Pb_t	Cr_t	Ni_t	Cu_t	Zn_t	pH	C-total
Depth	-0.39*	0.13	0.07	-0.26	-0.26	-0.32*	-0.10
Pb_t		0.14	0.04	0.25	0.17	0.17	0
Cr_t			0.03	0.06	0.06	0.30*	-0.15
Ni_t				0.07	-0.02	0.25*	-0.05
Cu_t					0.53*	0.26*	0.08
Zn_t						0.25*	0.06
pH							-0.60*

Key: n=85, correlation ratio significant at: *$p<0.05$, **$p<0.01$

The latter authors also emphasize a sudden decrease in the pH value, just a few years after a fire, due to migration of soil components induced by intensive precipitation and flowing water (Zaidelman et al. 2003). The accumulation of new acid products of plant decomposition on the soil surface may also intensify this process (Zaidelman et al. 1999). Higher pH values, resulting from neutral or alkaline reaction, are a consequence of heavy metal accumulation in post-fire soils (Dikici, Ylmaz 2006). This is confirmed by the correlation coefficient between the reaction of a soil horizon and its depth (r=-0.32*, $p<0.05$, n=85) – Table 3. Many studies on heavy metal translocation in organic post-fire soils (Zaidelman et al. 2003, Kutiell, Shavin 1993, Johnston, Eliot 1998, Lynham et al. 1998) clearly indicate a high rate of metal migration, mainly during the first years after a fire. In the present study, important changes were observed in the content of heavy metals in surface layers of forest and meadow soils, and differences were noticed relative to the time lapse after a fire. These changes were mainly noticeable in the

Table 4

Heavy metal pools in upper horizons of pyrogenic soils in meadow areas

Site No.	Layer (cm)	Heavy metals calculated (g m^{-2})									
		Pb		Zn		Cu		Cr		Ni	
12/LU	0-10	2.31	4.36	5.58	6.70	1.14	1.67	0.68	1.89	0.34	0.70
	10-20	2.05		1.12		0.53		1.21		0.36	
13/LU	0-10	1.43	3.63	1.47	3.44	0.18	1.20	0.64	2.63	0.22	0.87
	10-20	2.20		1.97		1.02		1.99		0.65	
14/LU	0-10	5.34	9.33	5.30	8.96	1.63	2.85	2.24	5.73	1.07	2.15
	10-20	4.01		3.66		1.22		3.49		1.08	
15/LU	0-10	2.65	4.87	3.40	4.95	0.96	1.48	1.29	2.49	0.73	1.33
	10-20	2.22		1.55		0.52		1.20		0.60	
16/SJ	0-10	2.83	4.92	1.69	2.63	3.70	4.87	0.37	0.77	0.34	0.80
	10-20	2.09		0.94		0.90		0.40		0.46	
17/SJ	0-10	1.40	2.93	1.40	2.07	1.54	1.90	0.40	0.88	1.51	2.44
	10-20	1.53		0.67		0.36		0.48		0.93	
18/SJ	0-10	1.75	3.41	1.09	1.88	1.38	3.08	0.44	0.90	1.04	2.06
	10-20	1.66		0.79		1.71		0.46		1.02	
19/SJ	0-10	4.60	9.10	2.92	3.52	3.33	3.75	1.19	1.38	2.30	3.24
	10-20	4.50		0.60		0.42		0.19		0.94	
20/SJ	0-10	2.58	5.95	1.45	3.38	1.88	3.09	0.61	1.35	2.65	5.44
	10-20	3.37		1.96		1.21		0.74		2.79	

Key: LU – Lubsko, SJ – Sobin - Jędrzychów

Table 5

Pools of heavy metals in post fire soils used as meadowand forests

Metal (n=18)	Type of use	x	SD	dx	CV
Pb	forest	3.24	0.294	2.15	0.022606
	meadow	5.39	0.177		
Zn	forest	1.79	0.232	2.38	0.002537
	meadow	4.17	0.225		
Cu	forest	0.86	0.129	1.80	0.000007
	meadow	2.65	0.203		
Cr	forest	0.46	0.182	1.54	0.000025
	meadow	2.00	0.282		
Ni	forest	0.71	0.368	1.40	0.001118
	meadow	2.11	0.302		

Key: x – arithmetic mean, SD – standard deviation, dx – difference of arithmetic means, CV – coefficient of variation, n – number of samples case of lead, zinc and copper (Tables 2 and 4). Statistically significant differences were observed between pools of heavy metals and land use type (Table 5), a relationship which was particularly strong for Cu, but the least significant for Pb.

CONCLUSIONS

A fire causes raises the soil reaction and enlarges pools and concentration of some heavy metals, mainly in surface layers. A decrease in organic matter is also observed, especially in surface layers with ash. Generally, the threshold values set up by the norms on soil quality standard and ground quality standard (*Ordinance*…2002) were not exceeded. In a few cases, however, a slight excess in heavy metals was noticed versus the norms established by the *Threshold quantities*... (1985) for arable soils. Forest soils had a lower content and poorer pools of heavy metals in surface layers than meadow soils. This is mainly due to changes in pH, organic matter loss and a different time lapse after a fire.

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THE ELEMENTS OF SPECIFIC BIOLOGICAL ACTIVITY IN THERAPEUTIC WATERS IN POLISH HEALTH RESORTS

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Abstract

The study characterizes specific therapeutic waters in Polish health resorts, which contain iodide, fluoride, silicon, sulphides, iron(II), carbon dioxide and radon in concentrations that ensure the therapeutic status of water and specify how it can be used for medical treatment.

Based on the physicochemical analysis, it has been verified that among 160 waters from 39 health resorts, 37 contain Fe(II) in concentrations > 10 mg dm^{-3}, which means that they can be classified as ferruginous. Twelve waters containing fluoride in concentrations > 2 mg dm^{-3} were recognized as therapeutic fluoride waters. Five waters were classified as siliceous ones owing to the content of silicon compounds above 70 mg dm^{-3} (H_2SiO_3). Twenty-eight of the analysed waters contain sulphides (H_2S+HS^-) in concentrations > 1 mg dm^{-3} and 64 waters contain iodide in concentrations > 1 mg dm^{-3}. Carbon dioxide, which occurs in 72 waters in concentrations above 1 g dm^{-3} (acidulous waters), proved to be the most widespread component. Waters which contain radon in an amount corresponding to the radiation of 74 Bq (2 nCi) in 1 dm^3 are classified as radon waters. Ten of the analysed waters were found to contain this element.

The waters were highly varied in chemical composition and type of specific components. Sulphurous waters are mainly used for mineral baths; ferruginous waters are drunk; fluoride and silicon-rich waters are taken for mineral baths and iodide waters are used for mineral baths and inhalations. Using these waters in balneotherapy is an important part of spa treatment and defines different healing profiles of Polish health resorts.

Key words: specific therapeutic waters, sulphides, iodides, fluorides, iron (II), silicon, radon, carbon dioxide.

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PIERWIASTKI O SWOISTEJ AKTYWNOŚCI BIOLOGICZNEJ W WODACH LECZNICZYCH POLSKICH UZDROWISK

Abstrakt

W pracy przedstawiono charakterystykę leczniczych wód swoistych polskich uzdrowisk. Są to wody zawierające jodki, fluorki, związki krzemu, związki siarki (II), żelazo (II), dwutlenek węgla i radon w stężeniach decydujących o statusie leczniczym wody i sposobie jej wykorzystania do zabiegów kuracyjnych.

Na podstawie wyników analiz fizykochemicznych stwierdzono, że spośród 160 wód pochodzących z 39 miejscowości uzdrowiskowych 37 wód zawiera żelazo (II) w stężeniu > 10 mg dm^{-3} wymaganym dla uznania wody za żelazistą; 12 wód zawierających fluorki w stężeniu > 2 mg dm^{-3} uznano za lecznicze wody fluorkowe; 5 wód ze względu na zawartość związków krzemu w stężeniu > 70 mg dm^{-3} (H_2SiO_3) zaliczono do wód krzemowych; 28 wód zawiera związki siarki (II) w stężeniu > 1 mg dm^{-3} (HS^- + H_2S), a 64 wody zawierają jodki w stężeniu > 1 mg dm^{-3}. Najczęściej występującym składnikiem swoistym wód leczniczych jest dwutlenek węgla, którego stężenie w 72 wodach przekracza 1 g dm^{-3} (szczawy). Wody zawierające radon w ilości, której odpowiada aktywność promieniotwórcza co najmniej 74 Bq dm^{-3} (2 nCi), są zaliczane do wód radonowych. Pierwiastek ten występuje w 10 badanych wodach.

Omawiane wody charakteryzują się dużym zróżnicowaniem podstawowego składu chemicznego oraz zawartością ww. składników swoistych. Wody swoiste siarczkowe są wykorzystywane głównie do kąpieli, wody żelaziste do kuracji pitnych, wody fluorkowe i krzemowe do kąpieli, a jodkowe do kąpieli i inhalacji. Stosowanie tych wód w balneoterapii stanowi ważną część działalności uzdrowiskowej i uzasadnia różne profile lecznicze polskich uzdrowisk.

Słowa kluczowe: swoiste wody lecznicze, związki siarki (II), jodki, fluorki, żelazo (II), krzem, radon, dwutlenek węgla.

INTRODUCTION

In the literature dealing with balneology and in spa practice, the following elements have significant influence on the human body: macroelements (sodium, potassium, calcium, chlorides, bicarbonates, sulphurs) and specific components (iodides, compounds with sulphur(II), silicon, fluorine, iron(II) and radon as well as carbon dioxide). In some waters, concentration of these components determines their therapeutic properties and specifies the type of treatment, e.g. drinking water, mineral baths or inhalation therapy. The minimum concentration of these components for healing applications is set in the Regulation of the Minister for Health of 13 April 2006 (Journal of Law, 2006.80.565).

The aim of the study has been to demonstrate some characteristics of the therapeutic properties of specific waters containing sulphlides, iodides, fluorides, iron, silicon, radon and carbon dioxide, which occur in some of the Polish spas and which are currently used.

MATERIAL AND METHODS

This review is based on the authors' own results of physical and chemical analyses of spa waters, which were performed during the certification of spa waters in 2007-2010. The results from the previous years, 1998 to 2006, originate from own analyses as well as from data supplied by other institutions authorized to analyse water.

The composition of 160 waters from 39 spas in four regions of Poland, i.e. the Carpathian Mountains, an area of Miocene marine sediments, the Sudety mountains and the Polish Lowland, was analyzed.

RESULTS AND DISCUSSION

Acidulous waters (over 1 g dm^{-3} CO_2) and waters containing carbon dioxide

The most common component of specific water is carbon dioxide, which occurs in 88 of all the analysed healing waters from the south of Poland. In 16, the concentration of CO_2 was 250-1.000 mg dm^{-3} (waters containing carbon dioxide) and in 72, the concentration was above 1.0 g dm^{-3}, which means that they belong to acidulous waters. The concentration of CO_2 in such water can reach 3,316 mg dm^{-3} (intake Zofia II in Żegiestów), but is normally in the range from 2,800 to 2,970 mg dm^{-3}, especially in the intakes in Wysowa and Krynica. Carbon dioxide usually occurs in the water types: HCO_3-Cl-Na/Ca, I (Rymanów, Wysowa, Szczawnica, Rabka, Kudowa); HCO_3-Ca-Mg, HCO_3-Ca (Muszyna, Krynica); HCO_3-Na (Krynica, Szczawno), and in low mineralized ferruginous and radon waters (Świeradów, Lądek). In balneochemistry, when characterising mineral water, names of dominant anions and cations are given in the order of decreasing concentrations. Concentration is expressed in milligram-equivalent per 1 dm^3.

Carbon dioxide is a natural stabilizer of mineral water composition. It maintains carbonic acid and solubility balance, thereby preserving bio-accessible forms of chemical compounds of calcium, magnesium, iron and manganese. This is very important when acidulous waters are used for drinking treatment, which is the main application of these waters.

It is less popular to use acidulous waters for mineral baths due to their limited resources. However, there are clinical observations confirming good localised effect as well as positive influence of carbon dioxide on the human body during a bath, as a result of the release of tissue hormones in the skin, expansion of capillaries, decreased heart rhythm, lowered blood pressure, improved diuresis, accelerated metabolism and improved blood rheology (Drobnik, Latour 2001, 2002, 2006, Gutenbrunner 1990). At present, the acidulous waters available in Polish health resorts are not available as bottled water to continue treatment outside a spa.

Ferruginous waters

There are 42 iron water intakes in 15 Polish spas which have water with at least 10 mg dm^{-3} of iron(II); this is the threshold level to assume that they are ferruginous waters. The highest concentration of iron(II) in the range from 217.7 to 270.0 mg dm^{-3} was found in the intakes in Świeradów and Wysowa; in the area of Krynica-Żegiestów, Kudowa, Goczałkowice and Świnoujście, it ranged from 47.5 to 95.1 mg dm^{-3} and in Konstancin, Busko, Świeradów and Wysowa, it varied from 20 to 40 mg dm^{-3}. Iron(II) usually occurs in the following water types: Na-Cl (brine with the general mineral content higher than 1.5%), (11 intakes), acidulous waters –HCO_3--Mg-Ca (8 intakes); – HCO_3-Na-Ca (6 intakes); –Cl-Na (4 intakes), all characterised by a different level of mineralization. Many ferruginous waters contain other specific components, mainly iodides and carbon dioxide.

Iron (II) in essential for haemoglobin production. It also has positive influence on the growth and development of muscles, as well as the formation and activity of enzymes which control the heart and improve the immune system (REINHOLD 1995).

When formulating therapeutic recommendations for waters containing iron(II), it is important to consider the general mineralization of water and concentration of other components. Iron in mineral water can have a medicinal effect only when administered *per os*. Consequently, the best results can be achieved by drinking such waters as acidulous waters HCO_3-Mg-Ca, HCO_3-Na-Ca, low mineralized waters, mineralized waters and Cl-Na with mineralization lower than 0.9%.

Iron occurs in many brine waters used for mineral baths (in significant concentrations, e.g. in Goczałkowice, Świnoujście), but then it does not have any influence on the human body.

Iodide waters

Waters from 61 intakes in 17 spas have been classified as iodide waters, containing at least 1.0 mg I^- dm^{-3}. Iodides in concentrations above 10 mg dm^{-3} occur in 2 intakes in Busko, 2 intakes in Iwonicz, 1 intake in Ustroń, and in all the intakes in Rabka (5 intakes) and Goczałkowice (3 intakes). The highest iodide concentration was found in Ustroń (31.9 mg I^- dm^{-3}), Goczałkowice (19.0-22.2 mg dm^{-3}) and Rabka (12.9-21.2 mg dm^{-3}). Iodide waters are generally Cl-Na waters (also brine), Cl-SO_4-Na waters, and carbonated waters or acidulous waters Cl-HCO_3-Na (low and medium mineralized).

Iodides produce various effects on the human body, depending on the type of treatment. Iodides from Cl-SO_4-Na waters (Busko), waters containing carbon dioxide or acidulous waters like Cl-HCO_3-Na (Iwonicz) used as drinking water affect production of the thyroid hormones and metabolism. Iodides from Cl-Na and brine waters used for mineral baths and inhalations have a beneficial influence on the skin and act as a disinfectant (Deutsch, Klieber 1982).

Fluoride waters

Eleven waters, mainly from Kłodzka Valley, contain fluorides in concentrations > 2.0 mg dm^{-3} (Jedlina, Cieplice, Lądek, and Busko). The water from Cieplice contains 11-12 mg F^- dm^{-3}, from Lądek-Długopole 7.6-10.0 mg F^- dm^{-3}, and from Busko 3.5 mg F^- dm^{-3}. In Poland, fluorides occur mainly in low mineralized waters: radon waters, siliceous waters and thermal waters (Cieplice), or radon and ferruginous waters (Lądek-Długopole).

The use of fluoride waters in Poland is inadequate. Nowadays, they are used for mineral baths, owing to other properties like the content of sulphur, hydrogen sulphide (Busko, Lądek, Jedlina), radon (Lądek), or as thermal waters (Cieplice). The effect of fluorides on the skin as well as their diffusion have not been fully recognised yet. It is known that fluorine is absorbed by mucous membranes of the mouth and has a positive influence on the structure of teeth (Ogaard 1999). Therefore, such water can be used as mouth wash (also as bottled water, outside a spa) or for manufacturing anti-decay dental products (Czajka et al. 1998, Drobnik 1999, Iracki, Wierzbicka 2005).

Siliceous waters

Waters with silicon concentration higher then 70 mg H_2SiO_3 dm^{-3} are classified as siliceous waters. Five waters from the spas: Cieplice (3 intakes), Świeradów (1 intake), and Kudowa (1 intake) are in this category. The maximum concentration of metasilicic acid of 106 mg dm^{-3} occurs in the intake called Jan II in Świeradów.

With the content of silicon compounds (silicon dioxide, metasilicic acid), the above waters belong to specific therapeutic waters but are not assigned any kind of therapeutic treatment. They are rather an additional factor in the therapeutic properties of mineral baths (Cieplice, Świeradów) or drinking treatment (Kudowa). Siliceous waters contain mainly silicon dioxide in the colloidal state, which participates in mineralization of bones and hardening of connective tissue. Silicon limits permeability of blood vessel walls and keeps the skin and collagen fibers elastic (Puzanowska-Tarasiewicz et al. 2009). For those reasons, siliceous waters are nowadays used more often in cosmetology (Dejneka, Łukasiak 2005, Czerpak, Jabłońska-Trypuć 2008).

Sulphurous waters

Twenty-eight of the analysed waters contain sulphur(II) compounds (H_2S, HS^-, $S_2O_3^{2-}$, HSO_3^-) in a concentration of 1.0 mg dm^{-3} determined idometrically. These compounds occur in concentrations from just a few to hundreds of mg dm^{-3} in 10 spas (Busko, Solec, Wieniec, Horyniec, Swoszowice, Wapienne, Wysowa, Lądek-Długopole, Kudowa, Przerzeczyn). The highest concentration was found in the water (3.97% Cl-Na) from the intake Malina near Solec (830 mg dm^{-3}), in therapeutic waters (1.6-2.0% Cl-SO_4-Na) from Solec (49.6-278.0 mg dm^{-3}) and in Cl-Na waters from Busko (29.7-40.9 mg dm^{-3}). The richest sulphide-hydrogen sulphide resources are in the spas Busko and Solec.

The concentration of sulphides, especially > 20 mg dm^{-3}, indicates that the water is suitable for mineral baths. Sulphurous waters containing <10 mg S(II) dm^{-3}, either low and medium mineralized, are also used as drinking cure. Sulphides diffused through the skin or taken *per os* result in an increased glutathione level in blood serum, -SH groups in tissues, mucosal secretions and histamine release. They also lower the blood sugar level and, by binding heavy metals, detoxify the organism. When in contact with the skin, they produce keratolytic and keratoplastic effects on the metabolism of cutaneous and subcutaneous tissues (Banaszkiewicz et al. 1992). Waters containing sulphide-hydrogen and sulphide are successfully used as a periodontium wash in the periodontitis therapy. These waters, in the raw form, are broadly used in cosmetology, both in spas and at home (added to creams, gels and emulsions).

Radon waters

Underground waters which contain radon in an amount in 1 dm^3 of water that corresponds to the radiation of 74 Bq (2 nCi) are classified as radon waters. Radon occurs in 4 low mineralized acidulous waters in Świeradów (from 96.2 to 592.0 Bq dm^{-3}) and 6 low mineralized waters, fluoride waters, and sulphurous waters in Lądek (from 179 to 1124 Bq dm^{-3}). Radon is found in concentrations from 113.6 to 193.1 Bq dm^{-3} in 0.13-0.22% of acidulous waters HCO_3-Ca-Na in Kudowa and Szczawno. Intakes of therapeutic radon waters, pursuant to the Mining Law classified as basic mineral deposits, occur in the Sudety Mountains and in the Fore-Sudetic Block, which is related to the geologic structure of Lower Silesia (Przylibski et al. 2004).

Radon is a gas with a short half-life time of 3.825 days. It easily evaporates from water during a bath. Therefore, during mineral baths in radon waters, a patient is exposed to radon through the skin and via inhalation. The biological action of radon has not been recognized yet. However, it is claimed that radon stimulates endocrine glands, mainly the pituitary, and stimulates biological processes. However, the latest research shows that radon waters should be used carefully, with each course of treatment adjusted

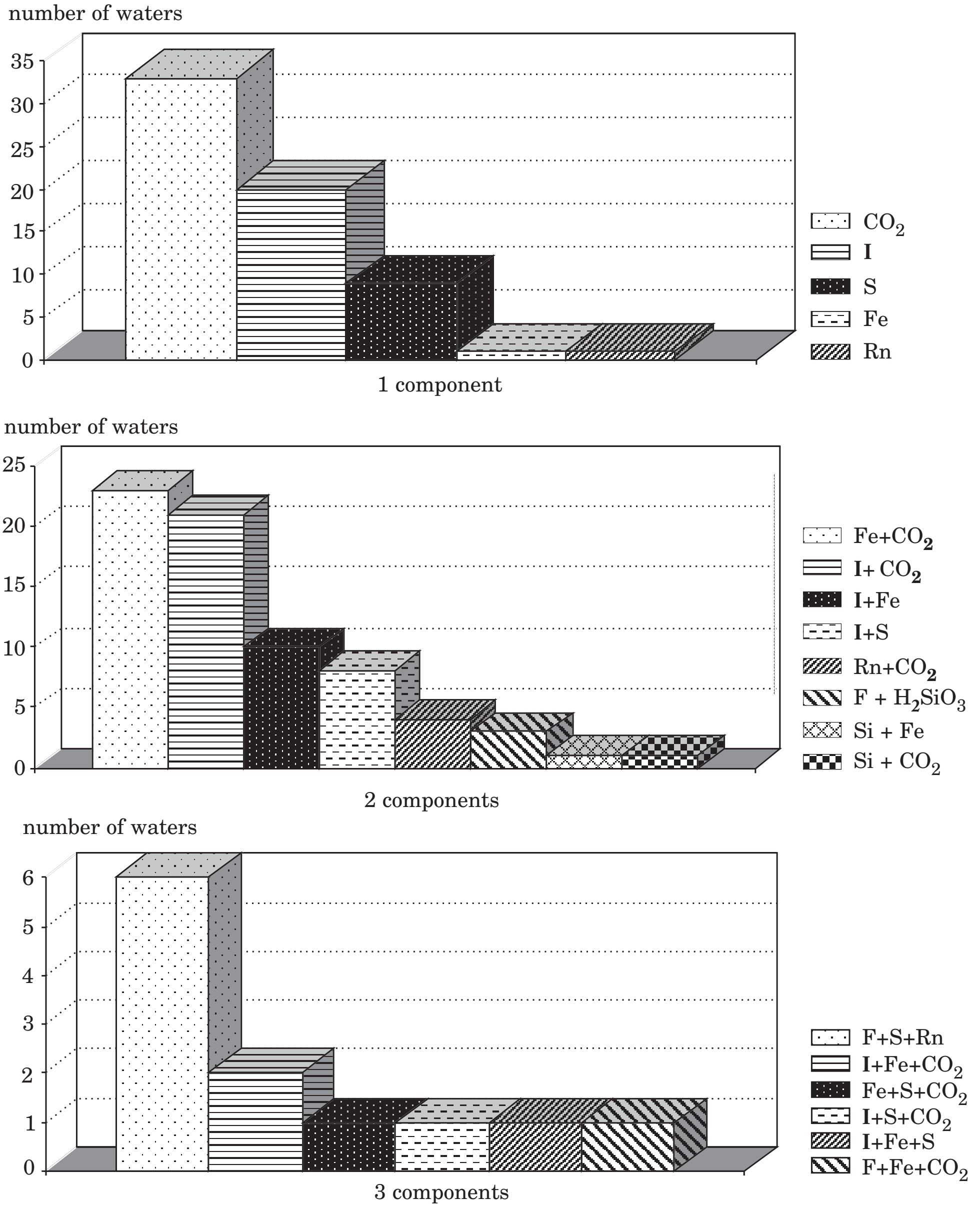

Fig. 1. Type and distribution of specific components in therapeutic waters in Polish health resorts

to an individual patient (Falkenbach 2000, 2001, Falkenbach et al. 2000, Deetjen 1992).

Specific components in significant concentrations suitable for therapeutic purposes occur in different combinations (Gundermann 2009, Schwarz, Morstadt 2010). Sixty-four waters contain one specific component, mainly iodides and carbon dioxide. In 71 waters there are two specific components and 12 contain three types of specific components. The distribution of the specific components is shown in Figure 1.

CONCLUSIONS

1. Mineral and low mineralized specific therapeutic waters from Polish spas are highly varied in the basic chemical composition as well as the type and concentration of specific components.

2. Sulphurous as well as siliceous waters in the raw form are nowadays used in spas most often for the skin care. These waters as well as fluoride waters are used for production of cosmetics (cream, gel, emulsion, paste) used in many spas, beauty parlours or at home for the oral prophylaxis, dental care, hair and skin treatment.

3. Owing to the specific biochemical properties and the number of known groundwater natural resources, sulphurous, iodide and acidulous waters deserve special attention.

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EFFECT OF LIMING AND MINERAL FERTILIZATION ON CADMIUM CONTENT IN GRAIN OF SPRING BARLEY (*HORDEUM VULGARE* L.) AND WINTER WHEAT (*TRITICUM AESTIVUM* L.) CULTIVATED ON LOESSIAL SOIL

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Abstract

In 1986-2001, a two-factor, strict experiment was conducted on a field subjected to static fertilization, situated in the Rzeszów Foothills (Pogórze Rzeszowskie, S-E Poland). The experiment was set up in a random sub-block design with 4 replications. The first variable was liming (A) and the second one consisted of different mineral fertilization variants (B). The basic level of fertilization (N_1 P_1 K_1) was 80 kg N ha^{-1}, 100 kg P_2O_5 ha^{-1} and 120 kg K_2O ha^{-1} under spring barley and 90 kg N ha^{-1}, 80 kg P_2O_5 ha^{-1}, 100 kg K_2O ha^{-1} under winter wheat. Liming did not prove to have any significant effect on the cadmium content in spring barley and winter wheat grain, although the latter tended to decline in response to the treatment.

Mineral NPK fertilization, applied in higher doses, significantly raised the content of cadmium, especially in spring barley grain, but less in winter wheat grain. No statistical correlation was proven between the influence of liming and mineral fertilization on the cadmium content in grain of the two cereals. However, the cadmium content was generally lower in grain of cereals cultivated on fertilized objects where liming was applied.

Key words: cadmium, liming, mineral fertilization, spring barley, winter wheat.

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WPŁYW WAPNOWANIA I NAWOŻENIA MINERALNEGO NA ZAWARTOŚĆ KADMU W ZIARNIE JĘCZMIENIA JAREGO (*HORDEUM VULGARE* L.) I PSZENICY OZIMEJ (*TRITICUM AESTIVUM* L.) UPRAWIANYCH NA GLEBIE LESSOWEJ

Abstrakt

W latach 1986-2001 przeprowadzono dwuczynnikowe statyczne doświadczenie polowe zlokalizowane na Podgórzu Rzeszowskim (południowo-wschodnia Polska). Doświadczenie dwuczynnikowe w 4 powtórzeniach założono metodą bloków losowanych z podblokami. Pierwszym czynnikiem było wapnowanie (A), drugim – niezależne od wapnowania zróżnicowane nawożenie mineralne (B). Podstawowy poziom nawożenia ($N_1P_1K_1$) dla jęczmienia jarego wynosił: 80 kg N ha^{-1}, 100 kg P_2O_5 ha^{-1} i 120 kg K_2O ha^{-1}, natomiast dla pszenicy ozimej: 90 kg N ha^{-1}, 80 kg P_2O_5 ha^{-1}, 100 kg K_2O ha^{-1}. Nie udowodniono istotnego wpływu wapnowania na zawartość kadmu w ziarnie jęczmienia jarego i pszenicy ozimej, jednakże zaobserwowano tendencję do zmniejszania się zawartości kadmu w ziarnie zbóż pod wpływem wapnowania.

Nawożenie mineralne NPK stosowane w wysokich dawkach miało istotny wpływ na wzrost zawartości kadmu, szczególnie w ziarnie jęczmienia jarego, w mniejszym stopniu w ziarnie pszenicy ozimej. Nie stwierdzono istotnych statystycznie interakcji wapnowania i nawożenia mineralnego w kształtowaniu się zawartości kadmu w ziarnie badanych zbóż, jakkolwiek zawartość kadmu była generalnie niższa w ziarnie zbóż uprawianych w obiektach nawozowych z zastosowaniem wapnowania.

Słowa kluczowe: kadm, wapnowanie, nawożenie mineralne, jęczmień jary, pszenica ozima.

INTRODUCTION

Accumulation of cadmium in soils and plants is determined by the subsoil geological type (GORLACH 1995, KANIUCZAK, HAJDUK 1995, GORLACH, GAMBUŚ 1996, KANIUCZAK, HAJDUK 2000, LIPIŃSKI 2000), as well as any use of waste for fertilization (MERCIK et al. 1997, KOČAŘ et al. 2008) and intensive phosphorus fertilization (GORLACH, GAMBUŚ 1997, KANIUCZAK, HAJDUK 2000). Besides, atmospheric emissions caused by natural processes and human activities add their share of cadmium. Industry, including power industry, and transportation are among more significant sources of trace elements emitted to the atmosphere (PACYNA, PACYNA 2001, FRIESL-HANL et al. 2009).

Cadmium is extremely readily taken up by plants, both by their roots and leaves, usually in proportion to the cadmium content in the environment. Contamination of soil with cadmium may reduce the mass of aerial parts and vary the concentration of macronutrients in plants (WYSZKOWSKI, WYSZKOWSKA 2009). Cadmium is assimilated by plants independently from soil properties. Such good phyto-assimilation of cadmium poses a risk of its direct intake in excessive quantities. Cereal grains are a potential threat, too, because bakery and flour products constitute approx. 20% of human diet. Cadmium accumulation in wheat grain is positively correlated with the quantity of silt and with its content in soil (LIPIŃSKI 2000), but is negatively affected by the soil pH value and sorptive capacity (WŁAŚNIEWSKI 2000). Much research has dealt with the cadmium concentration in grain of cereals (wheat, rye, barley, oats) cultivated on soils with natural content of this element. Wherever the soil lay in a zone affected by refineries and oil-industry, the cadmium content in harvested grain exceeded acceptable levels for edible plants (INDEKA, KARACZUN 2000). The results of this research indicate another source of cadmium in cereal grain, i.e. atmospheric precipitation. It is very important for human health to reduce cadmium in cereal grain. Cadmium in wheat grain can be limited by soil liming (OLIVER et al. 1996). Liming and manure fertilization also lower the cadmium content in wheat and spring barley grain (SINGH et al. 1989). In addition, liming and mineral NPK Mg fertilization restrict the phyto-assimilation of cadmium (KANIUCZAK 1997). Cadmium solubility is controlled by soil pH (JANSSON 2002).

The purpose of this research has been to determine the effect of agrochemical treatments (liming and mineral fertilization) on the behavior of cadmium in grain of spring barley (*Hordeum vulgare* L.) and winter wheat (*Triticum aestivum* L.).

MATERIAL AND METHODS

In 1986-2001, a two-factor, strict experiment was conducted on a field subjected to static fertilization, situated in the Rzeszów Foothills (Podgórze Rzeszowskie). The experiment was set up in a random sub-block design with 4 replications. The area of each plot (fertilization treatment) was 30 m^2. The first variable was liming (A) and the second consisted of different mineral fertilization variants (B), independent from liming. In this experiment, the first sub-block was left without liming (A_1) and the second one was limed (A_2). The following crops were cultivated in a four-year crop rotation cycle: potato, spring barley, fodder sunflower, (fodder cabbage in the 1st crop rotation cycle) and winter wheat. The study covered 4 crop rotation cycles. Spring barley was grown in 1989, 1993, 1997 and 2001, whereas winter wheat was cultivated in 1987, 1991, 1995 and in 1999. The soil on which the experiments were set up was formed from loess of grey-brown podzolic type (Haplic luvisols), of the grain-size distribution of fine silt, and was characterized by high acidification in the Ap arable humus layer and Bt enrichment layer. The total content of trace elements (Cu, Zn, Ni, Co, Cd, Pb) in the soil was on the natural level and did not differ from their content in loess soils in other regions of Poland.

The mean content of cadmium in the mineral fertilizers applied in this experiment ranged from 0.03 mg Cd kg^{-1} in magnesium sulfate, through 0.36 mg Cd kg^{-1} in limestone oxide fertilizer, to 5.36 mg Cd kg^{-1} in triple superphosphate.

Liming was applied every 4 years (in a dose calculated per 1Hh), prior to sowing a consecutive crop in the rotation. Different mineral NPK fertilization covered 14 fertilized plots in each sub-block. The basic level of fertilization (N_1 P_1 K_1) was 80 kg N ha^{-1}, 100 kg P_2O_5 ha^{-1} and 120 kg K_2O ha^{-1} under spring barley and 90 kg N ha^{-1}, 80 kg P_2O_5 ha^{-1}, 100 kg K_2O ha^{-1} under winter wheat. Potatoes, spring barley and winter wheat were fertilized with magnesium in a dose of 40 kg MgO ha^{-1}; the magnesium dose for fodder sunflower was 120 kg MgO ha^{-1} (in crop rotations 1986-1989 and 1990-1993), but was reduced to 40 kg MgO ha^{-1} in the two subsequent crop rotations (1994-1997 and 1998-2001). Grain samples were dried at 105°C and mineralized in a mixture of concentrated acids (HNO_3 : $HClO_4$: H_2SO_4) in 20:5:1 proportions, using a Tecator digestion system DS 40. The cadmium content was determined using flame spectrophotometry (FAAS), after APDC complexing and extraction of the formed complex to the organic phase of methyl-isobutyl ketone (MIBK). The data were statistically processed using two-factor analysis of variance and calculating the lowest significant difference (LSD) with Tukey's test at significance levels p=0.05 and p=0.01.

RESULTS AND DISCUSSION

The mean content of cadmium in spring barley and winter wheat grain varied largely depending on liming (A) and mineral fertilization (B) – Tables 1, 2. Cadmium in grain of barley and wheat cultivated on unlimed soil varied within a wider range compared to the cereals grown on limed soil. The maximum threshold level for cadmium in edible plants, as set by the Polish Institute of Soil Science and Plant Cultivation (IUNG) (i.e. not higher than 0.15 mg kg^{-1} of dry matter), was exceeded in the grain of barley fertilized with the double dose of NPK fertilizer (KABATA-PENDIAS et al. 1993). The cadmium content in wheat grain was within the above standard. Cadmium in cereal grain harvested from the plots fertilized with the higher dose of N, P, K and NPK did not exceed the values established as the maximum permissible concentrations by the Act on Food and Fodder Safety of 25 August 2006 (Act… 2006) with later amendments, which corresponds to the EU regulations such as the Commission Regulation (EC) No. 466/2001 (*Regulation…* 2001) with subsequent amendments.

Cadmium appeared in varied concentrations in cereal grains during the years of the experiment (Tables 1, 2) and ranged between 0.050 and 0.120 mg kg^{-1} in 1993 or between 0.000 and 0.054 mg kg^{-1} in 1997 in grain of spring barley cultivated on unlimed soil. In another study (OLIVER et al. 1996), cadmium concentrations in cereal grain were even more variable.

Statistically, the cadmium content in grain of spring barley and winter wheat depended highly significantly on mineral fertilization (B), irrespectively of liming, and higher levels of this element occurred after using higher NPK doses (Table 1). Increasing cadmium concentrations in cereal grain are associated with phosphorus fertilization (Gorlach, Gambuś 1997, Kaniuczak 1997) and with deposition of dust from the atmosphere on the soil surface and on plants (Indeka, Karaczun 2000, Kabata-Pendias 2002). Wíngstrand et al. (2007) confirmed that the grain Cd concentration increased with an increasing N fertilizing rate, irrespective of the Cd concentration in soil and grain.

No evidence was found to support any statistically significant effect of liming on the cadmium content in grain of barley and wheat. However, less cadmium was found in grain of cereals cultivated on limed soil. Cadmium solubility in soil depends on soil pH (Singh et al. 1989, Gorlach, Gambuś 1996, Oliver et al. 1996, Kaniuczak, Hajduk 2000, Jansson 2002). Some research-

Table 1

Content of cadmium in grain of spring barley (mg kg^{-1} d.m.) cultivated on loessial soil (mean for 4 years)

Treatments of fertilizers (B)	A_1		A_2		Mean (B)
	mean	range	mean	range	
N_0 P_0 K_0	0.097	0.02-0.16	0.082	0.03-0.15	0.089
N_0 P_1 K_1	0.098	0.02-0.17	0.064	0.01-0.10	0.081
$N_{0.5}$ P_1 K_1	0.083	0.03-0.17	0.078	0.05-0.13	0.080
N_1 P_1 K_1	0.061	0.00-0.09	0.047	0.02-0.10	0.054
$N_{1.5}$ P_1 K_1	0.111	0.09-0.21	0.051	0.01-0.10	0.081
N_1 P_0 K_1	0.086	0.09-0.13	0.066	0.02-0.10	0.076
N_1 $P_{0.5}$ K_1	0.099	0.09-0.18	0.063	0.02-0.12	0.081
N_1 $P_{1.5}$ K_1	0.121	0.04-0.24	0.047	0.01-0.08	0.084
N_1 P_1 K_0	0.069	0.06-0.18	0.030	0.00-0.05	0.049
N_1 P_1 $K_{0.5}$	0.050	0.06-0.11	0.047	0.05-0.10	0.048
N_1 P_1 $K_{1.5}$	0.131	0.03-0.17	0.093	0.04-0.26	0.112
$N_{0.5}$ $P_{0.5}$ $K_{0.5}$	0.150	0.09-0.18	0.119	0.05-0.31	0.134
$N_{1.5}$ $P_{1.5}$ $K_{1.5}$	0.145	0.04-0.27	0.138	0.04-0.25	0.141
N_2 P_2 K_2	0.171	0.05-0.32	0.165	0.05-0.30	0.168
Mean of A	0.100	-	0.076	-	-
LSD	A = ns, B*= 0.024 AB = ns				

A_1 – NPK fertilization + Mg constans, A_2 – NPK fertilization + Mg, Ca constans,
LSD – lowest significant difference for: A – liming, B – mineral fertilization (irrespective of liming),
AB – interaction between liming and mineral fertilization, *significant at $p = 0.01$,
ns – differences not significant

ers (Singh et al. 1989, Gorlach, Gambuś 1996, Oliver et al. 1996, Kaniuczak 1997, Jansson 2002) suggest that it is possible to reduce the content of cadmium in plants through liming. An increase in pH values from 4 to 5 as a result of applying burnt lime reduced the cadmium concentration in wheat grain (Oliver et al. 1996). In most of the experiments carried out by Jansson (2002), the cadmium content in plants depended significantly on soil pH. Our own studies did not demonstrate statistically significant interaction between liming and mineral fertilization (AB). Nonetheless, on all the fertilized objects that were limed, the cadmium content in grain of the analyzed cereals was lower, compared to grain harvested from on unlimed soil (Table 1, 2).

Table 2

Content of cadmium in grain of winter wheat (mg kg^{-1} d.m.) cultivated on loessial soil (mean for 4 years)

Treatments of fertilizers (B)	A_1		A_2		Mean (B)
	mean	range	mean	range	
N_0 P_0 K_0	0.077	0.05-0.10	0.040	0.02-0.06	0.058
N_0 P_1 K_1	0.091	0.07-0.12	0.033	0.00-0.05	0.062
$N_{0.5}$ P_1 K_1	0.085	0.03-0.14	0.034	0.00-0.06	0.059
N_1 P_1 K_1	0.089	0.04-0.14	0.023	0.00-0.07	0.056
$N_{1.5}$ P_1 K_1	0.107	0.09-0.14	0.099	0.08-0.11	0.103
N_1 P_0 K_1	0.067	0.00-0.11	0.062	0.00-0.12	0.064
N_1 $P_{0.5}$ K_1	0.099	0.04-0.17	0.095	0.07-0.14	0.097
N_1 $P_{1.5}$ K_1	0.103	0.07-0.14	0.068	0.01-0.11	0.085
N_1 P_1 K_0	0.062	0.00-0.10	0.045	0.01-0.09	0.053
N_1 P_1 $K_{0.5}$	0.070	0.03-0.09	0.046	0.04-0.08	0.058
N_1 P_1 $K_{1.5}$	0.056	0.01-0.08	0.036	0.03-0.06	0.046
$N_{0.5}$ $P_{0.5}$ $K_{0.5}$	0.078	0.02-0.10	0.031	0.02-0.06	0.054
$N_{1.5}$ $P_{1.5}$ $K_{1.5}$	0.102	0.07-0.12	0.096	0.06-0.14	0.099
N_2 P_2 K_2	0.120	0.04-0.16	0.100	0.07-0.15	0.110
Mean of A	0.086	-	0.058	-	-
LSD	A = ns, B*= 0.030 AB = ns				

A_1 – NPK fertilization + Mg constans, A_2 – NPK fertilization + Mg, Ca constans,
LSD – lowest significant difference for: A – liming, B – mineral fertilization (irrespective of liming),
AB – interaction between liming and mineral fertilization, *significant at $p = 0.01$,
ns – differences not significant

The cadmium content in grain of the examined cereals was within the range provided by some monitored research (Kabata-Pendias 2002, Szteke, Boguszewska 2000). Among the analyzed grain, wheat grain usually accumulates more cadmium (Indeka, Karaczun 2000, Wĺngstrand et al. 2007). These values are comparable with the ones found in other European countries and in the other parts of the world.

CONCLUSIONS

1. Liming did not prove to have any significant effect on the cadmium content in grain of spring barley and winter wheat. However, in response to this treatment, the cadmium content in cereal grain tended to decline.
2. Mineral NPK fertilization, applied in higher doses, had significant impact by raising the cadmium content, especially in spring barley grain and to a lesser degree in winter wheat grain.
3. No statistical relationship was proven between liming and mineral fertilization in shaping the cadmium content in grain of spring barley or winter wheat. However, the cadmium content was generally lower in grain of cereals cultivated on fertilized objects where liming was applied.
4. The cadmium content in grains of spring barley and winter wheat, fertilized with high doses of NPK, did not exceed the level (0.20 mg Cd kg^{-1} of fresh matter) considered as the maximum permissible one in crops intended for human consumption.

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CONTENT OF AVAILABLE MAGNESIUM, PHOSPHORUS AND POTASSIUM FORMS IN SOIL EXPOSED TO VARIED CROP ROTATION AND FERTILISATION

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Abstract

Soil richness in available magnesium, phosphorus and potassium forms is one of the key factors of fertility, which ensures the potential of soil for satisfying nutritional requirements of plants. The aim of the present research has been to determine the effect of crop rotation and varied mineral and organic fertilisation on the content of available Mg, K and P forms. Soil was sampled from a long-term experiment, carried out on Luvisol formed from sandy loam (soil valuation class IVa, very good rye complex). The experiment was performed in a 3-factor design, which included two types of crop rotation as well as FYM and nitrogen fertilisation. Basic physicochemical properties of soil were determined. The content of available forms of magnesium was defined with Schachtschabel method and the content of potassium and phosphorus – with Egner-Riehm method (DL). The reaction in the arable-humus horizon of the soils ranged from 4.7 to 6.2. It was found that the Corg to Nt ratio in the arable-humus horizon of soils of all the experiment variants was typical of biologically active soils. The content of available magnesium ranged from 27.8 to 58.3 mg kg^{-1} of soil, while its water-soluble forms varied from 3.5 to 6.8 mg kg^{-1} of soil. The highest content of magnesium, potassium and phosphorus forms available to plants was observed after the application of FYM in the doses of 60 and 80 t ha^{-1} combined with mineral nitrogen fertilisation, in both crop rotation regimes. The content of magnesium and phosphorus forms available to plants was significantly positively correlated with the content of organic carbon. Soil sampled from those plots demonstrated higher classes of the content of that element. The research data proved that the analysed soils showed moderate and high richness in nutrients available to plants. High doses of organic

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and mineral fertilisation, however, did not increase significantly the electrolytic conductivity of the soil solution.

Key words: soil, available nutrients (Mg, K, P), long-term experiment, fertilization.

ZAWARTOŚĆ PRZYSWAJALNYCH FORM MAGNEZU, FOSFORU I POTASU W GLEBIE POD WPŁYWEM ZMIANOWANIA I NAWOŻENIA

Abstrakt

Zasobność gleby w dostępne dla roślin formy magnezu, fosforu i potasu jest jednym z ważniejszych elementów jej żyzności, warunkujących możliwości gleby do zaspokojenia potrzeb pokarmowych roślin. Celem badań było określenie wpływu zmianowania i zróżnicowanego nawożenia mineralno-organicznego na zawartość przyswajalnych form Mg, K i P. Próbki do badań pobrano z wieloletniego doświadczenia statycznego, prowadzonego na glebie płowej wytworzonej z gliny lekkiej (IVa klasa bonitacyjna, kompleks żytni bardzo dobry). Doświadczenie prowadzono w układzie 3-czynnikowym, obejmującym: 2 typy zmianowania; nawożenie obornikiem i nawożenie azotem. Oznaczono podstawowe właściwości fizykochemiczne gleb. Zawartość przyswajalnych form magnezu określono metodą Schachtschabela, a potasu i fosforu metodą Egnera-Riehma (DL). Odczyn w poziomie orno-próchnicznym badanych gleb wahał się od 4,7 do 6,2. Stwierdzono, że stosunek Corg do Nt w poziomie orno-próchnicznym gleb we wszystkich wariantach doświadczenia był typowy dla gleb aktywnych biologicznie. Zawartość magnezu przyswajalnego wynosiła od 27,8 do 58,3 mg kg^{-1} gleby, natomiast jego form wodno-rozpuszczalnych od 3,5 do 6,8 mg kg^{-1} gleby. Najwyższą zawartość przyswajalnych dla roślin form magnezu, potasu i fosforu zaobserwowano po zastosowaniu obornika w dawkach 60 i 80 t ha^{-1} łącznie z nawożeniem azotem mineralnym, w obu rodzajach zmianowania. Zawartość form magnezu i fosforu przyswajalnych dla roślin była istotnie dodatnio skorelowana z zawartością węgla organicznego. Glebę z tych poletek można było zaliczyć do wyższych klas zawartości tego pierwiastka. Na podstawie wyników badań stwierdzono, że analizowane gleby charakteryzowały się średnią i wysoką zasobnością w składniki pokarmowe dla roślin. Wysokie dawki nawożenia organicznego i mineralnego nie wpłynęły w istotny sposób na wzrost przewodnictwa elektrolitycznego roztworu glebowego.

Słowa kluczowe: gleba, składniki pokarmowe (Mg, K, P), doświadczenie wieloletnie, nawożenie.

INTRODUCTION

Intensive agricultural use of soil frequently causes soil degradation. Unbalanced fertilisation, inadequate crop rotation or defective agricultural soil reclamation treatments can trigger unfavourable changes in the soil properties connected with their acidity, inferior sorption capacity, depleted organic matter resources as well as a lower content of nutrients available to plants. The soil richness in magnesium, potassium and phosphorus forms available to plants is a key fertility factor, which ensures the potential for satisfying the nutritional requirements of plants (ASKEGAARD et al. 2005). Numerous research centres perform long-term, multifactor experiments to determine the effect of crop rotation and varied organic and mineral fertilisation on the properties and parameters connected with soil fertility (Mercik et al. 2000, Jaskulska, Jaskulski 2003, Murawska, Spychaj-Fabisiak 2009). The chemical composition of soil solution and the concentration of particular elements determine the uptake of nutrients by plants. As for magnesium and potassium, a considerable share of these metals occurs in a form strongly bound to or incorporated in crystalline forms of minerals. Those are the resources which make up hardly available soil reserve of these elements (Błaszczyk 1998). Plants can use some amounts of potassium in the non-exchangeable form, derived from interpackage layers of clay minerals, mostly illites (Kobierski, Dąbkowska-Naskręt 2005). The content of nutrients available to plants is affected by the reaction and soil grain size composition (Lipiński, Bednarek 1998; Rutkowska et al. 2006). A considerable share of phosphorus taken up by plants occurs as ions HPO_4^{2-} and $H_2PO_4^-$ in the soil environment of pH 5.0. Alkaline and strongly acid reactions depress the availability of phosphorus to plants. The content of water-soluble forms of Mg, K, P and Ca in arable soils is usually higher than the content of those elements in forest soil solution (Smal, Misztal 1996). Fertilised soils contain relatively more nutrients than non-fertilised ones. By taking up nutrients, crops reduce their content in soil and determine their circulation. Most frequently, it is the circulation of phosphorus and potassium, which usually accumulate in the soil surface layer, that is affected (Jobbagy, Jackson 2001, Fotyma et al. 2005).

The aim of the present research has been to determine the effect of crop rotation and varied mineral-and-organic fertilisation on the content of nutrients available to plants as well as on selected physicochemical properties of soil. The following were determined: the content of magnesium, potassium and phosphorus available to plants and their water-soluble forms, as well as the electrolytic conductivity of soil solution for respective experimental variants.

MATERIAL AND METHODS

The research material was made up of soil sampled from the arable-humus horizon of soils after 22 years of a long-term experiment located at the IUNG Research Station in Grabów near Puławy. The experiment was set up in 1980 on Luvisol: Ap-Eet-Bt-C1-C2 (IUSS Working Group WRB 2007) of the grain size composition of loamy fine sand and fine sandy loam in surface horizons and sandy loam in the parent material. The soils belonged to the very good rye complex, soil valuation class IVa. The content of carbon in organic compounds (C_{org}) in the soil prior to the establishment of the experiment was 7.7 g kg^{-1}.

The field experiment was performed in a 3-factor design:

Factor I: crop rotation A – plants considered as humus-depleting ones (potato, winter wheat, spring barley, corn) and crop rotation B – plants considered as humus-enriching ones (potato, winter wheat + white mustard in intercrop, barley with an undersown mixture of red clover with grasses), factor II: different rates of FYM fertilisation under potato (0, 20, 40, 60, 80 t ha^{-1}); factor III: different rates of mineral nitrogen fertilisation (N0 – no nitrogen; N1 – 170 kg N ha^{-1} in crop rotation A and 275 kg N ha^{-1} in crop rotation B; N2 – 340 kg N ha^{-1} in crop rotation A and 550 kg N ha^{-1} in crop rotation B) per rotation. Throughout the experiment, phosphorus and potassium fertilisers were applied under respective plant species in the following doses: P_2O_5 – potato, spring barley and winter wheat – 60 kg ha^{-1}, corn and a mixture of red clover with grasses – 80 kg ha^{-1}; K_2O – potato: 140 kg ha^{-1}, spring barley: 90 kg ha^{-1}, winter wheat: 100 kg ha^{-1}, corn and a mixture of red clover with grasses: 160 kg ha^{-1}. The experiment was performed in four replications for each treatment.

The following were determined in the soil samples: the grain size composition with the Cassagrande aerometric method, modified by Prószyński; pH – potentiometrically in 1M KCl solution; the content of organic carbon and total nitrogen with a TOCN FORMACS^{TM} analyser provided by *SCALAR*. The content of available phosphorus – P_A and potassium – K_A was determined with Egner-Riehm method (DL), whereas the content of magnesium – Mg_A – with Schachtschabel method. The content of water-soluble (active) cations and the electrolytic conductivity (EC) were defined in distilled water extract in a 1:5 soil to water ratio. The content of elements in the extracts was determined using an atomic absorption spectrometer (Philips PU 9100X). The results were statistically verified with Statistica 8.0 software.

RESULTS AND DISCUSION

The arable-humus horizon of the analysed soils was found to represent the grain size composition of loamy fine sand and fine sandy loam (Table 1). The experiment was established on soils in which the sand fraction prevailed and the content of clay fraction was relatively low. According to the breakdown into agronomic categories, the soil was classified as light one. The soil reaction was acid and slightly acid and the highest acidity was recorded in the soil samples of crop rotation B. It was found that mineral fertilisation in crop rotation A, without FYM and with FYM in the doses of 20 and 40 t ha^{-1}, resulted in a decrease in the content of Corg in soil. It was not until the higher FYM doses (60 and 80 t ha^{-1}) were applied that the content of organic carbon in soil increased. In crop rotation B, an average 25% increase in the Corg content in soil was identified as compared with its content in soil prior to the experiment (7.7 g kg^{-1}). The content

Table 1

Selected soil properties of long-term field experiment soils

Experimental treatments	pH KCl	C_{org}	Nt	C_{org}/N_t	EC (μS cm^{-1})	Sand	Silt	Clay
		(g kg^{-1})				(%)		
A-0-N0	5.9	6.8	0.6	13.0	330.8	75	20	5
A-0-N1	6.0	6.5	0.6	10.8	342.5	72	23	5
A-0-N2	5.7	6.6	0.6	11.0	333.3	71	25	4
A-20-N0	5.8	6.2	0.6	10.3	333.6	70	24	6
A-20-N1	5.9	6.9	0.6	11.5	350.6	71	25	4
A-20-N2	6.0	6.4	0.6	10.7	343.2	75	23	4
A-40-N0	5.8	7.6	0.7	10.9	342.5	77	18	5
A-40-N1	5.7	7.2	0.7	10.3	412.3	78	18	4
A-40-N2	5.5	7.1	0.7	10.1	357.0	77	19	4
A-60-N0	6.2	8.7	0.7	12.4	428.0	61	34	5
A-60-N1	6.0	8.2	0.7	11.7	439.6	71	22	7
A-60-N2	5.9	7.8	0.7	11.1	419.8	78	16	6
A-80-N0	5.9	7.4	0.7	10.6	343.9	68	25	7
A-80-N1	5.9	7.8	0.7	11.1	522.3	70	25	5
A-80-N2	5.8	8.0	0.7	11.4	554.9	67	26	7
B-0-N0	4.9	8.8	0.8	11.0	386.5	71	23	6
B-0-N1	4.9	8.3	0.8	10.4	337.9	78	16	6
B-0-N2	4.7	9.3	0.9	10.3	352.8	69	23	8
B-20-N0	5.0	9.6	0.9	10.7	354.2	74	21	8
B-20-N1	5.0	9.6	0.9	10.7	361.6	77	15	8
B-20-N2	5.1	9.8	0.9	10.9	465.2	70	24	6
B-40-N0	5.1	9.3	0.9	10.3	347.5	66	26	8
B-40-N1	5.1	9.7	0.9	10.8	413.8	69	25	6
B-40-N2	5.0	9.6	0.9	10.7	395.3	75	20	5
B-60-N0	5.1	9.9	0.9	11.0	318.7	71	24	5
B-60-N1	5.4	10.1	0.9	11.2	329.7	70	24	6
B-60-N2	5.3	9.6	0.9	10.7	347.1	68	25	6
B-80-N0	5.3	10.1	0.9	11.2	397.8	74	22	4
B-80-N1	5.4	10.2	1.0	10.2	491.0	69	25	6
B-80-N2	5.3	10.9	1.0	10.9	390.7	73	22	5

A, B – crop rotation, 0-80 – FYM doses in t ha^{-1}, N0, N1, N2 – nitrogen dose kg ha^{-1},
EC – electrolytic conductivity

of total nitrogen (N_t) in the analysed soils was determined by all the three experimental factors. In crop rotation B, the N_t content in soil was higher than in soil from the crop rotation A plots. The ratio of carbon to nitrogen in the arable-humus horizon of soils in all the experimental variants was typical of biologically active soils.

Soil abundance in magnesium, potassium and phosphorus available to plants is one of the key soil fertility factors. The content of available magnesium forms in the tested soils ranged from 27.8 to 58.3 mg kg^{-1} (Table 2). It was slightly lower than reported in Luvisols by Krauze and Bowszys (1998). The statistical analysis showed that the FYM fertilisation had a significant effect on the content of available magnesium forms in both crop rotation variants. The content of available magnesium rose by 39.2% (the dose of 80 t ha^{-1}) compared with its content in soil from plots not amended with FYM (Table 3), while the statistical analysis did not demonstrate any significant effect of mineral fertilisation on the content of available magnesium.

The content of potassium available to plants ranged from 52.4 to 234.0 mg kg^{-1} (Table 2). In the samples from crop rotation A plots, its average content was 124.6 mg kg^{-1}, thus being 27.7% higher than recorded in the crop rotation B soil. A significant effect on the content of available potassium in soil was also noted for FYM fertilisation, especially with doses of 60 and 80 t ha^{-1}. The soil from these plots belonged to higher classes of the content of this element. According to Askegaard et al. (2005), the content of available potassium is mostly determined by the grain size composition, mineral composition, soil moisture and the amount of potassium supplied as mineral fertiliser and FYM. Long-term FYM application most considerably increases the content of exchangeable and active potassium and, to a much lesser extent, available potassium (Szymańska et al. 2005).

The statistical analysis did not show any significant effect of the crop rotation applied in the field experiment on the content of available phosphorus (Table 2). Its average content in the soil samples from the crop rotation A plots was 76.7, while in the soil sampled from crop rotation B it equalled 78.5 mg kg^{-1}. Likewise, no effect of mineral fertilisation on the content of available phosphorus forms was detected. However, FYM fertilisation was found to have produced a significant effect on the content of this form of phosphorus (Table 3). Sienkiewicz et al. (2009) claimed that a combined application of FYM and mineral fertilisers resulted in a significant increase in the content of Mg, P and K available to plants, as compared with the content recorded following the application of mineral fertilisation alone.

The content of water-soluble magnesium (Mg-H_2O), referred to as an active form of this element, ranged from 3.5 to 6.8 mg kg^{-1} (Table 2). The statistical analysis did not show any significant effect of the crop rotation variants on the content of water-soluble magnesium (Table 4). A different effect was reported for FYM fertilisation. The average Mg-H_2O content in the soil sampled from the plots exposed to organic fertilisation tended to

Table 2

Content of available nutrients and their water-soluble forms (mg kg^{-1})

Experimental treatments	Available forms			Content of water-soluble forms			
	Mg_A	K_A	P_A	Mg-H_2O	K-H_2O	Ca-H_2O	Na-H_2O
	(mg kg^{-1})						
A-0-N0	27.8	83.3	68.0	3.6	31.0	10.9	2.7
A-0-N1	28.8	74.9	56.2	4.0	38.1	10.7	2.4
A-0-N2	29.2	60.2	57.4	3.6	32.2	10.1	1.6
A-20-N0	35.2	90.0	58.8	4.4	36.7	11.0	
A-20-N1	35.3	83.2	68.6	4.3	35.8	13.2	4.2
A-20-N2	37.8	98.5	61.4	4.5	34.1	14.4	2.9
A-40-N0	39.4	151.5	73.3	4.4	33.0	11.0	3.3
A-40-N1	42.8	105.0	72.4	5.4	47.3	12.7	2.9
A-40-N2	31.3	82.6	77.7	3.8	34.3	12.0	2.9
A-60-N0	45.5	173.0	97.0	5.3	49.8	14.2	2.9
A-60-N1	51.0	166.1	98.0	5.7	56.3	14.4	5.2
A-60-N2	47.6	172.7	102.4	4.8	54.5	13.0	2.0
A-80-N0	48.5	151.2	89.0	4.3	38.2	15.6	4.7
A-80-N1	49.0	234.1	88.4	6.0	64.0	16.1	2.9
A-80-N2	49.4	142.8	82.1	5.7	47.0	16.5	2.7
B-0-N0	35.5	61.2	77.2	3.9	16.3	5.2	1.0
B-0-N1	31.9	53.4	68.3	4.3	18.0	4.9	1.3
B-0-N2	32.1	67.9	74.5	3.8	15.8	5.1	1.5
B-20-N0	36.7	61.3	70.2	3.5	23.9	6.0	1.2
B-20-N1	36.6	53.3	71.8	5.0	21.4	7.1	1.9
B-20-N2	38.6	52.4	70.9	4.3	20.2	7.8	2.2
B-40-N0	40.8	82.8	80.0	5.0	33.5	8.8	3.4
B-40-N1	42.8	83.1	68.0	4.3	28.5	7.6	2.3
B-40-N2	39.5	68.5	60.3	3.6	22.2	6.1	1.1
B-60-N0	48.3	105.2	86.1	5.7	44.0	13.8	8.5
B-60-N1	48.6	135.1	90.4	6.3	37.7	16.2	3.1
B-60-N2	46.7	98.0	82.5	4.9	35.2	10.5	6.8
B-80-N0	57.3	166.1	96.7	5.3	56.2	13.0	2.0
B-80-N1	58.3	157.7	94.8	6.8	56.2	15.8	2.8
B-80-N2	42.4	106.0	86.0	6.1	48.5	11.3	9.3

A, B – crop rotation, 0-80 – FYM dose in t ha^{-1} FYM, N0, N1, N2 – nitrogen doses kg ha^{-1}

Table 3

Results of the analysis of variance for the content of available magnesium, potassium and phosphorus forms in soil (mg kg^{-1})

Factors		Mg_A		K_A		P_A	
I – crop rotation	A	39.9	LSD n.s.	124.6	LSD 18.57	76.7	LSD n.s.
	B	42.4		90.1		78.5	
II – FYM (t ha^{-1})	0	30.9	LSD 7.17	66.8	LSD 43.99	66.9	LSD 9.36
	20	36.7		73.1		66.9	
	40	39.4		95.5		71.9	
	60	47.9		141.6		92.7	
	80	50.8		159.6		89.5	
III – nitrogen fertiliser (kg N ha^{-1})	N0	41.5	LSD n.s.	112.5	LSD n.s.	79.6	LSD n.s
	N1	42.5		114,5		77.6	
	N2	39.4		94.9		75.5	

A, B – crop rotation, 0-80 – FYM doses in t ha^{-1}, N0, N1, N2 – nitrogen doses kg ha^{-1}

increase when an FYM dose was applied. Analogously, mineral fertilisation has a significant effect on the content of active magnesium in soil. The highest content of the active form of this element was found for the doses of mineral nitrogen variant N1. The content of water-soluble magnesium in the analysed soils was twice as high as the one observed by MURAWSKA and SPYCHAJ-FABISIAK (2009) in soil under long-term field experiment.

Water-soluble potassium in soil was significantly affected by all the three experimental factors. A higher content of the water-soluble potassium form was reported in the soil samples where crop rotation A had been applied, as compared with the soil under crop rotation B, while organic fertilisation showed a similar effect on water-soluble potassium as in the case of the Mg-H_2O content. Similarly, the content of water-soluble potassium in soil tended to rise as the FYM doses increased (Table 4). The statistical analysis showed that mineral fertilisation affected the content of active potassium forms. The highest content of water-soluble potassium in soil was noted for nitrogen dose N1.

The content of water-soluble calcium in soil ranged from 4.9 to 16.5 mg kg^{-1} (Table 2). A significant effect of both crop rotation and organic fertilisation on the content of Ca-H_2O in soil was detected. The average content of active calcium in the soil samples exposed to crop rotation A was 13.0 mg kg^{-1}, being 28.5% higher than in the soil under crop rotation B (Table 4). It was also found that the content of Ca-H_2O in soil increased with the increasing FYM doses, from 7.8 mg kg^{-1} for the dose of 0 t ha^{-1} to 14.7 mg kg^{-1} for the dose of 80 t ha^{-1}. However, no significant effect of any of the experimental factors on the content of water-soluble sodium in the analysed soil samples was confirmed.

Table 4

Results of the analysis of variance for the content of water-soluble cations of Mg^{2+}, K^+, Ca^{2+}, Na^+ in soil (mg kg^{-1})

Factors		Mg-H_2O		K-H_2O		Ca-H_2O		Na-H_2O	
I – crop rotation	A	4.6	LSD n.s.	42.1	LSD 3.13	13.0	LSD 0.91	3.1	LSD n.s.
	B	4.8		31.8		9.3		3.2	
II – FYM (t ha^{-1})	0	3.8	LSD 0.85	25.2	LSD 7.43	7.8	LSD 2.14	1.7	LSD n.s.
	20	4.3		28.6		9.9		2.7	
	40	4.4		33.1		9.7		2.6	
	60	5.4		46.2		13.6		4.7	
	80	5.7		51.6		14.7		4.1	
III – nitrogen fertilisation (kg N ha^{-1})	N0	4.5	LSD 0.54	36.2	LSD 4.76	10.9	LSD n.s.	3.3	LSD n.s.
	N1	5.2		40.3		11.8		2.9	
	N2	4.5		34.4		10.6		3.3	

A, B – crop rotation, 0-80 – FYM doses in t ha^{-1}, N0, N1, N2 – nitrogen doses kg ha^{-1}

In biologically active soils, the content of elements available to plants depends mostly on the reaction, grain size composition, amount and quality of humus compounds, oxidation-reduction potential and on the interactions between individual parameters (Bronick, Lal 2005). The soil use is a limiting factor for available magnesium soil resources, although when we compare the analysed arable soils with Luvisols under orchards (Licznar, Licznar 2008) which had similar humus content, grain size composition and pH, the determined differences begin to blur. Rutkowska et al. (2006) report that the ion concentration in soil solution depends on the grain size composition, and especially on the content of silt and clay fractions. Similarly, Holmqvist et al. (2003) claim that the amount of potassium released to soil solution in mineral soil depends on its grain size composition and the intensity of mineral weathering. In the analysed soils, however, no such relationship was noted. The content of organic carbon was significantly positively correlated with the content of magnesium and phosphorus available to plants and with the concentration of water-soluble magnesium (Table 5). Significantly positive correlation was verified between the content of available magnesium and the content of potassium and phosphorus available to plants (r=0.75 and 0.80 for p<0.05). High organic and mineral fertilisation doses applied during the 22 years of the field experiment did not have any significant effect on the electrolytic conductivity of the soil solution. By analogy, Murawska and Spychaj-Fabisiak (2009) did not observe any effect of long-term intensive fertilisation with nitrogen and potassium (32 years of a field experiment) on soil salinity.

Table 5

Significant coefficients of correlation between the investigated parameters (n=35)

Parameters	Mg_A	K_A	P_A	$Mg\text{-}H_2O$	$K\text{-}H_2O$	$Ca\text{-}H_2O$	$Na\text{-}H_2O$
EC	0.54	0.52	0.42	0.49	0.50		
Corg	0.44		0.42	0.39			
Mg_A		0.75	0.80	0.79	0.71	0.61	
K_A			0.75	0.65	0.86	0.78	
P_A				0.68	0.66	0.53	
$Mg\text{-}H_2O$					0.73	0.66	0.40
$K\text{-}H_2O$						0.83	

EC – electrolytic conductivity; confidence level $p<0.05$

CONCLUSIONS

1. FYM fertilisation significantly affected the content of magnesium, potassium and phosphorus forms available to plants. High content of those elements was recorded following the application of 40, 60 and 80 t FYM ha^{-1}.

2. The crop rotation applied had a significant effect on the content of potassium available to plants. More potassium was noted following the humus-depleting crop rotation variant.

3. Significant effect of FYM fertilisation in the doses of 60 and 80 t ha^{-1} and mineral fertilisation in the dose of 170 kg N ha^{-1} on the content of water-soluble forms of magnesium and potassium was detected. The content of active potassium in soil was also significantly affected by crop rotation.

4. The content of magnesium and phosphorus forms available to plants was significantly positively correlated with the organic carbon content. Significantly positive correlation between the content of magnesium and potassium available to plants and the content of their water-soluble forms was confirmed.

5. The correlation results did not imply any significant effect of organic and mineral fertilisation on the electrolytic conductivity of the soil solution.

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CONTENT OF MACRONUTRIENTS AND VALUES OF MOLE RATIOS IN LEAVES OF THE WOOLLY FOXGLOVE (*DIGITALIS LANATA* EHRH.) CULTIVATED UNDER DIFFERENTIATED MINERAL FERTILISATION

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Abstract

The Woolly Foxglove (*Digitalis lanata*) is one of the herbal plants cultivated in Poland. It is grown as important plant material for production of cardenolides used in the pharmaceutical industry. The aim of the research has been to determine the chemical composition and mole ratios in leaves of cv. Victoria Woolly Foxglove affected by mineral fertilisation. For this purpose, a strict, one-factor, micro-plot field experiment was set up in randomised blocks with four replications. The experimental factor was NPK mineral fertilisation applied to soil in the following doses per hectare. A_0 – no mineral fertilisation, A_1 – 40 kg N + 17.48 kg P + 49.8 kg K, A_2 – 80 kg N + 34.96 kg P + 99.6 kg K, A_3 – 120 kg N + 52.44 kg P + 149.4 kg K. For chemical analyses, samples of 0.50 kg of leaves were taken from each plot. Dried, melted and wet mineralised in sulphuric(VI) acid, the samples were analysed and concentrations of the following elements were determined: nitrogen by Kjeldahl method, total phosphorus by the colorimetric method with ammonium molybdate, potassium, calcium and sodium by flame photometry, and magnesium by atomic absorption spectrometry (AAS). Mole ratios of the chemical elements in leaves were also calculated. In general, the use of mineral fertilisation induced a significant increase in the content of total nitrogen, total phosphorus and potassium, but reduced the sodium concentration in leaves of the Woolly Foxglove compared to the control. After the applied fertilisation doses, the K:Ca and K:Na ratios in leaves were significantly broadened and the Ca:P ratio was narrowed. Significantly higher values of the K:(Ca + Mg) ratio were observed in leaves after applying the medium (A_2) and highest NPK ha^{-1} (A_3) doses. The Ca:Mg ratio in leaves of the Woolly Foxglove was distinctly narrowed versus the control after using the

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lowest fertilisation dose (A1). However, after applying the medium dose of NPK (A2), the K:Mg ratio was broader than in the control treatment.

Key words: Woolly Foxglove, fertilisation, macronutrients, chemical composition, mole ratios.

ZAWARTOŚĆ MAKROELEMENTÓW I WARTOŚCI STOSUNKÓW MOLOWYCH W LIŚCIACH NAPARSTNICY WEŁNISTEJ (*DIGITALIS LANATA* EHRH.) UPRAWIANEJ W WARUNKACH ZRÓŻNICOWANEGO NAWOŻENIA MINERALNEGO

Abstrakt

Jedną z roślin zielarskich uprawianych w Polsce jest naparstnica wełnista. Stanowi ona ważny surowiec kardenolidowy wykorzystywany w przemyśle farmaceutycznym. Celem badań była ocena składu chemicznego oraz proporcji molowych w liściach naparstnicy wełnistej odmiany Victoria pod wpływem zróżnicowanego nawożenia mineralnego. W związku z powyższym założono ścisłe jednoczynnikowe doświadczenie mikropoletkowe, metodą losowanych bloków, w czterech powtórzeniach. Czynnikiem doświadczenia było nawożenie mineralne NPK zastosowane doglebowo w następujących dawkach na 1 ha: A_0 – bez nawożenia mineralnego, A_1 – 40 kg N + 17,48 kg P + 49,8 kg K, A_2 – 80 kg N + 34,96 kg P + 99,6 kg K, A_3 – 120 kg N + 52,44 kg P + 149,4 kg K. Do analiz chemicznych pobrano reprezentatywne próby w ilości po 0,50 kg liści z każdego poletka. Po wysuszeniu, zmieleniu i mineralizacji na mokro w kwasie siarkowym(VI) określono w nich zawartości: azotu ogólnego metodą Kjeldahla, fosforu ogólnego metodą kolorymetryczną z molibdenianem amonu, potasu, wapnia oraz sodu metodą fotometrii płomieniowej, a magnezu metodą absorpcyjnej spektroskopii atomowej. Obliczono również stosunki molowe pierwiastków w liściach. Zastosowane nawożenie mineralne, w odniesieniu do obiektu kontrolnego, powodowało na ogół istotny wzrost zawartości azotu ogólnego, fosforu ogólnego, potasu, natomiast zmniejszenie koncentracji sodu w liściach naparstnicy wełnistej. Pod wpływem stosowanych dawek stwierdzono istotne rozszerzenie wartości stosunków K:Ca i K:Na oraz zawężenie Ca:P w liściach. Znaczące rozszerzenie wartości stosunku K:(Ca + Mg) w liściach uzyskano po zastosowaniu dawek: średniej (A_2) oraz najwyższej NPK ha^{-1} (A_3). Wartość stosunku Ca:Mg w naparstnicy uległa wyraźnemu zawężeniu w porównaniu z obiektem kontrolnym, po zastosowaniu dawki najniższej (A_1). Natomiast po aplikacji średniej dawki NPK (A_2) stwierdzono rozszerzenie stosunku K:Mg w odniesieniu do obiektu kontrolnego.

Słowa kluczowe: naparstnica wełnista, nawożenie, makroelementy, skład chemiczny, stosunki jonowe.

INTRODUCTION

Growing herbs is one of the newest plant production branches. According to the FAO data, about 50 thousand plant species are used by man, including 700 grown by farmers. It has been estimated that about 15-20 thousand species, i.e. 3 to 5% of the global plant resources, are used for medicinal purposes (SEIDLER-ŁOŻYKOWSKA 2009). Poland produces around 50% of the herbal raw material made in Europe, which corresponds to about 20% of the global production. Thus, Poland belongs to the countries with high herbal production potential, verified by high quality of herbal raw material and herbal processing (MIKOŁAJCZYK-GRZELAK 2008). The Woolly Foxglove, an important raw material for cardenolides, is one of the plants cultivated in Poland. It is a biennial plant, which in the first year forms a rosette of lanceolate, dark green leaves that are harvested together with seeds in the following year, making tradable raw material. In the second year of vegetation, a foxglove plant produces a stem which is about 150 cm long. The flower head of *Digitalis lanata* forms a bunch consisting of numerous bell-shaped flowers white and creamy-brown in colour. The plant blooms in late June and early July. Seeds mature gradually from August to the end of September. Cardiac glycosides contained in leaves of the Woolly Foxglove (SUCHORSKA, WĘGLARZ 1988) are used for treating circulatory deficiency by improving the contractility of the heart muscle. Although the literature contains agronomic guidelines concerning the cultivation of this plant, we still lack precise recommendations on how to fertilise it. Moreover, there is no information about mole ratios in leaves of the plant as shaped by mineral fertilisation. Therefore, the present research has been undertaken to determine the chemical composition and mole ratios in leaves of cv. Victoria Woolly Foxglove as modified by mineral fertilisation.

MATERIAL AND METHODS

In 2006-2008, at the research station owned by the Faculty of Agriculture of the University of Technology and Life Sciences in Bydgoszcz and located in Wierzchucinek near Bydgoszcz, a strict, multiple, one-factor, micro-plot experiment was set up in four replications according to the method of randomised blocks. The experimental factor was NPK fertilisation applied to soil in the following doses per hectare:

0 – with no mineral fertilisation,

A_1 – 40 kg N + 17.48 kg P + 49.8 kg K = 107.3 kg NPK;

A_2 – 80 kg N + 34.96 kg P + 99.6 kg K = 214.6 kg NPK;

A_3 – 120 kg N + 52.44 kg P + 149.4 kg K = 321.8 kg NPK.

Phosphorus and potassium fertilisation was applied before seeding in the form of Polifoska 6 (6-20-30). Nitrogen fertilisation was applied in three dosages: before seeding as Polifoska 6, and – in two equal doses – after sprouting and three weeks later as ammonium nitrate (V).

The experiment was conducted on Luvisol typical soil (Pw), good rye complex, characterized by the following physicochemical parameters:

- pH_{KCl} – 6.6,
- content of bio-available forms of phosphorus – 55 P mg kg^{-1} of soil (mean fertility),

- content of bio-available forms of potassium – 55 K mg kg^{-1} of soil (mean fertility),
- content of bio-available forms of magnesium – 49 Mg mg kg^{-1} of soil (mean fertility).

The mean air temperature in the years when the research was conducted, during the vegetation period of the Woolly Foxglove (from March to September), did not diverge from the multi-year mean. However, some differences appeared in the precipitation (Figures 1). The highest rainfall during the plant's growth occurred in 2007 (on average 428.2 mm). In June and July, the mean total precipitation was by 50.6 mm and 33.6 mm higher, respectively, than the multi-year mean for these months. In contrast, June and July in 2006 had the lowest total precipitation (by 33.1 mm and 46.6 mm less, respectively, than the multi-year mean). The lowest precipitation was recorded in 2008, especially in May and June, when the mean total precipitation was lower than the multi-year mean by 31.5 mm and 39.4 mm, respectively.

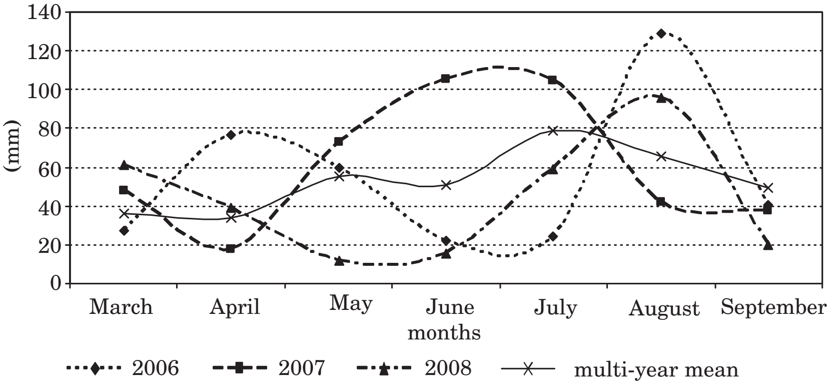

Fig. 1 Rainfall in the vegetation period of the Woolly Foxglove

Digitalis lanata, preceded by spring barley as forecrop, was sown every year. Its leaves were gathered manually from the 3 m^2 plots in the first decade of September. Representative samples of 0.50 kg of leaves were taken from each plot to make chemical analyses. Dried, melted and wet mineralised in sulphuric(VI) acid, the samples were analysed to determine the content of: nitrogen by Kjeldahl method, total phosphorus by the colorimetric method with ammonium molybdate, potassium, calcium and sodium by flame photometry, and magnesium by atomic absorption spectrometry (AAS). The mole ratios of chemical elements in leaves were also calculated.

The findings were statistically processed by the one-way analysis of variance (ANOVA), and the differences between the means were estimated by Tukey's test at the significance level $\alpha = 0.05$.

RESULTS AND DISCUSSION

The research revealed that the applied doses of nutrient components generally had a significant effect on the chemical composition of the Woolly Foxglove leaves (Table 1). The fertilisation affected most strongly the content of total nitrogen in leaves, which ranged on average from 18.54 g kg^{-1} to 23.29 g kg^{-1}. In response to the subsequently higher NPK rates, the content of this element in leaves rose considerably, i.e. by 12.3%, 20.8%, and 25.6%, respectively, compared to the control treatment. It should be emphasized that a significant increase in the content of total phosphorus in leaves of *Digitalis lanata* was observed every year during the research, in all the fertilised treatments except A_1 in 2007. The highest amount of total nitrogen (on average 23.89 g kg^{-1}) was determined in the leaves gathered in 2006, when it significantly surpassed the results obtained in the other years. The lowest amounts of total nitrogen were observed in the leaves harvested in 2007, which was also the driest year (the lowest total precipitation). Nitrogen belongs to elements which are easily transported in the plant (labile elements). The deficiency of this element retards the growth of plants and causes chlorosis on the oldest leaves. Proteins are products of the synthesis of nitrogen, which is the basic structural material in plants. This element participates in stimulating the synthesis of growth regulators, i.e. auxins and gibberellins. It is also essential for the biochemistry of herbal plants because some alkaloids and natural protective substances contain nitrogen (Pozsonyi 2002, Kordana et al. 1998).

The total phosphorus content in the Woolly Foxglove's leaves was also significantly modified by fertilisation. On average, it reached 2.65 g kg^{-1}. Most phosphorus (2.85 g kg^{-1}) was accumulated by leaves gathered from the plots fertilised with the highest dose (object A_3). The increase was 21.8% with reference to the control. Under the medium dose of NPK (object A_2), an increase in the total phosphorus content was slightly lower – 18.8% more than the control. The NPK fertilisation considerably increased the mean content of this macronutrient in leaves of *Digitalis lanata* in particular years of the research. The mean content of total phosphorus in leaves was significantly higher in 2007 than in the other years of the research.

The content of potassium in the air-dried leaves of *Digitalis lanata*, except the 3rd year of the research, was significantly modified by the doses of fertiliser. Generally, an increase in the content of this element in the dried mass of leaves was observed in all of the fertilized treatment. Significantly less potassium was accumulated by the leaves gathered in 2006 compared with 2007 and 2008. The differences were 6.5 and 7.9%, respectively. On average, during the three-year research period, the highest amount of potassium (20.21 g kg^{-1}) was accumulated by leaves of the Woolly Foxglove fertilised by the A_2 dose, where the increment was 4.6% compared to the control. A slightly lower content of potassium (19.72 g kg^{-1}) in leaves was

Table 1

The content of macronutrients in leaves of the Woolly Foxglove

Nutrient	Years	Fertilisation treatment				Mean
		A_0	A_1	A_2	A_3	
Total nitrogen ($g\ kg^{-1}$)	2006	20.53	23.92	25.64	25.46	23.89
	2007	16.25	17.38	18.79	20.74	18.29
	2008	18.85	21.17	22.77	23.68	21.62
	mean	18.54	20.82	22.40	23.29	21.26
	LSD: for years – 0.626 for fertilisation – 0.382			for fertilisation in years: 2006 – 2.041 2007 – 1.352 2008 – 1.233		
Total phosphorous ($g\ kg^{-1}$)	2006	1.92	2.23	2.47	2.56	2.29
	2007	2.72	2.98	3.05	3.10	2.96
	2008	2.37	2.66	2.82	2.89	2.68
	mean	2.34	2.62	2.78	2.85	2.65
	LSD: for years – 0.109 for fertilisation – 0.048			for fertilisation in years: 2006 – 0.106 2007 – 0.168 2008 – 0.155		
Potassium ($g\ kg^{-1}$)	2006	18.75	18.76	19.48	18.29	18.82
	2007	19.25	20.03	20.28	20.61	20.04
	2008	19.95	20.37	20.88	20.10	20.31
	mean	19.32	19.72	20.21	19.67	19.73
	LSD: for years – 0.298 for fertilisation – 0.354			for fertilisation in years: 2006 – 0.595 2007 – 0.679 2008 – n. s.		
Calcium ($g\ kg^{-1}$)	2006	5.96	5.85	5.98	5.79	5.89
	2007	4.48	4.31	4.35	4.31	4.36
	2008	5.07	4.93	5.02	4.90	4.98
	mean	5.17	5.03	5.12	5.00	5.08
	LSD: for years – 0.217 for fertilisation – 0.133			for fertilisation in years: 2006 – 0.187 2007 – n. s. 2008 – 0.165		
Magnesium ($g\ kg^{-1}$)	2006	3.72	3.62	3.30	3.13	3.44
	2007	3.64	4.01	4.09	4.18	3.98
	2008	4.60	4.77	4.62	4.57	4.64
	mean	3.99	4.13	4.00	3.96	4.02
	LSD: for years – 0.085 for fertilisation – 0.116			for fertilisation in years: 2006 – 0.283 2007 – 0.317 2008 – n. s.		
Sodium ($g\ kg^{-1}$)	2006	4.96	4.76	4.41	4.38	4.63
	2007	2.35	2.24	2.04	2.01	2.16
	2008	4.57	4.38	3.88	3.69	4.13
	mean	3.96	3.79	3.44	3.36	3.64
	LSD: for years – 0.192 for fertilisation – 0.081			for fertilisation in years: 2006 – 0.221 2007 – 0.141 2008 – 0.265		

achieved after applying the lowest fertilisation dose. Potassium content in herbal plants varies greatly depending on the region of cultivation (Ajasa et al. 2004, Sheded et al. 2006, Wojciechowska 2008), type of raw material (Singh, Garg 1997) and soil fertility (Sheded et al. 2006, Zaręba, Błoniarz 2008).

The mean content of calcium in leaves of *Digitalis Lanata* was significantly modified by fertilisation. The increasing level of mineral fertilisation induced a lower content of this chemical element in the air-dried matter of leaves, which proved significant for the treatments A_1 and A_3 with reference to the control combination (2.7% and 3.3% less, respectively). In 2007, no significant effect of the applied fertilisation on the calcium content in the Woolly Foxglove's leaves was observed. In 2007, the concentration of this element was significantly lower (by 35.1 and 14.2%) than in leaves collected in 2006 and 2008. Calcium in plants is responsible for the rate of the uptake of mineral components and their transport. Its deficiency in soil may disturb the uptake of ions. The content of calcium in plants depends considerably on the type of fertilisation, physicochemical characteristics of soil, air temperature and the amount of precipitation during the plant vegetation period, as well as on the age of the plant (Wójcik 1998).

Magnesium, a basic component of chlorophyll and an element that activates many enzymatic processes during the syntheses of proteins, nucleic acids, lipids and hydrocarbons, plays a key role in the plant metabolism. It has been demonstrated that after the fertilisation the mean content of magnesium in *Digitalis lanata* was the highest in the treatment fertilised with the lowest dose of NPK (A_1). The content of magnesium in the air-dried matter of leaves from the other treatments was similar to the control. Most magnesium was accumulated in leaves gathered in 2008.

The physiological role of sodium is less thoroughly recognised than that of the other macronutrients. Based on the response to fertilisation with this element, the plants are divided into natriophytes and non-natriophytes. The content of sodium in plants usually varies from 0.1 to 5 g kg^{-1} but in natriophytes it reaches from 15 to 25 g kg^{-1} (Zaręba, Błoniarz 2008). As seen from the above, the Woolly Foxglove does not belong to natriophytes and the content of sodium in its leaves may be modified by mineral fertilisation (Table 1). In comparison to the control, a significant decrease in the content of sodium in leaves of the Woolly Foxglove was observed after applying the experimental fertilisation doses. The lowest amounts of sodium were in leaves gathered from the plot fertilised with the highest NPK dose (A_3), where the decrease was 15.2%. The lowest sodium concentrations were typical of leaves gathered in 2007 (2.16 g kg^{-1}). Compared to 2006 and 2008, the differences were significant and equalled to 2.47 g kg^{-1} and 1.97 g kg^{-1}, respectively.

The optimum content of mineral components in herbal plants ensures proper quantitative ratios between the elements in herbal raw material and is beneficial for the bio-availability and efficacy of products extracted from

Table 2

The values of mole ratios in leaves of the Woolly Foxglove

Ratio	Year	Fertilisation object				Mean
		A_0	A_1	A_2	A_3	
1	2	3	4	5	6	7
Ca:P	2006	2.44	2.08	1.92	1.80	2.06
	2007	1.27	1.12	1.10	1.07	1.14
	2008	1.65	1.43	1.38	1.32	1.44
	mean	1.79	1.55	1.47	1.40	1.55
	LSD: for years – 0.133 for fertilisation – 0.037			for fertilisation in years: 2006 – 0.171 2007 – 0.008 2008 – 0.121		
Ca:Mg	2006	0.99	1.01	1.13	1.15	1.07
	2007	0.75	0.65	0.65	0.63	0.67
	2008	0.67	0.63	0.66	0.65	0.65
	mean	0.80	0.76	0.81	0.80	0.80
	LSD: for years – 0.066 for fertilisation – 0.037			for fertilisation in years: 2006 – 0.109 2007 – n. s. 2008 – n. s		
K:Ca	2006	3.17	3.21	3.26	3.16	3.20
	2007	4.42	4.77	4.80	4.91	4.72
	2008	4.05	4.24	4.27	4.21	4.19
	mean	3.88	4.07	4.11	4.10	4.04
	LSD: for years – 0.266 for fertilisation – 0.053			for fertilisation in years: 2006 – n. s. 2007 – 0.255 2008 – 0.123.		
K:Mg	2006	3.13	3.23	3.68	3.64	3.42
	2007	3.30	3.11	3.09	3.07	3.14
	2008	2.70	2.66	2.82	2.74	2.73
	mean	3.04	3.00	3.20	3.15	3.10
	LSD: for years – 0.069 for fertilisation – 0.122			for fertilisation in years: 2006 – 0.368 2007 – n. s. 2008 – n. s.		
K:Na	2006	2.23	2.32	2.60	2.46	2.40
	2007	4.82	5.26	5.85	6.02	5.49
	2008	2.57	2.73	3.17	3.21	2.92
	mean	3.20	3.44	3.87	3.90	3.60
	LSD: for years – 0.161 for fertilisation – 0.097			for fertilisation in years: 2006 – n. s. 2007 – 0.410 2008 – 0.313		

cont. Table 2

1	2	3	4	5	6	7
K:(Ca+Mg)	2006	1.57	1.61	1.73	1.69	1.65
	2007	1.88	1.88	1.88	1.89	1.88
	2008	1.62	1.63	1.70	1.66	1.65
	mean	1.69	1.71	1.77	1.75	1.73
	LSD: for years – 0.053 for fertilisation – 0.043			for fertilisation in years: 2006 – 0.106 2007 – n. s. 2008 – 0.069		
(K+Na):(Ca+Mg)	2006	2.28	2.30	2.39	2.38	2.34
	2007	2.27	2.24	2.20	2.20	2.23
	2008	2.25	2.23	2.23	2.17	2.22
	mean	2.27	2.26	2.28	2.25	2.26
	LSD: for years – 0.078 for fertilisation – n.s.			for fertilisation in years: 2006 – n. s. 2007 – n. s. 2008 – n. s.		

such plants. The present study has proven that the effect of mineral fertilisation doses on mole ratios in leaves of *Digitalis lanata* was not unidirectional (Table 2). The values of the Ca:P ratio varied from 1.40 to 1.79 and tended to narrow down as the doses of applied fertiliser rose. The optimum mole Ca:P ratios in crop plants, acc. to FILIPEK (1987), ought to be 1.5:1; the present research has showed similar values for treatments A_1 and A_2. It is worth noticing that mole ratios in herbal plants are rarely discussed in the literature

The use of NPK dose A_1 significantly narrowed the Ca:Mg ratio versus the control. Further increase in the fertiliser dose did not alter the ratio between these elements in leaves. The effect of the applied doses of fertiliser on the chemical composition of the Woolly Foxglove's leaves was also confirmed by changes in the K:Ca ratio. The research showed that the applied fertilisation significantly increased the above ratio in leaves of *Digitalis lanata* relative to the control, which resulted from a rise in the potassium content and a drop in the level of calcium in leaves when the NPK dose was higher. Assuming that the optimum mole ratios of these elements in crops are 2:1 (PANAK, WOJNOWSKA 1982), the K:Ca ratio in leaves of the Woolly Foxglove should be recognised as high.

A significant increase in the K:Mg ratio with reference to the control was observed after using the medium dose of NPK (A_2). It should be emphasized that this dose is recommended for *Digitalis lanata*. The fertilisation induced a statistically confirmed enlargement of the K:Na ratio from 3.20 in the control to 3.90 in the plants fertilised with the highest dose of NPK (A_3).

A significant growth in the ratio of potassium to the total of calcium and magnesium with reference to the object without mineral fertilisation was observed after using the highest (1.75) and the medium NPK dose (1.77). In 2006 and 2008, the mean value of this ratio significantly increased compared to the control. Mineral fertilisation did not change significantly the ratio of the total sum of monovalent ions to the total sum of bivalent ions compared with the control. The mean value of the above ratio was 2.26. Analogously, no such changes were observed in the particular years of the research. The value of total monovalent to total bivalent ion ratio is shaped by antagonisms, for example between calcium and potassium, which affect the plant's water management. The effect of calcium on transpiration may also depend on the proportion between the content of this element and the content of potassium and sodium.

The research has clearly demonstrated that the weather conditions, different during the vegetation season in the consecutive years, affected mole ratios of the elements in leaves of the Woolly Foxglove. The fact that the changes in the calculated ratio values were not unidirectional (Table 2.) was mainly the result of the unequal distribution and amount of precipitation.

In the above research, the highest NPK dose (A_3), exceeding the recommend one, caused significant changes in the chemical composition of leaves of *Digitalis lanata*, a development which was also reflected by changes in proportions of mineral compounds in leaves. This finding may imply that higher quality raw material can be obtained by optimised fertilisation.

CONCLUSIONS

1. The tested mineral fertilisation generally induced a significant increase in the content of total nitrogen, total phosphorus, and potassium, but depressed the sodium concentration in leaves of the Woolly Foxglove compared to the control.
2. After applying 107.3 kg NPK ha^{-1}, the content of calcium and magnesium increased, while the ratio between these chemical elements decreased in leaves of *Digitalis Lanata* in comparison to the control.
3. The increased mineral fertilisation significantly widened the ratios of K:Ca and K:Na, and narrowed the Ca:P ratio in leaves.
4. A significantly broader K:(Ca+Mg) ratio in leaves was observed after applying the medium and the highest NPK doses.
5. After applying 214.6 kg NPK ha^{-1}, an increase in the K:Mg ratio in leaves was observed versus the control.

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CONCENTRATION OF SELECTED METALS IN BUTTER LETTUCE (*LACTUCA SATIVA* L.) CONTAMINATED WITH ANTHRACENE AND PYRENE

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Abstract

PAHs (polycyclic aromatic hydrocarbons) are widespread in the natural environment. They are suspected to have mutagenic, carcinogenic and teratogenic effect on our organisms. Production of wholesome and high quality vegetables does not necessarily involve selection of adequate farmlands or substrates used for vegetable growing. Combustion processes are a source of PAHs in the soil and air. Excessive levels of PAHs are accumulated when the balance between their decomposition in soil and supply from other sources is disrupted. The objective of the present research has been to evaluate the effect of anthracene and pyrene on the concentration of selected trace elements in butter lettuce (*Lactuca sativa* L.), cultivar Vilmorin, grown on substrates with different nutrient abundance. A pot experiment in four replications was carried out twice in the spring of 2007 and 2008. The pots were maintained in a greenhouse at the University of Warmia and Mazury in Olsztyn. Lettuce was grown under the minimum (optimum) and triple (II fertilization level) substrate abundance in nutrients. In the second variant o the experiment, when the nutrient abundance was elevated, nitrogen was split into two doses: 2/3 of the whole rate were added before planting lettuce seedlings, and 1/3 of the rate was introduced 10 days afterwards. In the first variant, when the nutrient abundance was minimal, all of the nitrogen was added in a single dose. Spraying the lettuce plants with anthracene (ANT), pyrene (PYR0 or their mixture started 10 days after planting. Foliar introduction of the tested PAHs continued for 25 days (in the amount of 1.8 cm^3 day^{-1} at a concentration of 100 mg dm^{-3}) until the vegetative season finished. At the same time, an identical total amount of the PAHs was added to soil as aqueous solution, at 7-day intervals, according to the same pattern. Determinations of the concentrations of heavy metals (Cd, Cu, Ni, Cr, Zn, Mn) was performed with the ASA method, having first mineralised ($HClO_4$+HCl+HNO_3) the plant material which had been dried at 60°C. The determinations were compared with certified material (CTA-VTL-2). Concentrations of the trace elements in butter lettuce were

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significantly varied depending on the experimental factors. A more abundant substrate significantly raised the levels of Cd, Zn and Mn, but had no effect on concentrations of Cu, Ni and Cr. The way the PAHs were applied did not have any influence on modifications in the determined levels of the metals. Out of the two analysed PAHs, anthracene, especially when applied to soil, increased the concentrations of Zn nd Cr in lettuce leaves.

Key words: anthracene, pyrene, *Lactuca sativa* L., heavy metals, substrate abundance.

KONCENTRACJA WYBRANYCH METALI W SAŁACIE (*LACTUCA SATIVA* L.) SKAŻONEJ ANTRACENEM I PIRENEM

Abstrakt

WWA (wielopierścieniowe węglowodory aromatyczne) są związkami powszechnie występującymi w środowisku. Przypisuje się im działanie mutagenne, rakotwórcze i teratogenne na nasze organizmy. Produkcja zdrowych i dobrych jakościowo warzyw nie zawsze jest związana z wyborem odpowiednich terenów lub podłoży do ich uprawy. •ródłem WWA zarówno w glebie, jak i w powietrzu są procesy spalania. Do nadmiernego ich nagromadzenia dochodzi na skutek zachwiania równowagi między rozkładem w glebie a przychodem z innych źródeł. Celem badań była ocena wpływu antracenu oraz pirenu na zawartość wybranych pierwiastków śladowych w sałacie masłowej (*Lactuca sativa* L.) odmiany Vilmorin uprawianej w podłożach o zróżnicowanej zasobności. Eksperyment wazonowy w 4 powtórzeniach przeprowadzono dwukrotnie wiosną w latach 2007-2008 w hali wegetacyjnej UWM w Olsztynie. Sałatę uprawiano w warunkach o minimalnej (optymalnej) zasobności podłoża w składniki pokarmowe oraz 3-krotnie zwiększonej (II – poziom nawożenia). Azot wprowadzano w dwóch dawkach przy zwiększonej zasobności podłoża: 2/3 dawki przed posadzeniem rozsady, a 1/3 dawki po 10 dniach od posadzenia oraz w całości w przypadku pierwszego poziomu nawożenia. Oprysk roślin antracenem (ANT) oraz pirenem (PYR) i ich mieszaniną rozpoczęto po 10 dniach od posadzenia. Dolistne wprowadzanie wybranych WWA trwało przez 25 dni (w ilości 1,8 cm^3 doba^{-1} o stężeniu 100 mg dm^{-3}) do końca okresu wegetacji. Jednocześnie doglebowo dostarczono sumaryczną ilość WWA w takim samym układzie 1 raz na 7 dni w wodnym rozcieńczonym roztworze. Zawartość wybranych metali ciężkich (Cd, Cu, Ni, Cr, Zn, Mn) oznaczano metodą ASA po mineralizacji ($HClO_4$+HCl+HNO_3) wysuszonego w temp. 60°C materiału roślinnego. Oznaczenia wykonano wobec materiału certyfikowanego (CTA-VTL-2). Zawartość pierwiastków śladowych w sałacie masłowej była istotnie zróżnicowana w zależności od czynników doświadczalnych. Zasobniejsze podłoże wpływało istotnie na zwiększenie zawartości Cd, Zn i Mn, nie miało zaś wpływu na koncentrację Cu, Ni i Cr. Sposób aplikacji WWA nie miał wpływu na zmiany zawartości oznaczonych metali. Spośród testowanych WWA, szczególnie antracen aplikowany doglebowo zwiększał zawartość Zn i Cr w liściach testowanej rośliny.

Słowa kluczowe: antracen, piren, *Lactuca sativa* L., metale ciężkie, zasobność podłoża.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are among the substances which tend to appear in the natural environment in ever increasing quantities (TSAI et al. 2001). They are generated during incomplete incineration or combustion of various substances, caused by both natural forces (forest wild fires, mineralization of organic matter) or man (tobacco smoking, production and combustion of fuels, transport, etc.) (Henner et al. 1997, Samanta et al. 2002, Johnson et al. 2005, Ravindra et al. 2008). They are the compounds which re readily bioaccumulated in living organisms and have an adverse effect on our health, producing toxic, carcinogenic, mutagenic and teratogenic effects (Perera 1997, Jian et al. 2004). Plants grown on soil which contains PAHs, or otherwise exposed to these compounds, respond to contamination demonstrating certain disorders in physiological processes (photosynthesis, transpiration), biomass production, uptake and concentration of some nutrients (Marwood et al. 2001, Wieczorek et al. 2001, Witting et al. 2003, Wieczorek et al. 2004, 2006, Krzebietke, Sienkiewicz 2010a,b). In practice, the relevant literature contains no reports on the effect of PAHs on concentrations of heavy metals in plants. The objective of the present study has been to determine the effect of anthracene and pyrene and their mixture, sprayed over leaves or introduced to soil, on concentrations of some metals in butter lettuce (*Lactuca sativa* L.), cv. Vilmorin, grown on substrate with two different levels of nutrient abundance.

MATERIAL AND METHODS

A three-factor pot experiment was carried out in he spring of 2007 and 2008, in a greenhouse at the University of Warmia and Mazury in Olsztyn. The experiment was run in four replications. The substrate used in the experiment had the following chemical properties: 4.1 mg N-NO_3; 5.5 mg N-NH_4; 44.2 mg P; 173.3 mg K; 60.9 mg Mg; 921.9 mg Ca; 8.3 mg Na; 13.4 mg Cl^-; 71.7 mg S-SO_4 in dm^3 of the pH 6.5 and EC 0.11 mS cm^{-1}. The following does of nutrients were added to 1 dm^3 of the substrate: 60 or 180 mg N; 50 or 150 mg P; 50 or 150 mg K; 40 or 120 mg Mg; 20 or 60 mg Na and 30.8 or 92.4 mg Cl (1st factor – substrate abundance).

One gram of anthracene and 1 g of pyrene were disolved in 10 cm^3 of acetonitrile, filled up with deionised water to 100 cm^3 and diluted 100-fold by adding deionised water to the solution. For the fist time, lettuce plants were sprayed 10 days after planting, and the treatment was repeated twice a day for 25 consecutive days, using 1.8 cm^3 day^{-1} of the solution at a concentration of 100 mg dm^{-3}. The same amounts of the PAHs were introduced to soil, every 7 days, in an aqueous diluted solution. Control plants were sprayed or watered in the same way but without the PAHs (2nd factor – PAH application method).

The tested solutions comprised: anthracene (ANT), pyrene (PYR) or their mixture, and the control plants were sprayed or watered with deionised water mixed with acetonitrile organic solvent (ACN), added in the same amount as need for disolving the PAHs (3rd factor – type of PAHs).

Before the lettuce was planted, the surface of the soil was sprayed with propyzamide in the aound of 0.65 mg dm^{-3} of the substrate. In Kick Brauckmann pots of the capacity of 10 kg soil, six-week old seedlings of cv. Vilmorin butter lettuce (Lactuca sativa L.) were planted, one seedling per pot. The lettuce was harvested after 6 weeks. During the harvest, lettuce heads were weighed. Determinations of the selected metals (Cd, Cu, Ni, Cr, Zn and Mn) were performed with the ASA method, after mineralization ($HClO_4$+HCl+HNO_3) of the plant material dried at temp. 60°C. The determinations were verified with the certified material CTA-VTL-2. The results underwent statistical processing, employing the analysis of variance for a three-factor experiment, in a completely random design – Statistica v. 7.0.

RESULTS AND DISCUSION

The concentrations of trace metals (Mn, Cd and Zn) in butter lettuce were modified primarily by the abundance of the substrate (Tables 1-3). There are researchers, e.g. SAMSŘE-PETERSEN et al. 2002, WENNRICH et al. 2002, KHAN et al. 2008, who demonstrate that soil is the major route of transfer for PAHs to plants. In the present experiment, the way of introducing the PAHs (over leaves or to soil) did not significantly modify the concentrations of the analysed elements in butter lettuce leaves. However, statistically proven differences in the concentrations of chromium or zinc in the tested butter lettuce leaves were found following the application of anthracene or pyrene or their mixtures (Tables 1 and 2). The concentration of chromium rose 2.75-fold in lettuce leaves due to soil contamination with ANT in the substrate with elevated nutrient abundance. When the nutrient levels in the substrate were on the optimum level, the increase in chromium in lettuce leaves was 1.33-fold (Table 1).

In the substrate richer in nutrient, ANT contributed to increased concentrations of Cr in lettuce leaves; a reverse correlation was observed for pyrene. This effect was much stronger when pyrene was supplied to soil and when nutrient availability to plant was higher. A mixture of PAHs (ANT+PYR), regardless of an application method, led to a decrease in the concentration of Cr in butter lettuce plants.

Manganese in butter lettuce leaves occurred in concentrations between 31.16 and 59.17 mg kg^{-1} d.m. (Table 1). The substrate abundance produced a significant effect on this element (a 30.7% increase) only when butter lettuce was grown on the substrate with a three-fold lower abundance in P, K, Mg and Na.

Foliar application of pyrene, under the optimum substrate abundance in nutrients, raised the concentration of nickel in the analysed plant (Table 2). Reverse tendencies were observed in the case of ANT. Under the higher

Table 1

Effect of substrate abundance, soil or foliar application of PAHs, on the concentrations of chromium and manganese in butter lettuce (mg kg^{-1} d.m)

Treatments				Control		ANT		PYR		ANT+PYR		Mean	
				Cr	Mn	Cr	Mn	Cr	Mn	Cr	Mn	Cr	Mn
Substrate abundance	I level	application PAHs	foliar	3.27	34.91	3.25	35.61	3.49	38.90	2.45	31.16	3.12	35.15
			soli	3.16	47.31	4.21	49.08	3.12	45.45	3.35	44.39	3.46	46.56
	II level		foliar	3.65	43.86	3.20	50.34	3.39	53.19	3.78	59.27	3.51	51.67
			soil	3.75	50.71	10.30	59.18	1.02	52.77	0.93	57.98	3.99	55.16
Mean				3.45	44.20	5.23	48.55	2.76	47.58	2.63	48.20	–	
Substrate abundance		I level		3.21	41.11	3.73	42.34	3.30	42.18	2.90	37.78	3.29	40.85
		II level		3.70	47.28	6.73	54.76	2.21	52.98	2.36	58.63	3.75	53.41
Application PAHs		foliar		3.46	39.39	3.23	42.97	3.44	46.05	3.12	45.22	3.31	43.41
		soil		3.45	49.01	5.23	54.13	2.07	49.11	2.14	51.19	3.22	50.86

LSD$_{0.01}$		Cr – chromium	Mn – manganese
Substrate abudance	– factor I	n.s.	11.5401
Application PAHs	– factor II	n.s.	n.s.
PAHs	– factor III	1.1093	n.s.
Interaction	– factor I×II	n.s.	n.s.
	– factor I×III	1.5688	n.s.
	– factor II×III	1.5688	n.s.
	– factor I×II×III	2.2186	n.s.

Table 2

Effect of substrate abundance, soil and foliar application of PAHs on the concentrations of zinc and nickel in butter lettuce (mg kg^{-1} d.m)

Treatments				Control		ANT		PYR		ANT+PYR		Mean	
				Zn	Ni	Zn	Ni	Zn	Ni	Zn	Ni	Zn	Ni
Substrate abundance	I level	application PAHs	foliar	18.64	2.48	20.41	2.39	19.84	2.99	21.23	2.28	20.03	2.54
			soil	16.90	2.34	19.26	3.06	18.00	2.79	18.26	2.55	18.10	2.69
	II level		foliar	25.57	2.35	24.97	2.07	25.01	2.47	26.63	2.80	25.54	2.42
			soil	24.53	2.53	28.90	2.38	25.81	2.13	26.87	2.21	26.53	2.31
Mean				21.41	2.22	23.38	2.47	22.16	2.60	23.25	2.47	-	
Substrate abundance		I level		17.77	2.41	19.83	2.72	18.92	2.89	19.74	2.42	19.07	2.62
		II level		25.05	2.44	26.94	2.22	25.41	2.30	26.75	2.48	26.04	2.37
Application PAHs		foliar		22.10	2.42	22.69	2.23	22.42	2.73	23.95	2.54	22.79	2.48
		soil		20.71	2.44	24.08	2.72	21.90	2.46	22.57	2.39	22.32	2.50

LSD$_{0.01}$		Zn – zinc	Ni – nickel
Substrate abudance	– factor I	0.9446	n.s.
Application PAHs	– factor II	n.s.	n.s.
PAHs	– factor III	1.3358	n.s.
Interaction	– factor I×II	1.3358	n.s.
	– factor I×III	n.s.	0.7167
	– factor II×III	1.8892	n.s.
	– factor I×II×III	n.s.	1.0135

substrate abundance, application of ANT, PYR or ANT+PYR resulted in a 9.5% decrease in the Ni concentration in lettuce leaves.

The content of zinc in lettuce changed depending on the applied polycyclic aromatic hydrocarbons and nutrient abundance of the substrate (Table 2). Its concentration varied from 16.9 to 28.9 mg kg^{-1} d.m. of butter lettuce leaves. Under the higher nutrient abundance of the substrate, the Zn content in lettuce rose by 36.5%. The PAHs caused a higher concentration of zinc in the analysed lettuce. Analogously, positive correlation was found between the concentration of zinc and manganese and the concentration of cadmium in lettuce plants (Figure 1). The more Zn and Mn the plants contained, the more cadmium they accumulated, although the effect of manganese was much stronger. It is highly probable that an increase in the concentration of Mn and Zn in plants can also lead to increased levels of Cd – in the present experiment the determination coefficients were r=0.7845 for manganese and r=0.4924 for zinc.

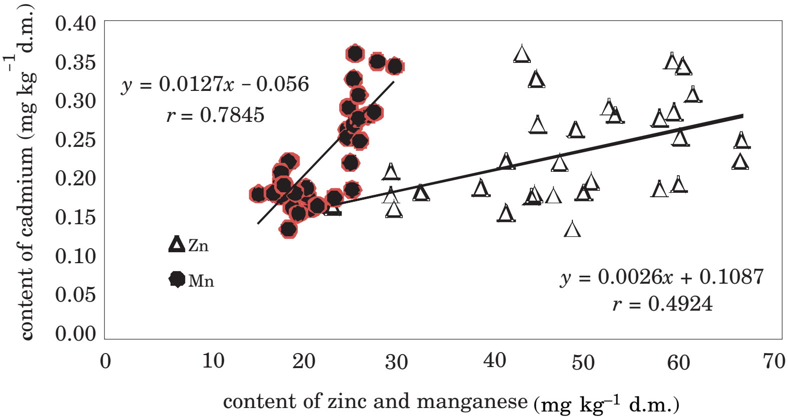

Fig. 1. Correlation between the concentration of cadmium and zinc versus manganese in butter lettuce

The concentration of copper in butter lettuce plants varied from 1.24 to 1.65 mg kg^{-1} d.m. (Table 3). No significant effect of the tested experimental factors was evidenced on the concentration of Cu in lettuce leaves. However, statistically significant differences in the concentration of copper in lettuce leaves were found out for the interaction of the three experimental factors. A mixture of the tested PAHs (ANT+PYR) sprayed over leaves, under the minimal substrate abundance in nutrients, was responsible for a decrease in the Cu content in lettuce leaves.

The concentration of cadmium in lettuce leaves depended mainly on the substrate abundance; the richer substrate raised its concentration by 55.5% (Table 3). The PAHs introduced to the substrate alongside its higher abun-

Table 3

Effect of substrate abundance soil and foliar application of PAHs on the concentrations of copper and cadmium in butter lettuce (mg kg^{-1} d.m.)

Treatments				Control		ANT		PYR		ANT+PYR		Mean	
				Cu	Cd	Cu	Cd	Cu	Cd	Cu	Cd	Cu	Cd
Substrate abundance	I level	application PAHs	foliar	1.47	0.17	1.45	0.16	1.59	0.16	1.24	0.18	1.43	0.17
			soil	1.31	0.19	1.38	0.20	1.65	0.20	1.51	0.20	1.46	0.20
	II level		foliar	1.41	0.34	1.41	0.28	1.61	0.24	1.63	0.27	1.52	0.28
			soil	1.60	0.24	1.40	0.35	1.46	0.29	1.46	0.28	1.48	0.29
Mean				1.45	0.24	1.41	0.25	1.58	0.22	1.46	0.23	-	
Substrate abundance		I level		1.39	0.18	1.42	0.18	1.62	0.18	1.38	0.19	1.45	0.18
		II level		1.51	0.29	1.40	0.31	1.54	0.26	1.54	0.27	1.50	0.28
Application PAHs		foliar		1.44	0.26	1.43	0.22	1.60	0.20	1.44	0.22	1.48	0.23
		soil		1.46	0.22	1.39	0.28	1.56	0.25	1.48	0.24	1.47	0.25

$LSD_{0.01}$		Cu – copper	Cd – cadmium
Substrate abudance	– factor I	n.s.	0.0190
Application PAHs	– factor II	n.s.	n.s.
PAHs	– factor III	n.s.	n.s.
Interaction	– factor I×II	n.s.	0.0270
	– factor I×III	n.s.	0.0381
	– factor II×III	n.s.	0.0381
	– factor I×II×III	0.3893	0.0539

dance in nutrients caused an increase in Cd in butter lettuce leaves. Anthracene raised the amount of cadmium in lettuce plants grown on the substrate richer in nutrients, whereas pyrene reduced its concentration. Foliar application of the PAHs (ANT, PYR, ANT+PYR) depressed the concentration of cadmium, whereas soil application resulted in higher levels of this metal in lettuce leaves.

CONCLUSIONS

1. The substrate richer in N, P, K, Mg, Na and Cl significantly increased concentrations of Cd, Zn and Mn in butter lettuce leaves, but had no effect on concentrations of Cu, Ni and Cr.
2. No significant effect of the PAH application path (foliar or to soil) on concentrations of the analysed metals in butter lettuce was demonstrated.
3. Increased content of anthracene in soil may raise the concentration of Zn and Cr in butter lettuce leaves.
4. An increase in the concentration of Mn and Zn in butter lettuce leaves may also entail a higher level of Cd in the tested plant.

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CHANGES IN THE ENZYMATIC ACTIVITY IN SANDY LOAM SOIL EXPOSED TO ZINC PRESSURE

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Abstract

One of the bioindicators most often applied to assess the quality of soil is its enzymatic activity. Undesirable changes in the activity of enzymes can imply excessive presence of substances which are harmful to soil environment, such as heavy metals. Being a heavy metal, zinc is also an element essential for maintaining proper functions of live organisms. The purpose of this study has been to determine the significance of changes occurring in moderately heavy soil under the influence of zinc.

The experiment was carried out in three replicates under laboratory conditions. Sandy loams of pH 5.5 and 7.0 were used for the trials. The soils were contaminated with zinc according to the following design: control (natural content), raised content (70 mg Zn^{2+} kg^{-1}), weakly polluted (200 mg Zn^{2+} kg^{-1}), moderately polluted (500 mg Zn^{2+} kg^{-1}), heavily polluted (1,500 mg Zn^{2+} kg^{-1}) and very heavily polluted soil (5,000 mg and 10,000 mg Zn^{2+} kg^{-1}). The soil samples prepared as above were brought to the moisture content of 50% maximum water capacity and incubated at 25°C for 120 days. On day 30, 60 and 120, the activity of dehydrogenases, β-glucosidase, urease, acid phosphatase and arylsulphatase was determined. Based on these determinations, the following indices were calculated: ED_{50}, the index for resistance (RS) and the index for resilience (RL).

The tests have demonstrated that as the rate of soil contamination with zinc increased, the activity of all the analyzed enzymes was significantly depressed. The negative influence of zinc contamination on the activity of particular enzymes, irrespective of the soil pH, persisted throughout the whole experiment. In respect of their sensitivity to zinc, the enzymes can be ordered as follows: arylsulphatase > dehydrogenases > acid phosphatase > urease > β-glucosidase. Zinc contamination caused lasting changes in the soil environment, but the return to the state of equilibrium was the quickest in the case of dehydrogenases (RL = 0.276), less rapid for arylsulphatase (RL = 0.173) and the slowest for acid phosphatase (RL = 0.064). In contrast, the activity of urease, instead of regenerating, was increasingly disturbed (RL = 0.350). Soil acidification was the factor that most evidently exacerbated the negative influence of zinc on the activity of β-glucosidase and arylsul-

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phatase. Values of ED50 for the activity of particular enzymes were varied. In the soil of pH 7.0, they ranged from 3,324 mg Zn^{2+} kg^{-1} for β-glucosidase to 412 mg Zn^{2+} kg^{-1} for dehydrogenases, and in the soil of pH 5.5, they varied from 1,008 Zn^{2+} kg^{-1} for β-glucosidase to 280 mg Zn^{2+} kg^{-1} for arylsulphatase.

Key words: zinc, ED_{50}, index for resistance (RS), index for resilience of (RL), soil contamination, activity of enzymes.

ZMIANY AKTYWNOŚCI ENZYMATYCZNEJ W GLINIE PIASZCZYSTEJ PODDANEJ PRESJI CYNKU

Abstrakt

Aktywność enzymatyczna gleby stanowi jeden z najczęściej wykorzystywanych biowskaźników do oceny jakości gleby. Niekorzystne zmiany w aktywności enzymów mogą świadczyć o nadmiernej zawartości substancji szkodliwych dla środowiska glebowego, do których należą również metale ciężkie. Cynk jest też niezbędnym składnikiem do prawidłowego funkcjonowania organizmów żywych. Celem badań było określenie istotności zmian zachodzących w glebie o średniej kategorii agrotechnicznej ciężkości pod wpływem cynku.

Doświadczenie wykonano w warunkach laboratoryjnych, w 3 powtórzeniach. Do badań wykorzystano gliny piaszczyste o pH 5,5 oraz o pH 7,0, które zanieczyszczono cynkiem wg następującego schematu: kontrola (zawartość naturalna), zawartość podwyższona (70 mg Zn^{2+} kg^{-1}), słabe zanieczyszczenie (200 mg Zn^{2+} kg^{-1}), średnie zanieczyszczenie (500 mg Zn^{2+} kg^{-1}), silne zanieczyszczenie (1500 mg Zn^{2+} kg^{-1}) i bardzo silne zanieczyszczenie (5000 mg oraz 10 000 mg Zn^{2+} kg^{-1}). Tak przygotowane próbki gleby uwilgotniono do poziomu 50% maksymalnej pojemności wodnej i inkubowano w temp. 25°C przez 120 dni. W 30., 60. oraz 120. dniu określono aktywność dehydrogenaz, β-glukozydazy, ureazy, fosfatazy kwaśnej oraz arylosulfatazy. Na ich podstawie obliczono wskaźniki ED_{50}, oporności (RS) i powrotu do równowagi (RL).

W wyniku badań stwierdzono, że wraz ze zwiększeniem stopnia zanieczyszczenia gleby cynkiem aktywność wszystkich badanych enzymów ulegała istotnemu zmniejszeniu. Negatywny wpływ zanieczyszczenia cynkiem na aktywność enzymatyczną gleby, niezależnie od jej pH, utrzymywał się przez cały okres trwania badań. Enzymy pod wpływem wrażliwości na zanieczyszczenie gleby cynkiem można uszeregować następująco: arylosulfataza > dehydrogenazy > fosfataza kwaśna > ureaza > β-glukozydaza. Zanieczyszczenie cynkiem powodowało długotrwałe zmiany w środowisku glebowym, ale najszybciej do stanu równowagi powracały dehydrogenazy (RL = 0,276), nieco wolniej arylosulfataza (RL = 0,173), i najwolniej fosfataza kwaśna (RL = 0,064). Natomiast aktywność ureazy nie tyko nie regenerowała się, ale stan zaburzenia ulegał pogłębieniu (RL = -0,350). Zakwaszenie gleby w największym stopniu wzmagało negatywne działanie cynku na aktywność β-glukozydazy i arylosulfatazy. ED_{50} dla aktywności poszczególnych enzymów było zróżnicowane. W glebie o pH 7,0 wahało się od 3324 mg Zn^{2+} kg^{-1} dla β-glukozydazy do 412 mg Zn^{2+} kg^{-1} dla dehydrogenaz, a w glebie o pH 5,5 od 1008 mg Zn^{2+} kg^{-1} dla β-glukozydazy do 280 mg Zn^{2+} kg^{-1} dla arylosulfatazy.

Słowa kluczowe: cynk, ED_{50}, wskaźnik oporności (RS), wskaźnik powrotu do równowagi (RL), zanieczyszczenie gleby, aktywność enzymów.

INTRODUCTION

The intensive growth of industries and civilization progress have raised levels of heavy metals in soil, a development which is now a serious problem (SEIFERT, DOMKA 2005) affecting not just plants and animals (BROOKES 1995) but microorganisms as well (KUCHARSKI et al. 2000, WYSZKOWSKA, KUCHARSKI 2003, WYSZKOWSKA et al. 2001). Excessively high concentrations of these elements have an adverse effect on soil fertility. Elevated levels of heavy metals produce negative influence on the physicochemical and biological properties of soils (GILLET, PONGE 2002, KUCHARSKI, WYSZKOWSKA 2004, WYSZKOWSKA et al. 2007), including the activity of enzymes and the nitrification process (KUCHARSKI et al. 2009).

Soil enzymes are produced by plants and animals, but the dominant ones are secreted by microorganisms (NIELSEN, WINDING 2002). Consequently, counts of microorganisms in soil are a very important indicator of soil quality. Excessive content of zinc has a negative impact on counts of oligotrophic bacteria, *Azotobacter* and *Arthrobacter* bacteria as well as fungi (WYSZKOWSKA, KUCHARSKI 2003).The harmful influence of heavy metals on soil ecosystems is compounded by the fact that in practice – unlike organic compounds – heavy metals do not undergo transformations, that is, they are not degraded and consequently their accumulation in soil is burdensome to each biotope and to the whole biocenosis as well (ADRIANO et al. 2004).

Soil enzymes are involved in the circulation of biogenic elements (NIELSEN, WINDING 2002). This fact is of great importance from the point of view of ecology, as the activity of microorganisms and enzymes they secrete enable the circulation of elements in nature (BROOKES 1995, TRASAR-CEPEDA et al. 2000) and the transformation of the substances they contain into forms available to other autochthonous organisms. A low level of soil contamination with heavy metals may just impede the metabolic processes of microorganisms, but amounts surpassing several-fold the permissible levels will become a sole reason why soils are degraded due to depressed microbial activity (KUCHARSKI, WYSZKOWSKA 2004, NIELSEN, WINDIG 2002, WYSZKOWSKA et al., 2008, 2009). Analysis of the enzymatic activity in soil is an important bioindicator of soil quality as it enables us to detect some unfavourable changes occurring in soil environment (VISSER, PARKINSON 1992, HINOJOSA et al. 2008).

Zinc is one of those heavy metals which living organisms need as a trace element (MICHALAK 2006). It is found in the structure of metalloenzymes (MCCALL et al. 2000) and it plays many important physiological functions, but when present in excessive amounts, zinc may cause destabilisation of ecosystems. Thus, it seems essential to gain thorough knowledge of the effects zinc exerts on soil, including its influence on the activity of soil enzymes.

The objective of the present study has been to determine changes in the activity of dehydrogenases, β-glucosidase, urease, arylsulphatase and acid phosphatase in sandy loam soil of different reaction, exposed to zinc pressure.

MATERIAL AND METHODS

The experiment was run in three replicates under laboratory conditions. Sandy loams of pH 5.5 and 7.0 were used for the trials. The soils were sampled from the arable humus horizon, from the depth of 5-25 cm. More detailed specification of the soils is presented in Table 1. A portion of 100 g air dry soil was placed in each of 100 cm^3 glass beakers. Zinc in the form of aqueous solution of $ZnCl_2$ salt was added to the soil samples in the following doses (in mg Zn^{2+} kg^{-1} d.m. of soil): 0 (natural content – 0°), 70 (raised content – I°), 200 (weak contamination – II°), 500 (moderate contamination – III°), 1,500 (heavy contamination – IV°), 5,000 (very heavy contamination – V°), 10,000 (a dose two-fold higher than very heavy contamination – VI°). Afterwards, the soil samples were thoroughly mixed and brought to the moisture content equal 50% of the maximum water capacity. The soil samples thus prepared were covered with plastic film and placed in a heater (25°C). The experiment was set up in three replicates. During the incubation, the soil moisture was monitored regularly and water losses were supplemented. The soil samples were incubated for 30, 60 and 120 days. On each of these dates, the activity of the following enzymes was determined in three replications: acid phosphatase (EC 3.1.3.2), arylsulphatase (EC 3.1.6.1), β-glucosidase (EC 3.2.1.21), urease (EC 3.5.1.5) and dehydrogenases (EC 1.1.1.1).

Table 1

Some physicochemical properties of soil used in the experiment

Kind of soil	Grain-size composition (mm)			C_{org}	pH_{KCl}	Hh	S	T	V
	2-0.05	0.05-0.002	<0.002	(g)		mmol(+) kg^{-1} of soil d.m.			(%)
Sandy loam	72	21	7	7.05	7.0	16.05	75.00	91.05	82.37
Sandy loam	74	16	10	5.75	5.5	33.50	35.33	68.83	51.33

C_{org} – organic carbon content per 1 kg of soil d.m., pH_{KCl} – soil reaction, Hh – hydrolytic acidity, S – exchangeable soil capacity, T – total exchangeable soil capacity, V – soil base saturation

The substrates used for determination of the activity of particular enzymes were 4-nitrophenylphosphate disodium for acid phosphatase, potassium-*p*-nitrophenylsulphate for arylsulphatase, *p*-nitrophenyl-β-D-glucopyranoside for β-glucosidase, urea for urease and 2,3,5-triphenyltetrazolium chloride

(TTC) for dehydrogenases. The activity of acid phosphatase, arylsulphatase and β-glucosidase was expressed in mmol p-nitrophenol (PNP) kg^{-1} d.m. h^{-1}; of urease – in mmol N-NH_4 kg^{-1} h^{-1}, and of dehydrogenases – in µmol triphenyl formazan (TPF) kg^{-1} d.m. h^{-1}. The activity of all the enzymes, except dehydrogenases, was determined according to the protocol described by ALEF and NANNIPIERI (1995). In turn, the activity of dehydrogenases was assessed as explained by ÖHLINGER (1996). The results of the analyses were processed statistically with a multi-factor analysis of variance Anova, using Statistica software. ED_{50} as well as the index for resistance (RS) and the index for resilience (RL) were calculated for each enzyme according to ORWIN and WARDLE (2004). The activity of the enzymes as well as RS were given as means from all the dates of analyses, whereas the RL index was calculated for the samples tested after 120 days of incubation.

RESULTS AND DISCUSSION

The results of the determinations suggest that the presence of zinc in soil significantly modifies the biological properties of soil. The extent to which zinc affects the enzymatic activity in soil largely depends on the soil contamination degree and soil reaction.

The activity of dehydrogenases in the soil of pH 7.0 containing a naturally occurring amount of zinc was 10.06 µmol TFF kg^{-1} d.m. h^{-1}, and in the soil of pH 5.5, it was 7.89 µmol TFF kg^{-1} h^{-1} (Figure 1). In the soil of pH 7.0, the activity of these enzymes fell by 12% when a dose of 200 mg Zn^{2+} kg^{-1} was added and by 65% – when the rate of zinc rose to 500 mg Zn^{2+} kg^{-1}. The same rates of zinc added to the soil of pH 5.5 inhibited the activity of dehydrogenases more strongly, i.e. by 30 and 71%, respectively. Higher doses of the contaminant (1,500 to 10,000 mg Zn^{2+} kg^{-1}) had a nearly identical influence on dehydrogenases in the soils of both reactions, by depressing their activity within 96 to 98%.

WYSZKOWSKA et al. (2006a, 2006b) demonstrated 30% inhibition of the activity of dehydrogenases in response to a rate of zinc as low as 50 mg Zn^{2+} kg^{-1}. The same experiment showed that the activity of dehydrogenases decreased as the zinc contamination of soil increased. The effect produced by this element on dehydrogenases, like that of other heavy metals (WYSZKOWSKA et al. 2001), depends not only on its concentration in soil but also on a whole range of soil properties, such as levels of heavy metals (HINOJOSA et al. 2004). The results obtained in the present study coincide with the report presented by BIELIŃSKA (2007) and confirm the suggestions made by NIELSEN and WINDIG (2002), who claimed that dehydrogenases might be a good indicator of the microbiological activity of soil since they are classified as intercellular enzymes (BROOKES 1995).

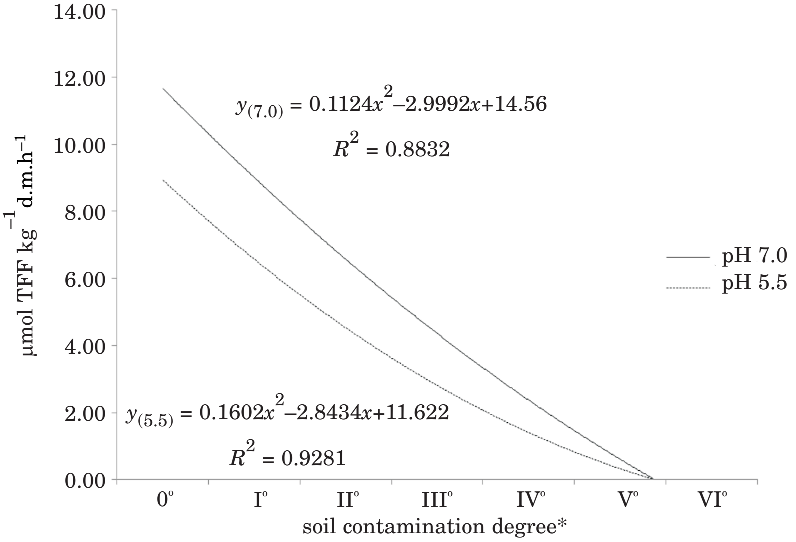

Fig. 1. Activity of dehydrogenases in soil contaminated with zinc, μmol TFF kg^{-1} d.m. h^{-1}
*zinc doses (mg Zn^{2+} kg^{-1} of soil d.m.): 0° – 0, I° – 70, II° – 200, III° – 500, IV° – 1,500, V° – 5,000, VI° – 10,000

Urease, another enzyme tested in our trials, was similar to dehydrogenases in that it was very sensitive to soil contamination with zinc and to soil acidification (Figure 2). In acid sandy loam not contaminated with zinc, the activity of this enzyme was up to 47% lower than in sandy loam of neutral reaction. In both soils, as the zinc concentration rose, the activity of urease decreased. However, the activity of that enzyme seemed more grossly disturbed in the soil of pH 7.0 than 5.5. In the former soil, zinc depressed the activity of urease by an average 58%, regardless of the degree of contamination, while in the latter type of soil – the average decrease was 49%. The negative effect of excess zinc on the activity of urease in soil has also been reported elsewhere (BALYAEVA et al. 2005, GÜLSER and ERDOĐAN 2008, STUCZYŃSKI et al. 2003, WYSZKOWSKA et al. 2006a,b). Both, the authors' own studies and the above references prove that excess zinc in soil can cause disorders in the nitrogen cycle, in which urease plays an important role (NIELSEN, WINDING 2002).

Acid phosphatase responded to soil acidification differently than urease or dehydrogenases, although its reaction to zinc contamination was similar to that of the other enzymes (Figure 3). The activity of phosphatase in uncontaminated sandy loam of pH 7.0 was 56% lower than in soil of similar grain size composition but of lower pH (5.5). Differences between the soils in the scale of the negative effect of zinc on acid phosphatase were significant up to the contamination dose of 200 mg Zn^{2+} kg^{-1} but became less evident under the influence of increasing rates of the pollutant (500-10,000

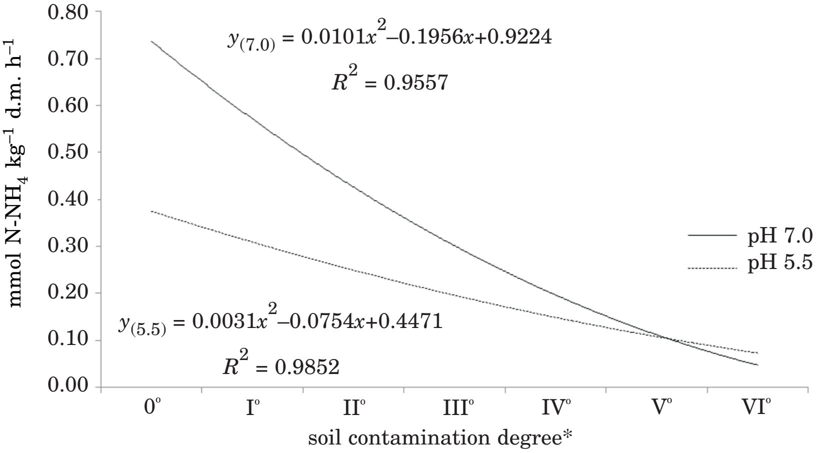

Fig. 2. Activity of urease in soil contaminated with zinc, mmol N-NH_4 kg^{-1} d.m. h^{-1}
*The key is given under Fig. 1

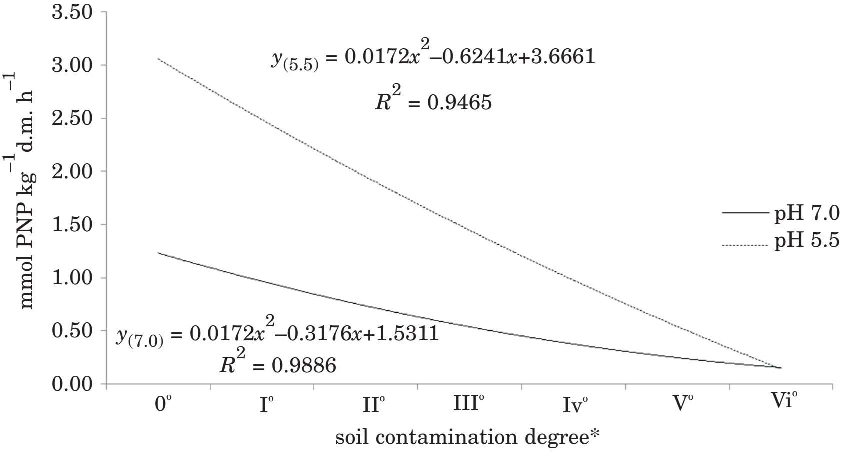

Fig. 3. Activity of acid phosphatase in soil contaminated with zinc, mmol PNP kg^{-1} d.m. h^{-1}
*The key is given under Fig. 1

mg Zn^{2+} kg^{-1}). In the soil of neutral reaction, a dose of zinc as low as 70 mg Zn^{2+} kg^{-1} depressed the activity of this enzyme by 26%, whereas a higher dose such as 200 mg Zn^{2+} kg^{-1} lowered it by 42%. In the soil of acid reaction, the activity of urease was depressed by 7 and 20%, respectively. About 50% inhibition of the activity of urease appeared in both types of soil in response to a dose of 500 mg Zn^{2+} kg^{-1}. Higher rates of the contaminant (1,500-10,000 mg Zn^{2+} kg^{-1}) depressed the activity of urease within the range of 74% to 88%.

Among the analyzed enzymes, acid phosphatase was characterized by the highest variability in respect to the soil reaction, analogously to its behaviour in an experiment reported by Stuczyński et al. (2003), and its response to zinc contamination was similar to the results obtained by Balyaeva et al. (2005), Gülser and Erdođan (2008) and Kuperman and Carreiro (1997).

The activity of β-glucosidase in sandy loam of pH equal 7.0, without additional zinc, was 0.466 mmol PNP and in sandy loam of pH 5.5 it reached 0.505 mmol PNP kg^{-1} d.m. h^{-1} (Figure 4). Evident inhibition of the activity of this enzyme occurred in moderately polluted soils (500 mg Zn^{2+} kg^{-1}): by 26% in soil of pH 7.0 and by 49% in soil of pH 5.5. Strong contamination (10,000 mg Zn^{2+} kg^{-1}) depressed the activity of β-glucosidase by 85% (pH 7.0) and 79% (pH 5.5). Likewise, Hinojosa et al. (2004) determined that the activity of β-glucosidase was 84% lower in response to heavy soil contamination with zinc, whereas Kuperman and Carreiro (1997) noted the inhibition of this enzyme reaching as much as 97%. The fact that the activity of β-glucosidase was more strongly inhibited in the experiments completed by the above authors than in the present study could be related to some interaction of zinc contamination with other heavy metals.

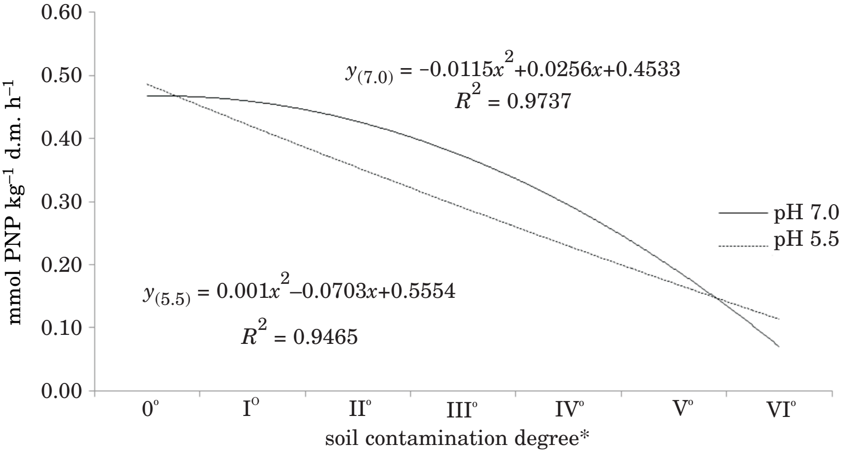

Fig. 4. Activity of β-glucosidase in soil contaminated with zinc, mmol PNP kg^{-1} d.m. h^{-1}
*The key is given under Fig. 1

The activity of arylsulphatase (Figure 5), in contrast to β-glucosidase, was higher in the soil of neutral (0.417 mmol PNP kg^{-1}) rather than acid reaction (0.203 mmol PNP kg^{-1}). In both soils, negative correlation appeared between the degree of zinc contamination and the activity of arylsulphatase. Depending on the concentration of zinc, the activity of this enzyme was inhibited by 36 to 98% in the former soil; in the latter one – it fell by 15 to 85%. These results are only partly congruent with the research reported by Stuczyński et al. (2003), who demonstrated 41% inhibition in the activity

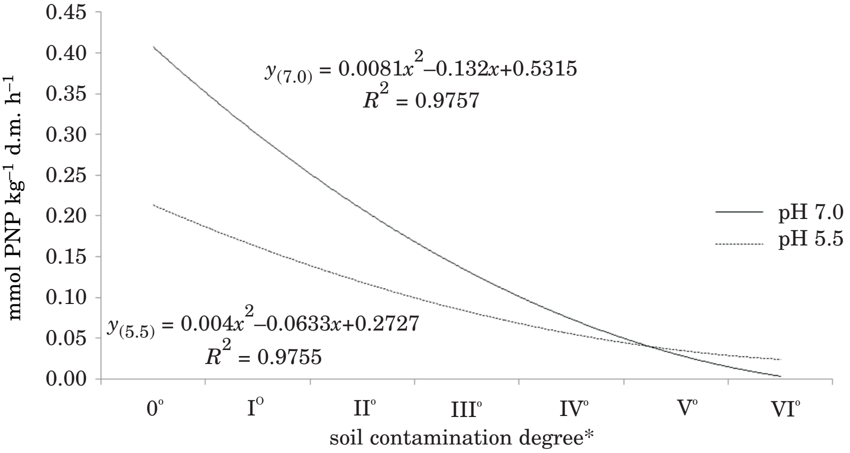

Fig. 5. Activity of arylsulphatase in soil contaminated with zinc, mmol PNP kg^{-1} d.m. h^{-1}
*The key is given under Fig. 1

of arylsulphatase under the influence of 980 mg Zn^{2+} kg^{-1} in soil of pH 7.4 and complete inhibition under the effect of 390 mg Zn^{2+} kg in soil of pH 5.1.

The value of the index for resistance (RS) to zinc proves that the sensitivity of particular enzymes to this metal is varied (Table 2). Irrespective of soil pH, in terms of RS the enzymes can be ordered as follows: β-glucosi-

Table 2

Index of soil enzyme resistance (RS) depending on zinc pollution

mg Zn^{2+} kg^{-1} of soil d.m.	Activity									
	dehydrogenases		urease		acid phosphatase		β-glucosidase		arylsulphatase	
	soil pH									
	7.0	5.5	7.0	5.5	7.0	5.5	7.0	5.5	7.0	5.5
	RS									
70	0.914	0.978	0.889	0.846	0.591	0.864	0.900	0.580	0.621	0.544
200	0.793	0.539	0.522	0.543	0.407	0.673	0.967	0.653	0.500	0.373
500	0.209	0.172	0.287	0.376	0.328	0.341	0.582	0.345	0.291	0.186
1,500	0.018	0.018	0.091	0.220	0.151	0.117	0.436	0.325	0.065	0.090
5,000	0.011	0.013	0.085	0.174	0.102	0.085	0.282	0.207	0.046	0.081
10,000	0.008	0.009	0.063	0.125	0.071	0.062	0.081	0.119	0.014	0.064
Average	0.326	0.288	0.323	0.381	0.275	0.357	0.541	0.372	0.256	0.223
r	-0.623	-0.582	-0.629	-0.696	-0.752	-0.684	-0.866	-0.818	-0.720	-0.641

r – correlation coefficient

dase (0.457) > urease (0.352) > acid phosphatase (0.316) > dehydrogenases (0.307) > arylsulphatase (0.240). However, dehydrogenases, β-glucosidase and arylsulphatase were more resistant to the contamination in neutral soil whereas acid phosphatase and urease – in acid one. Another study by WYSZKOWSKA et al. (2009) proves that resistance of particular soil enzymes to the influence of heavy metals is varied although there is a certain regularity that repeats, namely heavy metals inhibit more strongly the activity of dehydrogenases than that of urease.

ED_{50} for the analyzed enzymes was likewise varied Table 3). In the soil of pH 7.0, it ranged from 3,324 mg Zn^{2+} kg for β-glucosidase to 412 mg Zn^{2+} kg^{-1} for dehydrogenases, and in soil of pH 5.5. – from 1,008 mg Zn^{2+} kg^{-1} for β-glucosidase to 280 mg Zn^{2+} kg^{-1} for arylsulphatase. The above values demonstrate that the response of β-glucosidase and arylsulphatase was the highest to the zinc contamination dependent on the pH of the environment.

Table 3

The dose of zinc (mg Zn^{2+} kg^{-1} of soil) decreases by 50% the activity of soil enzymes (ED_{50})*

Soil pH	Activity				
	dehydrogenases	urease	acid phosphatase	β-glucosidase	arylsulphatase
	ED_{50}				
7.0	412*a*	422*b*	425*b*	3324*a*	432*a*
5.5	357*b*	508*a*	510*a*	1008*b*	280*b*

*homogenous groups in the columns labelled with identical letters

The values of RL suggest that zinc contamination causes lasting changes in soil environment (Table 4), but dehydrogenases are the first to return to equilibrium (RL = 0.276); arylsulphatase is somewhat less resilient (RL = 0.173) and acid phosphatase takes the longest to return to the normal state of balance (RL = 0.064). In contrast, the activity of urease, instead of regenerating, becomes increasingly disturbed (RL = -0.350). The fact that dehydrogenases return to the state of equilibrium sooner may be associated with the succession of microorganisms. Dead cells are replaced by new ones, more resistant to zinc contamination and dead microorganisms serve as energy substrate for new microbial assemblages. Disproportions between the resumed activity of dehydrogenases and the inferior activity of urease might be due to the fact that dehydroogenases express exclusive intercellular activity while urease shows both extra- and intercellular activity.

Table 4

Index of soil enzymes resilience (RS) depending on zinc pollution

mg Zn^{2+} kg^{-1} of soil d.m.	Activity									
	dehydrogenases		urease		acid phosphatase		β-glucosidase		arylsulphatase	
	soil pH									
	7.0	5.5	7.0	5.5	7.0	5.5	7.0	5.5	7.0	5.5
70	-0.291	0.405	-1.000	-0.667	0.663	0.307	-0.710	0.496	-0.101	0.391
200	0.909	0.427	-0.500	-0.643	0.435	-0.090	0.599	0.980	0.164	0.498
500	0.403	0.111	0.080	-0.444	0.462	-0.416	0.319	0.046	0.114	0.141
1,500	0.262	0.177	-0.111	-0.256	0.159	-0.375	0.049	-0.115	0.093	0.230
5,000	0.266	0.184	-0.111	-0.224	0.127	-0.323	0.142	0.002	0.043	0.237
10,000	0.268	0.187	-0.109	-0.216	0.133	-0.321	-0.007	0.045	-0.005	0.274
Average	0.303	0.248	-0.292	-0.408	0.330	-0.203	0.065	0.242	0.051	0.295
r	-0.062	-0.406	0.397	0.727	-0.711	-0.386	-0.022	-0.442	-0.267	-0.250

r – correlation coefficient

CONCLUSIONS

1. Soil contamination with zinc in doses from 70 to 10,000 mg kg^{-1} d.m. of soil causes highly significant inhibition of the activity of dehydrogenases, arylsulphatase, urease, acid phosphatase and β-glucosidae.

2. In respect of their sensitivity to soil contamination with zinc, the enzymes can be ordered as follows: arylsulphatase > dehydrogenases > acid phosphatase > urease > β-glucosidae.

3. Zinc contamination causes persistent changes in soil environment, but dehydrogenases are the first to return to the normal state of equilibrium (RL = 0.276), while arylsulphatase takes longer (RL = 0.173) and acid phosphatase is the least resilient (RL = 0.064). Urease, instead of having its activity improved in time, becomes increasingly disturbed (RL = -0.350).

4. Soil acidification reinforces the negative effect of zinc contamination most evidently in respect of the activity of β-glucosidase and arylsulphatase.

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EFFECT OF SUPPLEMENTATION WITH COPPER IN DIFFERENT CHEMICAL FORMS ON SELECTED PHYSIOLOGICAL BLOOD MARKERS AND CONTENT OF MINERALS IN SELECTED TISSUES OF TURKEYS

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Abstract

Scientific research has demonstrated that different compounds containing copper are capable of stimulating body weight gains, improving the health of animals and attaining high production performance without causing excessive accumulation of various chemical compounds in animal tissues. Besides, copper has been reported to exert a positive effect on the immune system, haematological blood markers and the microbiological balance in the gastrointestinal tract of turkeys.

The experiment was conducted on 60 BUT-9 line turkeys. The birds were divided into 5 experimental groups. Each group included 12 turkeys kept in cages. The bird were reared for 19 weeks. The first group served as control. Group II received $CuSO_4$ added to water in a dose of 10 mg Cu dm^{-3} H_2O. Group III was com posed of birds administered a Cu chelate with lysine in a dose of 10 mg Cu dm^{-3} H_2O. Group IV received $CuSO_4$ in a dose of 20 mg Cu dm^{-3} H_2O, whereas group V were given a Cu-lysine supplement in a dose of 20 mg Cu dm^{-3} H_2O. The birds were fed commercial complete feed mixes. The copper preparations were added to drinking water since the 3rd week of rearing. The objective of this study has been to determine the effect of Cu supplementation in the organic and inorganic form and in two doses on the health of birds and on accumulation of minerals in their breast muscle and liver. Further analyses involved the determination of haematological and biochemical markers in blood as well as assays of selected minerals in the birds' blood, breast muscle and liver. The analysis of the results indicated that the doses of copper improved the health of the turkeys, as verified by better haematological markers. Statistically significant difference appeared in the group receiving 20 mg Cu dm^{-3}

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H_2O irrespective of the chemical form in which copper was administered. The study also shows that Cu supplementation has a significant effect on the metabolism of lipids and a non-significant impact on the content of mineral elements in the analyzed tissues. It was not until the 20 mg dose of Cu as a chelate had been applied that the accumulation of copper in the turkey's liver was enhanced.

Key words: turkeys, copper, blood haematology and biochemistry, mineral elements in tissues, body weight gains.

WPŁYW SUPLEMENTACJI MIEDZIĄ PODAWANĄ W RÓŻNYCH FORMACH CHEMICZNYCH NA WYBRANE WSKAŹNIKI FIZJOLOGICZNE KRWI ORAZ ZAWARTOŚĆ PIERWIASTKÓW MINERALNYCH W WYBRANYCH TKANKACH INDYKÓW

Abstrakt

Z badań naukowych wynika, że różne związki miedzi można zaliczyć do substancji stymulujących przyrosty masy ciała oraz poprawiających zdrowotność zwierząt, co sprzyja uzyskiwaniu lepszych efektów produkcyjnych, nie powodując nadmiernego akumulowania związków chemicznych w tkankach zwierząt. Ponadto miedź korzystnie wpływa na funkcjonowanie układu immunologicznego, poprawia wskaźniki hematologiczne krwi oraz umożliwia utrzymanie równowagi mikrobiologicznej przewodu pokarmowego indyków.

Badania przeprowadzono na 60 indykach linii BUT-9 podzielonych na 5 grup doświadczalnych. Każda grupa liczyła po 12 indyków, które były utrzymane w klatkach. Odchów zwierząt trwał 19 tygodni. Pierwsza grupa stanowiła kontrolę. Grupa II otrzymywała dodatek $CuSO_4$ do wody w ilości 10 mg Cu dm^{-3} H_2O, grupa III – dodatek chelatu Cu z lizyną w dawce 10 mg Cu dm^{-3} H_2O, grupa IV – dodatek $CuSO_4$ w dawce 20 mg, grupa V – suplement Cu – lizynę w dawce 20 mg Cu dm^{-3} H_2O. Paszę stanowiła typowa mieszanka pełnoporcjowa dostępna na rynku. Preparaty miedzi dodawano do wody pitnej od 3. tygodnia odchowu. Celem badań było określenie wpływu suplementacji Cu stosowanej w formie nieorganicznej i organicznej oraz w dwóch różnych dawkach na zdrowotność indyków oraz stopień akumulacji elementów mineralnych w mięśniu piersiowym i wątrobie. W badaniach oznaczono wskaźniki hematologiczne i biochemiczne krwi indyków oraz zawartość wybranych elementów mineralnych we krwi, mięśniu piersiowym i wątrobie ptaków. Wykazano, że zastosowane dawki miedzi spowodowały poprawę stanu zdrowia indyków, co wyrażało się korzystnymi zmianami wskaźników hematologicznych. Statystycznie istotne wartości odnotowano w grupie ptaków, które otrzymywały 20 mg Cu dm^{-3} H_2O niezależnie od formy chemicznej. Z badań wynika, że suplementacja Cu ma znaczący wpływ na gospodarkę lipidową. Miedź miała nieznaczny wpływ na zawartość elementów mineralnych w tkankach. Jedynie dawka 20 mg Cu w formie chelatu spowodowała zwiększoną kumulację tego pierwiastka w wątrobach ptaków.

Słowa kluczowe: indyki, miedź, hematologia i biochemia krwi, elementy mineralne w tkankach, przyrosty.

INTRODUCTION

In poultry breeding practice, much attention is paid to the proper feeding of birds and rational use of feed supplements supposed to increase productivity, which is necessitated by legal regulations, economic considerations and protection of consumers' health (Jamroz, Potkański 2004). Changes in the law governing the use of drugs in animal breeding for human consumption have forced the breeders to apply supplementation which is consistent with the currently binding regulations and which helps to improve the health of animals and to attain high production performance without causing excessive accumulation of various chemical compounds in animal tissues (Olejnik et al. 2009). This fact has contributed to an increased interest in non-antibiotic growth stimulators, which are not a health hazard to consumers (Noyce et al. 2006). Scientific research has demonstrated that different compounds containing copper are capable of stimulating body weight gains and improving feed utilization (Harms, Buresh 1986, Pesti, Bakalii 1996, Smulikowska 1996). The stimulating effects of copper are claimed to be produced owing to improved hematological blood markers (Jamroz 1990, Makarski et al. 2002). Other authors additionally point to the contribution of copper to the synthesis and activation of many enzymes (Boguszewska et al. 2003). This element has been reported to exert a positive effect on the immune system by stimulating the biosynthesis of antibodies (Failla et al. 1998) as well as by inhibiting the growth of pathogenic microorganisms (Brunch et al. 1965, Jamroz, Potkański 2004, Noyce et al. 2006, Kuźlik-Wyrostek, Makarski 2009). In turn, a study conducted by Makarski (2002) demonstrated that the application of copper modified the composition of bacterial flora in the gastrointestinal tract of turkeys. Consequently, copper supplementation enabled the birds to maintain the microbiological balance, which has an indirect, positive effect on body functions, also improving body weight gains.

The objective of this study has been to determine the effect of copper supplementation in two chemical forms and in various doses on levels of selected hematological and biochemical blood markers of turkeys. In order to assess accumulation of minerals in tissues, levels of minerals were assayed in blood, breast muscle and liver of the birds.

MATERIAL AND METHODS

The experimental material were 60 BUT-9 line turkeys reared for 19 weeks. The birds were divided into 5 experimental groups. The first group served as control. Group II received $CuSO_4$ added to drinking water in a dose of 10 mg Cu dm^{-3} H_2O. Group III consisted of birds administered supplementary Cu chelate with lysine in a dose of 10 mg Cu dm^{-3} H_2O. Group IV received $CuSO_4$ in a dose of 20 mg Cu dm^{-3} H_2O, whereas the group V birds were given Cu-lysine supplement in a dose of 20 mg Cu dm^{-3} H_2O. The experimental preparations were administered with drinking water to eliminate the antagonistic effect of feed mix ingredients on the availability of this element (Noy et al. 1994).

Each group included 12 turkeys kept in cages. All birds were provided identical zoohygienic conditions, pursuant to the requirements for slaughter turkey rearing. The birds had an *ad libitum* access to feed and water. They were kept on straw litter and fed typical complete feed mixes available on the Polish market. The quality of water used in the experiment met sanitary requirements stipulated in the Regulation of the Ministry of Health and Environmental Protection (MZiOS) of 4th May 1990 (Journal of Law no 3, item 250/1990) and the requirements set up for water for drinking and household purposes.

Before slaughter, in the 19th week of life, blood was sampled from the brachial vein for haematological and biochemical analyses. Haematological markers, including red blood cells (RBC) count, haemoglobin (Hb) and haematocrit (Ht), were determined in the blood samples according to the clinical methods. In addition, an automatic blood analyzer Hitachi 704 was used to assay the following biochemical parameters in serum: aspartate aminotransferase (AST), alanine aminotransferase (ALT), total cholesterol (CHOL), uric acid (UA), triacylglycerols (TG), glucose (GLU) as well as the content of mineral elements in blood (Ca, Mg and P). All analyses were carried out with reagents produced by the company called Katal. The concentrations of Fe, Cu and Zn were determined directly from serum after dilution (1:4) by means of the ASA method.

The slaughter was followed by detailed dissection, during which samples of liver and breast muscle were collected for analyses. All samples were individually marked, packed in plastic bags, tagged and frozen at -18°C. According to the design of the experiment, samples of liver and breast muscle were determined for concentrations of Zn, Cu, Mn, Fe, P, K, Ca, and Mg. These determinations were carried out on standardized and thoroughly comminuted turkey tissues, from which 1-g samples were collected for analyses. Next, weighed portions of the analytical material were transferred into glass thimbles, to which 5 ml of 70% $HClO_4$ and 65% HNO_3 in a 1:5 ratio were added. Afterwards, the thimbles were placed in a mineralizer, and the samples were mineralized first at 80°C for 60 min, and then at 200°C for ca 90 min. The resultant solution was transferred to a measuring flask, which was filled up to 50 ml with redistilled H_2O. The solution thus prepared was assayed for the concentration of Cu using an atomic absorption spectrophotometer (AAS) on a UNICAM 939 apparatus. Concentrations of the other elements, i.e. Ca, Fe, Zn, and Mg, were assayed with the use of an SP9 AS atomic absorption spectrophotometer by Unicam. The complete feed mixes applied in the experiment were also analyzed for the content of major minerals, i.e. Cu, Zn, Mn, Fe, Ca, and Mg, following the above procedures.

The experimental data underwent statistical analysis with Statistica 5.0 PL 97 software, using a one-way analysis of variance at significance levels of 0.05 and 0.01.

RESULTS AND DISCUSSION

The biological experiment discussed in this paper has involved the application of five types of complete feed mixes, whose nutritive value was balanced depending on the age and physiological needs of turkeys. The composition of the feed mixes declared by the producer is presented in Table 1.

Table 1

Nutritive value of the complete mixes for turkeys

Feeding period	0-3 week	4-6 week	7-10 week	11-14 week	15-19 week
Metabolizable energy (kcal kg^{-1})	2800	2900	3000	3075	3150
Total protein (%)	27.00	25.50	23.00	21.00	18.00
Raw fibre (%)	4.00	3.50	3.50	4.00	4.00
Lysine (%)	1.70	1.60	1.45	1.30	1.10
Methionine (%)	0.62	0.62	0.57	0.54	0.50
Methionine + cystine (%)	1.10	1.02	0.96	0.91	0.82
Tryptophan (%)	0.32	0.26	0.25	0.22	0.19
Calcium (%)	1.30	1.30	1.15	1.10	1.00
Available phosphorus (%)	0.70	0.65	0.63	0.60	0.58
Sodium (%)	0.15	0.16	0.15	0.15	0.15

The feed mixes were tested for their content of selected mineral elements, i.e.: Cu, Zn, Mn, Fe, K, and Mg, and the results are given in Table 2. The analysis of these results demonstrated that the content of minerals in the feed mixes was above the recommended levels (KRATZER et al. 1994), but not toxic (NOY et al. 1994). According to SMULIKOWSKA (1996), the recommended dose of copper is 10 to 20 mg kg^{-1} whereas POLONIS et al. (1999) indicate that for growing turkeys this dose may be as high as 20-30 mg kg^{-1}.

Table 3 presents the final body weights of turkey hens after 19 weeks of rearing. The experimental factors applied in the particular groups did not cause increased body weight gains of turkey hens as compared to the control group. The results just imply a growing tendency along with an increasing dose of copper in particular groups. However, the recorded differences were statistically insignificant. Other investigations (MAKARSKI, ZADURA, 2006) demonstrated that copper addition in the organic form and in a dose of 10 mg dm^{-3} H_2O resulted in a significant increase in body weight of turkeys as compared to the control birds.

Haematological blood analyses are the basic source of information on the health of an animal. Table 4 presents levels of haematological blood

Table 2

Mineral elements in the feed mixes for turkeys

Feed mixes (feeding period)	Minerals					
	Cu (mg kg^{-1})	Zn (mg kg^{-1})	Mn (mg kg^{-1})	Fe (mg kg^{-1})	K (mg kg^{-1})	Mg (mg kg^{-1})
0-3 week	12.8	73.1	114.6	77.0	9.7	1.7
4-6 week	13.8	73.0	118.0	79.0	11.3	2.0
7-10 week	18.7	73.0	110.0	64.0	10.2	1.9
11-14 week	23.9	77.0	120.0	62.0	9.4	1.8
15-19 week	22.1	69.0	104.0	55.0	10.4	1.9

Table 3

Final body weights of turkey hens after 19 weeks of rearing

Indicator	Group I	Group II	Group III	Group IV	Group V
	control	$CuSO_4$	chelate Cu - Lys	$CuSO_4$	chelate Cu - Lys
	-	10 mg Cu dm^{-3} H_2O		20 mg Cu dm^{-3} H_2O	
Body weight (g)	13 235.00 ±770.00	12 180.00 ±706.80	12 340.00 ±1056.80	12 590.00 ±1103.50	13 220.00 ±1188.10

Table 4

The level of haematological indices in blood of 16-week-old turkeys

Index	Group I	Group II	Group III	Group IV	Group V
	control	$CuSO_4$	chelate Cu - Lys	$CuSO_4$	chelate Cu - Lys
	-	10 mg Cu dm^{-3} H_2O		20 mg Cu dm^{-3} H_2O	
RBC (10^{12} dm^{-3})	2.02^{b} ± 0.69	2.28^{ab} ± 0.73	2.35^{ab} ± 0.69	2.58a ± 0.14	2.55^{a} ± 0.14
Hb (g dl^{-1})	13.00b ± 4.30	13.80^{b} ± 4.55	14.77^{b} ± 4.31	16.00a ± 0.58	15.55^{a} ± 0.21
Ht (%)	34.13^{b} ± 11.62	38.60^{ab} ± 12.23	39.45^{ab} ± 11.66	42.84a ± 1.79	42.05^{a} ± 0.92

a, b – statistically significant difference at $p \leq 0.05$

markers of the turkeys in the 19th week of life. The results suggest that the Cu supplementation in doses of 10 mg as well as 20 mg Cu dm^{-3} H_2O had a beneficial influence on all analyzed markers. Statistically significant differences were noted in the group of birds receiving 20 mg Cu dm^{-3} H_2O irrespective of the chemical form in which Cu was administered. The above results confirm the usability of copper for the biosynthesis of haeme, which improves iron utilization from feed ingested by turkeys. Similar observations were made by other authors (Jamroz 1990, Makarski et al. 2002).

Table 5 contains levels of selected biochemical serum markers of turkeys in the 19th week of life. In order to assess toxicity of the applied copper doses, determinations were carried out on activities of blood serum enzymes. The analyses showed enhanced activity of AST in groups III and IV. With respect to the increased ALT activity, a statistically significant value was reported in the group receiving 20 mg Cu dm^{-3} H_2O in the organic form. The enhanced activity of both aminotransferases could be indicated by pathological lesions in livers of the birds. Their increased activity could have also been affected by other factors. The detailed *post-mortem* anatomopathological analysis did not demonstrate any adverse changes in the liver. It seems that in this case the enhanced activity of aminotransferases reflects enhanced biosynthesis of endogenous amino acids necessary for the synthesis of tissue proteins of turkeys. This is confirmed by the final body weight of the turkeys in the groups receiving the increased dose of copper, i.e. 20 mg Cu dm^{-3} H_2O.

Supplementation of feed mixes with copper was observed to have raised the content of total cholesterol in blood serum of the birds. Significant differences between the control and experimental birds did not appear in the case of birds administered 20 mg Cu dm^{-3} H_2O in the form of Cu-lysine

Table 5

The values of biochemical indices of turkeys' blood serum

Index	Group I	Group II	Group III	Group IV	Group V
	control	$CuSO_4$	chelate Cu - Lys	$CuSO_4$	chelate Cu - Lys
	-	10 mg Cu dm^{-3} H_2O		20 mg Cu dm^{-3} H_2O	
AST (U dm^{-3})	592.36^b 119.13	526.25^b 91.33	712.75^a 153.69	632.67^a 129.95	525.70^b 58.75
ALT (U dm^{-3})	18.18^b 4.51	16.25^c 4.65	21.50^b 6.76	20.92^b 4.64	27.00^a 7.44
CHOL (mg dl^{-1})	177.18^c 31.00	308.33^b 141.32	370.17^a 107.02	285.42^b 118.54	109.70^c 16.51
UA (mg dl^{-1})	8.05^a 2.38	6.35^b 0.87	7.71^a 2.01	7.70^a 1.17	4.94^c 0.97
TG (mg dl^{-1})	44.64^a 8.31	24.33^c 7.40	30.00^b 4.43	31.33^b 7.60	23.01^c 6.77
GLU(mg dl^{-1})	223.73^b 58.37	238.92^b 43.52	217.67^b 67.00	248.08^b 24.85	266.87^a 31.48

a, b – statistically significant difference at $p \leq 0.05$

chelate. Birds from all the experimental groups were characterized by a statistically significant decrease in the content of triacylglycerols. This is a desirable development as it is associated with the suppression of lipogenesis in the liver and adipose tissues, and at the same time confirms the role of copper in regulating the blood level of glucose. No significant changes were noted in the level of glucose, except the groups receiving the higher doses of copper.

It is worth mentioning that blood serum of the birds receiving Cu supplement, irrespective of its dose or chemical form, contained lowered levels of uric acid, which may be explained by Cu suppressing deamination processes of amino acids, which in turn seems to indicate their better utilization in the biosynthesis of tissue proteins.

Supplementation with copper preparations induced modification of the mineral composition of turkeys serum. The results of the analyses are presented in Table 6. Supplementation of feed mixes with copper, irrespective of its chemical form, was observed to induce insignificant changes in the serum level of Cu in the experimental turkeys compared to the control birds. A considerably higher level of Cu in blood serum was, in turn, observed in the group administered 20 mg Cu dm^{-3} H_2O in the organic form.

Table 6

The content of mineral elements in blood serum of turkeys receiving supplementation of 10 and 20 mg copper in different chemical forms

Index	Control	$CuSO_4$	Chelate Cu - Lys	$CuSO_4$	Chelate Cu - Lys
	group I	group II	group III	group IV	group V
	-	10 mg Cu dm^{-3} H_2O		20 mg Cu dm^{-3} H_2O	
Cu (mg dl^{-1})	0.37^{a} ± 0.07	0.31^{a} ± 0.02	0.39^{a} ± 0.09	0.36^{a} ± 0.13	0.14^{b} ± 0.06
Zn (mg dl^{-1})	1.56^{b} ± 0.28	2.17^{a} ± 0.38	1.47^{c} ± 0.56	2.04^{ab} ± 0.27	2.04^{ab} ± 0.27
Ca (mg dl^{-1})	10.13^{c} ± 1.37	10.10^{c} ± 1.19	10.44^{b} ± 1.21	10.66^{a} ± 1.06	11.05^{a} ± 1.26
Mg (mg dl^{-1})	2.24^{b} ± 0.48	2.30^{b} ± 0.47	2.58^{b} ± 0.43	2.68^{a} ± 0.45	2.89^{a} ± 0.66
P (mg dl^{-1})	8.05^{a} ± 0.97	7.52^{a} ± 1.21	8.86^{b} ± 1.44	7.90^{a} ± 1.73	8.40^{b} ± 1.66
Fe (µg dl^{-1})	362.18^{a} ± 39.11	277.42^{c} ± 49.58	367.00^{b} ± 79.01	332.17a ± 75.89	370.11^{b} ± 75.60

a, b – statistically significant difference at $p \leq 0.05$

However, these results are inconsistent with findings of other authors, who reported increased concentrations of this mineral element in blood serum of reared birds (Harms, Buresh 1986, Ewing et al. 1998, Ward et al. 1998, Polonis et al. 1999). Noteworthy is the accumulation of this element in the breast muscle and, above all, in the liver. Attention should also be paid to the low Fe level in the group receiving 10 mg of Cu in the inorganic form. Despite the reduced concentration of Fe, the level of haemoglobin in the blood serum of group II birds increased slightly compared to the birds not receiving supplementation.

In order to asses the accumulation of minerals in muscles of the birds receiving Cu supplements, chemical analyses were conducted on samples of their breast muscle. The results can be found in Table 7. They indicate that the level of Cu increased in the group receiving 20 mg Cu dm^{-3} H_2O in the form of $CuSO_4$ and in the one administered 10 mg Cu dm^{-3} H_2O as a chelate, in which the Cu concentration was the highest. In the other groups, the analyses demonstrated a decrease in the Cu concentration as compared to the control group.

Table 7

The content of mineral elements in turkeys' chest muscles

Index	Control	$CuSO_4$	Chelate Cu - Lis	$CuSO_4$	Chelate Cu - Lis
	group I	group II	group III	group IV	group V
	-	10 mg Cu dm^{-3} H_2O		20 mg Cu dm^{-3} H_2O	
Cu (mg kg^{-1})	1.20^{ab} ± 0.6	1.00^{ab} ± 0.1	1.70^{a} ± 0.1	1.40^{ab} ± 0.7	0.80^{b} ± 0.3
Zn (mg kg^{-1})	20.80^{b} ± 1.2	19.20^{bc} ± 0.3	19.50^{c} ± 0.4	19.40^{c} ± 0.5	30.40^{a} ± 3.7
Ca (%)	0.098^{c} ± 0.035	0.257a ± 0.021	0.122^{c} ± 0.007	0.209^{b} ± 0.008	0.225^{ab} ± 0.036
Mg (%)	0.3124^{ab} ± 0.0099	0.3143^{ab} ± 0.0123	0.3129^{ab} ± 0.0047	0.3423^{a} ± 0.0094	0.2984a ± 0.0324
Fe (mg kg^{-1})	10.70 ± 2.1	7.2 ± 0.7	10.20 ± 1.1	10.00 ± 3.3	9.8 ± 1.5
K (%)	2.82^{c} ± 0.19	3.47^{a} ± 0.05	2.97^{cb} ± 0.22	3.14^{ac} ± 0.071	2.83^{c} ± 0.27

a, b – statistically significant difference at $p \leq 0.05$

The liver serves a number of functions in the body's metabolism. It also aids excretion and secretion of metabolites. It is the site were detoxication processes occur and glucose is accumulated in the form of glycogen.

In order to determine the effect of the experimental preparations on the chemical composition of the turkey liver, samples of this organ were subjected to chemical determinations of the content of individual mineral elements. The results presented in Table 8 support the conclusion that Cu supplementation had a significant effect only on changes in Cu and Zn concentrations in a turkey's liver.

The application of copper, irrespective of its dose and chemical form, caused the enhanced accumulation of this element in livers of the turkeys. Despite the fact that copper competes with zinc for the site binding metallothioneins in cells of the intestinal mucosa (Brzozowska 1999, Makarski, Makarska 2009), the content of Zn was observed to increase significantly in the groups administered readily available Cu chelate with lysine.

Table 8

The content of mineral elements in the turkey liver

Index	Control	$CuSO_4$	Chelate Cu - Lis	$CuSO_4$	Chelate Cu - Lis
	group I	group II	group III	group IV	group V
	-	10 mg Cu dm^{-3} H_2O		20 mg Cu dm^{-3} H_2O	
Cu (mg kg^{-1})	4.971^{c} ± 0.447	6.613^{a} ± 0.735	17.308^{b} ± 1.534	10.692^{c} ± 2.334	25.325^{b} ± 0.933
Zn (mg kg^{-1})	38.669^{c} ± 8.211	33.050^{bc} ± 4.290	61.683^{a} ± 10.938	38.124^{c} ± 4.263	50.083^{b} ± 6.512
Ca (%)	0.022^{b} ± 0.001	0.024^{a} ± 0.001	0.020^{b} ± 0.001	0.023^{ab} ± 0.001	0.021^{b} ± 0.002
Mg (%)	0.024^{b} ± 0.002	0.028^{a} ± 0.003	0.025^{b} ± 0.001	0.023^{b} ± 0.003	0.024^{b} ± 0.002
Fe (mg kg^{-1})	106.542^{b} ±13.265	113.483^{b} ± 9.883	129.500^{a} ± 12.470	121.083^{b} ± 18.370	114.667^{b} ± 2.976
K (%)	0.345^{a} ± 0.016	0.326^{a} ± 0.018	0.338^{a} ± 0.017	0.338^{a} ± 0.014	0.283^{b} ± 0.029
Mn (mg kg^{-1})	2.992^{ab} ± 0.240	2.667^{ab} ± 0.436	2.765^{ab} ± 0.259	2.642^{b} ± 0.322	2.908^{a} ± 0.672
P (%)	0.274^{c} ± 0.018	0.359^{a} ± 0.037	0.303^{b} ± 0.014	0.294^{bc} ± 0.014	0.297^{b} ± 0.041

a, b, c – statistically significant difference at $p \leq 0.05$

CONCLUSIONS

1. Dietary supplementation of turkeys with Cu was observed to improve haematological blood markers, including RBC, Hb and Ht. These changes indicate improved health of the experimental turkeys.

2. The activity of transferase-group enzymes determined in the study proved that the supplementation did not cause any negative changes in internal organs.

3. The copper supplements added to drinking water were observed to significantly affect lipid markers in blood serum. The level of cholesterol was reduced only after the administration of increased doses (20 mg) of Cu in the organic form. In turn, the concentration of triacylglycerols in blood serum was positively affected by relatively low doses of this element. The above suggests the hypocholesterolemic properties of Cu.

4. The application of copper supplementation was found to reduce the concentration of uric acid.

5. The supplements did not cause any significant changes in the level of glucose. The level of this parameter was not raised until the highest, 20 mg dose of Cu in the organic form was applied.

6. Cu supplementation as a chelate and in a 20-mg dose caused enhanced accumulation of this element in the liver of turkeys.

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EVALUATION OF SOLID DISPERSIONS CONTAINING MAGNESIUM LEVULINATE SALTS WITH SELECTED CARRIERS

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Abstract

Among the methods applied to ensure optimal pharmaceutical availability of a drug is the incorporation of solid dispersions, i.e. combinations containing a therapeutic substance and a carrier deprived of its pharmacological activity. While manufacturing solid dispersions, special attention must be paid to carriers with a polymeric structure and hydrophilic properties, e.g. polyvinylpyrrolidone (PVP) and also phosphatidylcholine (PC).

The aim of this study has been to evaluate the influence of the carriers PVP and PC 45 on pharmacokinetic parameters of Mg^{2+} absorption from $Mg(Lev)_2$, Mg(LevGly), Mg(LevArg) as well as from solid dispersions containing these salts.

The o/w partition coefficient was determined and the log P value calculated for pure salts and for solid dispersions containing the salts during this study.

The process of Mg^{2+} absorption was examined *in vitro* on a model of the rat's small intestine. Our analysis of the results indicates that addition of PVP or PC 45 to solid dispersions (containing magnesium levulinate salts) significantly improves the degree of Mg^{2+} ion absorption. It has been found that addition of PVP and PC 45 to solid dispersions with magnesium levulinate salts significantly influences the rate of Mg^{2+} absorption from the formulations. Moreover, the results indicate that additional ligand (glycine or arginine) in the structure of magnesium levulinate triggers the effect consisting in depressed lipophilicity for these compounds.

Using the PVP or PC 45 carriers for making solid dispersions containing magnesium levulinate and derivatives with glycine or arginine ligands is quite a promising solution for attaining improved pharmaceutical availability of drugs.

Keywords: magnesium levulinate, solid dispersion, log P, absorption, amino acids: glycine, arginine.

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OCENA STAŁYCH ROZPROSZEŃ ZAWIERAJĄCYCH SOLE LEWULINIANU MAGNEZOWEGO Z WYBRANYMI NOŚNIKAMI

Abstrakt

Jedną z metod osiągnięcia optymalnej dostępności farmaceutycznej leku jest zastosowanie stałych rozproszeń, układów zawierających substancję leczniczą i nośnik, który jest pozbawiony własnego działania farmakologicznego. Przy wytwarzaniu stałych rozproszeń na szczególną uwagę zasługują nośniki o budowie polimerowej i właściwościach hydrofilowych. Należą do nich m.in. poliwinylopirolidon (PVP), a także fosfatydylocholina.

Celem badań była ocena wpływu wybranych nośników: poliwinylopirolidonu (PVP) i roztworu fosfatydylocholiny (PC 45) na poprawę parametrów farmakokinetycznych procesu wchłaniania jonów Mg^{2+} z soli lewulinianu magnezowego $Mg(Lev)_2$, Mg(LevGly), Mg(LevArg) oraz ze stałych rozproszeń zawierających te sole.

W badaniach wyznaczono współczynnik podziału o/w i wyliczono wartość log P dla czystych soli oraz dla stałych rozproszeń zawierających te sole.

Badania procesu wchłaniania jonów Mg^{2+} przeprowadzono metodą *in vitro* na modelu jelita cienkiego szczura. Wykazano, że dodatek PVP lub PC 45 do stałych rozproszeń zawierających sole lewulinianu magnezowego znacząco wpływa na poprawę stopnia wchłaniania. Na podstawie tych badań stwierdzono, że dodatek PVP lub PC 45 do stałych rozproszeń z solami lewulinianu magnezowego istotnie (p=0.01) wpływa na szybkość procesu wchłaniania jonów Mg^{2+} z otrzymanych formulacji. Ponadto wyniki badań wskazują, że dodatkowy ligand (glicyna, arginina) w strukturze cząsteczki lewulinianu magnezowego wywołuje efekt zmniejszenia lipofilowości dla tych związków.

Zastosowanie nośników PVP lub PC 45 do wytwarzania stałych rozproszeń zawierających lewulinian magnezowy i pochodne z ligandami glicyny lub argininy jest obiecujące w poprawie dostępności farmaceutycznej.

Słowa kluczowe: lewulinian magnezu, stałe rozproszenia, log P, wchłanianie, aminokwasy: glicyna, arginina.

INTRODUCTION

Magnesium is one of the essential nutrients necessary for maintaining functions of the human organism. It is one of the macronutrients which determine the course of fundamental biological processes (RAYSSIGUIER et al. 2001). Magnesium is a stabilizer of cell membranes, which affects their fluidity and permeability (KONRAD et al. 2004). Magnesium is essential in human diet and its shortage can cause many diseases (FEILLET-COUDRAY et al. 2005).

Studying inorganic and organic magnesium salts in compounds is interesting because of the high biological activity of magnesium and its supplements as well as their practical role in pharmacotherapy (SCHMITASCHEK, REMPIS 2001,VORMAN 2003, COUDRAY et al. 2005).

Magnesium creates complexes with amino acids, which are essential for the magnesium transport through membranes and blood vessel barriers into blood and tissues. A review of the current research on magnesium and amino acid compounds shows that the number of papers dedicated to such systems is highly varied (RANDE, SOMBERG 2001). Amino acids, such as glycine or arginine are components of live cells. Amino acids are convenient factors modifying functioning of pharmacologically active compounds.

Owing to numerous studies we have learnt that magnesium compounds containing organic anions (FIROZ, GRABER 2001) are the best supplements; they facilitate magnesium transport through walls of the intestines and release metal ions into the serum. Pursuing our studies on the absorption process of Mg^{2+} from magnesium organic salts (in order to obtain a drug with improved Mg^{2+} absorption), we have synthesized magnesium levulinate. Modification of the structure of organic magnesium salts by ligand of glycine or arginine had positive influence on the kinetics parameters of Mg^{2+} absorption both *in vitro* and *in vivo* as described in the previous paper (MARCOIN, SZULC 2002, MARCOIN 2006, MARCOIN, SZULC-MUSIOŁ 2009).

By defining the pharmaceutical availability we can determine the amount and rate of release of a therapeutic substance in a given form of a drug. Absorption of a pharmaceutical substance from the gastrointestinal tract depends on the process and rate of releasing a biologically active substance, thus indicating its potential availability.

The concept of modern pharmaceutical technology is to obtain the form of a drug with a desirable rate of releasing the substance which can have a therapeutic effect. Its effect depends on physicochemical properties of the substance as well as on the form of the drug and the applied auxiliary substances (CORRIGAN et al. 2002).

Creation of molecular solid dispersions is one of the methods used to improve pharmaceutical availability of medications. There are numerous studies concerning the problems of enhancing the pharmaceutical availability of medicinal preparations. A large number of studies in this field imply (among other factors) an appropriate selection of the carriers of absorption. In order to increase the dissolution rate of therapeutic substances from solid dispersions, substances with solubillising and moisturising properties such as polyvinylpyrrolidone (PVP), polyethylene glycols, cellulose derivatives, alginate, phospholipids are tested. Solid dispersion can improve the dissolution rate of poorly water-soluble drugs by dispersing the drug in a carrier (KARAVAS et al. 2006, PATEL, PATEL 2007).

The subject of this paper has been to evaluate the absorption of Mg^{2+} by an *in vitro* method from magnesium levulinate $Mg(Lev)_2$, including a modifying factor such as glycine or arginine. Furthermore, evaluation of the process of Mg^{2+} absorption from solid dispersions containing the analysed compounds has been made. Finally, the influence of the absorption of Mg^{2+} from solid dispersions containing magnesium salts has been examined. The effects of the influence of the carriers, PVP and PC 45, on the pharmacokinetic parameters of magnesium salts and solid dispersions containing $Mg(Lev)_2$, Mg(LevGly), Mg(LevArg) are presented in this paper.

MATERIALS AND METHODS

The study was carried out on salts:

- magnesium levulinate: Mg(Lev)$_2$, Mg($C_5H_7O_6$)$_2$, mol. wt. 254.33;
- magnesium glycine-levulinate: Mg(LevGly), Mg($C_7H_{11}O_5N$), mol. wt. 213.33;
- magnesium arginine-levulinate: Mg(LevArg), Mg($C_{11}H_{20}O_5N_4$), mol. wt. 312.37.

The auxiliary substances such as polyvinylpyrrolidone (PVP), (Serva) and phosphatidylcholine 45%, (PC 45), Lucas Meyer, Ltd) were used in order to produce solid dispersions containing the above magnesium salts. All the chemicals were of the analytical reagent grade. The synthesis of magnesium levulinate was carried according to the procedure described previously (Marcoin, Ryszka 1991).

Modification of the magnesium levulinate structure with ligand of glycine or arginine was achieved in a reaction of magnesium levulinate and an appropriate amino acid in water solution of the molar ratio 1:1. The synthesis was carried out by stirring the mixture vigorously at 60-70°C for 3 hrs.

The products were isolated from the solution by water evaporation (a Unipam 350 evaporator), crystallized from acetone and dried at room temperature. The content of magnesium in the salts was measured by atomic absorption spectrophotometry (Carl Zeiss Jena model AAF 3) at the wavelength 258.2 nm.

Solid dispersions were prepared in the granulated form. After previous micronization, each magnesium salt was mixed with the selected carrier (PC 45 or PVP) in a molar ratio (1:10) and dissolved in ethanol. After complete evaporation of ethanol and drying under vacuum, the solid dispersions thus obtained were unified with a sieve (1.0 mm).

For the solid dispersions, the o/w partition coefficient for the system of *n*-octanol/phosphate buffer was determined using the traditional shake-flask method. Lipophilicity was characterized by the log P.

The absorption of Mg^{2+} from magnesium salts was carried out on an *in vitro* model according to the method described previously (Marcoin, Szulc 2002), in which the absorption area was the small intestine (ileum) of a rat. The study had been approved by the Bioethics Committee of the Medical University of Silesia. The results consisting of the absorption rate constant (k) and absorption half time ($t_{50\%}$) were calculated. The measurements were repeated twelve times in order to minimize statistical errors. Standard deviation (SD) and variance (V) were determined. Statistical significance was tested by repeated measures using ANOVA followed by Kruskal-Wallis test or else Post Hoc multiple comparisons were done. $P<0.05$ was considered significant.

RESULTS AND DISCUSSION

Lipophilic properties of $Mg(Lev)_2$, Mg(LevGly) and Mg(LevArg) were determinedby means of the partition coefficient of log P. The calculated values of log P for the examined pure salts and the solid dispersions containing these salts with PVP or PC 45 are presented in Figure 1.

The results of our analysis showed that introduction of additional ligands of amino acids such as glycine or arginine into the molecule of $Mg(Lev)_2$ leads to a decrease in the log P value. In the case of Mg(LevGly), the value of log P decreases by about 2.6 times in comparison with the parent salt.

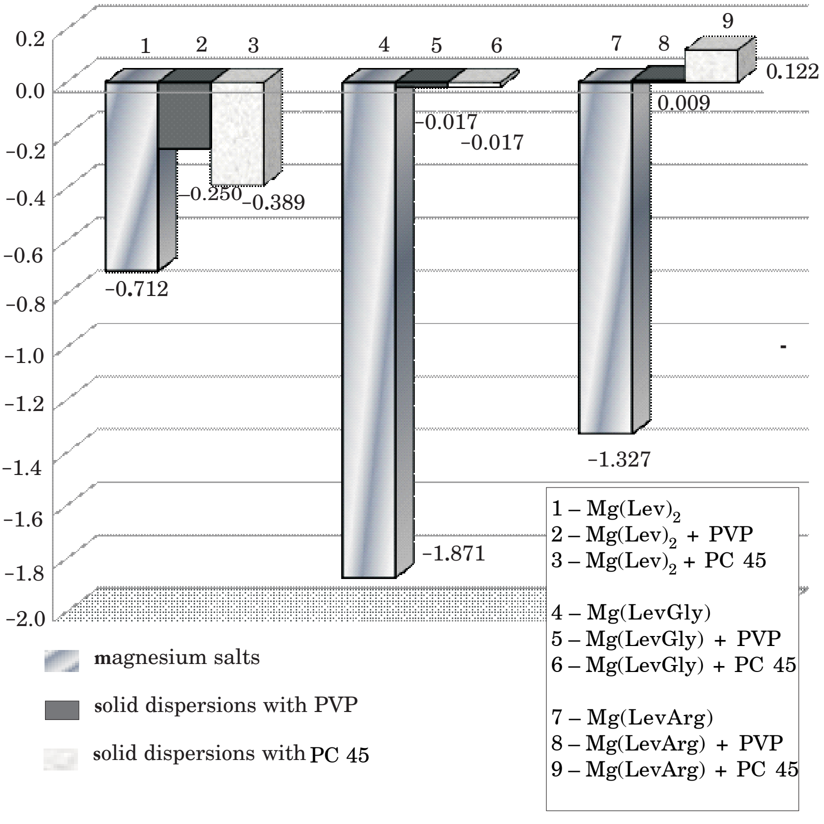

Fig. 1. The values of partition coefficient (logP) for pure magnesium salts and for solid dispersions containing these salts. Values are expressed as mean ± SD (*n*=12).

2,3 bars: $*P \leq 0.01$ vs. 1 bar; 5,6 bars: $*P < \leq 0.01$ vs. 4 bar; 8,9 bars: $*P < \leq 0.01$ vs. 7 bar.

Magnesium salts: $Mg(Lev)_2$, Mg(LevGly), Mg(LevArg). Solid dispersions: $Mg(Lev)_2$ + PVP, $Mg(Lev)_2$ + PC 45, Mg(LevGly) + PVP, Mg(LevGly) + PC 45, Mg(LevArg) + PVP, Mg(LevArg) + PC 45

Meanwhile, the modified structure of the parent salt with an additional ligand of arginine depresses log P values by about 1.8-fold. This value is statistically significant at $p=0.01$.

The influence of surface parameters, volume and molecule mass on the penetration parameters of a therapeutic substance through the lipid barrier became noticeable. Electrically charged compound particles were restrained inside the environment into which they had been introduced while electrically neutral complexes could easily penetrate the biological membranes. This phenomenon was described by BLAQUIERE, BERTHON (1987) and confirmed in this study.

The application of a PVP carrier to solid dispersions containing Mg(LevGly) and Mg(LevArg) salts causes increased lipophilicity, so that the calculated value of log P for Mg(LevGly) + PVP increased by 1.854 units and for Mg(LevArg) + PVP by 1.336 units compared with the log P values calculated for pure salts of Mg(LevGly) and Mg(LevArg). While using PC 45 for solid dispersions containing Mg(LevGly) and Mg(LevArg), the log P value increased by 1.854 and 1.449, respectively, compared with pure salts (Figure 1).

The mean values of pharmacokinetic parameters obtained for levulinate magnesium salts and solid dispersions containing these salts with PVP or PC 45 are presented in Table 1. It has been found that absorption of Mg^{2+} in the small intestine was in agreement with the first-order kinetics. As seen

Table 1

Kinetic parameters Mg^{2+} absorption from solid dispersions containing magnesium salts in the small intestine

Solid dispersions	$k \cdot 10^{-3}$ (min)	$t_{50\%}$ (h)	Total amount (%) of absorbed Mg^{2+} within 2 h of the experiment	W (%)	(±) SD
Mg $(Lev)_2$	2.584	4.469	24.90	9.66	2.407
Mg (Lev-Arg)	0.924^a	12.497^a	11.80^a	11.66	1.376
Mg (Lev-Gly)	0.681^a	16.963^a	9.80^a	9.61	0.942
Mg $(Lev)_2$ + PVP	2.565	4.503	28.42	8.23	2.340
Mg (Lev-Arg) + PVP	2.784^c	4.149^c	31.26^c	4.65	1.454
Mg (Lev-Gly) + PVP	2.638^b	4.381^b	29.30^b	4.41	1.295
Mg $(Lev)_2$ + PC 45	2.616	4.415	25.32	5.91	1.497
Mg (Lev-Arg) + PC 45	3.727^c	3.099^c	34.96^c	11.04	2.797
Mg (Lev-Gly) + PC 45	2.792^b	4.137^b	28.56^b	4.08	1.167

k – absorption rate constant;
$t_{50\%}$ – absorption half – time;
W – variance;
SD – standard deviation $^aP \leq 0.01$ vs. Mg$(Lev)_2$, $^bP \leq 0.01$ vs. Mg(Lev-Gly), $^cP \leq 0.01$ vs. Mg(Lev-Arg).

from Table1, the highest (k) value among the pure magnesium salts was found for $Mg(Lev)_2$ (k=2.584 · 10^{-3} min), while the k value for Mg(LevGly) was 0.681 · 10^{-3} min. For Mg(LevArg), (k) was equal to 0.924 · 10^{-3} min. By analysing the research results on Mg^{2+} absorption from the salts in an *in vitro* model, the negative influence of an amino acid ligand (Gly or Arg) on transportation of Mg^{2+} has been shown.

The amount of absorbed Mg^{2+} after 2 hours of the experiment was significantly higher (p=0.01) for $Mg(Lev)_2$ than for Mg(LevGly) and Mg(LevArg); these values were 24.90% for $Mg(Lev)_2$, 11.80% for Mg(LevArg) and 9.80%, for Mg(LevGly). As demonstrated above, the glycine or arginine ligand added into the structure of a molecule of $Mg(Lev)_2$ depressed the lipophilicity of the compound (Figure 2).

The results of our analysis (Table 1) showed that by adding an auxiliary substance such as PVP or PC 45 into solid dispersions containing the examined salts, the pharmacokinetic parameters are elevated. Application of PVP and PC 45 to manufacturing preparations of solid dispersions containing levulinate salts significantly influences (p=0.01) both the quantity and the rate of absorption of the tested medicinal preparations.

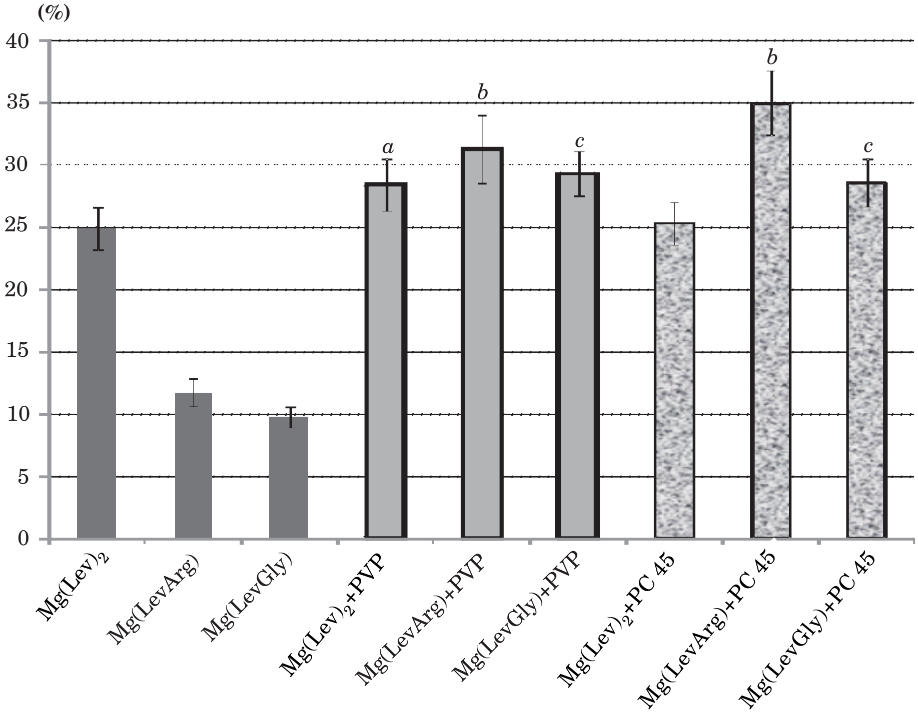

Fig. 2. Total amount (%) of absorbed Mg^{2+} through the rat's small intestine in 120 minutes of the experiment. Values are expressed as mean ± SD (n=12)

$^{a}P \leq 0.05$ vs. $Mg(Lev)_2$, $^{b}P \leq 0.01$ vs. Mg(LevArg), $^{c}P \leq 0.01$ vs. Mg(LevGly) at the same time point

Adding the PC 45 carrier into solid dispersions containing magnesium levulinate and its derivatives raised the rate of absorption. The highest values were obtained for Mg(LevArg) + PC 45 $k=3.727 \cdot 10^{-3}$ min.

In another study, JADHAY et al. (2009) aimed at developing microemulsion based on lecithin (PC) organogel formulations with fluconazol and investigating its suitability. The results of showed that of the surfactant action of the EO-lecithin organogels was safe enough for the purpose in question. The influence of lecithin (PC) on solubility of aceclofenac organogels was also described by SHAIKH et al. (2009), whose results indicate positive influence of lecithin (PC) as a pharmaceutically acceptable surfactant.

Besides, the addition of PVP into solid dispersion with levulinate magnesium salts positively influences the transportation of Mg^{2+} from these preparations. Application of magnesium levulinate salts as a solid dispersion improved the pharmacokinetic parameters, absorption rate and constant (k) of Mg ions, which was as follows: $k=2.784 \cdot 10^{-3}$ min for Mg(LevArg)+PVP, $k=2.638 \cdot 10^{3}$ min for solid dispersions with Mg(LevGly) + PVP and $k=2.565 \cdot 10^{-3}$ min for solid dispersions with $Mg(Lev)_2$ + PVP.

During the present research, it has been found that by adding PVP and PC 45 to solid dispersions containing magnesium salts their solubility is altered. These carriers for solid dispersions improve their pharmaceutical availability and, consequently, bioavailability. The results confirm that these auxiliary substances are of great importance to using the examined salts. Addition of the carrier PC 45 to a solid dispersion releases 4.96% if it contains Mg(LevArg) Mg^{2+}, and 28.56% Mg^{2+} it is has Mg(LevGly), compared to solid dispersion containing $Mg(Lev)_2$ – 25.32% Mg^{2+}. Similarly, the activity PC 45 significantly influences ($p=0.01$) dissolution and improves absorption of Mg^{2+}.

There was a correlation between the results of log P values as a factor characterizing lipophilicity and the total amount of absorbed Mg^{2+} ions during 2 hours of the experiment, confirmed by the results of a kinetic study of the absorption process. A higher value of the absorbed Mg^{2+} was found for the solid dispersion of Mg(LevArg) +PC 45, where the log P value was 0.122.

In order to improve the solubility of Tenoxicam or Flurinazine, PVP is used as a carrier of these substances in solid dispersion (EL-GAZAYERLY et al. 2000). Other researchers described the results of their studies (SILVA et al. 2010) in which they applied PVP K 15 to produce solid dispersions containing Simvastatin, which is practically insoluble in water. These inert carriers significantly improved the release profile of a drug from all solid dispersions. LATINEN et al. (2010) prepared a tablet readily dissolving in the mouth, which consists of a stable solid dispersion with PVP K 30, PEG 8000. Investigations have verified an improved dissolution profile of Valdecoxib when solid dispersion with PVP K 30 was used (MODI, TAYADE 2006). Dissolution of Valdecoxib improved significantly in solid dispersion products (< 85% in 5 minutes).

CONCLUSIONS

This study suggests that the analysed auxiliary substances (PVP, PC 45) are good carriers, which improve the lipophylic properties of the examined solid dispersions, thus facilitating the permeability of the medicinal substances thorugh the lipid barrier. Moreover, the magnesium salts improve the permeability of pharmaceuticals through the intestine.

It has been noticed that there is some correlation between the total amount of the absorbed Mg^{2+} and the lipophilic properties of the examined magnesium salts and their solid dispersions.

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PRELIMINARY EVALUATION OF THE INFLUENCE OF SOIL FERTILIZATION AND FOLIAR NUTRITION WITH IODINE ON THE EFFICIENCY OF IODINE BIOFORTIFICATION AND CHEMICAL COMPOSITION OF LETTUCE*

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Abstract

According to the World Health Organization, consumption of table salt (being a major carrier of iodine in human diet) should be reduced of 50%. Vegetables biofortified with iodine can become an alternative source of this element for humans. Agronomic recommendations with reference to biofortification have to be developed, including the evaluation of side-effects associated with iodine application to plants. Iodine is not an essential element for plants and hence its effect on crops has not yet been diagnosed. The aim of the study has been to assess the influence of soil fertilization with KI and foliar application of KIO_3 on the success of iodine biofortification as well as the mineral composition of lettuce. Lettuce cv. Melodion F_1 was cultivated in a field experiment in 2008-2009. Combinations with different soil fertilization and foliar nutrition with iodine were distinguished in the research including: control (without iodine application), three combinations with pre-sowing soil fertilization of iodine (in the form of KI) in doses of 0.5, 1.0 and 2.0 kg I ha^{-1} as well three combinations with four applications of foliar nutrition with iodine solution

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(as KIO_3) in the concentration of: 0.0005%, 0.005% and 0.05% after using 1000 dm^3 of working solution per 1 ha so that the following amounts of iodine were applied: 0.02, 0.2 and 2.0 kg I ha^{-1}, respectively. In lettuce heads, the iodine content as well as the content of: P, K, Mg, Ca, S, Na, B, Cu, Fe, Mn, Zn, Mo, Al, Cd and Pb were determined using the ICP-OES technique, while N-total was assayed by Kjeldahl method. In comparison to the control, only foliar nutrition with 0.05% solution of iodine significantly improved accumulation of this element in lettuce. At the same time, a lower level of nitrogen nutrition was observed in plants from this combination. A significant increase in the N-total content was found only in lettuce plants fed with 2.0 kg I ha^{-1} dose of KI. In reference to the control, both foliar and soil application of iodine contributed to a higher content of K, Mg, Ca, Mn and Cd as well as a decreased level of P, Cu and Zn in lettuce. Doses, forms and application methods of iodine were found to have produced diverse effects on the content of S, Na, B, Fe, Mo, Al and Pb in lettuce plants.

Key words: biofortification, iodine, mineral composition, lettuce.

WSTĘPNA OCENA WPŁYWU NAWOŻENIA I ODŻYWIANIA DOLISTNEGO JODEM NA EFEKTYWNOŚĆ BIOFORTYFIKACJI SAŁATY W JOD ORAZ JEJ SKŁAD CHEMICZNY

Abstrakt

Zgodnie z zaleceniem WHO spożycie soli kuchennej (głównego nośnika jodu w diecie) musi zostać ograniczone o 50%. Warzywa biofortyfikowane w jod mogą stać się głównym źródłem jodu dla człowieka. Zalecenia agrotechniczne dotyczące sposobów biofortyfikacji muszą być opracowane na postawie badań określających uboczne oddziaływanie jodu na rośliny. Wynika to z tego, że jod nie jest składnikiem pokarmowym roślin, a jego oddziaływanie na rośliny nie zostało dostatecznie zdiagnozowane. Celem badań było określenie wpływu doglebowego nawożenia jodem (w formie KI) i odżywiania dolistnego tym pierwiastkiem (w formie KIO_3) na efektywność biofortyfikacji w jod oraz na zawartość składników pokarmowych i balastowych w sałacie.

Sałatę odm. Melodion F_1 uprawiano w doświadczeniu polowym w latach 2008-2009. W badaniach uwzględniono kombinacje ze zróżnicowanym nawożeniem doglebowym i odżywianiem dolistnym jodem. Wyróżniono kontrolę (nienawożoną i nieodżywianą dolistnie jodem), kombinacje z przedsiewnym nawożeniem doglebowym jodem w dawkach 0,5, 1,0 i 2,0 kg I ha^{-1} oraz 4-krotne dolistne odżywianie roślin jodem w stężeniach 0,0005%, 0,005% i 0,05% – sumarycznie po zastosowaniu 1000 dm^3 cieczy roboczej na 1 ha zaaplikowano roślinom odpowiednio 0,02, 0,2 i 2,0 kg I ha^{-1}. W główkach sałaty oznaczono: zawartość jodu oraz P, K, Mg, Ca, S, Na, B, Cu, Fe, Mn, Zn, Mo, Al, Cd i Pb techniką ICP-OES oraz zawartość azotu metodą Kiejdahla.

W porównaniu z kontrolą, jedynie odżywianie dolistne jodem w stężeniu 0,05% wpłynęło na statystycznie istotne zwiększenie zawartości tego pierwiastka w sałacie. Jednocześnie stwierdzono pogorszenie stopnia odżywienia roślin w azot w tej kombinacji. Istotny wzrost zawartości azotu w sałacie uzyskano wyłącznie w wyniku nawożenia jodem w dawce 2,0 kg I ha^{-1}. W odniesieniu do obiektu kontrolnego, zarówno dolistna, jak i doglebowa aplikacja jodu wpłynęła na zwiększenie zawartości K, Mg, Ca, Mn i Cd oraz na zmniejszenie zawartości P, Cu i Zn w sałacie. Stwierdzono zróżnicowane oddziaływanie dawki, formy i sposobu aplikacji jodu na zawartość S, Na, B, Fe, Mo, Al i Pb w sałacie.

Słowa kluczowe: biofortyfikacja, jod, skład mineralny, sałata.

INTRODUCTION

Plant biofortification is defined as such an increase in the content of mineral element or biologically active substance in an edible part of crop plant that a notable improvement of human health can be achieved (White, Broadley 2005, 2009, Campos-Bowers, Wittenmyer 2007).

The need to undertake research on possible increase of the iodine content in vegetables results from the recommendation of the World Health Organization to significantly reduce (even by half) salt uptake by humans. Excessive consumption of table salt is considered as one of the major factors contributing to the development of numerous civilization diseases such as cardiovascular events or osteoporosis. At the same time, table salt is a major carrier of iodine in human diet (Szybiński et al. 2010). Reduced salt uptake can therefore pose a threat to the effective iodine prophylaxis in Poland as well an in other countries, resulting in increased incidence of iodine deficiency disorders (IDD). Consequently, search for alternative methods of iodine supplementation has been carried out in the last few years, indicating iodine biofortification of crop plants as the most promising direction (Nestel et al. 2006, White, Broadley 2009).

Current studies on iodine biofortification focus mainly on the effectiveness of increasing its content in edible parts of plants as well as on the influence of a dose, form and way of iodine application on biomass and yield (Maćkowiak, Grossl 1999, Zhu et al. 2003, Dai et al. 2004). It has been demonstrated that plants preferably uptake iodine by roots in the form of I^- (rather than IO_3^-) although excessive doses of this iodine form can negatively affect biomass (Maćkowiak, Grossl 1999, Zhu et al. 2003). What can influence the effectiveness of iodine biofortification is the method of its application. In the research conducted by Altinok et al. (2003), increased accumulation of iodine in alfalfa plants was observed as a result of foliar nutrition with potassium iodide in comparison to soil introduction of this compound. In just a few studies, evaluation of the interaction between increased content of iodine and biological value of crop plants has been undertaken (Blasco et al. 2008, Weng et al. 2008). No information, however, is available on the influence of iodine foliar or soil application on the uptake and accumulation of mineral elements in plants.

The aim of the study has been to determine the effect of iodine nutrition in reference to its form (KI and KIO_3), dose and method of introduction (foliar application or soil fertilization) on the effectiveness of iodine biofortification as well as chemical composition of lettuce heads.

MATERIAL AND METHODS

In 2008-2009, field cultivation of lettuce cv. Melodion F_1 was conducted in a crop rotation system on single soil complex at the Experimental Field Station of Agricultural University in Kraków (Poland). Lettuce was cultivated on silt loam soil (35% sand, 28% silt and 37% clay) with the content of organic matter in the 0-30 cm soil layer: 3.41% and 3.76% (in 2008 and 2009, respectively), and the following content of the available forms of nutrients soluble in 0.03 M acetic acid (in the two consecutive years): N (NO_3-N+NH_4-N) – 3.9-1.3, P – 27.8-46.1, K – 142.6-124.1, Mg – 118.3-113.3 and Ca – 1469.0-1062.5 mg dm^{-3} of soil. In 2008 and in 2009, the soil's pH$_{(H_2O)}$ was 6.99-6.73, while the salinity of soil (EC) was 0.13-0.12 mS cm^{-1}, respectively. Lettuce seeds were sown on 11 and 15 March (in 2008 and 2009, respectively). Seedlings were planted into soil in rows 30 cm apart with 30 cm plant spacing on 11 and 15 April of the following year. Nitrogen as ammonium nitrate in a dose of 100 mg N dm^{-3} soil as well as iodine soil fertilization in the form of KI were applied directly prior to lettuce planting.

Different iodine fertilization (in the form of KI) and foliar nutrition (as KIO_3) were applied in the experiment including: 1 – control (without soil fertilization and foliar nutrition with iodine); three combinations with presowing soil fertilization with iodine : 2 – 0.5 kg I ha^{-1}, 3 – 1.0 kg I ha^{-1} and 4 – 2.0 kg I ha^{-1} as well as three combinations with four treatments of foliar application of iodine in the following concentrations: 5 – 0.0005% (total 0.02 kg I ha^{-1}), 6 – 0.005% (0.2 kg I ha^{-1}) and 7 – 0,05% (2.0 kg I ha^{-1}). Foliar nutrition was performed using 1000 dm^3 of working solution per hectare on the following dates: 1st – 28 and 29 April, 2nd – 05 and 13 May, 3rd – 12 and 20 May, 4th – 23 and 27 May (in 2008 and 2009, respectively)

The experiment was arranged in a split-plot design with four replications. Each experimental treatment was randomized in four repetitions on 5 m × 1.5 m (7.5 m^2) plots. The total area used for experiment was 270 m^2.

Lettuce heads were harvested on 2 and 1 June (in 2008 and 2009, respectively). Soil samples from the 0-30 cm layer were collected during lettuce harvesting using a soil drill.

In lettuce samples, the iodine content was assessed after incubation with 25% TMAH according to the standard method (prEN 15111- R2-P5-F01). The total nitrogen was determined by Kjeldahl method (Persson, Wennerholm 1999). The content of P, K, Mg, Ca, S, Na, B, Cu, Fe, Mn, Zn, Mo, Al, Cd and Pb was assayed after mineralization of plant samples in 65% super pure HNO_3 (Merck no. 100443.2500) using a CEM MARS-5 Xpress microwave oven (Pasławski, Migaszewski 2006).

In soil samples, pH was determined by a potentiometer and the content of I, N-NH_4, N-NO_3, P, K, Mg, Ca, S and Na was determined after extraction of the soil with 0.03 M acetic acid (Nowosielski 1988). The content of B, Cu, Fe, Mn, Zn, Mo, Al, Cd and Pb was assayed after extraction with 1 M HCl (Gorlach et al. 1999).

The content of I as well as P, K, Mg, Ca, S, Na, B, Cu, Fe, Mn, Zn, Mo, Al, Cd and Pb in soil and plant samples was determined with the ICP-OES technique with a Prodigy Teledyne Leeman Labs USA spectrometer. The content of nitrogen forms in soil samples ($N\text{-}NH_4$, $N\text{-}NO_3$) was determined by the FIA technique [PN-EN ISO 13395: 2001; PN-EN ISO 11732:2005 (U)].

The results were statistically verified using an ANOVA module of Statistica 9.0 PL programme at the significance level $P<0.05$. Significance of changes was assessed with the use of variance analysis. In the case of significant changes, homogenous groups were determined on the basis of Duncan's test.

RESULTS AND DISCUSSION

RThe rsearch conducted by Dai et al. (2006) revealed that root application of oxidized form of iodine (IO_3^-) contributed to significantly higher (even by ten-fold) accumulation of this element in spinach leaves. However, some others studies with iodine applied to soil or solution culture indicate that iodine application in I^- form (when compared to IO_3^-) gives better results with regard to the uptake and bioaccumulation of this element in plants. What is more, increasing iodine doses improve the effectiveness of biofortification (Muramatsu et al. 1983, Zhu et al. 2003, Blasco et al. 2008, Weng et al. 2008). In the present work, relatively small differentiation of the iodine content in lettuce plants from particular combinations was found (Table 1). Statistically higher accumulation of iodine (in comparison to the control) was noted only for the foliar application of KIO_3 in the dose of 2 kg I ha^{-1}. It is worth noticing that significant build-up of the iodine content was observed owing to the application of a higher KIO_3 concentration used for foliar nutrition. Soil fertilization with KI did not result in iodine biofortification of lettuce when compared to the control plants not fertilized with this element (Table 1). Considering the total values of iodine, it could be concluded that application of higher doses of KI (1.0 and 2.0 kg I ha^{-1}) raised to a certain degree the iodine accumulation in lettuce plants. It should also be noted that the iodine content in soil after lettuce cultivation from individual combinations of the study (Table 2) did not correlate with the detected content of this element in lettuce heads.

Varied influence of soil fertilization and foliar nutrition with iodine was found in reference to the content of N, P, K, Mg, Ca, S, Na, B, Cu, Fe, Mn, Zn, Mo, Al, Cd and Pb in lettuce (Table 1). Slight changes were noted in the N-total content in lettuce heads resulting from the tested iodine treatments. Statistically significant increase in the N-total content, when compared to the control, was observed only in the case of soil fertilization with the high-

Table 1

Iodine biofortification effects and chemical composition of lettuce depending on soil fertilization and foliar application of iodine – means from 2008-2009

Combinations**	Iodine (mg I kg^{-1} d.w.)	(% d.w. of lettuce heads)						
		N	P	K	Mg	Ca	S	Na
1. Control	12.1 *ab*	3.99 *b*	0.637 *e*	4.90 *a*	0.286 *a*	0.97 *a*	0.262 *c*	0.052 *b*
2. Fertilization 0.5 kg I ha^{-1}	15.7 *ab*	3.93 *b*	0.593 *bc*	5.03 *c*	0.322 *d*	1.22 *e*	0.263 *c*	0.053 *c*
3. Fertilization 1.0 kg I ha^{-1}	19.9 *b*	3.94 *b*	0.579 *a*	5.01 *bc*	0.315 *c*	1.16 *d*	0.254 *a*	0.058 *e*
4. Fertilization 2.0 kg I ha^{-1}	18.4 *b*	4.06 *c*	0.597 *bc*	5.24 *d*	0.311 *c*	1.14 *c*	0.267 *d*	0.045 *a*
5. Foliar application 0.02 kg I ha^{-1}	8.5 *a*	3.97 *b*	0.591 *ab*	5.21 *d*	0.301 *b*	1.14 *c*	0.259 *bc*	0.046 *a*
6. Foliar application 0.2 kg I ha^{-1}	16.7 *b*	3.97 *b*	0.615 *d*	4.85 *a*	0.300 *b*	1.08 *b*	0.257 *ab*	0.055 *d*
7. Foliar application 2 kg I ha^{-1}	54.3 *c*	3.86 *a*	0.606 *cd*	4.96 *b*	0.316 *cd*	1.17 *d*	0.260 *bc*	0.061 *f*
Test *F*	*	*	*	*	*	*	*	*

	(mg kg^{-1} d.w. of lettuce heads)								
	B	Cu	Fe	Mn	Zn	Mo***	Al	Cd	Pb
1. Control	25.5 *ab*	9.05 *e*	292.1 *c*	41.2 *a*	73.6 *d*	0.294 *a*	229.5 *cd*	0.89 *a*	0.446 b
2. Fertilization 0.5 kg I ha^{-1}	26.7 *d*	8.24 *d*	284.3 *b*	51.8 *f*	70.2 *bc*	0.355 *bc*	235.7 *cd*	1.16 *d*	0.518 *bc*
3. Fertilization 1.0 kg I ha^{-1}	25.0 *a*	7.92 *c*	278.6 *b*	44.3 *d*	66.4 *a*	0.331 *ab*	225.6 *bc*	1.06 *b*	0.542 *c*
4. Fertilization 2.0 kg I ha^{-1}	26.1 *c*	7.77 *b*	280.3 *b*	43.0 *b*	67.4 *ab*	0.351 *bc*	227.4 *bc*	1.04 *b*	0.529 *bc*
5. Foliar application 0.02 kg I ha^{-1}	26.0 *bc*	7.88 *bc*	279.4 *b*	45.3 *e*	68.8 *abc*	0.299 *a*	217.6 b	1.05 *b*	0.766 *d*
6. Foliar application 0.2 kg I ha^{-1}	25.5 *ab*	7.45 *a*	254.0 *a*	43.7 *c*	71.0 *cd*	0.310 *a*	198.7 *a*	1.04 b	0.310 *a*
7. Foliar application 2 kg I ha^{-1}	26.1 *c*	8.29 *d*	298.5 *c*	45.7 *e*	70.0 *bc*	0.380 *c*	238.6 *d*	1.01 *b*	0.544 *c*
Test *F*	*	*	*	*	*	*	*	*	*

Means followed by the same letters are not significantly different for $P < 0.05$. Test *F*: * means are significantly different.
**in combinations 5-7 the total dose of iodine applied in four foliar nutrition treatments was given;
***results for Mo only from 2009

Table 2

Chemical properties of soil after lettuce cultivation – means for the 0-30 cm soil layer from 2008-2009

Combinations	$pH_{(H_2O)}$	(mg dm^{-3} soil)								
		I	$N\text{-}NH_4$	$N\text{-}NO_3$	P	K	Mg	Ca	S	Na
1. Control	7.31 *b*	2.88 *b*	1.41	17.39 *a*	33.40 a	97.8 a	145.9 bc	1384.3 abc	12.3 a	9.38
2. Fertilization 0.5 kg I ha^{-1}	7.07 *a*	1.76 *a*	0.25	44.89 *c*	45.44 *cd*	113.2 *b*	148.5 *c*	1452.7 *bcd*	25.5 *b*	8.85
3. Fertilization 1.0 kg I ha^{-1}	7.07 *a*	3.04 *bc*	0.34	29.28 *b*	47.95 *d*	143.1 *de*	131.8 *a*	1341.6 *ab*	19.0 *a*	8.15
4. Fertilization 2.0 kg I ha^{-1}	7.28 *b*	3.73 *c*	0.72	50.14 *d*	42.90 *cd*	150.6 *e*	149.0 *c*	1477.9 *cd*	13.2 *a*	9.17
5. Foliar application** 0.02 kg I ha^{-1}	7.25 *b*	3.42 *bc*	1.02	20.28 *a*	36.98 *ab*	121.3 *bc*	150.6 *c*	1557.6 *d*	17.9 *a*	8.30
6. Foliar application** 0.2 kg I ha^{-1}	7.28 *b*	3.21 *bc*	0.98	26.72 *b*	43.04 *cd*	132.2 *cd*	140.3 *abc*	1298.8 *a*	16.2 *a*	10.22
7. Foliar application** 2 kg I ha^{-1}	7.25 *b*	3.72 *c*	0.70	28.84 *b*	41.05 *bc*	122.5 *bc*	136.3 *ab*	1319.3 *a*	15.3 *a*	8.68
Test *F*	*	*	n.s.	*	*	*	*	*	*	n.s.
(mg kg^{-1} soil)										
		B	Cu	Fe	Mn	Zn	Mo**	Al	Cd	Pb
1. Control		1.60 *e*	6.19 *ab*	2495.9 *b*	301.9 *c*	59.29 *c*	2.20 *d*	1335.9 *cd*	0.988 *a*	29.71 *ab*
2. Fertilization 0.5 kg I ha^{-1}		1.54 *c*	6.11 *a*	2407.8 *a*	288.9 *a*	55.20 *a*	2.12 *bc*	1323.7 *bc*	1.002 *a*	30.14 *b*
3. Fertilization 1.0 kg I ha^{-1}		1.47 *a*	6.44 *d*	2448.8 *ab*	294.7 *ab*	54.77 *a*	2.07 *b*	1321.4 *bc*	1.020 *b*	30.24 *b*
4. Fertilization 2.0 kg I ha^{-1}		1.47 *a*	6.37 *cd*	2429.6 *a*	300.6 *bc*	54.30 *a*	1.94 *a*	1279.1 *a*	0.986 *a*	29.32 *a*
5. Foliar application** 0.02 kg I ha^{-1}		1.51 *b*	6.37 *cd*	2589.1 *c*	322.3 *d*	57.33 *b*	2.15 *cd*	1355.4 *d*	1.029 *b*	31.05 *c*
6. Foliar application** 0.2 kg I ha^{-1}		1.55 *cd*	6.26 *bc*	2457.0 *ab*	300.8 *bc*	58.16 *bc*	2.13 *bc*	1303.3 *b*	1.045 *c*	31.57 *cd*
7. Foliar application** 2 kg I ha^{-1}		1.57 *d*	6.33 *cd*	2584.3 *c*	305.2 *c*	63.21 *d*	2.33 *e*	1399.5 *e*	1.053 *c*	32.10 *d*
Test *F*		*	*	*	*	*	*	*	*	*

Means followed by the same letters are not significantly different for $P < 0.05$.
Test *F*: *means are significantly different, n.s. – not significant;
** For Mo results only from 2008

est dose of KI (2.0 kg I ha^{-1}), whereas foliar nutrition with the same dose but in the form of KIO_3 depressed this element. It should be mentioned that despite observed changes in the N-total content, no differences in lettuce yield obtained from individual combinations were observed – detailed data not presented.

As a result of iodine nutrition, irrespective of its dose, form and method of application, an increase in the content of K, Mg, Ca as well as Mn and Cd in lettuce plants was found in comparison to the control – with the exception of K in combination no 6 (Table 1). The highest content of K was recorded for soil fertilization with KI in a dose of 2 kg I ha^{-1} and for foliar nutrition with KIO_3 in the lowest dose (0.02 kg I ha^{-1}). Soil application of higher doses of KI (1.0 and 2.0 kg I ha^{-1}) contributed to a gradual decrease in the concentraions of Ca, Mg, Mn and Cd in lettuce in comparison to the lowest KI dose (0.5 kg I ha^{-1}) although they remained higher than their values detected for the control. It should be noted that introduction of the lowest amount of KI (0.5 kg I ha^{-1}) to soil intensified most profoundly (in the comparison to the other combinations) plant accumulation of Ca, Mg, Mn, as well as boron. This dependence can indirectly support the hypothesis of the positive influence of low iodine doses on plants (KABATA-PENDIAS, MUKHERJEE 2007). Increasing KI doses applied to soil reduced the content of Mn in lettuce heads (Table 1) along with increasing the soil content of this element to a level comparable with the control (Table 2). This observation suggests an antagonistic effect of higher doses of iodine applied as KI on the uptake of manganese by lettuce roots.

Nutrition with iodine generally depressed the accumulation of P, Cu and Zn in lettuce (except Zn in combination 6, cf. Table 1). Higher doses of iodine applied foliarly as KIO_3 (0.2 and 2.0 kg I ha^{-1}) raised the content of P in comparison to combination 5. In respect to soil fertilization with KI (irrespective of its dose), an increased amount of easily soluble forms of phosphorus was noted in soil (Table 2) along with a reduced content of this element in lettuce leaves. The effect could have been caused by the inhibition of P uptake by lettuce roots, which may have resulted from the introduction of the I^- form of iodine to soil. A similar observation was reported for Cu, but then an increasing KI dose was accompanied by a significant decrease in the uptake and accumulation of copper in lettuce plants.

A variable effect of the iodine form, dose and method of application was found in respect of S, Na, B, Fe, Mo, Al and Pb content in lettuce heads (Table 1). In comparison to the control, using higher concentrations of KIO_3 solution for foliar nutrition (combinations 6 and 7) raised the accumulation of sodium in lettuce leaves. Soil fertilization with iodine as KI (irrespective of its dose) reduced Fe but slightly increased the Mo and Pb content in lettuce in comparison to the control. No influence of soil application of iodine was observed on the Al content in plants. The highest accumulation of S was noted in plants fertilized with the biggest dose of KI (2.0 kg I ha^{-1}).

Foliar nutrition with KIO_3 slightly decreased the S content in lettuce but the iodine dose had no significant influence on the observed changes. Application of different iodine forms and doses contributed to small but statistically significant variation in the boron concentration in lettuce leaves.

It is particularly difficult to discuss our results on the influence of iodine introduction (through soil or foliarly) on the chemical composition of lettuce due to a very limited number of available publications referring to this issue. The results obtained by SMOLEŃ et al. (2010) in carrot cultivation carried out according to an analogous experimental design (iodine dose, form and method of application) revealed different relations than shown in the present study, which could have been caused by species-specific differences.

CONCLUSIONS

1. A statistically significant effect of iodine biofortification of lettuce was obtained only through foliar application of KIO_3 in a dose of 2.0 kg I ha^{-1}.

2. Iodine accumulation in lettuce heads tended to increase as a result of application of higher doses of KI through soil fertilization (1.0 kg and 2.0 kg I ha^{-1}).

3. An increased N-total content in lettuce heads (in comparison to the control) was noted in the case of soil fertilization with the highest dose of KI (2.0 kg I ha^{-1}), while a reduced content of this macronutrient occurred as a result of foliar application of the same dose but in the form of KIO_3.

4. Significantly higher content of K, Mg, Ca, Mn and Cd in lettuce was found after iodine introduction irrespective of its dose, form and method of application.

5. Nutrition with iodine (regardless of its dose, form and method of application) generally decreased the P, Cu and Zn accumulation in lettuce heads.

6. Varied influence of iodine application was observed in reference to the S, Na, B, Fe, Mo, Al and Pb content in lettuce heads.

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HEAVY METAL STATUS OF POLISH KONIK HORSES FROM STABLE-PASTURE AND OUTDOOR MAINTENANCE SYSTEMS IN THE MASURIAN ENVIRONMENT

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Abstract

Among domestic animals, cattle and horses kept on pasture and fed with hay and straw are exposed most prominently to environmental pollution. Determination of the status of heavy metals in bodies of horses is important for two reasons. Firstly, it helps to assess the level of environmental contamination. Secondly, heavy metals can have considerable influence on animals living outdoors. The aim of the study has been to verify whether the level of Cr, Mn, Cu, Cd, Pb and Ni in the coat hair and cornified structures of hooves may differ between horses maintained alternately in a stable and on pasture and living outdoors all year long, with Polish Koniks from Mazury (the Masurian Lake District) taken as an example. Possible effects of the feeding season and age of horses were considered.

In total, 35 Polish Konik horses were examined, either kept in a stable-pasture system in Popielno or outdoors in Wojnowo. Sixty-two samples of summer and winter coat hair, 28 samples of hoof horn, as well as 10 samples of pasture plants and water were analyzed. The data were elaborated with the use multi-factor analysis of variance, T-Tukey-Kramer test and Pearson's correlation.

The results show that Polish Konik horses bred in Mazury have a low heavy metal concentration in the coat hair and hoof horn. The stable-pasture and outdoor maintenance systems hardly differentiate the element content, whereas the winter feeding results in

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an elevated concentration of the elements in the hair. Yearlings show higher Mn and Cd content than mares and foals at foot. The dam's impact on the heavy metal concentration in the hair and hoof horn in their foals is usually non-significant.

Key words: horse, Polish Konik, heavy metal, coat hair, hoof horn.

ZAWARTOŚĆ METALI CIĘŻKICH U KONIKÓW POLSKICH W STAJENNO-PASTWISKOWYM I BEZSTAJENNYM SYSTEMIE UTRZYMANIA W ŚRODOWISKU MAZUR

Abstrakt

Spośród zwierząt gospodarskich bydło i konie utrzymywane na pastwisku oraz żywione sianem i słomą są najbardziej narażone na zanieczyszczenie środowiska. Zagadnienie koncentracji metali ciężkich u koni jest ważne pod dwoma względami: zanieczyszczenia środowiska i jego wpływu na zwierzęta utrzymywane w chowie bezstajennym. Celem pracy było ustalenie, czy poziom Cr, Mn, Cu, Cd, Pb i Ni w sierści i rogu kopytowym może różnić się u koni utrzymywanych w warunkach stajenno-pastwiskowych oraz żyjących przez cały rok w chowie bezstajennym, na przykładzie koników polskich na Mazurach. Uwzględniono możliwy wpływ sezonu żywieniowego i wieku konia.

Łącznie zbadano 35 koników polskich utrzymywanych w systemie stajenno-pastwiskowym w Popielnie i bezstajennym w Wojnowie. Przeanalizowano 62 próbki sierści letniej i zimowej, 28 próbek rogu kopytowego oraz 10 próbek roślinności pastwiskowej i wody. Dane opracowano wykorzystując wieloczynnikową analizę wariancji, test *T*-Tukeya-Kramera i korelacje Pearsona.

Wykazano, że koniki polskie hodowane na Mazurach mają niską zawartość metali ciężkich w sierści i rogu kopytowym. Stajenno-pastwiskowy i bezstajenny system utrzymania wpływa na niewielkie zróżnicowanie stężenia pierwiastków, natomiasy żywienie zimowe ma wpływ na wzrost poziomu badanych pierwiastków w sierści. U roczniaków wykazano wyższą zawartość Mn i Cd niż u klaczy dorosłych i sysaków. Wpływ klaczy matek na koncentrację metali ciężkich w sierści i rogu kopytowym źrebiąt jest zazwyczaj nieistotny.

Słowa kluczowe: koń, konik polski, metale ciężkie, sierść, róg kopytowy.

INTRODUCTION

Out of domestic animals, cattle and horses maintained on pasture and fed with hay and straw are most prominently exposed to the environment pollution (ZNAMIROWSKA et al. 2004). Chemicals affect animals through the digestive and respiratory systems, as well as through the skin, hair and horn. The concentration of heavy metals is analyzed most often in blood, since the material can be collected *in vivo* (SUTTLE et al. 1996). However, the plasma content changes frequently, being highly influenced by the physiological status of a given organism (JAŚKOWSKI et al. 1993). The material showing more persistent concentration of elements in horses and easily sampled is the hair and hoof horn (WICHERT et al. 2002, ASANO et al. 2005). The content of some heavy metals in hair is influenced by the feeding season and –

in some cases – by the age of an animal (Cieśla, Janiszewska 1997, 2000). The hoof horn grows continuously and special means would be necessary to determine which part appeared in a certain season of the year (Stachurska et al. 2009). However, the horn can probably absorb some elements from the ground at a rate that differs from season to season, depending on the presence of grass, mud, snow, etc.

The Polish Konik horses are a unique primitive breed, which originates from wild Tarpans living in the Central Europe and becoming extinct in the late 18th century. They are bred under stable-pasture conditions, outdoors on a pasture or paddock all year long or a free-ranging animals in Polish reserves, mainly in the regions called Mazury (N-E Poland) and Roztocze (S-E Poland). The environment around the largest Polish Konik breeding centers in Poland is dominated by forests and is assumed to be clean. The issue of the heavy metal status of horses is important for two reasons. First, it will attest whether the environmental contamination is low. Second, it will demonstrate how the given pollution affects animals living outside stables. According to Krumrych et al. (1995), the Polish Koniks are highly responsive to environmental changes. However, the latest study by Stachurska et al. (2009) performed on the Polish Koniks in Roztocze National Park indicates that the heavy metal concentration in hair is only slightly affected by seasonal changes in the feeding. The generally low concentration the elements does not reveal possible influence of the differences between the reserve and stable-pasture maintenance systems or the animals' age.

The aim of the study has been to determine if the levels of Cr, Mn, Cu, Cd, Pb and Ni in the coat hair and hoof horn may differ between horses kept in a stable and on pasture versus the ones living outdoors, on pasture, all year. The Polish Koniks in Mazury served as an example.

MATERIAL AND METHODS

In total, 35 Polish Konik horses were examined: 21 in Popielno (9 dams, 12 foals) and 14 in Wojnowo (8 dams and 6 foals) in the Mazury region. The samples were taken twice at six-month interval, hence sucklings became yearlings in the second year. In total, there were 17 dams, 12 yearlings and 18 sucklings. The horses were clinically sound during the collection of the material.

Popielno lies 20 km away from Wojnowo. The horses examined in Popielno were maintained under stable-pasture conditions, whereas those in Wojnowo were kept outdoors. The maintenance conditions could differ in three aspects: the length of the time when horses stayed outdoors (connected with a possible impact of heavy metals in the air, rain, snow and ground), the content of elements in the feed and their content in drinking water.

In summer, the horses are on pasture all day and night in both centers. In Popielno, during winter, they are mainly in a stable, released to a paddock for a few hours a day. In Wojnowo, during winter, they stay on pasture all day and night. With respect to their nutrition, in neither of the centers the horses are fed additionally in summer. In winter, they are given oats, hay and some straw in Popielno. They also receive some carrots in November and December. In winter, in Wojnowo, they eat dry grass which remained after autumn on the pasture. Sometimes they have to dig it out from under the snow cover. Hay and some straw are given when the snow cover is thick. In Popielno, the horses drink water taken up from a water supply system and poured to a bath, as well as rainwater collected in ditches. In winter, they are given water from the supply system. In Wojnowo, the horses drink water from natural small ponds and ditches all year long. If they are frozen, the horses are given water taken from a supply system.

Sixty-two samples of coat hair (34 from the stable-pasture and 28 from the outdoor system; 28 in summer and 34 in winter; 18 from sucklings, 12 from yearlings and 32 from dams) and 28 samples of hoof horn were collected at the beginning of October and in the April next year. The hoof horn was collected from dams and sucklings in October. Simultaneously, three samples of plants from different pastures and seven samples of water from various sources were taken. The coat hair was cut from the horses' neck under the mane. The autumn coat shedding in Polish Koniks living in reserves begins in late August and lasts till mid-October (DETKENS 1967, STACHURSKA et al. 2006). The winter coat is shed from January until June. Hence, two main seasons of feeding were distinguished: summer season and winter season. The October samples contained the hair grown during the summer feeding and the April samples during the winter feeding. The horn was trimmed from the toe of a front hoof during routine trimming. The pasture plants, the major feed in summer and used for making hay for the other seasons, as well as all the water sources available for the horses were examined to randomly assess the heavy metal pollution of the environment. The plants were cut from 1 m^2 squares from sites most often grazed by the horses. The water samples were collected in plastic vessels.

The coat hair and hoof horn samples (the latter after fragmenting) were washed in 1% Triton-X100 solution and acetone. Then, they were rinsed three times in redistilled water, air-dried, crushed and homogenized. 0.5 g samples weighted at ±0.0001 accuracy were digested in a Multiwave 3000, Anton Paar microwave stove. The level of Cr, Mn, Cu, Cd, Pb and Ni was determined in an atomic absorption spectrometer SpectrAA 220Z (electrothermal atomization, Zeeman background correction), Varian. Samples of plants were air-dried, crushed and homogenized. They underwent a similar mineralization procedure before determination of the heavy metal content as hair and horn samples. The water samples were filtered through a 0.45 μm Milipore filter. The AAS method was used directly after collecting the samples to define the heavy metal levels.

The SAS procedure, 9.1 version, was used to perform a multi-factor analysis of variance. The following effects were investigated: the maintenance system (stable-pasture, outdoor), the feeding season (summer, winter) and the horse's age (dam, yearling, suckling). The data were reported as means ±SD (Standard Deviation). Multiple *T*-Tukey-Kramer test was performed to identify homogenous groups. The heavy metal levels in the hair and hoof between dams and their sucklings, between dams and yearlings, as well as between the hair and the horn, were analysed with Pearson's correlation coefficients.

RESULTS

Mean concentrations of heavy metals in the coat hair and hoof horn in Polish Konik horses, as well as in pasture plants and water available for the horses, are shown in Table 1. The Ni content in the samples was below the detection level. Since the concentrations were determined in completely different materials, they were not compared statistically. The Mn level was higher in the hoof compared to the hair, whereas that of Cu was lower. The content of the other elements in the hair and hoof was in a similar range. In pasture plants, Mn and Cd concentrations were relatively higher than in the hair and hooves. The Mn level in pasture plants was particularly high.

Table 1

Mean concentration of heavy metals in Polish Konik horses, pasture plants and water

Sample	N	Cr		Mn		Cu		Cd		Pb	
		mean	SE	mean	SE	mean	SE	mean	SE	mean	SE
Coat hair (mg kg^{-1} d.m)	62	1.03	0.66	9.39	5.90	2.92	0.43	0.01	0.00	0.22	0.41
Hoof horn (mg kg^{-1} d.m.)	28	1.31	0.45	21.13	9.62	1.10	0.36	0.00	0.00	0.39	0.13
Pasture plants (mg kg^{-1} d.m.)	3	0.84	0.30	245.45	77.23	2.05	0.48	0.22	0.13	0.35	0.06
Water sources (μg dm^{-3})	7	0.44	0.55	54.07	27.29	4.73	2.71	0.63	0.10	1.53	1.02

SE – standard error
d.m. – dry matter

The mean Cr content in the coat hair in Polish Konik horses was higher in the stable-pasture system than in the outdoor one, but similar in the hoof horn from both groups (Figure 1). More Cr was found in the hair grown in winter than in summer. The levels of the elements in the hair did not differ between the age groups. The Cr concentration in hooves was higher

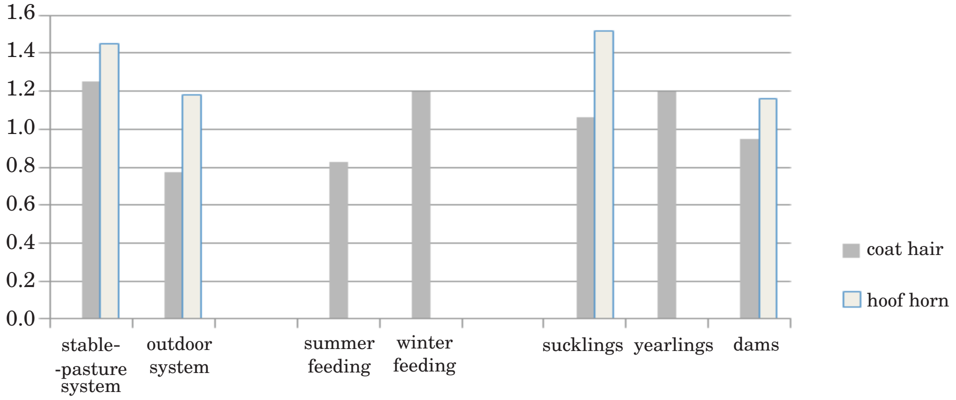

Fig. 1. Cr content (mg kg^{-1} d.m.) in coat hair and hoof horn in Polish Koniks. Significant differences in the hair between maintenance systems, feeding seasons and in the horn between age groups (*P*=0.05)

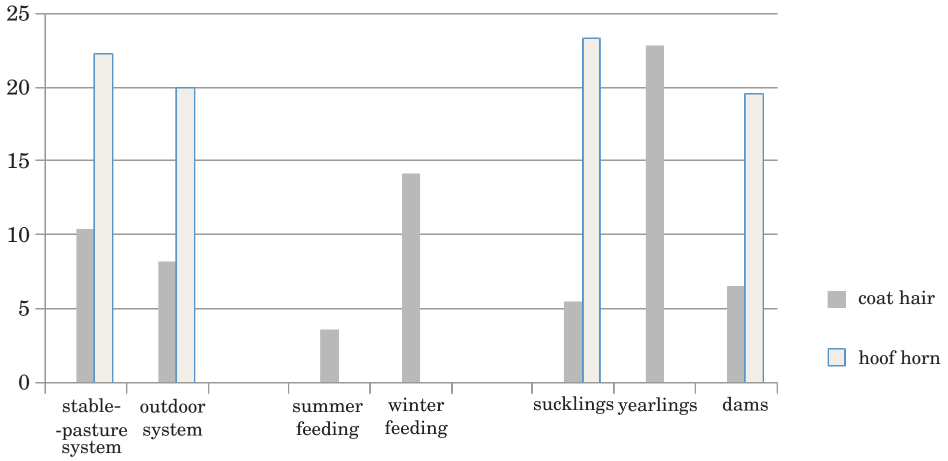

Fig. 2. Mn content (mg kg^{-1} d.m.) in coat hair and hoof horn in Polish Koniks. Significant differences in the hair between feeding seasons and age groups (*P*=0.05)

in sucklings than in dams. Differences in the Mn level occurred only in the hair between the summer and winter feeding and between the age groups (Figure 2). There was more Mn in winter hair and in yearlings compared to the other groups. The average Cu content was balanced except for the feeding seasons in the hair: it was higher in winter than in summer (Figure 3). In the coat hair, relatively more Cd occurred in the outdoor system, in winter and in yearlings (Figure 4). In the hoof horn, Cd was below the detection level. Considering the mean Pb content, differences were found with regard to the seasons, as well as to the maintenance systems

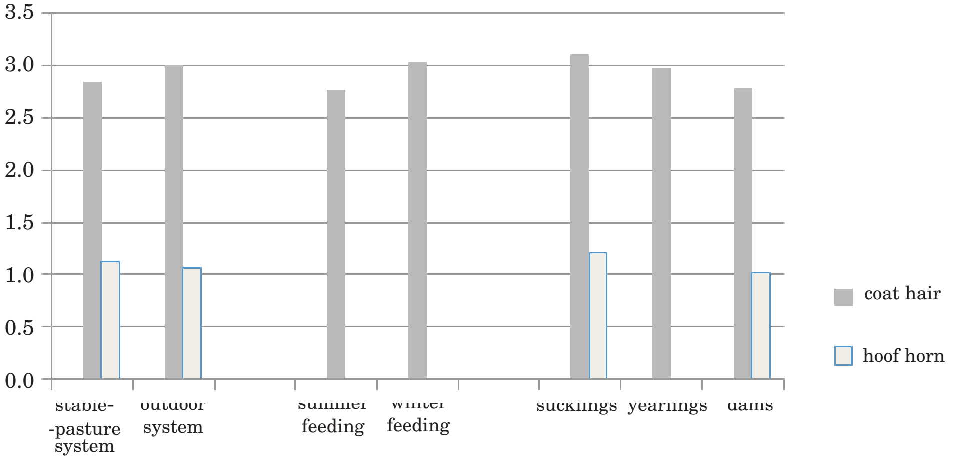

Fig. 3. Cu content (mg kg^{-1} d.m.) in coat hair and hoof horn in Polish Koniks. Significant differences in the hair between feeding seasons (P=0.05)

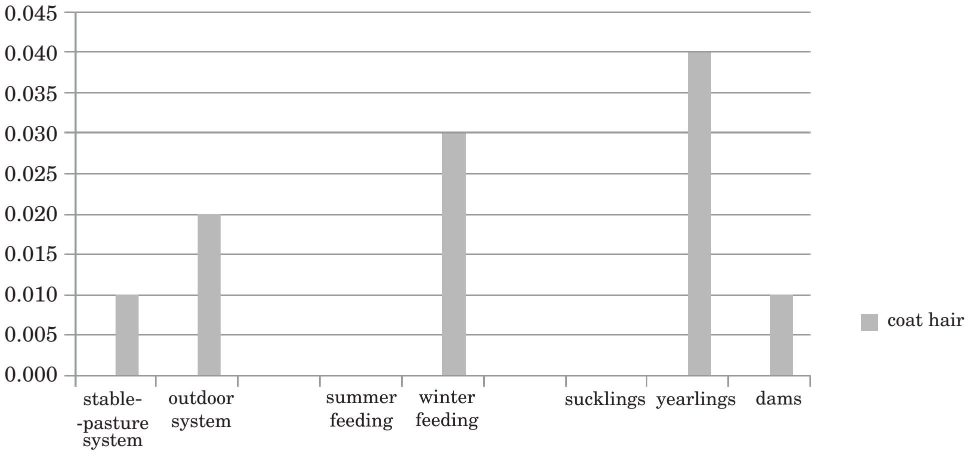

Fig. 4. Cd content (mg kg^{-1} d.m.) in coat hair in Polish Koniks. Significant differences in the hair between maintenance systems, feeding seasons and age groups (P=0.05)

(Figure 5). There was more Pb in winter hair and in the horn of horses kept in the outdoor system.

In the hair, significant correlations were found between the concentration of Cr versus Mn, Cd and Pb, of Mn versus Cd and Pb, as well as of Cd versus Pb (Table 2). In the hoof horn, it was only the correlation between Cr and Mn content that proved significant. The correlation between the hair and the hoof in their levels of particular elements was significant only in the case of Cr.

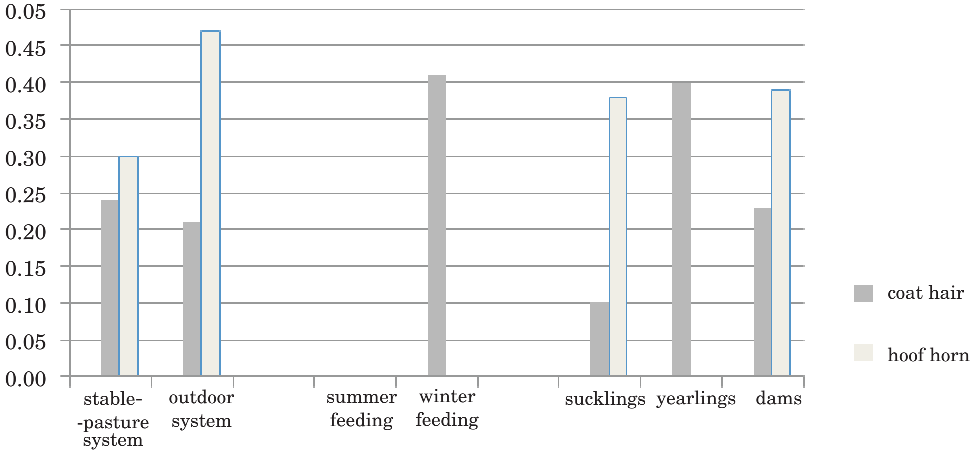

Fig. 5. Pb content (mg kg^{-1} d.m.) in coat hair and hoof horn in Polish Koniks. Significant differences in the hair between feeding seasons and in the horn between maintenance systems (*P*=0.05)

Table 2

Significant correlations (*P*<0.01) in heavy metal content within the coat hair (above the diagonal), within the hoof horn (below the diagonal) and between the coat hair and the hoof horn samples (along the diagonal)

Element	Hair-hoof	Cr	Mn	Cu	Cd	Pb
		coat hair				
Cr	hoof horn	0.52	0.43		0.31	0.61
Mn		0.64			0.66	0.38
Cu						
Cd						0.44
Pb						

The correlation of 0.68 (*P*<0.01) was found in the Cr level in the coat hair between dams and sucklings in summer. Correlations between the concentrations of the other elements in dams versus their sucklings and yearlings were not significant.

DISCUSSION

Generally, the content of heavy metals in coat hair and hoof horn of Polish Konik horses living in Mazury is low. The chromium levels found in the study are similar to the ones reported previously (Asano et al. 2005, STACHURSKA et al. 2009). The Mn content in the hair assessed in this study resembles the one determined in Roztocze (STACHURSKA et al. 2009), but is lower than 17.24 mg kg^{-1} reported by CIEŚLA and JANISZEWSKA (1997) or 14.19 mg kg^{-1} found by CIEŚLA (2002). However, it exceeds the concentration in the horse mane hair (1.24 mg kg^{-1}) observed by ASANO et al. (2005). According to KOWNACKI (1962), the Mn level in the hoof horn was 0.8 mg kg^{-1} in reserve horses and 1.0 mg kg^{-1} in stabled horses, hence it was several-fold lower than in the present study. SASIMOWSKI et al. (1987) also observed low Mn levels (2-5 mg kg^{-1}) in hooves in cold-blood and warm-blood horses. It should be pointed out that Mn plays an important role in bone mineralization and reproduction, hence its deficiency may cause disorders in the organism (CIEŚLA 2002). The Cu concentration in the hair is slightly lower and in the hoof horn similar as in Roztocze (STACHURSKA et al. 2009). Meanwhile, the content of copper in the hair is approximately two-fold lower and in the hoof horn several-fold lower than observed by other researchers (SASIMOWSKI et al. 1987, CIEŚLA, JANISZEWSKA 1997, 2000, WICHERT et al. 2002). It can be suggested that there is some Cu deficiency in Polish Koniks maintained in the two analysed systems. REIWALD and RIOND (2002) show that a traditional ration of oats and hay as well as many commercial feeds do not contain sufficient amounts of copper for adult horses. Cu and Mn are antagonistic elements, although in the present study it does not seem that the high Mn level caused a low Cu content in the environment. Copper deficiency in horses may result in *physitis*, *osteochondrosis, osteoporosis*, as well as *fracturae spont* (GEHRKE 1997). The Cd level in the hair was slightly lower or not detectable compared to the horses living in Roztocze (STACHURSKA et al. 2009) or in Dobrzyniewo in Wielkopolska (CIEŚLA, JANISZEWSKA 1997). Concentrations of Pb are of a similar order as determined in Roztocze and lower than found by ASANO et al. (2005).

Concentrations of heavy metals in the coat hair and hoof horn of horses maintained in stable-pasture and outdoor systems are in most cases similar. It seems that the element levels, except for Mn in the hoof horn, are too low to show significant differences between the two breeding centers. The two systems, i.e. stable and pasture or entirely outdoors, do not differ in the effect on the horse's status regarding heavy metals. This finding is similar to that determined for stabled and reserve Polish Konik horses in Roztocze (STACHURSKA et al. 2009).

In turn, the impact of the feeding season on levels of elements in hair shows that in winter the content of heavy metals is higher than in summer. This regularity appeared both centers (the data not presented in tables due to the paper's limited length). Probably, the differences result from the environmental seasonal changes. In Roztocze, it was only Mn that occurred in higher concentration in winter hair (STACHURSKA et al. 2009). CIEŚLA and JANISZEWSKA (2000) found a higher Cu level in the coat hair in halfbred horses in summer than in winter.

Heavy metals, particularly Mn and Cd, tend to accumulate in higher amounts in hair of yearlings compared to dams or sucklings. In the hoof, the element levels in dams and sucklings are similar in nearly all the cases. In Roztocze (Stachurska et al. 2009), the Mn concentration was higher in yearlings' hair as compared to dams. In a growing organism, the demand for nutritional components, including minerals, is the highest (Gehrke 1997). That may explain the elevated Mn content in the yearlings' coat. However, Asano et al. (2005) did not find the age effect on the content of heavy metals in the hair.

In general, the differences between levels of heavy metal in the feed versus the hair and hoof horn were small, except an exceptionally high Mn content in plants. It is likely that the Mn and Cd concentrations in the pasture flora are not high enough to accumulate in considerable amounts in the horses' hair and hoof. It has been documented that exposure to elevated levels of heavy metals in the environment is reflected by high concentrations of these elements in horses, although the path from ingestion or absorption to deposition in animals' tissues is not direct (Wichert et al. 2002, Stachurska et al. 2009). Therefore, differences in the degree of heavy metal contamination between the feed and the animal can appear.

In both centers, the water drunk by the horses can be classified as class one (the highest) in the Polish water purity classification system with regard to Cr, Cu and Pb, between class one and two considering the Mn content and class two in respect of Cd (*Regulation of the Minister for the Environment, 11 February 2004*). Hence, the water does not pose a threat to the horses in terms of toxicology.

The correlations between levels of different elements are mainly found in the hair, therefore suggesting that in the hooves they can be more strongly dependent on external factors, e.g. concentrations of metals in the ground. Since the concentrations of the analysed elements in the hair and in the hoof were most often not correlated between dams and their foals, it seems that the effect produced by dams is weak.

CONCLUSION

In conclusion, the Polish Konik horses bred in Mazury have low heavy metal concentrations in the coat hair and hoof horn. The stable and pasture or the outdoor maintenance system hardly differentiated the content of elements, whereas the winter feeding resulted in elevated concentrations of the elements in the hair. Yearlings showed a higher Mn and Cd content than mares and foals at foot. The dams' impact on the heavy metal concentration in the hair and hoof horn in their foals is usually negligible.

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EFFECT OF FOLIAR MAGNESIUM FERTILISATION AND STORAGE ON SOME PARAMETERS OF THE NUTRITIVE VALUE OF CARROT STORAGE ROOTS

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Abstract

The nutritive value and wholesomeness of carrot are highly appreciated owing to such biologically active compounds as carotenoids, vitamin C and mineral compounds found in its roots.

In 2007-2009, field experiments were performed on the effect of foliar fertilisation of carrot with magnesium sulphate during its vegetative growth on selected nutrients (total carotenoids, vitamin C, mineral magnesium). The field experiment was set up in a split-plot design with three replications on light soil, of slightly acid reaction, low in available P and K forms and very low in Mg. Magnesium was applied in rates of 0, 45 and 90 kg MgO ha^{-1} by spraying carrot plants with a 3% solution during their intensive growth in the vegetative season, accompanied by constant N, P, K fertilisation. The experiment involved five carrot cultivars: medium-late Berjo, and late Flacoro, Karotan, Koral and Perfekcja, all characterized by good storage life. The content of selected nutrients in carrot roots was determined immediately after harvest and after six-month storage in a traditional earthen mound.

Foliar application of magnesium significantly increased the nutritive value of roots of the carrot cultivars after harvest, raising the content of total carotenoids, vitamin C and magnesium in edible parts. The rate of 45 kg MgO ha^{-1} was most favourable. The six-month root storage led to a decrease in the biologically active compounds. The recorded loss (mean for cultivars and fertilisation) reached 20% of carotenoids, 50% of vitamin C

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and 1% of magnesium. The highest nutritive value was determined for roots of the cultivars Karotan and Perfekcja and the lowest one in roots of cv. Flacoro.

Key words: carotenoids, vitamin C, magnesium, carrot cultivars, magnesium fertilisation, storage.

WPŁYW DOLISTNEGO DOKARMIANIA MAGNEZEM I PRZECHOWYWANIA NA WYBRANE PARAMETRY WARTOŚCI ODŻYWCZEJ KORZENI SPICHRZOWYCH MARCHWI

Abstrakt

Wartość odżywcza i zdrowotna marchwi jest wysoko ceniona za względu na występujące w jej korzeniach związki biologicznie czynne, jak karotenoidy, witamina C, oraz związki mineralne.

W latach 2007-2009 przeprowadzono doświadczenia polowe dotyczące wpływu dolistnego dokarmiania marchwi siarczanem magnezu w okresie jej wegetacji na wybrane składniki odżywcze (sumę karotenoidów, witaminę C, magnez mineralny). Doświadczenie polowe założono w układzie zależnym split-plot w 3 powtórzeniach na glebie lekkiej o odczynie lekko kwaśnym, małej zasobności w przyswajalne formy P i K oraz bardzo małej zasobności w Mg. Nawożenie magnezem zastosowano w dawkach 0, 45 i 90 kg MgO ha^{-1} – w formie oprysku 3% roztworem w okresie intensywnego wzrostu roślin w trakcie sezonu wegetacyjnego na tle stałego nawożenia N, P, K. Obiektem badań było 5 odmian marchwi: średnio późna Berjo oraz późne: Flacoro, Karotan, Koral, Perfekcja – o dobrej trwałości przechowalniczej. Zawartość wybranych składników w korzeniach marchwi oznaczono bezpośrednio po zbiorze i po 6 miesiącach przechowywania w kopcu tradycyjnym.

Dolistne nawożenie magnezem wpłynęło istotnie na wzrost wartości odżywczej korzeni badanych odmian marchwi bezpośrednio po zbiorze wskutek zwiększenia w częściach jadalnych zawartości sumy karotenoidów, witaminy C oraz magnezu. Najkorzystniejsza okazała się dawka 45 kg MgO ha^{-1}. Po 6 miesiącach przechowywania korzeni stwierdzono zmiejszenie zawartości związków biologicznie czynnych. Wystąpiły straty (średnio dla odmian i nawożenia) 20% karotenoidów, 50% witaminy C i 1% magnezu. Największą wartość odżywczą miały korzenie odmian Karotan i Perfekcja, a najmniejszą Flacoro.

Słowa kluczowe: karotenoidy, witamina C, magnez, odmiany marchwi, nawożenie magnezem, przechowywanie.

INTRODUCTION

Carrot (*Daucus carota* L.) is one of the key vegetables grown in Asia and Europe. With the carrot yield of 270-300 dt ha^{-1}, Poland is the second European producer (Gajewski et al. 2007). In 2009, the carrot consumption was 21.4 kg per person, including fresh carrot consumption of about 10 kg, which shows how important this vegetable is in human nutrition, as the total annual consumption of vegetables is about 115-120 kg per capita. Most of the carrot harvest supplies the Polish market, although because of the large scale of production and a growing demand, the vegetable has become an important export commodity (Filipiak, Maciejczak 2010).

Over the recent years, much attention has been paid to the presence of natural antioxidants found in food, which inhibit or delay the oxidation of other molecules and protect the cells from harmful action of reactive oxygen forms. Fruit and vegetables, e.g. carrot, are rich sources of antioxidants such as carotenoids and phenolic compounds as well as ascorbic acid (Velioglu et al. 1998, Paulauskiene et al. 2006, Podsędek 2007, Pokluda 2008, Domaradzki et al. 2010). With different sowing dates, high yields as well as good storage life, carrot roots are available on the market all year. The quality of storage roots in carrot is cultivar-specific and depends on climate and soil conditions, agronomic practice as well as environmental conditions during prolonged storage (Seljasen et al. 2001, Karkleliene et al. 2009, Domaradzki et al. 2010).

There is relatively much information on the effect of organic and basic mineral (N, P, K) fertilisation on the chemical composition of carrot roots, but little is known about the effect of Mg fertilisation on transformations in carrot roots (Smoleń, Sady 2007). Magnesium participates in the synthesis of carbohydrates, proteins and provitamin A. It is also a structural component of chlorophyll. Therefore, it can be assumed that supplying carrot with such an important macronutrient can affect the accumulation of chemical compounds and their transformations in its roots.

The aim of the present research has been to determine the effect of foliar fertilisation of plants with magnesium as well as of long-term storage on selected parameters of the nutritive value of roots in selected carrot cultivars.

MATERIAL AND METHODS

The research material was obtained from a field experiment carried out at the Experimental Station in Mochełek (2007-2009), owned by the Faculty of Agriculture and Biotechnology, the University of Technology and Life Sciences in Bydgoszcz (the Kujawy and Pomorze Province). The field experiment was performed on light soil of slightly acid reaction, low in available P and K forms as well as a very low in Mg (Table 1).

The experiment was set up in a split-plot design with three replications. The experiment design covered:

- date of analysis (after harvest, after storage);
- cultivars (medium-late Berjo, late Flacoro, Karotan, Koral and Perfekcja);
- magnesium rates (0, 45, 90 kg MgO ha^{-1}) in the form of magnesium sulphate (16%) alongside constant fertilisation with nitrogen (70 kg N ha^{-1}), phosphorus (80 kg P_2O_5 ha^{-1}) and potassium (100 kg K_2O ha^{-1}). Foliar fertilisation with magnesium was applied twice during the intensive plant growth (July, August) with a 3% solution of magnesium sulphate in the rate of 300 dm^3 ha^{-1}.

Table 1

Chemical composition of soil prior to the experiment

Parameter	Unit	Growing year			Richness
		2007	2008	2009	
pH H_2O	-	6.6	6.5	6.7	slightly acid soil
pH KCl	-	6.0	5.9	6.1	
Organic carbon	(mg kg^{-1})	7650	7800	7550	-
Total nitrogen	(mg kg^{-1})	720	690	750	-
Available phosphorus forms	(mg kg^{-1})	24.0	23.0	25.0	low abundance
Available potassium forms	(mg kg^{-1})	42.0	43.0	45.0	low abundance
Available magnesium forms	(mg kg^{-1})	18.5	20.0	17.0	very low abundance

Cultivation treatments and protection against diseases and pests were in line with the requirements established for carrot, i.e. seeds were seed-dressed with Funaben T and the herbicide Stomp 330 EC was applied each year after sowing and prior to plant emergence. During the vegetative period, hand-weeding was performed. Carrot storage roots were harvested at full maturity (first ten days of October). During the harvest, root samples were taken from each plot for analysis and storage studies. The samples were stored in a traditional earthen mound for 6 months. The parameters of the chemical composition of carrot were determined applying the following methods:

- total carotenoids – with the colorimetric method following PN-90/A-75101/12;
- vitamin C – with the titration method following PN-90A-75101/11;
- magnesium – with the atomic absorption method after mineralization.

In the present paper, an attempt has been made to present model determination of daily consumption of total carotenoids, vitamin C and magnesium, assuming that the daily consumption of carrot storage roots equalled 55 g per capita. The data were compared with the Recommended Dietary Allowance. The content of vitamin A was calculated from total carotenoids, assuming 18 mg of carotenoids as equivalent to 1mg retinol RE (Retinol Equivalent).

The results of the 3-year research were statistically verified using the analysis of variance. The significance of differences was evaluated with the Tukey's multiple confidence intervals for the significance level of α=0.05. Coefficients of linear correlation between the quality characteristics in carrot were calculated.

RESULTS AND DISCUSSION

Carotenoids and fibre mostly determine the wholesomeness and nutritive qualities of carrot (Alasalvar et al. 2005, Fik et al. 2008, Domaradzki et al. 2010). Carotenoids are red, orange or yellow pigments soluble in plant lipids, and give the characteristic coloration of roots (Skrede et al. 1997). Present in edible parts of carrot, carotenoid compounds are mostly α- and β-carotene (Holden et al. 1999, Mayer-Miebach, Spiess 2003, Nawirska, Krol 2004). Being a vitamin A precursor (Handelman 2001, Nawirska, Król 2004) and highly active antioxidants, carotenoids are essential in controlling proper functions of the human body (Rock 1997, Velioglu et al. 1998, Paulauskiene et al. 2006, Podsędek 2007). According to Holden et al. (1999), depending on the cultivar, carrot contains from 57 to 84 mg kg^{-1} of fresh weight of carotenoids, while Poklud (2008) reported that an average content of carotenoids calculated for 28 carrot cultivars was 86.0 ±25.0 mg kg^{-1} of fresh weight, with the lowest concentration of 42.0 mg kg^{-1} for cv. Fuk and the highest one of 168.0 mg kg^{-1} for cv. Kortina. Some other data imply that the content of carotenoids in carrot roots, being cultivar-specific, ranges even more broadly, e.g. from 10 to 140 mg kg^{-1} of fresh weight (Gajewski et al. 2007), from 55.8 to 138.9 mg kg^{-1} of fresh weight (Nawirska, Król 2004) and from 136 to 854 of fresh weight (Lachman et al. 2000). In the present research, the results of the content of carotenoids in roots of five carrot cultivars are given in Table 2 and confirm the earlier results reported by the above authors. The analysis of variance showed significant differences in the content of carotenoids between the cultivars. The highest content of this nutrient was found in cv. Karotan: on average 136.1 mg kg^{-1} of fresh weight; the lowest one appeared in cv. Flacoro: 106.8 mg kg^{-1} of fresh weight. Such results correspond to the root coloration since out of all the cultivars, cv. Karotan had roots in the most intensive dark-orange colour, a finding which coincides with the results reported by Skrede et al. (1997). A similar relationship was reported by Gajewski et al. (2007), who analysed six carrot cultivars and the root colour ranged from yellow (cv. Yellowstone) through orange (cv. Florida) to purple (cv. Purple Haze). These authors claimed that the darker the root colour, the higher the content of carotenoids.

Magnesium fertilisation significantly increased the content of carotenoids in carrot roots of the cultivars (Table 2). The magnesium rates applied during the growing season increased the content of carotenoids in storage roots immediately after harvest, except for cv. Karotan, which – when exposed to the rate of 45 kg MgO ha^{-1} – did not respond with a significant increase of this nutrient. Carotenoid pigments are thought to be stable as in adequately stored plant products their changes are negligible (Koca, Karadeniz 2008). They can only undergo oxidation (Seljasen et al. 2001), which can lead to a decrease in their content and to sensory changes in the stored plant material, e.g. paler colour, unpleasant flavour and smell. The present results confirm the above, since after 6-month storage a slight decrease in the content of carotenoid was recorded although the determination date did not have a significant effect on any such changes (Table 2). The smallest decrease in the content of carotenoids occurred in roots of cv. Karotan (17.8%), and the biggest one – in roots of vc. Perfekcja (20.5%). Contrary results were reported by Kopas-Lane and Warthesen (1995), Kidmose et al. (2004), who found a slight increase in the content of carotenoids after storage. Gajewski et al. (2010) examined 8 carrot cultivars of various root colour and concluded that after 3 months of storage the content of carotenoids in edible parts of the carrot cultivars rose by 19.2%. In the present research, the magnesium rates slightly stimulated the carotenoid loss in carrot storage roots (Figure 1).

Carrot contains relatively small amounts of vitamin C (L-ascorbic acid). Reports on the content of vitamin C in carrot roots provide various results, e.g. 59.0 mg of vitamin C per 1 kg of fresh root weight (Holden et al. 1999), 60.0 mg (Wierzbicka, Kuskowska 2002) or 65.2-97.8 mg (Majkowska-Gadomska, Wierzbicka 2010), depending on the cultivar. Alasalvar et al. (2001), who determined vitamin C in fresh carrot, reported the following results: 53.3 mg in orange, 19.8 mg in yellow and 12.5 mg in white carrot. Different results

Table 2

Content of carotenoids in the fresh weight of carrot roots; means for 2007-2009 (mg kg^{-1})

Date of determination (A)	Cultivars (B)	Fertilisation MgO kg ha^{-1} (C)			Mean
		0	45	90	
After harvest	Berjo	100.7	107.9	114.5	107.7
	Flacoro	98.5	108.9	113.0	106.8
	Karotan	132.3	133.9	142.1	136.1
	Koral	112.8	120.0	124.7	119.2
	Perfekcja	126.2	131.4	137.6	131.7
	mean	114.1	120.4	126.4	120.3
After storage	Berjo	82.7	86.4	94.7	87.9
	Flacoro	80.1	86.9	92.4	86.5
	Karotan	105.9	112.9	116.9	111.9
	Koral	94.7	94.1	101.0	96.6
	Perfekcja	102.9	104.2	106.8	104.6
	mean	93.3	96.9	102.4	97.5
Mean		103.7	108.7	114.4	108.9

$LSD_{\alpha=0.05}$

A = n.s.* B = 16.8 C = 2.8
B/A = n.s. A/B = n.s. C/A = n.s.
A/C = n.s. C/B = n.s. B/C = n.s.

*n.s. – no significant difference

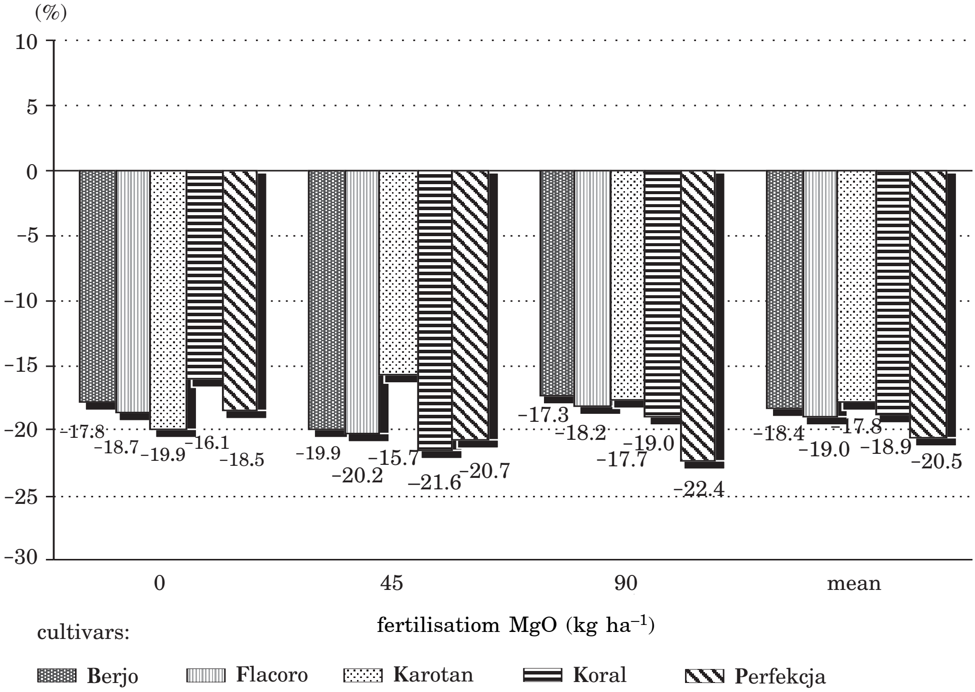

Fig. 1. Percent loss of carotenoids in carrot roots depending on the cultivar, fertilisation and storage period; means for years 2007-2009

were noted by Favell (1998), who claimed that the content of vitamin C in fresh weight ranged from 28.0 to 45.0 mg. The present research showed that vitamin C in roots after harvest ranged from 91.1 to 107.4 mg kg^{-1} of fresh weight (Table 3), being slightly higher than reported by the cited authors. This may have been caused by the foliar fertilisation of plants with magnesium. The magnesium rate of 45 kg MgO ha^{-1} increased the content of vitamin C in carrot roots after harvest, as compared with the control, by an average of 13.9% (Table 3). Majkowska-Gadomska and Wierzbicka (2010), while studying various carrot cultivars and the effect of additional plant fertilisation, recorded a lower content of vitamin C in roots in cv. Deep Purple and Purple Haze but a higher one in cv. Florida after an application of multicomponent fertiliser Crop Care in a dose of 30 kg per ha The same authors, and likewise Alasalvar et al. (2001), observed that storage roots of the more colour-saturated cultivars contained more ascorbic acid. In the present research, the content of vitamin C was higher in storage roots of the cultivars with the most intensive red colour: cv Karotan had on average of 107.1 mg of vitamin C per kg^{-1} of fresh weight (Table 3). Rogozińska et al. (2005) in their earlier experiment with another vegetable (potato), recorded similar effects when applying identical magnesium fertiliser rates as in the

Table 3

Content of vitamin C in the fresh weight of carrot roots; means for 2007-2009 (mg kg^{-1})

Date of determination (A)	Cultivars (B)	Fertilisation MgO kg ha^{-1} (C)			Mean
		0	45	90	
After harvest	Berjo	91.8	90.6	90.8	91.1
	Flacoro	88.0	102.0	93.3	94.4
	Karotan	94.6	117.9	108.8	107.1
	Koral	89.2	105.9	112.0	102.4
	Perfekcja	84.2	94.0	98.4	92.2
	mean	89.6	102.1	100.7	97.4
After storage	Berjo	49.0	48.8	49.0	48.9
	Flacoro	52.1	55.9	59.9	56.0
	Karotan	49.3	44.2	56.9	50.1
	Koral	49.7	51.7	51.9	51.1
	Perfekcja	50.6	53.2	58.8	54.2
	mean	50.1	50.8	55.3	52.1
Mean		69.9	76.4	78.0	74.8

$LSD_{\alpha=0.05}$

A = 17.3 B = n.s.* C = 5.8

B/A = n.s. A/B = n.s. C/A = 8.2.

A/C = 18.0 C/B = n.s. B/C = n.s.

*n.s. – no significant difference

present research,. The most favourable rate was 45 kg MgO ha^{-1}, for which the content of vitamin C in potato tubers was the highest.

Vitamin C is the least stable nutrient and can be destroyed by ultraviolet radiation, high temperatures, the presence of oxygen, oxidation enzymes, metal ions and long-term storage (WIERZBICKA, KUSKOWSKA 2002, GĄSIOROWSKA, MARKIEWICZ 2004, DOMARADZKI et al. 2010). GĄSIOROWSKA and MARKIEWICZ (2004) demonstrated a linear dependence between the vitamin C content and the storage period in another vegetable (potato). According to the above authors, the longer the storage period, the higher the vitamin C loss. It is also common knowledge that the content of vitamin C benefits from low but stable temperature. The present study confirms such dependence since carrot storage roots were stored in an earthen mound, in which maintaining stable temperature is very difficult. After long storage (6 months) a very high vitamin loss was reported: from 40.6 to 52.7% on average (Figure 2). The highest vitamin C loss was reported in cv. Karotan (62.5%) fertilised with 45 kg MgO ha^{-1} and the lowest one in cv. Flacoro (35.8%) which received 90 kg MgO ha^{-1}. NAWIRSKA and KRÓL (2004) recorded a very low vitamin C content after 60 days of storage of 4 carrot cultivars: from 16.0 mg in cv. Nantejska to 24.5 mg kg^{-1} of fresh weight in cv. Flacoro. As reported

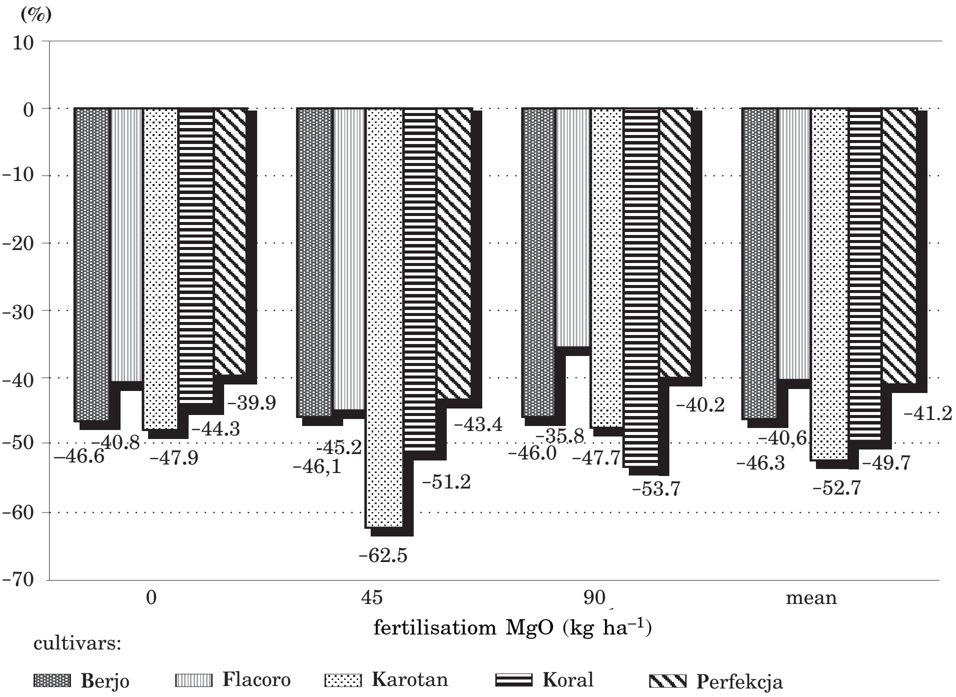

Fig. 2. Percent loss of vitamin C in carrot roots depending on the cultivar, fertilisation and storage period; means for years 2007-2009

by Wierzbicka and Kuskowska (2002), vitamin C loss after 60 days of storage ranged from 12.9% to 52.8% in 20 different vegetables, including carrot. Magnesium is an element which is not readily absorbable from our diet (just 50% of magnesium in food is absorbed). On the other hand, since magnesium partakes in most biochemical processes in the body, it can be perceived as one of the most essential cell cations; hence the importance of the presence of magnesium in food products. The results of our analyses on the magnesium content in dry matter of five carrot cultivars are given in Table 4. The cultivars did not differ significantly in their magnesium content. Majkowska-Gadomska and Wierzbicka (2010), who examined three other cultivars than the ones discussed here, recorded contrary results. Interestingly, the average content of magnesium in the dry matter of carrot roots was much higher in the present research (1.79 g kg^{-1}) than reported by Majkowska-Gadomska and Wierzbicka (2010), which was 1.0 g kg^{-1}. Such a big difference must have been induced by the positive effect of foliar fertilisation of carrot with magnesium sulphate. The authors cited above also applied additional fertilisation (multicomponent fertiliser Crop Care, containing magnesium). However, they did not record any significant effect of this fertiliser on the content of magnesium in carrot roots. In the present research, the statistical analysis showed that the magnesium fertilisation rate

Table 4

Content of magnesium in the dry weight of carrot roots; means for 2007-2009 (mg kg^{-1})

Date of determination (A)	Cultivars (B)	Fertilisation MgO kg ha^{-1} (C)			Mean
		0	45	90	
After harvest	Berjo	1.72	1.83	1.99	1.85
	Flacoro	1.53	1.75	1.89	1.72
	Karotan	1.53	1.70	1.99	1.74
	Koral	1.46	1.81	2.04	1.77
	Perfekcja	1.74	1.76	2.12	1.87
	mean	1.60	1.77	2.01	1.79
After storage	Berjo	1.70	1.82	1.97	1.83
	Flacoro	1.51	1.74	1.88	1.71
	Karotan	1.52	1.68	1.97	1.72
	Koral	1.44	1.79	2.02	1.75
	Perfekcja	1.73	1.74	2.10	1.86
	mean	1.58	1.75	1.99	1.77
Mean		1.59	1.76	2.00	1.78

LSD$_{\alpha=0.05}$

A = n.s	B = n.s.	C = 0.07
B/A = n.s.	A/B = n.s.	C/A = n.s.
A/C = n.s	C/B = 0.14	B/C = 0.23

*n.s. – no significant difference

had a significant effect of on the content of this element in carrot roots. As expected, each rate applied increased the content of magnesium in the dry matter of roots. The rate of 45 kg MgO ha^{-1} resulted in a 10.6% increase in magnesium content and the rate of 90 kg MgO ha^{-1} raised it by 25.8%, as compared with the control.

Minerals, including magnesium, demonstrate high thermal stability and, as such, they do not undergo extensive modifications. The present research confirms high stability of this cation in that the magnesium loss during storage was negligible, varying from 0.8% to 1.2% on average (Figure 3).

Numerous studies emphasise the positive effect of a diet rich in vegetables on human health. According to various sources, vegetable consumption should be about 500 g served with 4-5 meals a day. Since carrot is one of the most popular vegetables, this study has included an attempt at defining daily consumption of the total carotenoids, vitamin C and mineral magnesium, assuming that the average consumption of carrot was 55g per person a day (Table 5). By comparing the present data with the norm contained in the Recommended Dietary Allowance, it can be concluded that the daily requirement for vitamin C and magnesium will not be covered much by eating 55 g of carrot a day, but the daily consumption of carotenoids will be

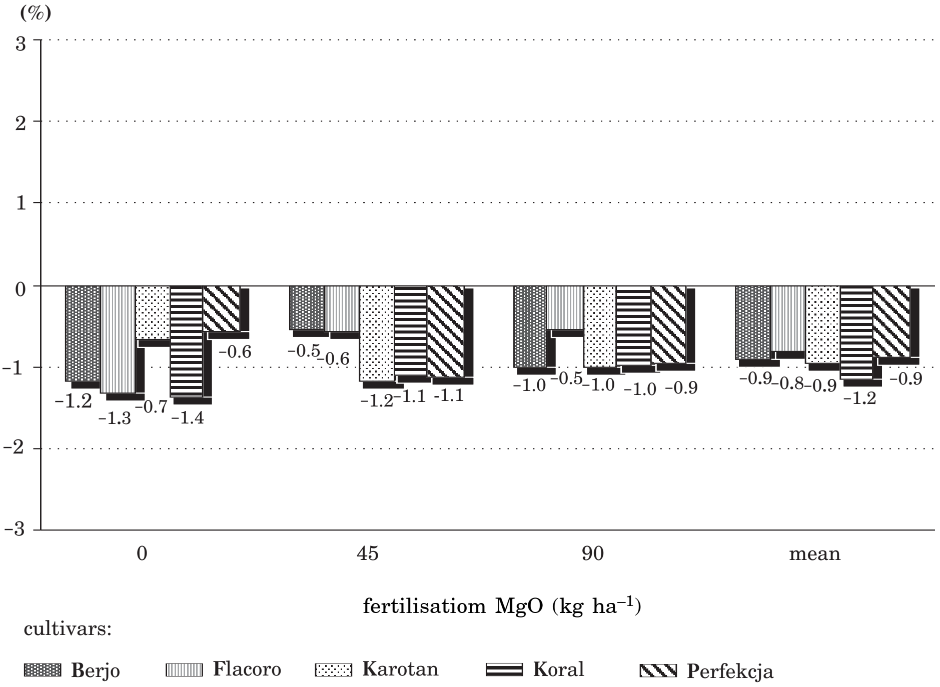

Fig. 3. Percent loss of magnesium in carrot roots depending on the cultivar, fertilisation and storage period; means for years 2007-2009

considerable. Converting the total carotenoids into vitamin A, on the assumption that 18 mg of carotenoids is equivalent to 1mg of retinol RE (Retinol Equivalent), the daily intake of vitamin A equals 43% of the required amount when eating 55 g of carrot right after harvest and 35 % when eating the same amount of stored carrot.

The results of our analysis of the linear correlation are given in Tables 6 and 7. As seen from Table 6, positive correlation between the content of vitamin C and the content of carotenoids in carrot roots appeared (r=0.50), whereas after storage (Table 7) vitamin C correlated positively with the content of magnesium, which means that the higher the content of vitamin C in carrot roots, the higher the content of carotenoids directly after harvest and magnesium after 6 months of storage.

Table 5

Daily nutrient requirements covered by consumption of 55 g of carrot daily* – mean for cultivars in 2007-2009

Dose of MgO (kg ha^{-1})	Carotenoids				Vitamin C				Magnesium			
	content (mg kg^{-1} fr.m.)		consumption (µg day^{-1})		content (mg kg^{-1} fr.m.)		consumption (mg day^{-1})		content (mg kg^{-1} fr.m.)		consumption (mg day^{-1})	
	1	2	1	2	1	2	1	2	1	2	1	2
0	114.1	93.3	6275	5129	8.96	5.01	0.49	0.28	202.4	204.3	11.13	11.24
45	120.4	96.9	6623	5328	10.21	5.08	0.56	0.28	230.6	235.4	12.68	12.95
90	126.4	102.4	6951	5631	10.07	5.53	0.55	0.30	268.1	271.6	14.75	14.94

*mean consumption of fresh and processed carrot (without juices) in Poland is 20 kg year^{-1} per capita.
1 – after harvest, 2 – after storage (6 months)

RDA (Recommended Dietary Allowance):
Vitamin C – 90 mg day^{-1}
Vitamin A – 900 µg day^{-1}
Magnesium – 350 mg day^{-1}

Table 6

Significant correlation coefficients (r) between the analysed characteristics determined after harvest

Characteristics		2.	3.
1.	Vitamin C content	0.503	
2.	Carotenoids content		
3.	Magnesium content		

$P_{0.05}$ $r = 0.497$

Table 7

Significant correlation coefficients (r) between the analysed characteristics determined after storage

Characteristics		2.	3.
1.	Vitamin C content		0.508
2.	Carotenoids content		
3.	Magnesium content		

$P_{0.05}$ $r = 0.497$

CONCLUSIONS

The wholesomeness of carrot roots was significantly affected by fertilisation at the rate of 45 kg MgO ha^{-1}. A significant increase in the content of carotenoids, vitamin C and mineral magnesium in the edible parts of carrots appeared after that treatment.

The cultivars differed in their content of nutrients. Irrespective of the experimental factors, the best nutritive value was recorded in roots of cv. Perfekcja and Karotan.

Analysing carrot roots after 6-month storage, on average, an average 19% loss of carotenoids, 46% vitamin C loss and 0.94% magnesium loss were recorded.

Consumption of 55 g a day of the analysed carrot fulfils much of the daily demand for vitamin A but fails to provide the required amounts of vitamin C and magnesium.

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EFFECT OF THE WAY SET-ASIDE LAND IS MAINTAINED ON THE CONTENT OF AVAILABLE FORMS OF SELECTED MICRONUTRIENTS IN SOIL

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Abstract

In 1996, a field experiment was set up on class IIIa soil, which consisted of 6 trials: 1 – bare fallow, 2 – fallow land seeded with annual plants, 3 – field swarded with goat's rue (*Galega orientalis* Lam.), 4 – traditional fallow, 5 – field swarded with a mix of goat's rue (*Galega orientalis* Lam.) and aweless brome (*Bromus inermis*), 6 – field swarded with aweless brome (*Bromus inermis*). During the tests, no agrotechnical measures were carried out apart from periodical mechanical weeding of the bare fallow and the sowing of annual plants. Plant samples for chemical analyses were taken from the swarded fields; the remaining biomass was left on the fields. After each growing season, soil samples were collected in four replicates from the 0-25 cm and 25-50 cm soil horizons, for determination of the content of Cu, Zn and Mn available forms. This paper discusses the results obtained in 2000-2007.

The experiment has demonstrated that the way farmland was laid fallow or set-aside had an effect on concentrations of the available forms of Cu, Zn and Mn in the 0-25 and 25-50 cm soil horizons. Particularly large changes occurred in the levels of Zn and Mn. The way set-aside arable land was kept had a weaker influence on the concentrations of easily soluble forms of copper. By seeding annual plants and leaving the grown biomass on a set-aside field, it was possible to maintain the soil abundance of available Zn on a high level and that of Cu and Mn – moderately high. Keeping soil as bare fallow favoured better accumulation of available forms of copper and manganese.

Key words: set-aside, fallow, available forms of Zn, Cu and Mn.

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WPŁYW SPOSOBU UTRZYMANIA GRUNTÓW WYŁĄCZONYCH Z UPRAWY NA ZAWARTOŚĆ PRZYSWAJALNYCH FORM WYBRANYCH MIKROELEMENTÓW W GLEBIE

Abstrakt

W 1996 r. na glebie klasy bonitacyjnej IIIa założono doświadczenie łanowe, które obejmowało 6 obiektów: 1 – ugór czarny, 2 – ugór obsiewany roślinami jednorocznymi, 3 – obiekt zadarniony rutwicą wschodnią (*Galega orientalis* Lam.), 4 – odłóg klasyczny, 5 – obiekt zadarniony mieszanką rutwicy wschodniej (*Galega orientalis* Lam.) ze stokłosą bezostną (*Bromus inermis*), 6 – obiekt zadarniony stokłosą bezostną *(Bromus inermis*). W czasie trwania badań, oprócz okresowego mechanicznego odchwaszczania czarnego ugoru i obsiewu obiektu jednorocznego, nie wykonywano żadnych zabiegów agrotechnicznych. Z obiektów zadarnionych pobierano tylko próbki materiału roślinnego do analiz chemicznych, a biomasę pozostawiano na polu. Po zakończeniu każdego okresu wegetacyjnego pobierano próbki gleby z każdego obiektu, w 4 powtórzeniach z warstw 0-25 cm i 25-50 cm, w których oznaczono zawartość przyswajalnych formy metali (Cu, Zn i Mn). W pracy zamieszczono wyniki badań z lat 2000-2007.

W badaniach wykazano, że sposób odłogowania i ugorowania gleby wpływał na zawartość przyswajalnych form Cu, Zn i Mn w warstwach 0-25 cm i 25-50 cm. Szczególnie duże zmiany wystąpiły w odniesieniu do Zn i Mn. W mniejszym zakresie sposób zabezpieczenia gleby wyłączonej z uprawy wpływał na koncentrację łatwo rozpuszczalnych form Cu. Coroczne obsiewanie pola wyłączonego z produkcji bez zbierania wyrosłej biomasy pozwoliło utrzymać zasobność gleby w Zn przyswajalny na poziomie wysokim, a Cu i Mn na poziomie średnim. Utrzymywanie gleby jako czarnego ugoru sprzyjało większemu nagromadzeniu przyswajalnych form miedzi i manganu.

Słowa kluczowe: odłóg, ugór, przyswajalne formy Zn, Cu i Mn.

INTRODUCTION

Being the most important component of agricultural production, soil should be ensured special protection (FIRBANK et al. 2003, MERTZ et al. 2008). Having fulfilled certain prerequisites, periodical cessation of agricultural production may lead to some improvement in soil chemical properties. Modification of soil's fertility largely depends on plant cover (or its lack). Organic matter left on a field and undergoing mineralization is an important link in the cycling of elements, as it facilitates the return of the elements previously taken up by the plants (ŻARCZYŃSKI et al. 2008). On farmland set aside for some time it is one of the few ways of improving the soil's abundance in available nutrients (SIENKIEWICZ et al. 2011).

It is very important to know the levels of available forms of Cu, Zn and Mn in soil. These micronutrients are essential for the life of animal and plant organisms, which need their adequate supply (SPIAK 2000). When trace elements are deficient, it is necessary to supplement the soil abundance. However, despite the application of balanced doses of nutrients and very precise fertilization techniques, fertilization is not indifferent to the natural environment (Anguelov, Anguelova 2009). Excessive concentrations of Cu, Zn and Mn in soil create a risk of plant contamination and poisoning of animals (McBride et al. 2004, Rattan et al. 2005, Diatta 2008).

The purpose of this study has been to evaluate how the way in which set-aside land is maintained affects concentrations of the available forms of copper, zinc and manganese in soil.

MATERIAL AND METHODS

A field experiment was set up in the spring of 1996, in Knopin, the community of Dobre Miasto, the Province of Warmia and Mazury. It was established on good wheat complex soil, class IIIa. This paper discusses the results obtained in 2000-2007. An area of 0.96 ha was divided into six equal parts:

1) bare fallow,
2) fallow seeded with annual plants,
3) plot swarded with goat's rue (*Galega orientalis* Lam.),
4) traditional fallow,
5) plot covered with a mixture of goat's rue (*Galega orientalis* Lam.) and aweless brome (*Bromus inermis*),
6) plot swarded with aweless brome (*Bromus ineris*).

The bare fallow was kept free from plant cover by mechanical weeding treatments carried out at regular intervals. On the seeded fallow, after the soil was mechanically tilled, cereals were sown. Since 2000, this plot was alternately cropped with oat and winter triticale. No agrotechnical treatments were carried out on the other plots. Samples of plants for chemical analyses were collected from the plant-covered plots, leaving all the remaining biomass on the field. Soil samples for analyses were taken once a year, i.e. in the autumn, when the growing season was over, from two soil horizons: 0-25 and 25-50 cm. The available forms of Cu, Zn and Mn were extracted in 1 mol HCl dm^{-3} solution and determined with the ASA method. The results were submitted to statistical analyses, including Duncan's test.

RESULTS AND DISCUSSION

The content of available forms of copper, zinc and manganese evidently depended on the way the set-aside fields were managed. Presence or absence of plant cover as well as the type of plants had a significant effect on the concentration of the analyzed metals in soil (Table 1). This dependence held true for both the topmost and deeper layer of soil (Tables 1-3).

Table 1

Content of available zinc in soil (mg Zn kg^{-1})

Layer	Treatment						Mean
	bare fallow	seeded fallow	goat's rue	traditional fallow	goat's rue + brome grass	brome grass	
0-25 cm	20.19	24.10	25.21	22.56	24.43	22.07	23.09
25-50 cm	19.43	20.23	20.51	19.47	18.69	18.65	19.50
Mean	19.81	22.16	22.86	21.01	21.56	20.36	

$LSD_{0.05}$ factor I 0.28
$LSD_{0.05}$ factor II 0.48
$LSD_{0.05}$ factor I×II 0.68

Most of the available zinc (25.21 mg kg^{-1}) was determined in the soil from the plot swarded with goat's rue (Tabela 1). Slightly less zinc appeared in the soil under the mixture of goat's rue and aweless brome. Similar quantities of available Zn as in the soil covered with goat's rue mixed with aweless brome were detected in the soil seeded every year with cereals. According to CHUDECKA and TOMASZEWICZ (2001), absence of soil cultivation treatments leads to an increase in the content of available forms of heavy metals, the finding which these authors attribute to the fact that there is more organic matter, originating from the plants growing on set-aside and left there. This procedure reduces erosion and migration of micronutrients. Our own studies partly confirm the hypothesis formulated by the above authors. It can be claimed that discontinuation of soil cultivation, or rather the lack of plant cover, may result in a depressed content of available zinc in soil. Our results, however, suggest that the presence of plant cover has a particularly strong influence on the concentration of available zinc in the topmost layer of soil. Soil samples collected from the 0-25 cm horizon in the plant-covered plots contained significantly more of this micronutrient than the soil from a bare field. Thus, by keeping a set-aside field covered with plants it was possible to maintain its abundance in zinc on a high level, whereas when soil was left as bare fallow, the abundance of available zinc fell to a moderate level. Permanent sward as well as annual plant cover could have had some effect such as reduced zinc leaching from soil. The humus compounds which appear in such soil, as DZIADEK and WACŁAWEK (2005) suggest, may favour the formation of salts of simple chelates of complex compounds. This in turn might protect this element from excessive leaching from the top layer of soil. As STĘPIEŃ et al. (2004) claim, the solubility of zinc in soil solutions depends on the content of humus.

In the 25-50 cm layer, the impact of the tested ways of maintaining fallow land on the concentration of zinc in soil was much weaker than in the 0-25 cm horizon. In the subsoil, the content of available zinc was 15% lower than in the upper layer.

The way set-aside fields were managed had a significant effect on the concentration of available copper in the topmost soil layer (Table 2). The highest copper abundance was determined in the soil which was subjected to annual agrotechnical treatments (bare fallow and seeded fallow). Periodical weeding by ploughing or soil cultivating most probably stimulated the processes of mineralization and favoured the release of copper from more complex compounds. Dziadek and Wacławek (2005) state that organic substance plays the predominant role in binding copper compounds in soil. Soil sorptive capacity for Cu^{2+} is very high and in most soils it is a decisive factor affecting the mobility and plant availability of this metal. Thus, it is highly probable that once the mineralization conditions were improved owing to agrotechnical treatments, a significant increase in the concentration of available copper occurred in the topmost layer of the soil under bare fallow or seeded annually. Soil under permanent grass cover, compared to the former two variants, contained significantly less copper. This tendency appeared both in the upper soil horizon and in the subsoil. For comparison, it can be noticed that the arable layer of soil cropped with brome contained over 28% less available Cu^{2+} than that under bare fallow. According to Sienkiewicz-Cholewa and Wróbel (2004), copper is second to boron among all the micronutrients as the most heavily exhaustible and present in the smallest amounts in Polish soils. For this reason and in the light of the present results, it is recommendable to consider well how set-aside farmland should be managed. In our own studies, despite distinct differences caused by the different ways of maintaining fields temporarily excluded from agricultural production, the content of available Cu in the 0-25 cm layer of soil in all the treatments was on a moderate level. The content of available copper in the 25-50 cm horizon was on a slightly lower level and changed analogously to that in the 0-25 cm layer.

Table 2

Content of available copper (mg Cu kg^{-1})

Layer	Treatment						Mean
	bare fallow	seeded fallow	goat's rue	traditional fallow	goat's rue + brome grass	brome grass	
0-25 cm	3.81	3.73	3.30	3.62	2.68	2.49	3.27
25-50 cm	3.52	3.07	3.25	3.42	2.56	2.21	3.00
Mean	3.66	3.40	3.28	3.52	2.62	2.34	
$LSD_{0.05}$ factor I 0.08 $LSD_{0.05}$ factor II 0.14 $LSD_{0.05}$ factor I×II 0.19							

Manganese is directly responsible for environmental contamination, but at the same time it is an essential microelement for plants (Jakubus 2004). Therefore, it is particularly important to monitor concentrations of this element in set-aside fields. In the soil maintained as bare fallow, the concentration of plant available manganese was the highest (Table 3). Higher accumulation of the available form of manganese in soil lacking any plant cover may have been an effect of the most strongly progressing soil acidification. These results correlate with the tests reported by Chudecka and Tomaszewicz (2001), who emphasized that fact that the soil's sorptive complex in a fallow field undergoes negative changes, such as an increased share of H^+ cations and decreased share of alkaline cations, mainly Ca^{2+}. Chudecka and Tomaszewicz (2001) suggest that levels of available forms of heavy metals rise in set-aside soil once all or some of the agrotechnical treatments are discontinued. In our experiment, significantly more available manganese was determined in soil under a mix of goat's rue and aweless brome than in soil covered with monocultures of these two species or by natural plants. Irrespective of the way fallow land was maintained, the concentration of plant available manganese in soil, with respect to the threshold values, was on an average level.

Table 3

Content of manganese (mg Mn kg^{-1})

Layer	Treatment						Mean
	bare fallow	seeded fallow	goat's rue	traditional fallow	goat's rue + brome grass	brome grass	
0-25 cm	127.64	121.03	116.27	98.39	124.01	113.22	116.76
25-50 cm	119.91	120.05	102.00	84.82	120.64	98.58	107.66
Mean	123.78	120.54	109.13	91.61	122.32	105.90	
$LSD_{0.05}$ factor I 1.27 $LSD_{0.05}$ factor II 2.20 $LSD_{0.05}$ factor I×II 3.11							

The concentration of manganese in the subsoil (25-50 cm) was just as evidently affected by the way fallow land was managed as in the upper soil layer. The average concentration of Mn in the subsoil was nearly 8% lower than in the arable soil horizon. The highest supply of plant available manganese in the 25-50 cm layer was determined in the plots covered by annual plants and a mixture of goat's rue and aweless brome.

CONCLUSIONS

1. Long-term or annual sward of grass growing on fallow fields contributed to higher abundance of plant available copper in the upper soil layer.

2. When soil was kept as bare fallow, it tended to accumulate more of the available forms of copper and manganese.

3. Traditional fallow limited the availability of manganese in the two soil layers (0-25 and 25-50 cm) and fallow land swarded with aweless brome produced an analogous effect on copper.

4. Long-term fallowing or setting-aside may change concentrations of available forms of Cu, Zn and Mn in soil to the depth of 50 cm.

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REVIEW PAPER

THE ROLE OF BIOELEMENTS IN IMPROVING THE QUALITY OF LIFE OF PATIENTS SUFFERING FORM THE PREMENSTRUAL SYNDROME (PMS)

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Abstract

The Premenstrual Syndrome (PMS) is described as a cyclical disorder related to the hormonal changes during a menstrual cycle, which affects the emotional and physical health of many women during their reproductive period of life. The PMS can obviously change the quality of life. The syndrome is characterized by a complex group of symptoms, such as depression, irritability, mood swings, anxiety, abdominal discomfort. These signs occur during the luteal phase of a menstrual cycle and disappear after the onset of menses.

Some studies suggest that a variety of nutrients may play an important role in the mood swings which occur cyclically during the course of the premenstrual syndrome and that some can have a beneficial impact, especially on the estrous phase of a menstrual cycle.

The aim of the paper is to review the results of some studies concerning the role of bioelements in patients with the PMS.

Concentrations of magnesium, zinc, selenium and manganese are the highest during menses and the lowest in the ovulatory phase.

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Fluctuations of the magnesium concentration during a menstrual cycle and the involvement of this element in many cellular pathways and neuromuscular activities obviously affect the incidence or intensity of the PMS symptoms. However, we lack firm evidence that magnesium supplementation can have a positive effect on alleviating the the PMS-related ailments.

Some relationship between the PMS and bone demineralization or depressed calcium concentration in blood has been identified. However, further studies are necessary to examine how the calcium concentration in a human body can decrease the intensity of the PMS symptoms.

Key words: premenstrual syndrome (the PMS), bioelements.

ROLA BIOPIERWIASTKÓW W POPRAWIE JAKOŚCI ŻYCIA PACJENTEK Z ZESPOŁEM NAPIĘCIA PRZEDMIESIĄCZKOWEGO

Abstrakt

Zespół napięcia przedmiesiączkowego jest opisywany jako cykliczne zaburzenie związane ze zmianami hormonalnymi w cyklu miesiączkowym, które w istotny sposób wpływa na stan emocjonalny i somatyczny wielu kobiet będących w wieku reprodukcyjnym. Zespół napięcia przedmiesiączkowego może negatywnie wpływać na jakość życia. Na zespół ten składa się grupa objawów, takich jak depresja, drażliwość, wahania nastroju, niepokój, dyskomfort w jamie brzusznej. Objawy te pojawiają się podczas fazy lutealnej cyklu menstruacyjnego i znikają tuż po pojawieniu się miesiączki.

Opublikowano kilka badań, których wyniki wskazują na to, że różne biopierwiastki mogą odgrywać istotną rolę w cyklicznie pojawiających się w przebiegu zespołu napięcia przedmiesiączkowego zaburzeniach nastroju, a niektóre z nich mogą nawet korzystnie wpływać zwłaszcza na fazę estrogenową cyklu.

Celem artykułu jest przegląd wyników badań dotyczących znaczenia biopierwiastków u pacjentek z zespołem napięcia przedmiesiączkowego.

Okazuje się, że stężenie magnezu, cynku, selenu i manganu jest najwyższe podczas menstruacji, a najniższe podczas owulacji.

Wahania stężenia magnezu w trakcie cyklu miesiączkowego oraz udział tego pierwiastka w wielu przemianach komórkowych oraz w funkcjonowaniu układu nerwowego i mięśniowego wpływają z całą pewnością na pojawienie się oraz nasilenie objawów zespołu napięcia przedmiesiączkowgo. Jednak nie ma do tej pory przekonujących dowodów na to, że suplementacja magnezu może mieć pozytywny wpływ na złagodzenie dolegliwości związanych z omawianym zespołem.

Stwierdzono zależność między zespołem napięcia przedmiesiączkowego a demineralizacją kości i obniżeniem stężenia wapnia we krwi. Konieczne są jednak dalsze badania dotyczące tego, jak stężenie wapnia w organizmie może wpłynąć na zmniejszenie nasilenia objawów zespołu napięcia przedmiesiączkowego.

Słowa kluczowe: zespół napięcia przedmiesiączkowego, biopierwiastki.

INTRODUCTION

The Premenstrual Syndrome (PMS) is described as a cyclical disorder related to the hormonal changes in the menstrual cycle, which affects the emotional and physical health of many women during their reproductive period of life. The syndrome is characterized by a complex group of symptoms that occur during the luteal phase of the menstrual cycle but disappear soon after the onset of menses (THYS-JACOBS 2000).

The symptoms are limited to the luteal phase of the menstrual cycle, abate shortly after the onset of menses, and commonly include depression, irritability, mood swings, bloating, breast tenderness and abdominal discomfort. Women with predominant affective symptoms may also meet the criteria for the premenstrual dysphoric disorder, a more severe form of the PMS associated with significant impairment of normal functions; recent studies estimate that 5 to 8% of premenopausal women meet the criteria set for the premenstrual dysphoric disorder (BERTONE-JOHNSON et al. 2005).

Because of the number and diversity of the symptoms, many theories and mechanisms have been proposed to explain this syndrome, with an array of therapeutic approaches offered. Most of these approaches have proven disappointing and scientifically unfounded (THYS-JACOBS 2000).

There are some studies that suggest that a variety of bioelements may play an important role in the phase when mood swings and behavioural disturbances of the premenstrual syndrome appear, and some of these micronutrients can even level their cyclic fluctuations during the estrous and menstrual cycles.

The aim of the paper is to review the results of some studies concerning the role of bioelements in patients with the PMS.

There is some firm evidence that the PMS is related to hormonal fluctuations during a menstrual cycle and occurs only in women with ovulatory cycles. The PMS does not occur prepubertally or during a menopause (FERRIN et al. 1993).

The ovarian activity is cyclical and variations in the metabolism of bioelements may accompany hormonal fluctuations during a normal menstrual cycle and may help explain some characteristics of the PMS. Cyclical fluctuations during the estrous cycle have been demonstrated in a few animal investigations. The cyclic hormonal changes can affect a variety of physiological and biochemical processes. It has been reported that oestrogen induces hypercalcemia through the action of the parathyroid gland. Withdrawal of oestrogen is reported to cause a significant loss of bone calcium. It has been observed that an increase in the basic metabolic rate and oxygen consumption during the luteal phase is associated with increased carbohydrate utilization. This elevated metabolism requires magnesium ions and oxidative enzymes, which have been found to increase significantly during the luteal phase. It has also been found that the phosphate concentration falls more rapidly than the calcium rise after administration of the parathyroid hormone. This decline in the phosphate concentration is caused by a strong effect of the parathyroid hormone on the kidney, causing renal phosphate excretion (DULLO, VEDI 2008). PANDYA et al. (1995) have reported that serum inorganic phosphorus levels were higher in the menstrual phase as compared to the other phases. These ions are regulated by various hormones but predominantly by the sex hormones.

Concentrations of magnesium, zinc, selenium and manganese are the highest during menses and the lowest in the ovulatory phase. There is a rise in ionized magnesium and selenium levels, with a fall in zinc and manganese during the luteal phase. MUNEYYIRCI-DELALE and NACHARAJU (1999) noted a significant increase in the serum Ca^{2+}/Mg^{2+} ratio both in the ovulatory and luteal phases in healthy women.

In thirty-two women with the PMS, supplementation with 360 mg day^{-1} of magnesium (during the second half of a menstrual cycle) significantly reduced the general PMS symptoms and specially those related to mood changes. It should be noted that the experimental design resulted in the placebo group receiving only two months of supplementation at crossover, whereas the magnesium group received the supplement for four months (FACCHINETTI et al. 1991a). In 20 patients with premenstrual migraine, prophylactic supplementation with magnesium (360 mg day^{-1} or placebo during the second half of a menstrual cycle) significantly reduced the number of days with a headache (FACCHINETTI et al. 1991b).

CHUONG and DAWSON (1994) in their trial determined that the level of zinc in blood in patients with the PMS was significantly lower during the luteal phase than during the follicular phase, whereas in the controls zinc values were not significantly different between the follicular and the luteal phases. Copper levels were noted to be higher during the luteal phase in the PMS patients compared with the controls. Because copper competes with zinc for intestinal absorption and serum protein binding sites, the zinc/copper ratio can reflect the availability of zinc in the body. The calculated values of this ratio revealed that it was significantly lower during the luteal phase in treated patients than in the controls.

Thus, fluctuations in the metabolism of bioelements, especially calcium and the calciotropic hormones, have been observed during both the menstrual and estrous cycles, and these cyclical changes may help explain some of the features of the PMS.

Magnesium fluctuates throughout a menstrual cycle and is involved in many cellular pathways and neuromuscular activities which affect the PMS. The clinical evidence on magnesium supplementation, although promising, remains limited. A double blind randomized study examined the effect of magnesium (360 mg day^{-1}) compared to placebo during two cycles. Magnesium was administered during the luteal phase of a menstrual cycle until the onset of menstrual flow. Although magnesium was found to reduce total symptom scores and the negative group of symptoms, the baseline symptom scores between the treatment groups was significantly different and the expected placebo effect was lacking in this trial. In another study, benefits of magnesium supplementation for the PMS patients were investigated in a double blind crossover trial over four menstrual cycles (THYS-JACOBS 2000).

Magnesium is an important element that helps to form bones, relaxes muscle spasms, activates cellular enzymes, regulates nerve and muscle function (including the heart) and acid-alkaline balance. It is often referred to as an anti-stress element. Deficiency of magnesium is known to cause many symptoms usually associated with the PMS, such as irritability, depression, confusion and muscle aches. However, determining if a woman is deficient in magnesium can be a challenge.

Many sources of food that are thought to contain magnesium according to old nutrient charts (grains, legumes, vegetables, nuts and seeds) are actually low due to magnesium deficit in soil and its depletion during processing and cooking. Measuring magnesium levels in the blood plasma will often fail to demonstrate low levels, although erythrocyte magnesium levels have been shown to be low in the PMS patients (ROSENSTEIN, ELIN 1994). Therefore, woman who want to have a true picture of their magnesium levels should be encouraged to have an erythrocyte magnesium level test done. Oestrogen enhances the utilization of magnesium. However, if oestrogen levels are high, which is often the case in the PMS, and magnesium intake is less then optimal, oestrogen-induced shifts of magnesium can be deleterious, leading to muscle spasms, migraine, and other PMS disorders. In one study, serum levels of magnesium were inversely related to the serum level of oestrogen (SEELIG 1993).

Magnesium supplementation can help to alleviate many measurable parameters of the PMS, including cramps, irritability, fatigue, depression and water retention. Magnesium citrate, gluconate, and lactate are better absorbed than magnesium oxide. Magnesium glycinate is a well absorbable form of magnesium, with minimum laxative effects (WALKER, DE SOUZA 1998).

Two investigations have identified a relationship between the PMS and bone loss, further indicating a derangement in calcium metabolism in the PMS as a potential biologic trigger. In 1994, LEE and KANIS (1994) examined the relationship of premenstrual and postmenopausal symptoms with vertebral osteoporosis by means of a retrospective case control questionnaire.

Three calcium trials have demonstrated the efficacy of calcium treatment. Another randomized, double blind crossover trial was conducted to assess the effectiveness of calcium in women with the PMS (THYS-JACOBS et al. 1998).

Continuing Survey of Food Intakes by Individuals (CLEVELAND et al. 1996) shows that among menstruating women (ages 12 to 50 years) the mean daily intake of calcium ranged from 607 to 809 mg, suggesting that most of the population at risk of the PMS are not receiving the recommended intake levels. Therefore, since most women consume far less than 1.0 mg day^{-1} of calcium from food, they would not exceed the safety limit if they added 1.0-1.2 mg day^{-1} of supplemental calcium to their normal dietary intakes. Calcium, unlike some other supplements tested for the PMS relief efficacy, is safe even to women who may become pregnant. It is also relatively inexpensive, especially in comparison with prescription medications.

The PMS shares many of the features of depression, anxiety and the dysphoric states. Recent evidence has suggested that the PMS may be associated with some disorder in calcium homeostasis and parathyroid hormone dysregulation. In addition to the abnormalities in calciotropic hormones and the clinical response observed on calcium supplementation in the PMS, a relationship between the PMS and bone loss has been identified, further supporting a derangement in calcium metabolism occurring during the PMS. Clinical illness such as the PMS could be a reflection of an important physiological disruption in calcium regulation, while an adequate treatment of the PMS could help to restore bone mineral homeostasis and reverse the associated neuropsychiatric disturbances (Thys-Jacobs 2000).

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