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ul.Michała Oczapowskiego 8, 10-719 Olsztyn, tel. +48 089 5233231
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Autor strony internetowej: Sławomir Krzebietke

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SPIS TREŚCI

CONTENT

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Prace oryginalne

CONTENT OF MANGANESE IN SERUM, ERYTHROCYTES, AND HAIR OF MEN – AIRPORT EMPLOYEES

Maria Długaszek[1], Mirosława Szopa[1], Andrzej Sałacki[2]

[1]Instytut Optoelektroniki, Wojskowa Akademia Techniczna w Warszawie
[2]Klinika Chorób Wewnętrznych i Kardiologii
Wojskowy Instytut Medyczny w Warszawie

Abstract

Manganese is a component and cofactor for many important enzymes. In blood Mn is complexed to transferrin, and it quickly passes through the body to be extracted mainly in the bile and urine. Almost all Mn pool in blood is located in erythrocytes. Content of manganese in serum, erythrocytes, and hair of 26 men, workers of an airport, was determined. The control group consisted of administrative workers and the test group was composed of airplane servicemen. Hair samples of 0.5 g and 3–4 cm in length measured from the scalp were taken from some places on a head, washed with a detergent solution, rinsed with deionized water, acetone, and dried. Samples of blood were spun. All the samples were mineralized in a mixture of spectrally clean acids HNO_3 and $HClO_4$. Concentration of Mn was analyzed by electrothermal atomic absorption spectrometry GFAAS. The concentrations of Mn in the samples of erythrocytes were statistical significantly higher in the test group. In samples of hair, Mn concentrations were comparable between both groups of men. The coefficients of correlation between Mn concentrations in serum and hair, erythrocytes and hair, and between serum and erythrocytes did not imply significant correlations between Mn concentration in the analyzed clinical samples. In contrast, in the erythrocytes of men exposed on aviation fuel the content of Mn was significantly higher.

Key words: manganese, serum, erythrocytes, hair, atomic absorption spectroscopy.

dr inż. Maria Długaszek, Instytut Optoelektroniki, Wojskowa Akademia Techniczna, ul. S. Kaliskiego 2, 00-908 Warszawa, e-mail: mdlugaszek@wat.edu.pl

ZAWARTOŚĆ MANGANU W SUROWICY, ERYTROCYTACH I WŁOSACH MĘŻCZYZN – PRACOWNIKÓW LOTNISKA

Abstrakt

Mangan jest kofaktorem i składnikiem wielu enzymów. We krwi jest związany głównie z transferyną, szybko ulega dystrybucji i szybko jest wydalany z organizmu, przede wszystkim z żółcią. Niewielkie ilości tego pierwiastka są wydalane w moczu. Prawie cała pula Mn we krwi jest zlokalizowana w erytrocytach. W pracy oznaczono zawartość manganu w surowicy, erytrocytach i włosach 26 mężczyzn będących pracownikami lotniska. Grupę kontrolną stanowili pracownicy administracyjni, natomiast grupę badaną – mężczyźni obsługujący samoloty. Włosy pobrano z kilku punktów głowy, w ilości ok. 0.5 g i długości 3–4 cm, licząc od skóry. Próbki włosów umyto w roztworze detergentu, wodzie dejonizowanej i acetonie, a następnie wysuszono. Próbki krwi odwirowano i oddzielono masę erytrocytarną od surowicy. Tak przygotowane próbki mineralizowano w mieszaninie spektralnie czystych kwasów HNO_3 i $HClO_4$. Zawartość manganu oznaczono metodą spektrometrii absorpcji atomowej z atomizacją w piecu grafitowym GFAAS. Oznaczona zawartość Mn w badanych próbkach erytrocytów była statystycznie istotnie wyższa w grupie kontrolnej. W próbkach włosów zawartość Mn była porównywalna w obu grupach mężczyzn. Wyznaczone współczynniki korelacji między zawartością Mn w surowicy i we włosach, erytrocytach i we włosach oraz w surowicy i erytrocytach nie wskazują na występowanie istotnych zależności między zawartością Mn w badanych próbkach klinicznych. W badaniach, zarówno w surowicy, erytrocytach i włosach mężczyzn z grupy kontrolnej, jak i w badanej, nie stwierdzono, aby w znaczący sposób kumulował się mangan. Jednak w erytrocytach pracowników obsługujących lotnisko ilość Mn była znamiennie wyższa.

Słowa kluczowe: mangan, surowica, erytrocyty, włosy, spektrometria absorpcji atomowej.

INTRODUCTION

Manganese is a cofactor for many important enzymes such as kinases, hydrolases, and trasferases. Mn is also a component of superoxide dismutase (SOD), arginase, pyruvate carboxylase, glutamine synthetase, and katalase. This element participates in carbohydrate and lipid metabolism, synthesis of proteins, hormones (e.g. thyroxine), and also nucleic acids, steroids, hemoglobin, erythrocytes, neurotransmitters, cartilaginous and bone tissue. In blood Mn is complexed to transferrin, and quickly passes through the body to be extracted mainly in bile and urine. Across the cell membrane Mn is transported by transferrin, carrying DMT1 protein, and via Ca^{2+} gated channels. DMT1 is responsible for the cellular uptake of divalents cations (Fe^{2+}, Cd^{2+}, Co^{2+}, Ni^{2+},Cu^{2+}, Pb^{2+}). Almost all Mn pool in blood is located in erythrocytes. Chronic Mn deficiency can be linked to lipid metabolism disturbances, skeletal mineralization, dermatitis, and teratogenic effect. Depressed serum Mn levels have been reported in osteoporosis, epilepsy, and diabetes (ROTH, GARRICK 2003). Mn overexposure (mainly by the respiratory tract) results in damage of Fe absorption and metabolism, generation of reactive oxygen species (ROS) and oxidative stress, slow growth, respiratory

and neurological disorders. Neurological symptoms of Mn overload are similar to those observed in Parkinson's disease. Elevated Mn levels in many tissues were observed in many patients suffering from this disease. Deficiency of Fe promotes manganese accumulation in the body, specially in the brain and liver. In a biological medium manganese exists mainly as Mn(II) and Mn(III) cations (Roth, Garrick 2003, Zhang et. al 2003, Hussain, Syed 1999). Up to now, inverse relationships between manganese and magnesium, calcium, iron, mercury, and cadmium (Mn-SOD inhibition) were reported (Roth, Garrick 2003, Casalino et. al 2002).

Simultaneous exposure to As, Pb, and Mn can produce synergistic interactions, which result in elevated level of those elements, mainly in the brain. They also cause depressed brain dopamine level, and intellectual and neurological dysfunctions (Wright et. al 2006). Both lead and manganese are gasoline additives used as anti-knocking agents and they are components of many fuels (Davydowa 2005).

The aim of the work was to evaluate Mn contents in serum, erythrocytes, and hair of men aging 30-45 years, occupationally exposed to aviation fuel.

MATERIAL AND METHODS

Samples of hair and blood were taken from 26 men aged 30–45. Sixteen men were airplane servicemen exposed to aviation fuel. The control group was made of men working in the administration section of the airport. Hair samples weighing 0.5 g and 3–4 cm in length measured from the scalp were taken from few places on the head. Samples of hair were washed with a detergent solution, rinsed with deionized water, acetone and dried.

Blood was collected by venipuncture into plastic tubes. Next, blood samples were centrifuged at 3000 rpm for 10 minutes; erythrocytes and plasma were separated and kept at – 20°C until analyses. About 1 g of the prepared samples was mineralized in a mixture of spectrally clean acids HNO_3 (65%) and $HClO_4$ (65%) (3 + 1).

Concentration of Mn was analyzed by electrothermal atomic absorption spectrometry (GFAAS) using a spectrometer AVANTA Σ (GBC) equipped with a graphite furnace GF3000 fitted with a deuterium lamp for background correction and an autosampler PAL3000. The wavelength applied was λ=279.5 nm, the temperature program was 700/2400°C, and the intensity of lamp current was 5.0 mA with a slit width 0.2 nm.

RESULTS AND DISCUSSION

Analytical characterization of the method and results of certificated material analysis (human hair NCS ZC 81002) is presented in Table 1.

Table 1

Analytical characterization of used method

Characteristic mass (pg)	Limit of detection (ng ml^{-1})	Precision (%)	Recovery (%)	Certificated Mn concentration $x \pm s$ (µg g^{-1})	Found Mn concentration $x \pm s$ (µg g^{-1})
0.32	0.17	1.4	102.3	2.94 ± 0.2	2.84 ± 0.17

Concentration of Mn in serum of both groups exceeded 2 g/kg. Smaller contents of Mn were received by other authors: 11 nmol l^{-1} by KUCERA et. al (1995) and 0.5 g l^{-1} by CORNELIS et. al. (1995). The Mn concentration obtained from the analyzed samples of erythrocytes is statistically significantly higher in the test group – 11.8 µg kg^{-1}. According to IUPAC (CORNELIS et. al 1995) a typical content of Mn in erythrocytes is about 15 µg kg^{-1}. In samples of hair Mn concentrations were comparable between both groups of men: 0.07–0.42 µg kg^{-1}. Similar quantities of this element were received by other authors (Table 2). The coefficients of correlation between Mn concentration in serum and hair (r = -0.10871), erythrocytes and hair (r = -0.1393) and between serum and erythrocytes (r = 0.1764) did not suggest any significant relationships between Mn concentrations in the analyzed clinical samples.

Interactions between Pb and Mn in blood of children form Sydney were analyzed by GULSON et. al (2006) after enriching fuel with an admixture of Mn compound instead of Pb as an anti-knocker. In these children the content of Pb was much lower, but the content of Mn was the same. Similarly, the Mn concentration in blood of mothers living in Paris, where Pb is added to fuel, was identical as that in blood of mothers living in Montreal, where Mn is used (about 23 µg L^{-1}). Somewhat larger contents of Mn were in samples of umbilical cord blood of mothers living in Canada, but without statistical significance. However, differences were observed in the case of Pb. Lower contents were found in blood of women living in Canada and in umbilical cord blood (AUDREY et. al 2002). One statistically proven significant reverse correlation was found between neuropsychological functions as well as intelligence quotient between children whose hair had higher quantities of Mn and As in comparison to the control group (WRIGHT et. al 2006).

Table 2

Concentration of Mn in serum, erythrocytes and hair, $x \pm s$ (μg kg^{-1})

Tested group (n = 16)	Serum	Erythrocytes	Hair	Authors
	2.5 ± 0.9 (1.0 – 4.7)	11.8 ± 1.7 (4.3 – 13.5)	201 ± 110 (120 – 420)	own research
Control group (n = 10)	2.2 ± 0.9 (1.1 – 3.7)	7.4 ± 2.8, p <0.05 (8.5 – 14.0)	231 ± 90 (70 – 370)	own research
		165 ± 51* nmol l^{-1}		Kristiansen et al. 1997
	11 ± 3 nmol l^{-1}			Kucera et al. 1995
		7.40 (1.5 – 22.0)*μg l^{-1}		White, Sabbioni 1998
	0.5 μg l^{-1}	15 μg kg^{-1}		Cornelis et al. 1995
		2 – 27* μg l^{-1}		Apostoli 2002
			0.03 – 1.2 μg g^{-1}	Bermejo--Barrera et al. 1998
		5.0 –12.8* μg l^{-1} 0.63 – 2.26** μg l^{-1}	0.016 – 0.57 μg g^{-1}	Goulle et al. 2005
		0.0037 – 0.0140 μg ml^{-1}	0.10 – 2.4 μg g^{-1}	Teresa et al. 1997
		1.8 – 45.0* μg l^{-1}		Gulson et al. 2006
		6.3 – 151.2* μg l^{-1} (mother) 14.9 - 92.9 *μg l^{-1} (child)	0.1 – 3.24 μg g^{-1} 0.05 – 13.33 μg g^{-1}	Takser et al. 2003
			0.383 μg g^{-1}	Violante et al. 2000
			0.02 – 35.48 μg g^{-1}	Pereira et al. 2004
			89.1 – 2145.3 ng g^{-1}	Wright et al. 2006
		1.2 - 4.0* μg l^{-1}		Audrey et al. 2002

* blood
**plasma

CONCLUSIONS

In the analyses presented here serum and hair of men from the test and the control groups were not found to accumulate essential amounts of Mn. In contrast, in the erythrocytes of men exposed on aviation fuel the content of Mn was significantly higher. With regard to increasing environmental exposition of human to Mn, there is a justified need to continue research on interactions between Mn and other elements in biological media.

REFERENCES

APOSTOLI P. 2002. *Elements in environmental and occupational medicine.* J. Chromatogr. B., 778: 63-97.

AUDREY S., TAKSER L., ANDRE M., MARTIN S., MERGLER D., ST-AMOUR G., BLOT P., HELLIER G., HUEL G. 2002. *A comparative study of manganese and lead levels in human umbilical cords and maternal blood from two urban centers exposed to different gasoline additives.* Sci. Total. Environ., 290:157-164.

BERMEJO-BARRERA P., MOREDA-PINEIRO A., MOREDA-PINEIRO J., BERMEJO-BARRERA A. 1998. *Determination of aluminium and manganese in human scalp hair by electrothermal atomic absorption spectrometry using slurry sampling.* Talanta, 45:1147-1154.

CASALINO E., CALZARETTI G., SBLANO C., LANDRISCINA C. 2002. *Molecular inhibitory mechanisms of antioxidant enzymes in rat liver and kidney by cadmium.* Toxicology, 179: 37-50.

CORNELIS R., HEINZOW B., HERBER R.F.M., CHRISTENSEN J.M., PAULSEN O.M., SABBIONI E., TEMPLETON D.M., THOMASSEN Y., VAHTER M., VESTERBERG O. 1995. *Sample collection guidelines for trace elements in blood and urine.* Pure & Appl. Chem., 67: 1575-1608.

DAVYDOVA S. 2005. *Heavy metals as toxicants in big cities.* Microchem. J., 79:133-136.

GOULLE J.P., MAHIEU L., CASTERMANT J., NEVEU N., BONNEAU L., LAINE G., BOUIGE D., LACROIX C. 2005. M*etal and metalloid multi-elementary ICP-MS validation in whole blood, plasma, urine and hair. Reference values.* Forensic Sci. Int., 153: 39-44.

GULSON B., MIZON K., TAYLOR A., KORSH M., STAUBER J., DAVIS J.M., LOUIE H, WU M, SWAN H. 2006. *Changes in manganese and lead in the environment and young children associated with the introduction of methylcyclopentiadienyl manganese tricarbonyl in gasoline-preliminary results.* Environ. Res., 100:100-114.

HUSSAIN S., ALI S.F. 1999. *Manganese scavenges superoxide and hydroxyl radicals: an in vitro study in rats.* Neuroscience Letters, 261: 21-24.

KRISTIANSEN J., CHRISTENSEN J.M., IVERSEN B.S., SABBIONI E. 1997. *Toxic trace element reference levels in blood and urine: influence of gender and lifestyle factors.* Sci. Total. Environ., 204: 147-160.

KUCERA J., BENCKO V., SABBIONI E., VAN DER VENNE M.T. 1995. *Review of trace elements in blood, serum and urine for the Czech and Slovak populations and critical evaluation of their possible use as reference value*s. Sci. Total. Environ., 166: 211-234.

PERREIRA R., RIBEIRO R., GONCALVES F. 2004. *Scalp hair analyses as a tool in assessing human exposure to heavy metals.* Sci. Total. Environ., 327:81-92.

ROTH J.A., GARRICK M.D. 2003. *Iron interactions and other biological reactions mediating the physiological and toxic actions of manganese.* Biochem. Pharm., 66:1-13.

Takser L., Mergler D., Hellier G., Sahuquillo J., Huel G. 2003. *Manganese, monoamine metabolite levels at birth, and child psychomotor development.* Neurotoxicology, 24:667-674.

Teresa M., Vasconcelos S.D., Tavares H.M.F. 1997. *Trace elements concentrations in blood and hair of young apprentices of a technical-professional school*. Sci. Total. Environ., 205: 189-199.

Violante N., Senofonte O., Marsili G., Meli P., Soggiu M.E., Caroli S. 2000. *Human hair as a marker of pollution by chemical elements emitted by a thermoelectric power plant.* Microchem. J., 67:397-405.

White M.A., Sabbioni E. 1998. *Trace element reference values in tissues from inhabitants of the European Union. X. A study of 13 elements in blood and urine of a United Kingdom population*. Sci. Total. Environ., 216: 253-270.

Wright R.O., Amarasiriwardena C., Woolf A.D., JIM R., Bellinger D.C. 2006. *Neuropsychological correlates of hair arsenic, manganese, and cadmium levels in school-age children residing near a hazardous waste site.* Neurotoxicology, 27:210-216.

Zhang S., Zhou A., Fu J. 2003. E*ffect of manganese chloride exposure on liver and brain mitochondria function in rats.* Environ. Res., 93:149-157.

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SPECIATION ANALYSIS OF INORGANIC FORMS OF SELENIUM IN CONFECTIONERY PRODUCTS

Agnieszka Gawłoska-Kamocka

Wyższa Szkoła Zawodowa w Łodzi

Abstract

Owing to its biological properties the microelement selenium has attracted enormous interest. It has been established that selenium stimulates the human immune system and has anti-carcinogenic effect. The main sources of selenium are high-protein foodstuffs of plant and animal origin, as well as high-protein dairy products.

The aim of this study was to detect selenium content in confectionery products using speciation analysis in order to determine inorganic forms of selenium such as Se^{2-} SeO_3^{2-} and SeO_4^{2-} anions.

The hydride generation method combined with the atomic absorption spectroscopy was used for the final determination of selenium forms. The determination of selenium was conducted using aqueous extraction and digestion of samples with concentrated acids.

The speciation determination of selenium was conducted in ten confectionery products.

The correlation between the total content of selenium and its individual forms (-II), (IV),(VI) of different oxidation degree was also examined. It was shown that there was no correlation between the total selenium and inorganic forms of selenium. That means that speciation analysis is the only correct analysis of selenium content in foods.

K e y w o r d s: selenium, analysis, speciation, mineral confectionery products.

ANALIZA SPECJACYJNA NIEORGANICZNYCH FORM SELENU W WYROBACH CUKIERNICZYCH

Abstrakt

Jednym z mikroelementów, który w ostatnich latach wzbudza ogromne zainteresowanie ze względu na swoje biologiczne właściwości, jest selen. Stwierdzono, że w organizmie człowieka selen stymuluje układ immunologiczny oraz działa przeciwnowotworowo. Głów-

Agnieszka Gawłoska-Kamocka, Wyższa Szkoła Zawodowa w Łodzi

nym źródłem selenu jest żywność bogata w białko pochodzenia zwierzęcego i roślinnego oraz wysokobiałkowe produkty mleczne.

Celem pracy było poznanie specjacji selenu w próbkach wyrobów cukierniczych zmierzającej do określenia nieorganicznych form selenu w postaci anionów: selenkowych Se^{2-}, selenianowych (IV)SeO_3^{2-}, selenianowych (VI) SeO_4^{2-}.

Do oznaczeń końcowych zastosowano technikę generowania wodorków w połączeniu z metodą atomowej spektroskopii absorpcyjnej HG-AAS. Oznaczenie selenu opiera się na połączeniu ekstrakcji wodnej próbki z roztwarzaniem próbki stężonymi kwasami.

Oznaczenie specjacyjne selenu wykonano w 10 próbkach wyrobów cukierniczych.

Zbadano również korelację między zawartością selenu a poszczególnymi formami selenu na (-II), (IV),(VI) stopniu utlenienia. Wykazano brak korelacji między zawartością całkowitą selenu a nieorganicznymi formami selenu. Oznacza to, że analiza specjacyjna jest jedyną analizą zawartości selenu w żywności.

Słowa kluczowe: selen, analiza, specjacja, wyroby cukiernicze.

INTRODUCTION

Investigations on speciation of selenium most often concern samples of physiological liquids such as urine, blood and serum (PYRZYŃSKA 1998). At present speciation of this element is also examined in soils and in plants.

Speciation analysis of selenium deals mainly with two groups of relationships: volatile alkyl derivatives of selenium $(CH_3)_2Se$ and $(CH_3)_2Se^2$ and non-volatile selenium compounds including inorganic selenium oxoanions Se(IV) and Se(VI), as well as selenoamino acids, e.g. selenomethionine or selenocysteine (PYRZYŃSKA 1998, 2000). Volatile organic compounds of selenium are usually separated from the main components of a sample concentration on a solid sorbent such as glass wool or active carbon, packed in chromatographic columns or compressed in dishes with liquid nitrogen, a then thermally desorbed or extracted with organic solvents. PYRZYŃSKA (2000) established that speciation analysis of non-volatile compounds of selenium occurring in natural waters involved determination of the content of selenium in three samples following their appropriate preparation: 1. In a primary sample the content of Se(IV) is determined with oxidation methods suitable for this stage of analysis. 2. Following the mineralization of samples and oxidation of Se to organic forms (IV), the sum of Se(IV) + Se(-II) was determined. 3. In the mineralised sample Se(VI) was reduced to Se(IV) with an aid of hot concentrated HCl to determine the content of total selenium [the sum Se(IV) + Se(VI) + Se(-II)]. Concentrations Se(VI) and Se(-II) are defined by a difference between individual signs. High performance liquid chromatography (HPLC) is used in investigations of speciation of selenium. Other universally applied methods of detection, e.g. UV-VIS spectrophotometry or conductometry cannot be applied because of interference caused by typically large quantities of nitrates, sulphates or the phosphates as well as

many organic compounds. The recommended determination systems join separation techniques with detection methods specific for selenium, e.g. GF-AAS, ICP-AES, ICP-MS. Unlike wide-range investigations on total content of selenium, little information has been published on inorganic speciation and organic form of this chemical element. Studies on speciation of selenium in Poland have up to now been very scarce. The articles by ŚWIETLIK (1998), HULANICKI (1997) and PYRZYŃSKA (1995, 1998,1999, 2000) contain mainly theoretical considerations. DEJNEKA'S work (2000) is exceptional in that the author carried out speciation of selenium in cereals, herbs and nutrients for babies, which revealed insignificant content of Se(IV) and Se(VI) in the above products. Thus, it seems advisable to carry out more studies involving speciation analyses of selenium, an essential trace element, in organic samples, including foodstuffs.

The aim of this study was to run speciation analyses of selenium in confectionery products in order to determine inorganic forms of selenium such as Se^{2-} SeO_3^{2-} and SeO_4^{2-} anions.

MATERIAL AND METHODS

The technique of generating hydrides (HG-AAS) combined with the HG--AAS method can be applied for final determination of selenium in biological samples, e.g. foodstuffs or sewage (BENEMARIYA et al. 1993, CSER et al. 1996, DIAZ et al. 1996, 1997, GILECKI 1997, ASTRUC 1998, GAWŁOSKA and MASŁOWSKA 2000) as it enables researchers to eliminate the influence of the matrix as well as to obtain samples rich in the analyte.

The speciation analysis of selenium in samples of confectionery products, presented in this paper, was based on determination the content of selenium in three samples. The Se determination was preceded by an appropriate preparation of the samples including the following stages:

– in a primary sample selenium (IV), i.e. SeO_3^{2-}, was determined by separating it from the mixture via aqueous extraction in a Soxhlet apparatus;
– the primary sample was mineralised using concentrated nitric acid and Se was oxidised to organic forms (IV) in order to determine Se(IV) + Se(-II), i.e. the sum SeO_3^{2-} + Se^{2-};
– the sample was treated with hydrochloric acid (10% solution) in order to reduce SeO_4^{2-} to SeO_3^{2-} and to determine the total content of selenium as well as (SeO_4^{2-} + SeO_3^{2-} + Se^{2-});
– the content SeO_4^{2-} and Se^{2-} was established from the differences between the results obtained at the above determination stages.

The hydride generation method combined with the atomic absorption spectroscopy was used for the final determination of selenium forms. The determination were performed in 6 replications (n=6).

The limit of detection was at 0.01 μg kg^{-1}. The analyte was checked with the use of the certified reference material CRM 402, which showed the total content selenium of 6.70 ± 0.25 mg kg^{-1}. The total content of selenium in the mineralised reference material subjected to reduction with hydrochloric acid was 5.50 ± 0.15 mg kg^{-1}, which was lower than the total selenium content in reference material determined by DEJNEKA (2002).

RESULTS AND DISCUSION

The results of determinations of three different forms of selenium as well as total selenium concentration in several confectionary products can be found in Table 1.

Table 1

Results of determination of three of different forms of selenium and total selenium content in samples of confectionery products; n=6

Sample of product	Selenium content (μg kg^{-1})			
	Se^{2-} ± SD	SeO_3^{2-} ± SD	SeO_4^{2-} ± SD	Se total ± SD
Tiki-Tak chocolates	0.09±0.02	1.35±0.42	1.20±0.22	2.65±0.59
Malaga chocolates	0.05±0.01	2.05±0.24	1.42±0.38	3.53±0.46
Kasztanki chocolates	0.04±0.01	1.28±0.31	1.10±0.14	2.42±0.34
Prince-Polo wafers	0.03±0.01	2.30±0.47	1.63±0.87	4.00±0.55
Sesame biscuits	0.06±0.01	2.50±0.35	1.91±0.53	4.43±0.38
Salted breadsticks	0.03±0.01	1.94±0.93	1.83±0.75	3.82±0.74
Delicje biscuits	0.04±0.01	2.43±0.40	1.35±0.32	3.83±0.89
Alibi chocolate bar	0.07±0.01	1.88±0.95	1.50±0.54	3.47±0.55
Amaranth bar	0.03±0.02	1.38±0.47	1.30±0.23	2.71±0.63
Likwor rumowy chocolates	0.08±0.02	1.22±0.33	1.10±0.15	2.41±0.46

SD – standard deviation

The highest total selenium content was found in sesame biscuits (4.43 ± ± 0.38 μg kg^{-1}), which can be attributed to high selenium content in areas where sesame seeds are harvested. The data in Table 1 show that the remaining confectionery products are characterised by lower SeO_3^{2-} content, within the in range of 1.22 ± 0.33 to 2.50 ± 0.35 μg kg^{-1}. Comparatively rich in selenium were Prince-Polo wafers, salted breadsticks and Delicje biscuits. In general, the analysed sweets and biscuits were low in Se^{2-} (0.03 to 0,09 μg kg^{-1}), SeO_3^{2-} (1.22±0.33 to 2.43±0.40) and especially SeO_4^{2-} (1.10–1.91 μg kg^{-1}). The highest SeO_4^{2-} content was determined in sesame biscuits (1.91 μg kg^{-1}). Tiki-Tak chocolates possess the highest content of Se^{2-} ions (0.09 μg kg^{-1}). The highest content of SeO_3^{2-} was found in Delicje biscuits (2.43 μg kg^{-1}).

The results on determination of selenium ions in confectionary products obtained in the course of the present work cannot be compared to other data as the available references do not contain comparable works.

The correlation between the total content of selenium and its individual forms (-II), (IV),(VI) of different oxidation was also examined. However, no correlation between the total selenium and inorganic forms of selenium was established, which implies that speciation analysis is the only correct method for determination of selenium content in foods. This conclusion is in agreement with the opinion expressed by DEJNEKA (2002).

The experimental investigations proved that with the HG-AAS method it is possible to divide the mixture into three ion forms and simultaneously determined Se^{2-}, SeO_3^{2-} and SeO_4^{2-} in samples of confectionery products. The determination results suggest the content of selenium in confectionery products is low and it does not cover selenium daily demand. Therefore, it seems recommendable to enrich diet with selenium-high products or use dietary supplements, such as selenium yeast or pharmaceuticals including selenium preparations.

CONCLUSIONS

1. The confectionery products examined differ in proportions of selenium ion forms: Se^{2-}, SeO_3^{2-} and SeO_4^{2-}.

2. It was proven that there was no correlation between the total selenium and inorganic forms of selenium. That means that speciation analysis is the only correct analysis of selenium content in foods.

3. In the analysed food products selenium occurred as SeO_3^{2-} (22%), SeO_4^{2-} (12%) and Se^{2-} (only 2%).

REFERENCES

ASTRUC M. 1998. *Recent advances in speciation methods: arsenic and selenium*. J. Trace Elem. Exper. Med., 4: 333.

BENEMARIYA H., ROBBERECHT H., DEELSTRA H. 1993. *Daily dietary intake of copper, zinc and selenium by different population groups in Burundi.* Africa. Sci. Total Environ., 136: 49-76.

CSER M.A., SZIKKLAI I., LASZLO I., MENZEL H., LOMBECK I. 1996. *Selenium and glutathione peroxidase activity in Hungarian children.* J. Trace Elem. Med. Biol., 10: 167-173.

DEJNEKA W. 2002. *Analiza specjacyjna nieorganicznych form selenu w środkach spożywczych.* Bromat. Chem. Toksykol., 35 (3).

DIAZ J. P. , NAVARRO M., LOPEZ H., LOPEZ M.C. 1996. *Determination of selenium in cereals, legumes and dry fruits from southeastern Spain for calculation of daily dietary intake*. Sci. Total Environ., 184: 183-189.

Diaz J.P., Navarro M., Lopez H., Lopez M.C. 1997. *Determination of selenium levels in dairy products and drinks by hydride generation atomic absorption spectrometry: correlation with daily dietary intake.* Food Addit. Contam., 14: 109-114.

Gawłoska A., Masłowska J. 2000. *Specjacja jonów pierwiastków chemicznych w próbkach naturalnych.* Zesz. Nauk. Chemia Spoż. i Biotech., (62): 39-51.

Gilecki J. 1997. *Rola selenu w diecie.* Gazeta Farmaceutyczna, 9: 46-52.

Hulanicki A. 1997. *Analiza specjacyjna próbek biologicznych.* Wiad. Chem., (3/4): 189-203.

Pyrzyńska K. 1995. *Recent development in speciation analysis of selenium.* Chem. Anal., 40:677-685.

Pyrzyńska K. 1998. *Speciation of selenium compounds*. Anal. Sci., (14): 479-483.

Pyrzyńska K. 1998. *Preconcentration and separation of inorganic selenium species on activated alumina.* Anal. Chim. Acta., 36: 141-146.

Pyrzyńska K., Bulska E., Gucer S., Hulanicki A. 1999. *Aluminium speciation in natural waters.* Chem. Anal., 44: 1-14.

Pyrzyńska K. 2000. *Związki selenu w środowisku naturalnym.* Wiad. Chem., (1/2): 139-150.

Świetlik R. 1998. *Znaczenie pojęcia specjacja w chemii środowiska*. Wiad. Chem., (7/8): 587-595.

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INFLUENCE OF DEUTERIUM CONTENT OF WATER ON THE GROWTH OF PLANT EMBRYO AND FREE RADICAL PRODUCING PROCESSES

Sándor A. Kiss[1], Ilona Szollosi Varga[2]

[1]Hungarian Magnesium Society, Hungary,
[2]University of Szeged, Department of Genetics and Molecular Biology, Free Radical Res. Lab., Szeged

Abstract

In the course of germinating plant seeds - if the deuterium (D) concentration in the germinating medium (water) is lower or higher than in natural water (150 ppm) – the length and mass of seedlings (coleoptil, epi-cotyl, root) proportionally decrease. The cause of the decrease owing to the changed D concentration is explained partly on energetic-partly on free radical basis and proved by measuring. In waters with lower or higher than na-tural D concentration the energy supply decreases while the free radical formation in-creases. Free radicals exert their influence by inactivating the bios substances (e.g. auxin) through oxidation. This seems to explain the mechanism of the ensuing changes.

INTRODUCTION

Earlier we gave an account on the effect exercised by the medium of changed deuterium content (D20, heavy water) on the division of cells and growth of seedlings (Kiss et al. 1996, 1997, 1998, Kiss 2003).

Deuterium is a nonradiating mass number 2 isotope of hydrogen. The 100% mass difference appears in chemical reactions and biological effects of the two hydrogen atoms (H and D), e.g. *Aspergillus niger* raised in "light water" is black, while moulds grown in heavy water are alabaster white.

Sándor A. Kiss, tel.: +36-62-432-298; fax: +36-62-432-298, H-6726 Szeged, Fofasor 73/A/2. Hungary, e-mail address: [1]zgalbacs@chem.u-szeged.hu [2]szvarga@bio.u-szeged.hu

The rate of reaction of deuterated compounds is lower, since compounds of greater mass have a higher requirement of activation energy. According to the investigations of SIMMON and PALM (1966) the activation energy of the deuterated succinate dehydrogenase is 13% higher compared to the enzyme which contains natural hydrogen. Plants grown in culture media of increasing D content are consequently depressed in growth e.g. germination of maize and growth of coleoptiles (SACCHI and COUECHI, 1992).

SOMLYAI et al. (1993) found that under the influence of a decreasing D concentration the division of cells in various animal tissue cultures started with a 5–10 hour delay. The inhibitory effect can be experienced in tumorous animal tissues too. This was the first case when a D content lower than the natural deuterium concentration (150 ppm) was found to inhibit the propagation of cells.

Subsequently we also began to study the influence of a slight (50–130 ppm) change in the D content of a culture solution on the development of plants, using various seeds for that purpose. Our work differed essentially from earlier investigations (UPHANS et al 1975), where the effect of 30–70% D20 contents on cell propagation was studied, as we kept close to the natural (150 ppm) concentration.

We observed that when the D content in a germinating medium was either lower of higher than the deuterium concentration in natural waters, the growth of mono- and dicotyledonous seedlings was inhibited in proportion to the difference (Fig. l., KISS et al. 1998).

The present paper gives an account on the causes and possible mechanism of the inhibitory effect.

MATERIALS AND METHOD

In our germination experiments we used distilled water of 20-300 ppm D content that we produced by mixing "light water" (20 ppm) with heavy water of 99.689% D content at an appropriate ratio, and storing it in dark glass containers. The contamination was controlled by atomic absorption spectrophotometry.

GERMINATION

Maize and pea seeds of 95% germinability were germinated in high covered plastic Petri dishes on filter paper wetted with identical quantities of water. Each treatment consisted of 5 replications, with 20 seeds per dish.

DETERMINATIONS

We measured the length and mass of epicotyl and coleoptile as well as the mass of root during germination (after 6 days). To determine the organic matter (energy) utilization and the respiratory loss we measured dry weight of maize coleoptiles and roots, and the decrease in dry matter content of seed during germination. The samples were dried at 95°C to standard weight, and then measured.

The total activity of the "bios material" was determined by yeast test method. (Bios material = growth stimulating hormones such as auxin, gibberellin, etc). The bios materials exercise activity stimulated the propagation of yeast cells → the decomposition of sugar in the culture medium. The CO_2 produced leaves the culture medium resulting in a decrease of mass.

Fixed volumes of the supernatant obtained after centrifuging maize coleoptile homogenate were added to the yeast culture. The release of CO_2 was possible through a plug (0.1 mm) closing the vessels. We kept the cultures in a thermostat at 30°C and recorded every day the total weight of vessels and cultures; from the loss of weight we assessed the activity of the bios material.

To characterize free radicals of oxygen we determined the lipid peroxidation (LP), the hydroxyl radical ($OH\cdot$), the superoxide dismutase (SOD), catalase (Cat) enzyme activities as well as the total antioxidant capacity (FRAP).

PREPARATION OF THE SAMPLE

A predetermined quantity of shoots was homogenized in a Potter homogenizer in cold phosphate buffer of 1:9 (w/w) and 7.4 pH. Aliquots of the supernatant obtained after centrifuging (2000 rpm) were used to determine various parameters.

The superoxide dismutase [(SOD, EC 1.15.1.1) activity was determined by the method of Misra and Fridovich (1972) modified by Matkovics et al. (1977).

The rate of lipid peroxidation (LP) was measured by thiobarbituric acid colour reaction after Placer (Placer et al. 1966).

For the determination of the hydroxyl radical ($OH\cdot$) we employed Cheeseman's method (Cheeseman et al., 1988).

Catalase (Cat: EC 1.11.1.6) activity was measured after Beers and Sizer (1952), and the activity was given in Bergmeyer unit (BU), where 1 BU = 1000 mg H_2O_2 decomposed in 1 minute.

The protein concentration was determined as described by Lowry et al. (1951).

The total antioxidant activity was determined by the method of BENZIE and STRAIN (1996).

RESULTS

When germinating maize, rice, gourd and wheat seeds in media of 25, 150, 300 ppm deuterium concentration we found that the length and mass of shoots (coleoptile, epicotyl) were the largest at a D concentration of 150 ppm, i.e. corresponding to that in natural waters. Lower and higher deuterium concentrations had an inhibitory effect on growth (Fig. l.)

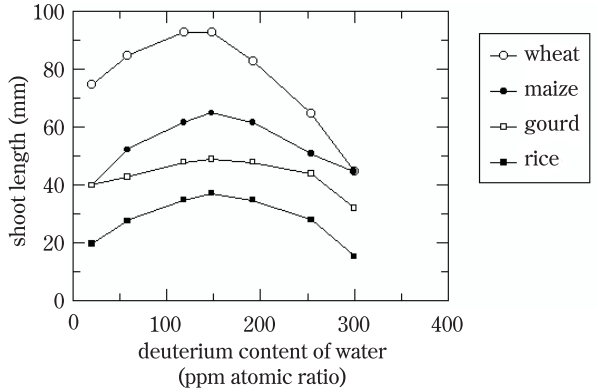

Fig. 1. Shoot length of seedlings as a function of the D content of water (KISS 1998) (the data represent mean values of several species of wheat, maize, gourd and rice seedlings)

The decrease in the mass of seeds during germination is the largest in the medium containing 150 rpm D (Table 1), while in the case of 25 and 300 ppm it is lower. The differences for the three maize hybrids, though considerable, were consistent.

The increase in the total shoot + root mass of seedlings in the first 10 days is, according to WILLIAMS-PETERSON (1973), proportional to the α-amilase activity of seeds, that is to the composition of yeast. According to our investigations, the increase in the mass and α-amilase activity was the highest in the medium of 150 rpm concentration. This is in harmony with a decrease in the mass of germinated seeds. In the course of germinating, comparing the decrease in the mass of seeds with that in the total dry mass of shoots and roots we found the latter to be lower in every case (Table 2). The difference is the so called respiratory loss, which has been spent on the

Table 1

Mass decrease of seeds of *Zea mays* L. varieties during germination as a function of the deuterium con-centration in a medium (water). The data are given as percentages of the original mass and in comparison to 150 ppm, means from 5x20 seeds

Species/Mass decrease (%)	Concentration of deuterium (ppm)		
	25	150	300
Ida			
absolute decrease, %	10.5	11.6	10.0
decrease compared to D-150, %	90.5	100.0	86.2
Raissa			
absolute decrease, %	22.4	24.1	22.1
decrease compared to D-150, %	92.9	100.0	91.7
Randa			
absolute decrease, %	19.8	21.4	19.6
decrease compared to D-15.0, %	92.5	100.0	91.5
Mean decrease, %	17.6	19.0	17.2

Table 2

Correlation between the coleoptile + root mass of *Zea mays* L. var. Ida germinated in media of various deuterium contents and the weight loss of seeds (mg/weight of 20 seeds), as percentage value of the respiratory (energy) loss, average of three varieties and in comparison to 150 ppm D content

Quantity (mg) and loss of quantity	Concentration of deuterium (ppm)		
	25	150	300
Coleoptil	184	210	182
%/D-150	88.0	100.0	87.1
Root	604	706	640
%/D-150	85.4	100.0	90.6
Coleoptil+root	788	916	822
%/D-150	86.0	100.0	90.6
Ratio of coleoptil/root	3.25	3.26	3.50
Mass decrease of seeds	1206	1350	1156
Respiratory loss, mg	414	434	382
Respiratory loss, %	34.3	32.1	33.0
%/D-150	106.8	100.0	102.8

Respiratory loss = mass decrease of seed - (coleoptile + root) mass

energy transformation. The lowest respiratory loss was measured at a 150 ppm D concentration. From this we have drawn the conclusion that the energy supply (transformation) varies with the deuterium concentration.

The assumption that owing to the changed D concentration the energy supply decreases in the course of germination seems to be supported by the fact that ATP (3 mg 100 ml^{-1}) added to the germinating medium influences the growth of the epicotyl as a function of the D content (Table 3). The growth of the epicotyl was proportionally higher in a medium of either decreased (25 ppm) or increased (300 ppm) D concentration than in the case of a natural (150 ppm) level of D content. This proves that with a deviation from 150 rpm the energy (ATP) production decreases and so does the transfer entropy (HASTEN et al. 1974), and its substitution resulted in a spectacular growth.

Table 3

Changes in the shoot (epicotyl) length, oxygen free radical- and antioxidant activity of germinating pea (*Pisum sativum* var. Hanka) as a function of the deuterium and ATP content of a medium (average of 5 replications)

Parameters	Deuterium (ppm)						Deuterium (ppm)+ ATP (mg ml^{-1})					
	28		150		300		28 + 30		150 + 30		300 + 30	
		%		%		%		%		%		%
Epicotyl, mm	12.3	67	18.3	100	14.0	76	19	+54	24.0	+31	22.1	+58
LP, nM mg^{-1}	18.1	111	16.2	100	17.9	110	18.2	0	17.2	+10	19.2	+7
$\cdot$OH nM mg^{-1}	23.2	123	18.9	100	21.2	112	22.1	-5	20.4	+8	21.8	+3
SOD, E mg^{-1}	14.8	98	15.1	100	15.0	99	15.2	+2	14.9	-2	16.0	+6
Cat, E $mg \cdot 10^{-4}$	0.8	108	0.7	100	0.7	100	1.28	+66	1.30	+83	1.20	+74

Effects of ATP in % = in proportion to the ATP-free, deuterated solution

The determination of the so called bios substances (auxin, gibberellin, etc.) in maize coleoptiles shows that their activity is a function of the deuterium concentration in a germinating medium (Table 4). The data are fully consistent with the growth of coleoptiles and the change of the α-amilase activity.

Values for the concentration of oxygen free radicals and the activity of antioxidants eliminating them are seen in Table 5. Lower hydroxyl radical and lipid peroxidation (LP) values as well as higher antioxidant enzyme (SOD, Cat) and total antioxidant (FRAP) activities are obtained in the case of a concentration corresponding to the deuterium concentration of natural waters.

Table 4

Comparison of *Zea mays* L. var. Ida coleoptiles grown in media of different deuterium concentrations for bios activity, on the basis of mass decrease in sugar-yeast solution, versus the mass decrease of seed during germination and the mass of coleoptil (mg n^{-1})

Parameters	Concentration of deuterium (ppm)		
	25	150	300
Quantity of colepotil, mg n^{-1}	56.58	126.53	77.36
%/D-150	44.7	100.0	61.1
Mass decrease, mg n^{-1}	42.48	46.50	41.04
%/D-150	89.5	100.0	88.2
Mass decrease of sugar-solution mg n^{-1} coleoptil			
During 12 hrs	1.72	2.00	1.55
+ 13 hrs	3.28	4.11	4.04
Total: during 25 hrs	5.00	6.11	5.59
%/D-150	81.8	100.0	90.7

5 parallel, mean for 20-20 seeds

Table 5

Oxygen free radical and antioxidant enzyme ac-tivities of coleoptiles of maize (var. lda) germinated in media of various deuterium concentrations

Parameters	Concentration of deuterium (ppm)		
	25	150	300
Protein, mg g^{-1}	22.70	23.25	22.50
%/D-150	97.6	100.0	96.7
LP, nM MDA mg^{-1} prot.	4.06	3.75	4.15
%/D-150	108.3	100.0	110.6
·OH, nM mg^{-1} prot.	43.6	34.5	39.3
%/D-150	126.4	100.0	113.9
Cat, E mg^{-1} prot. $\cdot 10^{-4}$	1.059	0.972	0.969
%/D-150	108.9	100.0	99.7
SOD, E mg^{-1} prot.	14.3	15.6	13.4
%/D-150	91.6	100.0	85.9
FRAP values, µM L^{-1}	252	276	239
%/D-150	91.3	100.0	86.6

DISCUSSION

According to SIMMONDS and DUMBROFF (1974) germination of seeds starts only at an ATP level (EC = energy charge) characteristic of a given species. In our experiments, the EC level in seeds was raised by an ATP treatment, germination started earlier and was more intensive than without such a treatment (KISS 1983). With D concentrations lower or higher than 150 ppm the effect of ATP was essentially more profound than in the case of 150 ppm.

HAILSTONES and SMITH (1988) pointed out that while the number of oxygen free radicals increase, the germinabi1ity of seeds decreased, e.g. in soya bean a 40% increase in the H_2O_2 inhibited germination. LEE (1972) attributed this phenomenon to a decreasing effect of peroxidation on the auxin level and consequently on growth. Similar conclusions were drawn by GASPAR et al. (1985): increasing peroxidation oxidizes auxin whereby it becomes inactive.

Increasing lipid peroxidation (LP), which is related to the damaging effect of oxygen radicals, is due to the fact that in a medium of low D content the SOD activity decreases, and so does the possibility of eliminating radicals (AUROMA et al. 1989). According to our investigations, with ATP added the quantity of oxygen radicals did not increase, neither did that of LP. The oxidative circumstances did not diminish the auxin level, therefore the growth of seedlings was intensive.

In our experiments the concentration of the hydroxyl radical and the rate of LP increased in a medium lower in D. The cause is that a lower D_20 concentration increases the disorder of the structures. Increased disorder means increased entropy (S) too, which tends to make the reactions spontaneous. Thus, the hydroxyl radical formation and the process of LP take place more easily and rapidly. A similar result was attained by HANSTEN et al. (1974), who found oxidation of the NADH-Coenzyme Q increased with a decrease in the D content of a medium.

Besides, with a decrease of the D concentration, the ATP-ase activity in mitochondria and NADH formation increase (GOMEZ-PUJOU et al. 1978). The increasing NADH concentration promotes formation of free radicals (PUNTARULO et al. 1988), which results in an increase of LP.

Higher production of free radicals in a medium of lower D content raises the activity of Cat, which takes part in their elimination. Supplementary ATP addition contributes by 60–80% higher Cat activity, probably by increasing the de novo synthesis. With increasing deuterium concentration oxygen uptake by plants decreases, and so does the ATP formation (SACCHI, COUECHI 1992). As a joint effect of energy decrease and free radical formation increase, the growth of seedlings under higher D content in a medium is inhibited. D_2O has a selective inhibitory effect on the mitochondrial energy transfer (MARGOLIS et al. 1966).

CONCLUSION

Germination of plant seeds and growth of shoots and roots depend on the deuterium content in a germinating medium. If the D content of a medium is lower or higher than the 150 ppm D concentration in natural waters, the growth or seedlings will be inhibited.

The growth inhibition or seedlings is partly caused by a decrease in energy or its transfer, in part due to the inactivation or auxin through a raised level or oxygen free radicals. The latter conclusion is supported by our "bios material" measurments.

If the energy level is raised by adding ATP, the growth inhibition caused by a change in the D concentration will cease. We can declare that the division of cells and the growth of plants is optimum when the deuterium content is around 150 ppm, a level developed in the course of evolution.

REFERENCES

AUROMA OI., HALLIWELL B., GAJEWSKI E., DIZDAROGLU M. 1989. *Damage to the bases in DNA induced by H_2O_2 and ferric ion chelates.* J. Biol. Chem., 264: 20509-20512.

BEERS RF., SIZER IW. 1952. *Spectrophotometric method for measuring the breakdown of hydrogen peroxyde by catalase.* J. Biol. Chem., 195: 133-140.

BENZIE IIF., STRAIN JJ. 1996. *The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay.* Anal. Biochem., 239: 70-76.

GASPAR T., PANEL C., CASTILLO FJ., GREPPIN H. 1985. *A two-step control of basic and acidic peroxidases and significance for growth and development.* Physiol. Plant., 64: 418-423.

GOMEZ-PUJOU TT., GOMEZ-PUJOU A., CERBON J. 1978. *Increased conformational stability of mitochondrial soluble ATP-ase (F1) by substitution of H_2O for D_2O.* Archiv. Biochem. Biophys. 187: 72-77.

HAILSTONES MD., SMITH MT. 1988. *Lipd peroxidation in relation to declining vigour in seeds of soya and cabbage.* J. Plant Physiol., 133: 452-456.

HANSTEN W.G., DAVIS K.A., HATEFI Y. 1974. *Solvent isotope effects on the structure and function of mitochondrial membranes in aqueous media*. Archiv. Biochem. Biophys., 163: 482-490.

KISS AS. 2003. *Deutérium az új mikroelem és annak biokémiai hatásai.* Magyar Kémikusok Lapja, 58: 41-45 (in Hungarian).

KISS AS. 1983. *Magnéziumtrágyázás, magnézium a biológiában.* Mezogazdasági Kiadó, Budapest, 110-115 (in Hungarian).

KISS AS., GALBÁCS Z., GALBÁCS G. 1997. *Magnesium ions hinder the growth on coleoptyl in deuterium depleted water.* In: *Current status and new developments.* THEOPHANIDES T., ANASTASSOPOULOU J. Eds. Kluver Acad. Publ., 77-80 pp.

KISS AS., GALBÁCS Z., KAMASZ-SÁRI V., GALBÁCS G. 1996. *Impact of the deuterium concentration of water and magnesium salts on the germination seeds.* Magnes. Res., 9: 226-227.

LEE TT. 1972. *Interaction of cytokinin, auxin, and gibberellin on peroxydase isoenzymes in tobacco tissue cultured in vitro.* Canad. J. Botany, 50: 2471-2477.

LOWRY OH., ROSEBROUGH NJ,. FARR AC., RANDALL RJ. 1951. *Protein measurement with Folin phenol reagent.* J Biol. Chem., 193: 265-275.

MARGOLIS SA., BAUM H., LENAZ G. 1966. *Observations on the effect of D2O on energy linked reactions of the mitochondrion.* Biochem. Biophys. Res. Commun., 25: 133-141.

MATKOVICS B., NOVÁK R., HOANG DUC HAHN, SZABÓ L., VARGA SI. 1977. *A comparative study of some more important experimenta animal peroxide metabolism enzymes.* Comp. Biochem. Physiol., 56B: 31-37.

MISRA HP., FRIDOVICH I. 1972. *The role of superoxide anion in the autoxidaton of Epinephrine and simple assay for superoxide dismutase.* J Biol. Chem., 247: 3170-3176.

PLACER ZA., CUSHMANM L., JOHNSON BC. 1966. *Estimation of product of lipid peroxidation (malonyl dialdehyde) in biochemical system.* Anal. Biochem., 16: 359-364.

PUNTARULO S., SANCHEZ RA., BOVERS A. 1988. *H_2O_2 metabolism in soybean embryonic axes at the onset of germination.* Plant Physiol., 86: 626-630.

SACCHI GA., COUECHI M. 1992. *Effect of D_2O on growth, proton extrusion, potassium influx and in vitro plasma membrane activities in maize root segments.* Plant Physiol., 100: 192-196.

SIMMON H., PALM D. 1966. *Isotope effects in organic chemistry and biochemistry.* Angewandte Chemie (Int. ed. in English), 5: 920-933.

SIMMONDS JA., DUMBROFF EB. 1974. *High energy charge as requirement for axis elongation in response to GA and kinetin during stratification of Acer saccharum seeds.* Plant Physiol., 53: 91-95.

SOMLYAI G., JANCSÓ G., JÁKLI G., VASS K., BARNA B., LAKICS V, GAÁL T. 1993. *Natural occuring deuterium is essential for normal growth rate of cells.* FEBS Lett., 317: 1-4.

SZALAY I., FRENYÓ V. 1962. *Növényélettani Kisérletek.* Tankönyvkiadó Budapest, 356-357 (in Hungarian).

UPHANS RA., BLAKE MI., KATZ JJ. 1975. *Deutrium isotope effects in Nicotiana tabacum.* Canad. J. Botany, 53: 2128-2131.

WILLIAMS JF., PETERSON ML.. 1973. *Relations between alfa-amylase activity and growth of rice seedlings.* Crop Sci., 51: 612-615.

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EFFECT OF A LAND RECLAMATION SYSTEM ON THE VOLUME AND SEASONALITY OF NITRATE RUNOFF FROM CROPLANDS

Józef Koc, Kamil Solarski, Andrzej Rochwerger

Katedra Melioracji i Kształtowania Środowiska
Uniwersytet Warmińsko-Mazurski w Olsztynie

Abstract

The paper contains the results of eight-year-long studies on the runoff of nitrates from heavy soils used as croplands. The runoffs of nitrates from a drainage catchment and a catchment drained with ditches were compared. The drainage system was found to carry away twice as much water, with a five-fold higher concentration of nitrates and 20--fold higher load of nitrates, than the system of ditches. High runoff of nitrates (22 kg ha^{-1} annually) from the soils drained by drains was distributed quite evenly throughout the year, with maximum peaks in March and June. Nitrate runoff through the system of ditches was low (1.15 kg ha^{-1} annually), reaching maximum peaks in March and April (62% of the load), but disappearing in the summer.

Key words: nitrates, runoff from soils, draining system.

WPŁYW SYSTEMU MELIORACYJNEGO NA WIELKOŚĆ I SEZONOWOŚĆ ODPŁYWU AZOTANÓW Z GLEB UPRAWNYCH

Abstrakt

W pracy przedstawiono wyniki 8-letnich badań odpływu azotanów z gleb ciężkich użytkowanych ornie. Porównywano odpływ azotanów ze zlewni drenarskiej i zlewni odwadnianej rowami. Stwierdzono, że system drenarski odprowadza 2-krotnie więcej wody o 5-krotnie wyższym stężeniu azotanów i 20-krotnie wyższym ich ładunku niż system rowów. Z gleb odwadnianych drenami wysoki odpływ azotanów (22 kg rocznie z 1 ha) rozkładał się równomiernie w ciągu roku, z maksimum w marcu i czerwcu. Odpływ azotanów systemem rowów był niewielki (1,15 kg rocznie z 1 ha), z maksimum w marcu i kwietniu (62% ładunku) i zanikiem odpływu latem.

Słowa kluczowe: azotany, odpływ z gleb, system odwodnienia.

prof. dr hab. Józef Koc, pl. Łódzki 2, 10-727 Olsztyn, e-mail: jozef.koc@uwm.edu.pl

INTRODUCTION

The natural circulation of water and chemical components has been seriously disturbed in the past century due to the rapid human population growth, development of civilisation and, mainly, the growth of industries, civil engineering, transportation, and intensive agricultural practices including widespread application of mineral fertilizers, chemical pesticides and herbicides, large densities of farm animals, and errors in agronomic techniques. All those factors have lead to excessive accumulation of noxious substances in soil. Land reclamation carried out for the last 150 years, with a preference for water draining systems, has accelerated such unwanted results.

Contemporary agriculture has disrupted the natural, harmonious and relatively closed matter cycling in ecosystems by introducing additional amounts and new substances (fertilizers, plant protection chemicals), as well as by using techniques to loosen soil, which accelerate water circulation. Nitrogen is among the elements whose natural circulation has been subjected to biggest modifications, as it is one of the essential constituents in agricultural production and is sensitive to habitat-related conditions. High productivity of nitrogen favours its application at rates exceeding the optimum nutritional requirements of crops, as a result of which it is not used up completely (Fotyma 1996, Fotyma 1997, Sapek 1996). This means that one of the early signs of environmental contamination caused by agriculture is the occurrence of large, frequently dangerous amounts of nitrates in ground and surface waters in rural areas (Fotyma 1996).

Often there are differences between the volume of nitrogen required by crops at a given time and the actual amount of available nitrogen forms present in soil. Because of their good availability to plants, nitrates are willingly used as N fertilizers, especially when the release nitrogen from soil resources and organic fertilizers is slow. Nitrates which have not been taken up by plants undergo other types of sorption and can easily transfer in soil. In our climate, water in soils travels down the profile for most of the year (except summer) and through a draining system reaches open water bodies. Nitrates are unwanted in water reservoirs due to their contribution to eutrophization and secondary contamination as well as the fact that nitrates and nitrate derived metabolites are harmful to aquatic organisms.

For many years we have been aware of the relationship between the concentration of nitrates in waters and intensive agricultural production with high levels of mineral fertilization or large animal farms with inadequate storage of manure (Koc et al. 1996). The code of good agricultural practice is expected to prevent this (Fotyma 1996), but will it suffice? The runoff of nitrates to surface waters should be related to systems of land reclamation, which were created with an aim of facilitating the fastest possible draining of water, which in turn is responsible for quicker warming of soils and initi-

ation of biological process as well as the runoff of biogens (Koc, Szymczyk, 2003, Lipiński 2003, Miller et al. 2001). The role of land reclamation systems in the removal of biogenic substances to waters is still debatable. Stabilization of groundwater on a level that is optimal for plants and earlier warming of soils in spring are favourable for the development of the root system and the uptake of nutrients from deeper layers of soil. Lack of water draining not only limits the runoff of biogenic compounds from soil but it is also responsible for oxygen deficit and favours denitrification of nitrates. The problem lies in the regulation of water draining and water retention within agricultural ecosystems during summer water deficits. This would enable biogens to return to the agricultural circulation. Implementation of these concepts in practice and their efficiency depend on the recognition of runoff of biogens through land reclamation systems.

The aim of this study has been to determine the dynamics of nitrates runoff from agricultural catchments and to recognize the effect of water draining systems on the dynamics of this process.

METHODS

The study was carried out in the Olsztyn Lake District in 1992–1999. The district lies in the postglacial area, with typically diversified land relief and varied geomorphologic and soil structure. Hills, often with steep slopes and largely diversified soils, are a characteristic physiographic factor in the area. Depending on the origin (boulder or fluvioglacial sediments) soil differs in granulometric composition and the resulting physicochemical properties or agricultural usability. Two catchments, both under agricultural use, were selected for the tests. The catchments where the observations and tests were conducted lie directly behind the fourth row of terminal moraines, which stretches from Morąg via Gietrzwałd, Stawiguda (10 km south of Olsztyn) and Barczewo towards Mikołajki (Solarski et al. 1996, Uggla 1956).

Catchment 1. The area of 72.8 ha drained with a ditch. The land configuration – hilly, 69% of the area covered with slopes at an angle up to 6%; 21% of the area has slopes from 6 to 12%. Slopes from 12 to 18% cover about 9% of the total area and 1% of the catchment lies on slopes reaching over 18%. Agricultural use. Typical brown soils are dominant (approximately 40%). They are mainly compact soils, formed from clay and silty clay, difficult to cultivate and not easily aerated. Pseudopodzolic soils (silty), which make up 37% of the area, are mainly composed of medium and light clays and weak loamy sands on clay or silty clay. The soils found in land depressions, which constitute 10% of the area, are used as grassland and belong to muck soils. Forests occupy 8% of the catchment and lie on light loamy sands. Arable land covers 75% of the area; other types of land use make up 7% of the area.

Catchment 2. The area of 8 ha, drained by an unsystematic system of drains. It is basin-shaped, with rather long slopes, inclined towards the line of water runoff. The fall of the ground of less than 6% angle occurs on 18% of the catchment's area; 6 to 12% – on 55% of the area; 12–18% on 20% of the area, and >18% on 7% of the area. The soils in the catchment belong to medium soils – strong loamy sands (42%) and light clay and loamy sands (43%), light sandy soils (15%).

Crop rotation systems tested in both catchments were identical, involving the following crops (in brackets – the average yield in t/ha): spring barley (2.6), winter wheat (4.9), winter triticale (3.7), winter rye (3.6), oats (2.5), mixed cereals (2.5), winter oilseed rape (3.3), lupine (2.5), horse bean (2.8), maize (3.0) and potatoes (25.0). Depending on the type of soil texture and fertility, type of soil management, water conditions and agricultural use, the following fertilization was applied to each crop (plants): from 120 to 215 kg NPK ha^{-1} on average, with the lowest fertilization rate, from 40 to 60 kg NPK ha^{-1} under winter rye and spring barley, to around 350 kg NPK ha^{-1}, supplied to winter oilseed rape (SOLARSKI 2002).

In both catchments, measurements of the water runoff were made every ten days; water samples for analyses were collected once a month. The water samples were analysed to determine nitrates by colorimetric method, using disulfophenol acid.

RESULTS

Transformation of nitrogen compounds in soil, their uptake by plants and transfer into deeper layers of the soil profile, they all depend on the current meteorological conditions. The course of the weather conditions was analysed in hydrological years, i.e. from 1st November to 31st October. The meteorological conditions during the years under study (1998–1999) were highly varied, which is also typical of the area analysed, situated in a transitional zone of the Atlantic and Continental climates (Tab. 1).

The average annual precipitation for one hundred years was 605 mm. The period under study was similar to the long-term data in terms of the total rainfall, but slightly warmer, with the mean annual temperature of 7.4°C, versus the mean annual temperature for one hundred years of 6.8°C. Some differences between the years occurred, from an extremely dry and cold year 1996 to warm and humid year 1995. Only one year was close to the long-term average for precipitation (1992), whereas three years were closer to a humid year (1993, 1994 and 1999), and two were drier (1997 and 1998). In respect of temperatures, only one year could be considered as a warm one (1995) and one – to be cold (1996). The other years were slightly warmer than the long-term average.

Table 1

Meteorological conditions during the years under study (data supplied by the meteorological station in Olsztyn)

Hydrological year	Parameter	Year 11-10	Winter 11-04	Summer 05-10	Spring 03-04	08-09	10-11
1992	*a*	628.0	288.0	340.0	115.0	188.0	108.0
	b	8.0	1.7	14.4	4.3	16.1	4.2
1993	*a*	679.0	252.0	427.0	52.0	146.0	36.0
	b	7.4	1.7	13.1	4.3	13.0	2.2
1994	*a*	690.0	378.0	312.0	175.0	97.0	155.0
	b	7.4	0.9	13.9	5.2	15.8	4.9
1995	*a*	701.0	321.0	380.0	102.0	152.0	58.0
	b	8.4	2.3	14.4	4.7	15.0	5.2
1996	*a*	415.0	125.0	290.0	24.0	71.0	84.0
	b	5.7	-2.1	13.5	2.6	14.1	5.5
1997	*a*	556.1	136.2	419.9	59.5	44.0	95.4
	b	7.1	0.2	13.9	3.0	15.7	4.2
1998	*a*	550.0	196.7	353.3	80.0	101.4	91.9
	b	7.8	2.2	13.5	4.7	13.9	1.9
1999	*a*	671.3	268.5	402.8	155.0	87.2	82.3
	b	7.5	0.5	14.5	6.1	15.8	5.0
Mean from 1992-1999	*a*	611.3	245.7	365.6	95.3	110.8	88.8
	b	7.4	0.9	13.9	4.4	14.9	4.1
Long-term mean 1881-1995	*a*	605.2	225.9	379.3	70.7	129.0	90.7
	b	6.8	0.1	13.4	3.2	14.4	3.9

a – rainfall in (mm)
b – air temperature in (°C)

According to the data presented in Table 1, the years 1992–1994 and 1997–1998 were warm with the total precipitation close to the normal level, and can therefore be considered as favourable to denitrification. The year 1996 was the least favourable to denitrification due to lower temperatures, whereas the year 1995 was wet, which reduced the availability of oxygen in soil. Transfer of nitrates in soil should be encouraged mainly by large amounts of rainfall at normal or depressed evapotranspiration, which depends on the amount of energy in the environment, as indicated by temperature. Transfer of substances in soil, including nitrates, is not favoured by a period of dry weather prior to the period under study. Consequently, the largest runoff should be expected in 1994 (a wet year after wet six months including summer).

Denitrification and nitrate runoff from soil are influenced by the course of weather conditions in each shorter period of time. Thus, the process of nitrate formation and runoff was favoured by warm and wet winter half-year in 1991/92, 1992/93,1993/94,1994/95 and 1998/99, whereas cold and dry winter such as in 1995/96, 1996/97 and 1997/98 was unfavourable to these processes.

In the six months of wintertime, the most important for nitrate runoff is the early spring (March-April), when snow cover melts and waters retained in winter begin to flow away. As regards this time of year, the seasons of 1993, 1995, 1997 and 1999 were favourable to nitrate runoff, in contrast to 1992 and 1998.

In summer half-year, especially unfavourable conditions migration of nitrates appear when evapotranspiration is high and drought conditions prevail while plants uptake nitrates – such conditions prevail in August and September. October and November, on the other hand, are favourable to the creation and transportation of nitrates, because soil is still warm and the uptake of nitrates is limited to winter crops and perennial plants. Such favourable conditions for nitrate runoff from soils occurred in 1994.

Based on the eight-year-long study, the test objects were characterised in terms of their hydrological properties and nitrate runoff (Figures 1–3). Significant differences in the levels of the characteristics investigated were discovered. In the catchment with a drainage system the runoff of water was twice as high as in the catchment drained by a system of ditches. Furthermore, the concentration of nitrates was 15-fold higher and the load of nitrates was 20-fold larger. It is typical for water discharge to grow from the beginning of a hydrological year (November) until March in an area drained by ditches or until April if drainage systems are used, which is when water flow reaches its maximum to decline afterwards. In the case of ditches, the March maximum is 50-fold as high as the minimum water discharge observed in August. In the drainage catchment, the water discharge in summer was significantly higher than in the catchment with ditches, reaching 45% of the April maximum. Draining with ditches causes large differences in seasonal water discharges, much larger than drainage systems (Figure 1).

Concentration of nitrates in water obtained its maximum levels from January to April in winter, and the maximum discharge of nitrates occurred in March and April, while the minimum water runoff was observed in August (Figures 2 and 3). These relationships were analysed against the background of meteorological conditions in each year, with special attention paid to the periods of March-April and August-September. The results of the analyses, presented in Fig. 1 and 3, indicate that the runoff of nitrates from soils to surface waters is directly connected with the efficiency of water draining systems. The measurements showed an indisputable relationship between the volume of water discharged from soils with the land reclamation system and with the weather conditions (Table 2).

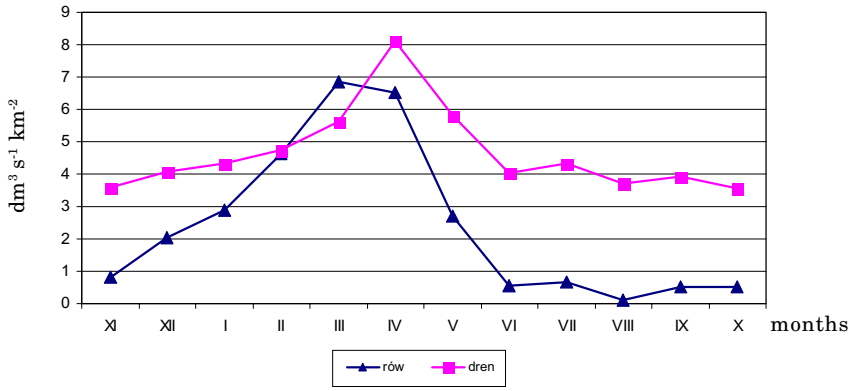

Fig. 1. Effect of a draining system on the mean unit runoffs in 1992–1999

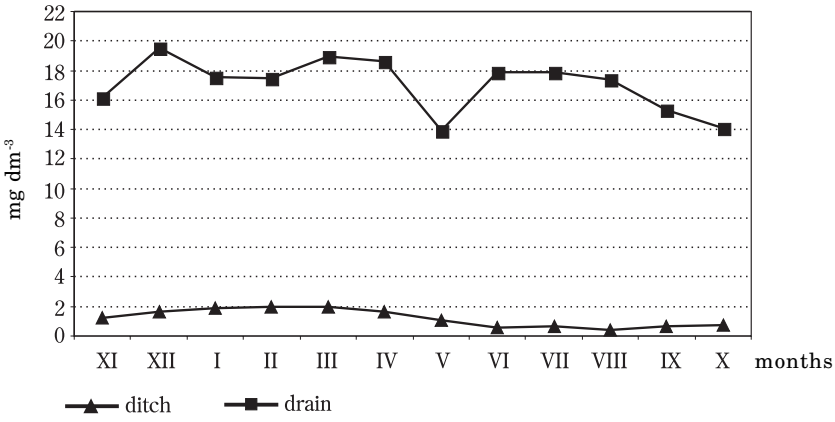

Fig. 2. Effect of a draining system on the mean concentration of nitrates in 1992–1999

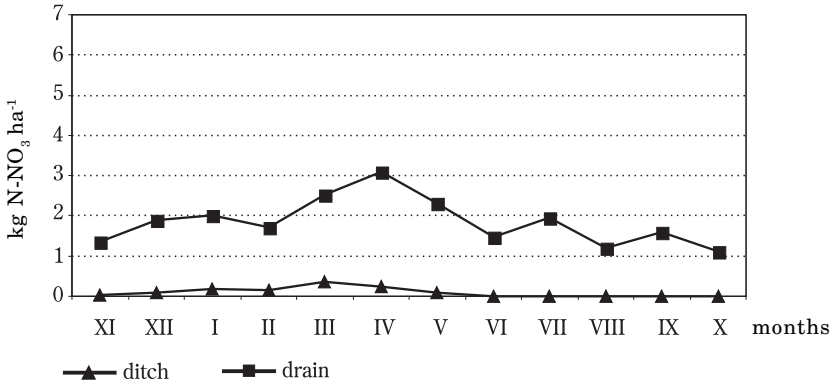

Fig. 3. Effect of a draining system on the runoff of nitrate load in 1992–1999

Table 2

Runoff of water from agricultural catchments in mm

Year	Draining system	Time period in months					
		11-10	11-04	05-10	03-04	08-09	10-11
1992	ditches	35	23	12	15	3	5
	drains	64	34	30	14	11	10
1993	ditches	86	74	12	38	6	2
	drains	105	52	53	21	19	19
1994	ditches	202	172	30	137	1	2
	drains	222	112	110	62	29	29
1995	ditches	73	58	15	20	3	2
	drains	228	119	109	49	32	30
1996	ditches	39	25	14	22	0	1
	drains	143	87	56	44	16	15
1997	ditches	31	12	19	7	1	9
	drains	82	43	39	16	13	14
1998	ditches	105	94	11	36	1	2
	drains	178	90	88	39	25	25
1999	ditches	34	34	0	9	0	0
	drains	151	95	56	48	17	9
Mean from 1992-1999	ditches	76	62	14	36	2	3
	drains	147	79	68	37	20	19

Volumes of discharged water from the two neighbouring catchments, with identical natural conditions and the crop cultivation system, differed two-fold. For the period of eight years, the system of ditches carried away 12% of the annual rainfall, and the drainage system – 24%. The differences in the draining capacity of the land reclamation systems were small in winter, but in the summer half-year, the underground drains carried away five as much water as open ditches. This is due to the fact that the drainage system is denser and can penetrate the soil more efficiently. Besides, in a drainage system there is no risk of halting the runoff.

Moreover, in summer, water in the ground between ditches (which is larger in area that that between drains) evaporates, and the runoff of the water which does flow into ditches is halted by plants. The effect of these two factors becomes stronger in late summer, when drains carry away ten times as much water as ditches.

Because of such differences between the two types of water draining systems, the runoff of water through drains is more uniform throughout the whole year, while the water discharge through ditches was not only smaller, but in 82% occurred in the winter half-year.

To assess the influence of meteorological conditions on water runoff we compared the runoffs from particular years, for example the years 1993 and 1994. With the precipitation and temperatures being similar, the water runoff in 1994 was 2.5-fold higher through ditches and 2-fold higher through drains than in 1993. The difference can be attributed to the fact that the year 1993 was preceded by a drier and warmer summer in 1992, as a result of which the soil contained smaller reserves of water. The year 1994, in contrast, was preceded by a wet summer. For further comparison, the year 1995 was wet and came after a wet year, but at the same time it was the warmest year, which meant that the evapotranspiration was the highest and the water runoff through ditches was smaller. The precipitation-runoff route is longer in a draining system with ditches, thus evapotranspiration is more likely to take place. In the case of drains, the precipitation-runoff route is shorter, therefore evapotransipartion occurs less readily and the water runoff through drains was similar in both years.

Among the shorter time periods compared (two-month), the largest water runoff was observed in early spring (March-April). The runoff was significantly higher than in the other bimonthly periods considered. The early spring water discharge is caused by the melting of snow cover, current precipitation and lack of plant cover. This was particularly evident in 1994, when early spring was extremely wet, and the soil permeated with water could not retain further rainfall.

Water runoff from soil was accompanied by improved soil aeration and nitrification of nitrogen, which meant that higher concentration of nitrates was found in the waters from the drained objected (Table 3).

On the other hand, a positive correlation was discovered between the mean annual temperature and concentration of nitrates. An exception was the year 1995, when large rainfall was accompanied by the highest temperature. It was therefore possible to observe some inhibition of oxygen supply to soil for soil transformations, and oxygen consumption for carbon mineralization, especially in summer, at high temperatures. This finding is confirmed by the fact that a decrease in the concentration of nitrates in water in the drains was lower, as the water transport was more efficient.

In the same year, i.e. in 1994, winter and early spring in particular were warm compared to the long-term data, which was favourable to the runoff of nitrates, because at the temperature of around 5^{o}C nitrification was not slowed down. The concentration of nitrates resulted from the combination of two factors - temperature and oxygen supply. Concentrations of nitrates in water from the drainage system were similar in the winter and summer half-year periods. Some larger dissimilarities in nitrate concentra-

Table 3

Mean concentration of nitrates in waters flowing from cropland soils (mg $N\text{-}NO_3$ dm^{-3})

Year	Draining system	Time period in months					
		11-10	11-04	05-10	03-04	08-09	10-11
1992	ditches	2.36	3.53	0.95	4.21	0.34	2.42
	drains	10.56	12.76	8.37	22.00	2.00	12.94
1993	ditches	1.94	3.01	0.87	2.63	0.48	0.48
	drains	19.41	19.45	19.37	23.58	22.73	21.58
1994	ditches	1.23	1.71	0.74	1.83	0.42	0.89
	drains	24.09	24.36	22.59	18.13	23.04	20.11
1995	ditches	0.94	1.54	0.35	1.47	0.36	0.36
	drains	14.89	16.35	13.43	18.58	13.70	16.35
1996	ditches	0.52	0.43	0.63	0.42	0.67	0.47
	drains	24.24	23.97	24.50	19.95	25.94	17.96
1997	ditches	1.03	1.18	0.89	2.72	0.96	0.99
	drains	16.44	21.35	11.53	2.17	12.36	7.03
1998	ditches	0.90	0.97	0.77	0.65	0.42	0.18
	drains	10.55	11.19	9.49	12.56	9.55	7.50
1999	ditches	0.54	0.36	0.71	0.04	1.63	3.34
	drains	11.13	11.07	11.19	13.12	12.90	0.06
Mean from 1992-1999	ditches	1.18	1.59	0.74	1.75	0.66	1.14
	drains	16.41	17.56	15.06	16.26	15.28	12.94

tions between certain years can be attributed to soil processes and dilution with water. As regards waters carried away through ditches, higher concentration of nitrates was usually determined in winter. The ratio between the concentration of $N\text{-}NO_3$ in winter and summer waters was on average 2.1:1, and 1:1.4 in summer half-year (in two years only the ratio was reverse, but this was due to the fact that those two winters were freezing cold, which slowed down nitrification, and in the following summers the water flow was minimal).

The following relationship appeared. Efficient draining favoured oxygen processes and nitrification. This was compounded by the effect of temperatures. In warm winters, and especially in warm early spring seasons, temperatures above zero favour nitrification and nitrate runoff with waters. On the other hand, some obstacles to water runoff as well as excessive amounts of water in soil slow down nitrification. Should the latter be combined with high temperatures (summer), oxygen deficits and depressed levels of nitro-

gen are likely to occur. The concentration of nitrates in drainage waters exceeds the safety limit, which is 50 mg NO_3 dm^{-3} for potable water (*Ordinance of the Ministry of Health*, 2000).

The runoff of nitrates from soil is an aggregate of the amount of the water discharged and the amounts of nitrates it contains. As a result, meteorological conditions often had similar influence on both factors (runoff and concentrations). Consequently, some significant interdependence was observed between these factors and their effect on the amounts of nitrates carried away from the croplands examined (Table 4).

The annual runoff ranged from 0.04 kg per ha to 49.44 kg N-NO_3 per ha. Variations in the runoff were caused by both the type of a draining system and the weather conditions. The drainage system carried away on average 22.2 kg N-NO_3 per ha annually, ranging from 7.1 to 49.6 N-NO_3 per ha. Under the same conditions, soils drained by ditches had an outflow of 1.2 kg N-NO_3 per ha annually, ranging from 0.04 to 3.64. These figures confirm our previous studies (Koc, Szymczyk 2003). The runoff of nitrates in hydro-

Table 4

Mean concentration of nitrates in waters flowing from cropland soils (mg N-NO_3 dm^{-3})

Year	Draining system	Time period in months					
		11-10	11-04	05-10	03-04	08-09	10-11
1992	ditches	1.06	0.91	0.15	0.61	0.01	0.18
	drains	7.13	4.93	2.21	2.95	0.47	1.45
1993	ditches	2.43	2.32	0.10	1.12	0.06	0.01
	drains	20.7	10.32	10.40	4.79	3.67	3.81
1994	ditches	3.64	2.26	0.38	2.52	0.01	0.04
	drains	49.55	25.49	24.06	11.27	6.91	5.71
1995	ditches	1.00	0.95	0.05	0.29	0.02	0.01
	drains	34.55	19.38	15.17	8.75	7.56	4.55
1996	ditches	0.18	0.11	0.07	0.09	0.02	0.01
	drains	34.01	20.55	13.46	8.61	4.49	1.09
1997	ditches	0.31	0.16	0.15	0.12	0.01	0.04
	drains	9.16	6.50	2.66	1.62	0.01	0.51
1998	ditches	0.79	0.68	0.11	0.23	0.01	0.01
	drains	13.86	9.40	4.45	4.97	1.52	0.01
1999	ditches	0.04	0.04	0.00	0.00	0.00	0.00
	drains	8.88	4.54	4.34	2.17	0.01	0.01
Mean from 1992-1999	ditches	1.18	0.93	0.13	0.62	0.02	0.04
	drains	22.23	12.64	9.59	5.64	3.08	2.14

logical half-years was highly variable. In soils drained by ditches, 87% of the annual load of nitrates was carried away in winter, with just 13% discharged in summer, which was similar to the annual distribution of water outflow. As regards waters drained by the drainage system, the load of nitrates carried away in summer reached 43% of the average annual runoff, and the water outflow equalled 46% of the annual total. The influence of meteorological conditions was such that in dry years the runoff from summer half-year decreased to 30% of the average annual runoff in the object with the drainage system. In the case of open ditches, the summer outflow was hardly measurable. Nitrates from ditch water in the summer half-year were taken up by plants, as a result of which water which flew into the ditches was used up by plants rather than discharged further.

Around 50% of the nitrate runoff in the winter half-year fell to March and April in the case of drains versus 60% in the ditches. Later on, in late summer (August-September) and autumn (October-November), the runoff of nitrates decreased, although in the drains, the runoff measured amounted to 15% of the annual runoff late summer and 10% in autumn. At the same time, the runoff of nitrates through ditches decreased to the minimum. Such a relationship occurred generally in all the years. However, nitrate runoff through the drains was favoured by high precipitation. As for ditches, the runoff of nitrates from soils is small, less than 10% of the fertilization rate, and occurs mainly in early spring (March-April). Draining ditches function like a barrier which prevents the runoff of nitrates from croplands. As regards drainage systems, the amount of nitrates carried away varies from over 10% to 40% of the fertilization rate and occurs throughout the whole year, but is more intense in early spring (March-April). The load of nitrates from croplands drained through a drainage system is dangerous to water reservoirs (VOLLENWEIDER 1968).

In the light of the nitrogen balance studies in soils of croplands, it can be assumed that most of the excessive amounts of nitrogen is removed from croplands with drainage waters. In the case of soils drained with ditches, the runoff of nitrates is minimal, and most of nitrogen is transferred to deeper groundwaters, where it is reduced due to lack of oxygen.

CONCLUSIONS

1. The runoff of nitrates from croplands depends on meteorological conditions and type of a draining system. In drained heavy soils, nitrate runoff amounts to an average 22 kg $N\text{-}NO_3$ per 1 ha annually, although it can reach as much as 50 kg, posing a threat to open surface waters. From an identical area drained with ditches, the runoff of nitrates is 20-fold lower and does not threaten open waters.

2. The runoff of nitrates is connected with the volume of waters flowing off the land and the intensity of nitrification, as indicated by the concentration of nitrates in waters. In drainage waters, nitrates can exceed 50 mg $N\text{-}NO_3$ per 1 dm^3, which means that the water is unsuitable for human or animal consumption.

3. Waters from draining ditches contain less than 10 mg $N\text{-}NO_3$ per 1 dm^3, declining to under 1 mg $N\text{-}NO_3$ per 1 dm^3 in summer. This is due to biological sorption of nitrates.

4. The runoff of nitrates with drainage waters from heavy soils continues throughout the whole year, being more intense in March and April. The runoff of nitrates from croplands drained with ditches occurs in winter, reaching the maximum level in March and April, and the minimum (down to complete disappearance) in August.

REFERENCES

FOTYMA E. 1996. *Zastosowanie metody Nmin do oceny środowiskowych skutków nawożenia azotem*. Zesz. Probl. Post. Nauk Rol., 440: 89-100.

FOTYMA E. 1997. *Wyznaczanie dawek nawozów azotowych na podstawie analizy gleb*. Zesz. Eduk. IMUZ, 4/97: 49-56.

FOTYMA M. 1996. *System doradztwa nawozowego z uwzględnieniem ochrony środowiska, a szczególnie ochrony wód gruntowych*. Zesz. Eduk. IMUZ, 1/96: 55-66

KOC J., ĆIECKO CZ., JANICKA R., ROCHWERGER A. 1996. *Czynniki kształtujące poziom mineralnych form azotu w wodach obszarów rolniczych*. Zesz. Probl. Post. Nauk Rol. i Leśn., 440: 175-184.

KOC J., SZYMCZYK S. 2003. *Wpływ intensyfikacji rolnictwa na odpływ azotu mineralnego.* Zesz. Probl. Post. Nauk Rol., 494: 175-181.

LIPIŃSKI J. 2003. Drenowanie gleb mineralnych, a środowisko przyrodnicze. Wiad. Mel. Łąk, 2: 74-76.

MILLER A., LIBERADZKI D., PLEWIŃSKI D. 2001. Jakość wód gruntowych w różnych siedliskach położonych wzdłuż transektów odpływu. Zesz. Probl. Post. Nauk Rol., 477: 93-100.

Rozporządzenie Ministra Zdrowia z dnia 4 września 2000 r. w sprawie warunków, jakim powinna odpowiadać woda do picia i na potrzeby gospodarcze, woda w kąpieliskach, oraz zasad sprawowania kontroli jakości wody przez organy Inspekcji Sanitarnej (Dz.U. z 2000 r. Nr 82, poz. 937).

SAPEK A. 1996. *Udział rolnictwa w zanieczyszczaniu wody składnikami nawozowymi.* Zesz. Eduk. IMUZ, 1/96: 9-34.

SOLARSKI H., KOC J., SOLARSKA J., BŁASZCZYK M., BIENIEK B. 1996. *Charakterystyka przyrodnicza doświadczalnych zlewni rolniczo-leśnej w Tomaszkowie na Pojezierzu Mazurskim*. PIOŚ, Warszawa, Bibl. Monitoringu Środowiska, 185-195.

SOLARSKI K. 2002. *Stosunki hydrologiczne i odpływ biogenów ze zlewni rolniczych i leśnych na Pojezierzu Mazurskim* (praca doktorska, ss. 144).

UGGLA H. 1956. *Ogólna charakterystyka gleb Pojezierza Mazurskiego.* Zesz. Nauk WSR Olsztyn, 1: 15- 5.

VOLLENWEIDER R. A. 1968. *Scientific fundamentals of the entrophication of lakes and flowing waters, with particular reference to nitrogen and phosphorus as factors in eutrophication.* DAS/CSIO/68.27, OECD, Paris pp. 192.

J. Elementol. 2007, 12(2): 135–148

Prace przeglądowe

IMPACT OF CROP PROTECTION CHEMICALS ON PLANTS AND ANIMALS

Małgorzata Baćmaga, Jan Kucharski, Jadwiga Wyszkowska

Chairof Microbiology
University of Warmia and Mazury in Olsztyn

Abstract

Crop protection chemicals are chemical compounds of high biological activity and are used on a large scale in agriculture. Their influence on crop planning and storage quality is mostly positive. Crop protection chemicals, on the other hand, may cause environmental pollution. Due to errors in agronomic practice, such chemicals may occur in various ecosystems, causing threat to people, animals and plants. Adverse effects of these products are attributed to their inappropriate use, decomposition time and the ability to accumulate in the environment. Their long-lasting presence has a negative effect on living organisms, including humans. Biocides enter the human body mainly through the digestive tract, causing life-threatening disorders, which, in some extreme cases, may be fatal.

K e y w o r d s: crop protection chemicals, plants, animals, human.

WPŁYW ŚRODKÓW OCHRONY ROŚLIN NA ROŚLINY I ZWIERZĘTA

Abstrakt

Środki ochrony roślin to związki chemiczne o dużej aktywności biologicznej, powszechnie stosowane w rolnictwie. Wywierają one przede wszystkim pozytywny wpływ na plonowanie roślin oraz jakość przechowywanych płodów rolnych. Środki ochrony roślin mogą

Professor Jadwiga Wyszkowska, Chair of Microbiology, University of Warmia and Mazury in Olsztyn, pl. Łódzki 3, 10–727 Olsztyn, Poland
e-mail: jadwiga.wyszkowska@uwm.edu.pl

być także przyczyną zanieczyszczenia środowiska przyrodniczego. Preparaty te w wyniku nieprawidłowych zabiegów agrotechnicznych przedostają się do różnych ekosystemów, zagrażając ludziom, zwierzetom i roślinom. Negatywne działanie tych substancji jest związane z ich niewłaściwym stosowaniem, trwałością oraz zdolnością do kumulowania w środowisku. Ich długotrwałe zaleganie wpływa negatywnie na organizmy żywe, w tym człowieka. Biocydy przedostają się do organizmu ludzkiego głównie drogą pokarmową, powodując zaburzenia w funkcjach życiowych, a w skrajnych przypadkach śmierć.

S ł o w a k l u c z o w e: środki ochrony roślin, rośliny, zwierzęta, człowiek.

INTRODUCTION

Crop protection chemicals have been used since ancient times, when people understood the importance of combating plagues and epidemics. Copper sulphate, for instance, was first applied as a fungicide in ancient Egypt and Babylonia. The earliest studies on crop protection chemicals are associated with the work of Alexis Millardet, who in 1895 used the Bordeaux mixture, a combination of copper sulphate and lime milk, to control downy mildew. Major development in for crop protection chemicals occurred after World War Two, for example large-scale production of DDT began in 1946 (BZIUK 2001, PRACZYK, SKRZYPCZAK 2004).

Chemical crop protection provides a basis for agricultural practices and measures aimed at generating the highest yields of best-quality farm products. Intensive agricultural production as well as mass occurrence of pests and weeds encourage increased use of crop protection chemicals (WYSZKOWSKI, WYSZKOWSKA 2004a). Pest organisms can cause yield losses ranging from 20 to 90%, depending on crop types (BANASZKIEWICZ 2003). Herbicides and insecticides are crucial chemicals applied in agriculture, as they can effectively control weeds and pests, thus increasing the quantity and quality of harvested crops (MICHALCEWICZ 1995, NOWAK et al. 1999, WYSZKOWSKI, WYSZKOWSKA 2004b). Crop protection chemicals are applied in agriculture not only to control pest organisms but also to disinfect storage space and to protect animal feeds, foods, plant raw materials and products (BANASZKIEWICZ 2003).

Biocides also play an important role in the protection of human life. BZIUK (2001) reported that the use of crop protection chemicals in the winter of 1944 allowed to curb a typhus fever epidemic, which nevertheless caused a large death toll in and around Naples. Crop protection chemicals have also been used to control malaria.

The basic component of any given crop protection chemical is a biologically active substance, also referred to as an active component. Biologically active substances are characterized by high biological activity against specific organisms (BANASZKIEWICZ 2003, PRZYBULEWSKA 2004). At present, 10,000 active compounds, components of crop protection chemicals, are known and applied throughout the world (BIESZCZAD, SOBOTA 1993).

IMPACT OF CROP PROTECTION CHEMICALS ON THE NATURAL ENVIRONMENT

Crop protection chemicals used to remove and destroy weeds, to fight parasites as well as to prevent crop losses during storage, have a negative impact on the environment and threaten many ecosystems (ANDERSON et al. 1994, WYSZKOWSKA 2002, BOJAKOWSKA, GLIWISZ 2005, WG et al. 2005). Due to their common use and environmental persistence, crop protection chemicals may be found in all environmental components, i.e. in water, soil, air as well as in plants, foods and in human and animal organisms (MCDONALD et al. 1999) – Figure 1.

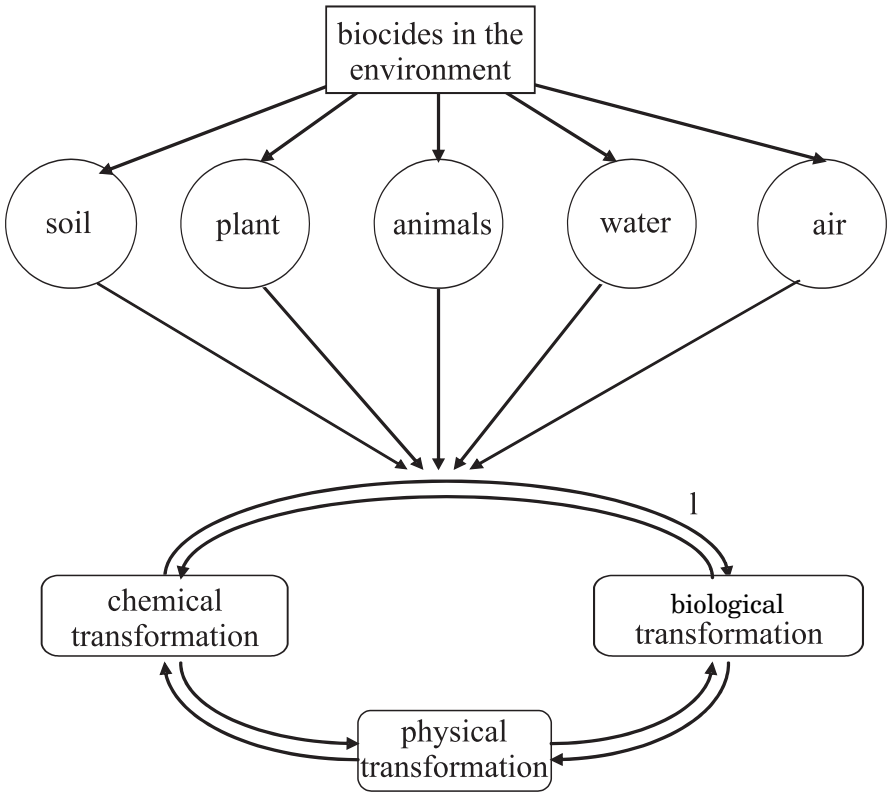

Fig. 1. Biocide transformations in the environment

Biocides are characterized mainly by their selective toxicity and environmental persistence, but they can also be grouped according to bioaccumulation potential and mobility. Many crop protection chemicals undergo bioaccumulation, i.e. they accumulate in living organisms (BZIUK 2001). Bio-

accumulation is usually higher in aquatic than in terrestrial organisms. Since crop protection chemicals may accumulate in water and land organisms, their amounts in environment constantly grow and their flow through a food chain accelerates. This is particularly dangerous to organisms at the end of a food chain, such as predators or humans (BOJAKOWSKA, GLIWISZ 2005). Biocide breakdown takes place mainly through biochemical processes, but may also be caused by photochemical and chemical reactions such as oxidation, reduction, hydrolysis and interactions with free radicals. Unfortunately, breakdown products may be more toxic than the original co protection compounds (GRIFFITHS et al. 2001). Many biocides that are no longer used, in particular chloroorganic biocides, remain in soil and water for many years or decades, and their very low water concentrations may multiply biologically in tissues of aquatic organisms (BOJAKOWSKA, GLIWISZ 2005).

Soil, the outermost layer of the Earth, is subject to the effects of chemical compounds, including crop protection chemicals. Due to its properties, soil is a fundamental component of the biosphere, which conditions food production and sustains human and animal life on land. Besides, it is a very diversified ecosystem teeming with a variety of living organisms, which perform many environmentally vital functions (KOBUS 1995, KUCHARSKI et al. 2004, RUSSEL 2005).

Soil fertility and biological activity may be limited if soil becomes contaminated by various toxic substances, including crop protection chemicals (MICHEL 1999). Presence of crop protection chemicals in soil is related to environmentally damaging human activities. Soil contamination by xenobiotics depends primarily on application doses and frequency, as well as on the soil physicochemical properties, sorption capacity, temperature, humidity and pH (STRZELEC 1986, NOWAK 1996, MICHALCEWICZ 2004, PRZYBULEWSKA et al. 2004, SWĘDRZYŃSKA 2004). Soil is contaminated mostly by compounds whose active substance is characterized by high resistance to soil borne microorganisms (NOWAK 1996, SÁNCHEZ et al. 2004). All biocides remaining in soil may constitute a danger to organisms living in this environment, primarily to soil microbes (NOWAK et al. 1999). As some biocides are toxic to soil microorganisms, quantitative composition and enzymatic activity of microbial populations change, which ultimately lowers soil fertility and causes soil degradation (MEGHARAJ et al. 2000, DAS et al. 2003, DURSKA 2004, TRASAR-CAPEDA et al. 2004).

According to literature (ANDERSON et al. 1994, KASZUBIAK et al. 1994, NOWAK 1996, BERGER 1998, JOHNSEN et al. 2001, SŘRENSEN et al. 2003), microbiological and biochemical properties of soil depend on the length of time a crop protection chemical persists in the environmental (half-life). According to BALICKA (1983) and WYSZKOWSKA (2004), the impact of biocides on the growth of microbial populations is reflected mainly by cell metabolism disorders. The effect of these compounds on microbial metabolism is primarily related to their penetration into cells. However, some authors claim that not all meta-

bolic changes impede microbial proliferation. According to KASZUBIAK et al. (1994), crop protection chemicals are not always toxic to microorganisms; in fact some provide nourishment for heterotrophic microbes. Chemical preparations applied as recommended by the manufacturer and at optimal rates have no significant influence on the activity of microbes. Exceeding the optimal dosage may lead to modifications in the biological activity of soil (WĘGOREK 1994, FURCZAK, KOŚCIELNA 1997, WYSZKOWSKA, KUCHARSKI 2004). Long-term use of crop protection chemicals affects the persistence time of compounds in soil colloids as well as soil microbial activity (OSTROWSKI 1996).

Soil degradation may also occur as a result of the use of biocides which contain heavy metals such as arsenic, copper and zinc. Heavy metals found in crop protection chemicals are very difficult to remove from soil since they are accumulated mainly in the root system of plants. Elements absorbed by roots penetrate into all parts of plants, pass through a food chain and ultimately reach humans (ZABOROWSKA et al. 2005).

The problem resulting from application of crop protection chemicals on soil surface is that they often block cultivation for many years and generate toxic residues in agricultural products. Non-monitored long-term use of biocides increases the risk of soil contamination with these compounds (DURSKA 2004).

Crop protection chemicals belong to those environmental pollutants that are very often present in surface and underground waters (DUGAN 1969, KOTRIKLA 1997). They penetrate into surface waters primarily via runoffs from fields and atmospheric precipitations. Maximum concentrations of crop protection chemicals are recorded during melt-water runoffs and following certain agricultural practices. Biocides may also accumulate in bottom deposits and living organisms (BOJAKOWSKA, GLIWISZ 2005).

Crop protection chemicals used intensively to produce high yields may be transported by rainwater and rivers and then filtered down into underground waters. The danger related to contamination with these chemicals increases in regions with high rainfalls during the vegetative growing season. Water contamination with crop protection chemicals is also observed in areas subject to erosion, where chemical compounds are readily transferred from contaminated soil to surface waters. Thus, great caution is advised when using crop protection chemicals as their improper application causes contamination (BŁAŻEWICZ 2003).

Herbicides are the main threat to surface and underground waters in Poland and in other developed countries. Crop protection chemicals accumulated in bottom deposits are distinguished by their high toxicity and persistence in aquatic habitats, particularly in bottom deposits, river and lake silt. Biocides may be present in potable water due to insufficient purification of surface waters. They enter a food chain, destroying particular links of this chain. Among the three environmental components: air, soil and water, water is most prone to contamination. Relatively small amounts of crop pro-

tection chemicals can deteriorate the organoleptic properties (taste and odour) of water, which disqualifies its use for consumption and household purposes. They can also diminish populations of fish and other aquatic organisms, while higher concentrations result in mass fish mortality and dying out of entire water bodies. Crop protection chemicals, in particular DDT, very often impoverish aquatic herbivorous fauna. Lakes and other water bodies which supply water for municipal purposes are subject to special control and protection.

In accordance with the Council Directive 91/414/EEC of 15 July 1991, the content of crop protection chemicals and substances of similar properties in potable water may not exceed 0.1 $cm^3\ l^{-1}$ and 0.5 $cm^3\ l^{-1}$ for total harmful compounds.

Due to high vapour pressure, most crop protection chemicals easily escape into the atmosphere from soil, surface waters and waste dumping sites (Totten et al. 2003, Shen et al. 2005). Research results show that the highest air concentrations of biocides are recorded over areas in which they were produced or intensively used in the past as well as over urbanized areas. Elevated levels of these xenobiotics were also observed over the southern and eastern parts of Europe (Jaward et al. 2004).

It was found (Bziuk 2001) that over 90% of crop protection chemicals present in the atmosphere are in the gaseous phase. Birds are at higher toxicological risk than humans due to crop protection chemicals released to air because of their more developed respiratory system as well as longer and more intensive exposure to those toxic substances.

IMPACT OF CROP PROTECTION CHEMICALS ON PLANTS

Mass emergence of harmful organisms in arable fields has stimulated increased use of chemicals in agriculture. The method applied most frequently to protect crops and improve their overall health is the use of crop protection chemicals (Wyszkowska 2002, 2004). Despite many advantages, crop protection chemicals may also have a negative impact on plant production, such as inhibition of plant growth and development (Klimach, Wieczorek 1998, Sukul 2006).

Chemical compounds applied in agriculture often penetrate soil, where they undergo complex transformations leading to their breakdown (Johnsen et al. 2001). Biologically active substances contained in biocides are transported deeper into the soil profile and then absorbed by field plants and weeds (Sadowski et al. 2001). The amount of absorbed biocides is related primarily to the properties of a given plant species as well as to the chemical structure of active substances (Praczyk, Skrzypczak 2004). The negative

influence of crop protection chemicals on soil properties may also involve inferior nutrient availability to plants, leading to mineral imbalance (Wyszkowska 2002). Biocides used for agricultural purposes not only contribute to pest and weed control, but also modify plant growth and development, thus changing the technological value of raw materials. Crop protection chemicals are mobile and can accumulate in plants, affecting the physiological, biochemical and nutritional properties of foods (Sawicka 2004). Disruptions in nutrient uptake by plants lead to yield decline and quality deterioration, which in turn depresses quality of feeds and foods produced from these plants (Braschi et al. 2000).

The rate of biocide absorption largely depends on the type and granulometric composition of soil, fertilization levels as well as organic substance content (Wyszkowska 2002, Wyszkowski, Wyszkowska 2004a). Crop protection chemicals penetrate into plants through roots of young seedlings. Absorption takes place mainly through root hairs and phellem cells, which are a fundamental component of the root apex region. Chemical compounds undergo biotransformation under the influence of microorganisms and plant enzymes. These preparations are absorbed by plants similarly to water, together with dissolved nutrients (Praczyk, Skrzypczak 2004). The response of some plants to herbicides may vary widely, from growth stimulation to yield decrease. Yield decline often results from plant damage caused by spraying with crop protection chemicals (Błażewicz et al. 2003). Negative impact of crop protection chemicals may involve morphological changes in plants, including leaf discoloration, turgor loss, leaf wilting and necrosis, plant growth inhibition as well as death of whole plants. Although some plants show no external symptoms, they respond to the use of these preparations by a decline in yield (Urban 2000). Symptoms of the phytotoxic effects of crop protection chemicals on field plants may be observed during emergence, growth or harvest. Damage may occur to an entire plant or to some of its parts (Praczyk, Skrzypczak 2004).

By disrupting the physiological processes in cultivated plants, crop protection chemicals may lead to changes in quality and reduce the activity of amylolytic, cellulolytic and proteolytic enzymes (Błażewicz et al. 2003, Kawka et al. 1998). According to Wyszkowski and Wyszkowska (2004ab), biocides exert a considerable influence on the chemical composition of plants, dependent mainly on a plant species and type of an active substance applied. This was confirmed by studies conducted by Rola and Kieloch (2001).

Sawicka (2004) demonstrated that certain active substances in crop protection chemicals inhibit photosynthesis and damage the chloroplast structure. Plants that possess a large surface area relative to their mass absorb a greater amount of biocides, which accumulate mainly in the peel of fruit, particularly citrus fruit (Bziuk 2001).

Research carried out in the year 2000 by the Institute of Plant Protection in Poznań, Poland, has shown that residues of crop protection chemi-

cals can be found primarily in fruits (22.6%), greenhouse vegetables (17.7%) and field-grown vegetables (10.7%). Biocide residues were not found in field crops such as cereals, potatoes and sugar beets. The maximum permissible amounts of biocide residues were most frequently exceeded in greenhouse vegetables (BANASZKIEWICZ 2003).

IMPACT OF CROP PROTECTION CHEMICALS ON HUMANS AND ANIMALS

Biocides pose a serious toxicological threat. They are toxic by nature, which means that they affect both harmful and beneficial organisms. Along with other properties, such as their environmental persistence and bioaccumulation capacity, they represent one of the most toxic groups of chemicals which humans are in contact with. Practically speaking, all biocides are toxic but their toxicity varies. In Poland, crop protection chemicals are divided into four toxicity classes, depending on the value of LD_{50}, i.e. the lethal dose expressed in milligrams of a toxic substance per kilogram of body weight which results in the death of 50% of the test population of animals following single administration. This pertains to experiments conducted on animals and is related to determining acute toxicity (ACT ON THE Protection of Plants of 18 December 2003, Journal of Laws 2006.171.1225).

Table 1

Classification of crop protection chemical toxicity with regard to mammals according to the Act on the Protection of Plants of 18 December 2003 (Journal of Laws, 2006.171.1225)

Toxicity class	Toxicity description	Acute oral toxicity LD_{50} ($mg \cdot kg^{-1}$ body weight)	Acute dermal toxicity (rat or rabbit) LD_{50} ($mg \cdot kg^{-1}$ body weight)	Acute inhalation toxicity LC_{50} ($mg \cdot dm^3 \cdot 4h$)
I	very toxic	≤ 25	≤ 50	≤ 0.25 – aerosols ≤ 0.50 – gases and vapors
II	toxic	$25 < LD_{50} \leq 200$	$50 < LD_{50} \leq 40$	$0.25 < LC_{50} \leq 1$ – aerosols $0.50 < LC_{50} \leq 2$ – gases and vapors
III	harmful	$200 < LD_{50} \leq 2000$	$400 < LD_{50} \leq 2000$	$1 < LC_{50} \leq 5$ – aerosols $2 < LC_{50} \leq 20$ – gases and vapors

Acute toxicity – capacity of the substance to produce a toxic effect in the body following a single exposure or the administration of a single dose.
LD_{50} – amount of the chemical substance, statistically calculated based on the results of research, that leads to the death of 50% of organisms following its administration in a given manner.
LC_{50} – statistically calculated concentration of the chemical substance in an environmental medium leading to the death of 50% of organisms of a given population under certain conditions.

Crop protection chemicals are very toxic to living organisms, with their actual toxicity depending on an organism in question, environmental conditions as well as on the type, form and method of biocide application. The main route of exposure of humans to crop protection chemicals is the digestive tract. Chloroorganic biocides, which the most toxic substances, can enter the human body through the digestive tract, mainly following consumption of fish and crustaceans (BZIUK 2001, BANASZKIEWICZ 2003).

Many crop protection chemicals accumulate in living organisms. Bioaccumulation is usually higher in aquatic than in terrestrial organisms. Biocides may affect living organisms very differently depending on metabolism, toxicity and concentration. Humans are at risk of ingesting residues of crop protection chemicals with food.

ATANIYAZOVA et al. (2001) reported that the concentrations of biocide residues in food products range from 0.1 to 1 mg, compared to 0.1–1 μg in underground waters.

Taking into account the persistence of crop protection chemicals in environment as well as human dependence on food, it has been found that these compounds are mostly accumulated in human tissues (mainly in adipose tissue). These substances often enter the human body through the skin and respiratory system. They may remain in the skin for a few months since exposure. Chloroorganic compounds, including crop protection chemicals, accumulate primarily in fat and milk of animals as well as humans, but they are also found in the brain, liver and kidneys, resulting in malfunction of these organs (MILLER, SHARPE 1998).

Even low amounts of these xenobiotics may cause negative effects, such as reduction of the reproductive performance of young animals and deterioration of their health. These substances are one of the reasons for developmental disorders in children, which manifest themselves at a later period (JUBERG 2000). Research results show that crop protection chemicals have an enormous impact on the immune (WEISGLAS-KUPERUS et al. 1995) and hormonal systems (BIRNBAUM 1994), and can lead to tumours (MILLER, SHARPE 1998). The group at highest risk of disease resulting from consumption of food and water contaminated with biocides are pregnant women, infants, elderly people as well as people with hypoimmunity (BANASZKIEWICZ 2003). Acute effects of crop protection chemicals may cause many symptoms, from light skin irritation to death. Children that consume relatively large amounts of fruit and vegetables originating from intensive agriculture may be at risk of nervous system dysfunction and disorders caused by toxins (DUGAN 1969, MALLATOU et al. 2002). Additionally, as such toxins can bioaccumulate, they enter a food chain and reach high concentrations in tissues of birds and mammals. The saddest evidence is a continually increasing level of these substances in the human body caused by consumption of crop protection chemicals dissolved in vegetable and animal fat from fish, poultry and beef (NANCY 1999). The highest quantities of these preparations are present in

food products such as cereal grains or vegetables, but primary sources of contamination for humans are milk and milk products, eggs and meat (MALLATOU et al. 2002).

In accordance with the Order of the Ministry of Health and Social Care of 15 April 1997, the maximum permissible levels of residues of crop protection chemicals used in the cultivation, protection, storage and transportation of plants (Journal of Laws, No. 43, item 273) may not exceed 0.005 – – 0.1 mg kg^{-1} of food products. The negative effects of crop protection chemicals have gained increasing interest recently, which resulted in the development of environmental monitoring programs, aimed primarily at human health protection (BIESZCZAD, SOBOTA 1993).

SUMMARY

Crop protection chemicals are natural or synthetic substances that are applied primarily in agriculture to fight weeds as well as to control plant diseases and pests. They play a key role in producing high yields, storing farm produce, fighting pests as well as maintaining proper sanitation and hygiene standards. These chemicals have become a very significant part of human life. Aside their advantages, biocides are also known for negative effects. When improperly applied, they pose a serious threat not only to animals, but also to the natural environment. Due to their properties, they possess the ability to accumulate in various ecosystems, resulting in contamination followed by degradation. Crop protection chemicals are capable of impacting all living organisms – including species that are not their target. Beneficial organisms may be destroyed and biodiversity diminished through the use of xenobiotics, which in consequence may upset ecosystem balance.

Intensive use of crop protection chemicals in agriculture, observed nowadays, leads to environmental pollution. However, more and more attention is paid to environment-friendly crop protection practices. The use of chemicals in agriculture requires supervision, monitoring and prevention of potential negative consequences. The range of crop protection chemicals has been changing over the last few decades. Some of them have been withdrawn from the market due to their toxicity, a long half-life as well as the development of resistance in target populations – this pertains in particular to insecticides. The amount of biocides used is increasing drastically, which in many cases makes it very difficult to determine their toxicity. Today a major global problem are disposal sites for hazardous wastes, including crop protection chemicals. These sites have not been properly prepared to store this kind of waste, so pesticides may leak out and cause significant

damage to the natural environment. Numerical data concerning this problem are usually difficult to estimate.

There is no doubt that the use of crop protection chemicals should be limited or maybe they should be eliminated entirely. A precise analysis of expected side effects should be performed prior to the introduction of these toxic substances on the market. The threat posed by pesticides may be minimized by strict observance of the relevant regulations as well as by their proper use. The establishment of legal provisions which would regulate all issues related to crop protection chemicals is also very important.

From the ecological perspective, improving agricultural efficiency via chemicalization is a serious mistake, which may have dire consequences in the future. Appropriate steps should be taken to prevent the undesirable side effects of crop protection chemicals on the natural environment.

REFERENCES

ANDERSON T. A., KRUGER E. L., COATS J. R. 1994. *Enhanced degradation of mixture of three herbicides in rizosphere of a herbicide – tolerant plant*. Chemosphere, 28(8): 1551 - 557.

ATANIYAZOVA O. A., BAUMANN R. A., MUKHOPADHYAY U. A., VOGELAAR E. F., BOERSMA E. R. 2001. *Leveis of certain metals, organochlorine pesticides and dioxins in cord blood, human milk and some commonly used nutriens of the Aral Sea* (*Karakalpakstan Republic of Uzbecistan*). Acta Pediatr., 90: 801-808.

BALICKA N. 1983. *różne formy wzajemnego oddziaływania drobnoustrojów z herbicydami*. Post. Mikrob., 22(3/4): 291-299.

BANASZKIEWICZ T. 2003. *Chemiczne środki ochrony roślin – zagadnienia ogólne*. Wyd. UWM, Olsztyn, 114 ss.

BERGER B. M. 1998. *Parameters influencing biotransformation rates of phenylurea herbicides by soil microorganisms.* Pestic. Biochem. Physiol., 60: 71- 82.

BIESZCZAD S., SOBOTA J. (red.) 1993. *Zagrożenia, ochrona i kształtowanie środowiska przyrodniczo-rolniczego*. Wyd. Akademii Rolniczej, Wrocław, s. 15-24.

BIRNBBAUN L. S. 1994. *Endocrine effects of prenatal exposure to PCBs, dioxins and other xenobiotics. Implication for policy and future research*. Environ. Health Perspect., 102: 676-679.

BŁAŻEWICZ J., JUREK K., HORCZAK K. 2003. *Wpływ herbicydów stosowanych w uprawie jęczmienia browarnego na cechy użytkowe ziarna*. Acta Sci. Pol., 2(1): 53-61

BOJAKOWSKA I., GLIWICZ T. 2005. *Chloroorganiczne pestycydy i polichlorowane bifenyle w osadach rzek Polski.* Prz. Geol., 53(8): 646-655.

BRASHI I., PUSINO A., GESSA C., BOLLAG J. M. 2000. *Degradation of primisulfuron by a combination of chemical and microbiological processes*. J. Agric. Food Chem., 48: 2565-2571.

BZIUK M. Red. 2001. *Pestycydy, występowanie, oznaczanie i unieszkodliwianie*. WNT, Warszawa, 275 ss.

DAS A. C., DEBNATH A., MUKHERJEE D. 2003. *Effect of the herbicides oxadiazon and oxyfluorfen oh phosphates solubilizing microorganisms and their persistance in rice fields*. Chemosphere, 53: 217-221.

DUGGAN R. E. 1969. *Pesticide residues in foods.* Food and Drug Administration, Department of Health, Education and Welfare. Washington D. C., 160(1): 173-182.

DURSKA G. 2004. *Wpływ zaprawy nasiennej Baytan Uniwersal na występowanie drobnoustrojów w glebie pod uprawą pszenicy ozimej*. Acta Agr. Silv. ser. Agr. 42: 75-85.

Dyrektywą Rady *91/414/EEC z dnia 15 lipca 1991 dotycząca wprowadzenia do obrotu środków ochrony roślin* (Dz. U. L 230 z 19. 8. 1991).

FURCZAK J., KOŚCIELSKA D. 1997. *Ocena ubocznego oddziaływania fungicydu tetrakonazolu na grzyby saprofityczne oraz aktywność biochemiczna gleby piaszczystej i gliniastej*. Rocz. Gleb., 48(1/2): 49-58.

GRIFFITHS B. S., RITZ K., WHEATLEY R., KUAN H. L., BOAG B., CHRISTENSEN S., EKELUND F., SŘRENSEN S. 2001. *Response of sorption process of MCPA to the amound and origin of organic matter in a long-term experiment*. Europ. J. Soil Sci., 52: 279-286.

JAWARD F. M., FARRAR N. J., HARNER T., SWEETMAN A. J., JONES K. C. 2004. *Pasive air sampling of PCBs and organochlorine pesticides across Europe.* Environ. Sci. Technol., 38: 34-41.

JOHNSEN K., JACOBSEN C. S., TORSVIK V., SORENSEN J. 2001. *Pesticide effects on bacterial diversity in agricultural – a review*. Biol. Fertil. Soil., 33: 443-453.

JUBERG D. R. 2000. *An evaluation of endocrine modulators. Implications for human health*. Exotox. Environ. Safety., 45: 93-105.

KASZUBIAK H., MUSZYŃSKA M., DURSKA G. 1994. *Evaluation of microbial response to herbicides using various methods*. Pol. J. Soil Sci., 27(2): 131-136.

KAWKA A., KLOCKIEWICZ-KAMIŃSKA E., ANIOŁA J., CIERNIEWSKA A., GĄSIOROWSKI H. 1998. *Ocena niektórych wyróżników jakościowych odmian jęczmienia uprawnego w Polsce.* Pam. Puławy. Mater. Sem., 112: 85-91

KLIMACH A., WIECZOREK W. 1998. *Ocena wpływu kilku środków ochrony roślin na wybrane organizmy glebowe*. Post. Ochr. Roś., 38(2): 587-589.

KOBUS J. 1995. *Biologiczne procesy a kształtowanie żyzności gleby*. Zesz. Probl. Post. Nauk Rol., 421a: 209-219.

KOTRIKLA A., LEKKAS T. D., BLETSA G. 1997. *Toxity of the herbicyde atrazine, two of its degradation products and the herbicyde metolachlor on photosythetic microorganisms*. Fresenius Environ. Bull., 6: 502-507.

KUCHARSKI J., KARUZO – WANKIEWICZ L., KUCZYŃSKA L. 2004. *Wpływ zanieczyszczenia gleby Starane 250 EC na jej mikrobiologiczne właściwości*. Acta Agr. Silv. ser. Agr., 42: 257-263.

MALLATOU H., PAPPAS C., ALBANIS T. A. 2002. *Behaviour of pesticides lidanes, lindane and methyl parathion during manufacture, repening and storage of feta cheese*. Soc. Dairy Technol., 55(4): 211-216.

MCDONALD L., JEBELRIE S. JMADRAMOOTOO C. A., DOODS G. T. 1999. *Pesticide mobility on a hillside soil in St. Lucia*. Agr. Ecosyst. Environ., 72: 181-188.

MEGHARAJ M., KANTACHOTE D., SINGLETON I., NAIDU R. 2000. *Effect of long – term contamination of DDT on soil microflora with special reference to soil algae and algae transformation DDT*. Environ. Pollut., 109: 35-42.

MICHALCEWICZ W. 1995. *Wpływ pestycydów stosowanych w chemicznej ochronie roślin uprawnych na niektóre właściwości biologiczne gleby*. Rocz. Glebozn., 46(1/2): 53-64.

MICHALCEWICZ W. 2004. *Wpływ temperatury i wilgotności na oddziaływanie niektórych herbicydów na biomasę drobnoustrojów w glebie*. Acta Agr. Silv. ser. Agr., 42: 317-325.

MICHEL M. 1999. *Wykrywanie pozostałości środków ochrony roślin w glebie techniką HPLC*. Progr. Plant Protect., 39(1): 253-257.

MILLER W. R., SHARPE R. M. 1998. *Enviromental oestrogens and human reproductive cancers*. Endocr.-Relat. Cancer., 5: 69-96.

NANCY N. RAGSDALE. 1999. *The role of pesticides in agricultural crop protection.* Ann. NY Acad. Sci., 894(1): 199-205.

NOWAK A., ZBIEĆ I., GAWIŃSKA H., HREBIEŃ T. 1999. *Wpływ niektórych herbicydów pirydynowych na liczebność drobnoustrojów oraz zawartość biomasy żywych mikroorganizmów w glebie.* Fol. Univ. Agric. Stetin., 201 Agric., 78: 243-252.

NOWAK J. 1996. *Interakcje między biodegradacą tetrachlorwinfosu i chlorfenwinfosu a ilością biomasy żywych mikroorganizmów w różnych warunkach temperatury i wilgotności gleby*. Zesz. Nauk. AR Szczec. 173. Roln., 63: 191-199.

OSTROWSKI J. 1996. *Influence of soil macropores on amide herbicide leaching.* Rocz. Gleboz., 47(1/2): 5-10.

PRACZYK T., SKRZYPCZAK G. 2004. *Herbicydy*. PWRiL, Poznań, 274 ss.

PRZYBULEWSKA K., NOWAK A., HOPPEN B. 2004. *Wpływ temperatury na działanie pestycydów na przykładzie aktywności enzymatycznej wybranych bakterii glebowych.* Fol. Univ. Agric. Stetin., 234 Agr., 93: 333 - 340.

ROLA H., KIELOCH R. 2001. *Wpływ herbicydów na plonowanie, zdrowotność i skład chemiczny nasion wybranych odmian łubinu białego i zółtego*. Biul. Nauk. UWM, 12: 47-55.

Rozporządzenie Ministra Zdrowia z dnia 16 kwietnia 2004 roku w sprawie najwyższych dopuszczalnych pozostałości chemicznych środków ochrony roślin, które mogą znajdować się w środkach spożywczych lub na ich powierzchni (Dz. U.2006.192.1422).

RUSSEL S. 2005. *Znaczenie badań enzymów w środowisku glebowym*. Acta Agroph. Rozpr. i Monogr., 3: 5-6.

SADOWSKI J., KUCHARSKI M., ROLA H. 2001. *Pozostałości herbicydów w środowisku glebowo-wodnym*. Biul. Nauk. UWM, 12: 23-32.

SÁNCHEZ M. E., ESTRADA I. B., MARTINEZ O., MARTIN-VILLACORTA J., ALLER A., MORÁN A. 2004. *Influance of the application of sewage sludge on the degradation of pesticides in the soil*. Chemasphere, 57: 673-679.

SAWICKA B. 2004. *Jakość bulw Helianthus tuberosus L. w warunkach stosowania herbicydów*. Ann. UMCS, 59(3): 1245-1257.

SHEN L., WANIA F., TEIXINERIA G., MUIR D., BIGLEMAN T. 2005. *Atmospheric distribution and long-range transport behaviour of organochlorine pesticides jn North America*. Environ. Sci. Technol., 39: 409-420.

SŘRENSEN S. R., DING G. D., JACOBSEN C. S., WALKER A., AAMAND J. 2003. *Microbial degradation of isoproturon and related phenylurea herbicides in and below agricultural fields.* FEMS Microbiol. Ecol., 45: 1-11.

STRZELEC A. 1986. *Wpływ właściwości gleb na reakcje ich mikroflory na herbicydy*. Rocz. Glebozn., 37(1): 129-138.

SUKUL P. 2006. *Enzymatic activities and microbial biomass in soil as influenced by metaxyl residues*. Soil Biol. Biochem., 38: 320-326.

SWĘDRZYŃSKA D. 2004. *Wpływ karboksyny i tiuramu na aktywność nitrogenazy i rozwój wybranych grup drobnoustrojów glebowych ze szczególnym uwzględnieniem bakterii z rodzaju Azospirillum*. Acta Agr. Silv. ser. Agr., 42: 437-449.

TOTTEN I. L. A., GIGLIOTTI C. L., OFFENBERG J. H., BAKER J. E., EISSNREICH S. J. 2003. *Revaluation of air-water exchange fluxes of PCBs in Green Bay and Southern Lake, Michigan*. Environ. Sci. Technol., 37: 1739-1743.

TRASAR-CAPEDA C., LEIRÓS M. C., SEOANE S., GILSOTRES F. 2000. *Limitats of soil enzymes as indicators of soil pollution*. Soil Biol. Biochem., 32: 1867-1875.

URBAN M. 2000. *Ocena wrażliwości odmian jęczmienia i pszenicy na jarej na herbicydy*. Post. Ochr. Rośl., 40(1): 387-394.

Ustawa O Ochronie Roślin z dnia 18 grudnia 2003 roku (Dz.U.2006.171.1225)

WĘGOREK W. 1994. *Badanie wpływu pestycydów na środowisko rolnicze*. Post. Nauk Rol., 2: 59-64.

Weisglas-Kuperus N., Sas T. C. J, Koopman-Esseboom C., van der Zwan C. W., Ridder M. A. J., Keishuizen A., Hooijkaas H., Sauer P. J. J. 1995. *Immunological effects of background prenatal and postnal exposure to dioxins and polychlorinated biphenyls in Dutch infants*. Pediatr. Res., 38: 404-410.

Wg T. J., Fleet G. H., Heard G. M. 2005. *Pesticides a source of microbial contamination of salad vegetables*. J Food Microbiol., 1001: 237-250.

Wyszkowska J. 2002. *Effect of soil contamination with Treflan 480 EC on biochemical properties of soil*. Pol. J Environ. Stud., 11(1): 71-77.

Wyszkowska J. 2004. *Właściwości mikrobiologiczne gleby zanieczyszczonej herbicydem Triflurotox 250 EC*. Acta Agr. Silv. ser. Agr., 42: 463-473.

Wyszkowska J., Kucharski J. 2004. 311-319. *Biologiczne właściwości gleby zanieczyszczonej Chwastoxem Trio 540 SL*. Rocz. Gleboz., 55(1): 311-319.

Wyszkowski M., Wyszkowska J. 2004a. *Skład chemiczny rzepaku jarego i gorczycy białej a aktywność enzymatyczna gleby zanieczyszczone Treflenem 480 EC*. Ann. UMCS, 54(4): 1631-1638.

Wyszkowski M., Wyszkowska J. 2004b. *Współzależność między zawartością makroelementów w jęczmieniu jarym a aktywnością enzymatyczną gleby zanieczyszczonej Chwastoxem Trio 540 SL i Granstarem 75 WG*. Ann. UMCS, 54(4): 1639-1649.

Zaborowska M., Wyszkowska J., Kucharski j. 2005. *Rola cynku w organizmach roślinnych i zwierzęcych*. J. Elementol., 10 (3): 653-664.

J. Elementol. 2007, 12(2): 149–152

„OŁOWIANY DIABEŁ”

Jerzy Oleszkiewicz

Abstrakt

Prawie każda patologia jest wieloprzyczynowa. W dążeniu do opanowania agresji młodzieży, należy uwzględnić możliwość toksycznego wpływu ołowiu i kadmu na mózg. Wynika to z analizy pierwiastkowej włosów metodą spektroskopii absorpcji atomowej wykonanej w Wojskowej Akademii Technicznej w Warszawie. Udokumentowano, że nadmiar ołowiu i niedobór magnezu, który jest neutralizatorem tej neurotoksyny – jest znamienny i powszechny. Dlatego konieczna jest masowa suplementacja magnezem młodzieży szkolnej.

LEAD EVIL

Abstract

Nearly all pathologies are caused by more than one factor. While striving to subdue agression among adolescents one should take into consideration possible toxic effect of lead and cadmium on human brain. This conclusion is grounded on AAS assays of elements in hair, which were conducted at the Military University of Technology in Warsaw. The results proved that elevated levels of lead alongside insufficient amounts of magnesium, which can neutralize lead, were widespread and symptomatic. Thus, mass supplementation of magnesium among school children is needed.

Ołów to jedno z największych zagrożeń dla życia biologicznego na ziemi. Niestety, jest wszechobecny w naszym środowisku. Przenika do organizmów ludzkich i zwierzęcych drogą pokarmową, oddechową oraz przez skórę.

Być może, ten metal, znany od kilku tysięcy lat, odegrał istotną rolę w upadku cywilizacji, m.in. imperium faraonów i cesarstwa rzymskiego: starożytni używali ołowianych kotłów do produkcji wina gronowego, za pośrednictwem którego ołowiana trucizna przenikała do ich organizmów. Jest również hipoteza, wg której przyczyną degeneracji władców Kremla, znanych z dewiacji psychicznych i okrucieństw, mogło być korzystanie z zainstalowanych w XVII w. wodociągów z ołowianymi zbiornikami na wodę i rurami odprowadzającymi.

Ołów był od dawna znany jako niebezpieczna substancja, lecz na początku XX w. prawie każdy produkt codziennego użytku zawierał jakąś dawkę tego metalu. Poważne konsekwencje zdrowotne spowodowała dopiero rozpo-

częta w USA w 1923 r. produkcja ołowiu na skalę przemysłową, stanowiącego dodatek do paliw silników samochodowych. Amerykański badacz Patterson udokumentował, że przed 1923 r. w atmosferze nie było prawie w ogóle związków ołowiu, a od tego czasu ich stężenie stale i niebezpiecznie rosło. Usunięcie ołowiu z benzyny stało się jego życiowym celem. Doprowadził do uchwalenia słynnej ustawy Clean Air Act (uchwała o czystym powietrzu) z 1970 r. i do wprowadzenia w 1986 r. zakazu sprzedaży benzyny ołowiowej w Stanach Zjednoczonych. Niemal natychmiast poziom ołowiu we krwi Amerykanów obniżył się o 80%. Mimo to żyjący dzisiaj Amerykanie mają ok. 625 razy wyższe stężenie ołowiu we krwi niż ich przodkowie 100 lat temu. Konserwy i inne pojemniki lutowane z użyciem materiałów zawierających ołów wycofano ze sprzedaży w Ameryce dopiero w 1993 r.

W krajach uprzemysłowionych ilość ołowiu w atmosferze nadal rośnie, całkowicie legalnie, głównie za sprawą kopalń, przetwórstwa metali oraz kilku innych gałęzi przemysłu.

Od dawna wiadomo, że ołów jest neurotoksyną, może powodować zaburzenia zachowań, halucynacje, itp. (Bryson 2003)

W latach 70. prof. Bożena Hager-Małecka u 300 dzieci zamieszkałych w pobliżu Huty Metali Nieżelaznych w Szopienicach stwierdziła ołowicę. Z ogólnej liczby tych dzieci aż 10% uczęszczało do szkół specjalnych (Oleszkiewicz 1996).

W latach 80. badano zawartość metali toksycznych u dzieci z zaburzeniami psychosomatycznymi leczonych w Zespole Psychosomatycznym przy Wojewódzkim Szpitalu Dziecięcym w Warszawie. Niemal powszechne było, nawet wielokrotne, przekroczenie dopuszczalnych poziomów ołowiu oraz kadmu (zawartego w dymie tytoniowym). Równocześnie udokumentowano drastyczne niedobory naturalnych neutralizatorów powyższych toksyn, tj. magnezu i cynku. Niedobory ilościowe tych neutralizatorów powodują, że metale toksyczne włączając się w procesy metabolizmu przenikają do mózgu. Długotrwałe uzupełnianie magnezu (odtrutki ołowiu) i cynku – jako neutralizatora kadmu – najczęściej powodowało poprawę w zakresie nadpobudliwości psychomotorycznej dzieci. Wyniki tych prac były przedstawiane na Europejskim Kongresie Psychosomatycznym (Oleszkiewicz 1988) oraz na Światowej Konferencji Toksykologicznej w New Delhi (Darzynkiewicz 1993)

Badania laboratoryjne włosów prowadzono metodą spektroskopii absorpcji atomowej w Wojskowej Akademii Technicznej w Warszawie. Metodą tą przebadano już ponad 30 tys. Polaków ze wszystkich grup wiekowych. Nadmiar ołowiu i niedobór magnezu jest znamienny i powszechny (Dunicz-Sokołowska 2006). Inni autorzy również donoszą o pozytywnych wynikach suplementacji magnezem dzieci z nadpobudliwością psychoruchową i zaburzeniami koncentracji (Mousain-Bosc 2006, Starobrad-Hermelin 1997, Kozielec 2002)

Bardzo skutecznie prowadzą do utraty magnezu stres i narkotyki.

Wydaje się, że u dzieci z opóźnieniem sprawności intelektualnej i u których testy psychologiczne nie wykazują organicznego uszkodzenia mózgu, na-

leży uwzględniać celowość prowadzenia działań detoksykacyjnych. Detoksykacja i dożywienie mózgu również mogą się przyczyniać do polepszenia wyników pracy psychologów.

Niedobory substancji odżywczych w mózgu są trudno wykrywalne, gdyż nie manifestują się objawami niedożywienia. Ważny dla mózgu jest selen, witaminy: E, B1, B6, kwas nikotynowy i kwas foliowy. Według aktualnych poglądów, niedobór każdego z tych składników zaburza funkcjonowanie mózgu i może prowadzić do pogorszenia pamięci, reakcji depresyjnych i innej patologii. Zaskakujący jest wysoki odsetek mieszkańców Stanów Zjednoczonych i Anglii z niedoborami tych składników pożywienia.

Celowe jest zapoznanie z tym zagadnieniem pedagogów, zwłaszcza szkół specjalnych, gdzie uczą się dzieci z zaburzeniami psychosomatycznymi.

Wydaje się, że zaistniała obiektywna i pilna potrzeba masowej suplementacji magnezem młodzieży szkolnej, na podobnej zasadzie jak „szklanka mleka dla ucznia".

Szczecińska lekarka swoją pracą doktorską udokumentowała, że suplementacja preparatami magnezu wpływa w sposób istotny na układ psychiczny młodzieży z upośledzeniem umysłowym stopnia lekkiego, przez co zbliża ich do młodzieży normalnej (Drybańska-Kalita 1992)

Współczesna szkoła, m.in. dzięki komputeryzacji, zaburzyła równowagę między wysiłkiem intelektualnym i pamięciowym a rozwojem ruchowym. A przecież ćwiczenia fizyczne usprawniają m.in. dopływ krwi do mózgu oraz stymulują jego aktywność. Taki skutek aktywności fizycznej stał się już bezdyskusyjnym faktem.

Autor przekazuje wyrazy wdzięczności za dyskusje i pomoc w uzyskaniu piśmiennictwa Pani prof. Alfredzie Graczyk i Pani dr Aldonie Dunicz-Sokołowskiej.

PIŚMIENNICTWO

Bryson B. 2003. *Krótka historia prawie wszystkiego.* (*A Short History of Nearly Everything).* Wyd. Zyski i s-ka, ss. 162-173.

Darzynkiewicz K., Graczyk A., Oleszkiewicz J., Radomska K., Sokołowska M., Świątkowska E., Watras J. 1993. *Magnesium deficiency in Polish children and psychosomatic disturbances: Medical and psychological aspects.* J. of Toxicology, Occupational and Environmental Health., 2 (1): 84.

Drybańska-Kalita A. 1992. *Wpływ różnych sposobów suplementacji magnezem na stan zdrowia dzieci specjalnej troski.* Zakł. Med. Rodzinnej Inst. Chorób Wewnętrznych Pomorskiej Akademii Medycznej w Szczecinie (rozprawa doktorska).

Dunicz-Sokołowska A., Radomska K., Długaszek M., Graczyk A. 2006. *Contens of bioelements and toxic metals in the Polish population determined by hair analysis.* Part 1. *Children aged 1 to 10 years.* Magnes. Res., 34-35.

Dunicz-Sokołowska A., Graczyk A., Radomska K., Długaszek M., Wlaźlak E., Surkont G. 2006. Part 2. *Young persons aged 10 - 20 years.* Magnes. Res., 1-13.

Kozielec T., Radomska K., Kędzierska E., Graczyk A.,,Durska G., Karakiewicz B., Fabian W. 2002. *Wpływ suplementacji magnezem na stężenie ołowiu i kadmu w erytrocytach i we włosach u dzieci.* Pediatria Polska, LXXXVII, 4.

Mousain-Bosc M., Roche M., Polge A., Pradal-Prat D., Rapin J., Bali J.P. 2006. *Improvement of neurobehavioral disordes in children supplemented with magnesium – vitamin B6.* Magnes. Res., 46-52.

Oleszkiewicz J. 1996. *Zaburzenia koncentracji i nadpobudliwość to uleczalne choroby cywilizacji.* Warszawa, ss. 47

Oleszkiewicz J., Sokołowska M., Darzynkiewicz K., Watras J., Świątkowska E. 1988. *The deficit of magnesium and the superexcitability in children with psychosomatic troubles.* 17th Europ. Conf. on Psychosomatic Research, Marburg, Abstracts, 156.

Starobrad-Hermelin B., Kozielec T. 1997. *The effects of magnesium physiological supplementation on hyperativity in children with Attention Deficit Hyperactivity Disorder (ADHD). Positive response to magnesium oral loading test*. Magnes. Res., 10(2): 149-156.

J. Elementol. 2007, 12(2): 153–156

Z życia PTMag

ZAGROŻENIA W ZIEMI

Barbara Olszewska,

Urząd Miasta Olsztyna, Wydział Ochrony Środowiska

Rozwój przemysłowy i konieczność ochrony wód podziemnych często są trudne do pogodzenia. Dla wielu miast zanieczyszczenie wód podziemnych na terenach miejsko-przemysłowych stanowi niezwykle ważny problem, ponieważ w przeszłości miasta z reguły powstawały w dolinach rzecznych lub zlewniach rzek i wykorzystywały na swoje potrzeby wody podziemne z płytkich systemów wodonośnych.

Zmiany w zagospodarowaniu powierzchni ziemi i strukturze własnościowej, jakie miały miejsce w kilku ostatnich dekadach, przyczyniły się do powstania źródeł różnorodnych zanieczyszczeń wód podziemnych, objawiających się niejednorodnym rozprzestrzenianiem substancji zanieczyszczających na dużych obszarach.

Obecnie zarówno publiczne, jak i prywatne środki finansowe przeznacza się na identyfikację i analizę punktowych źródeł zanieczyszczeń. Podejmowane wysiłki nie zapewniają jednak wiarygodnej i kompleksowej ilościowej oceny wpływu tych źródeł na jakość wód podziemnych. Niedoskonałości klasycznego monitoringu wód podziemnych polegają na niedokładnym wykrywaniu punktowych ognisk zanieczyszczeń. Stwarza to trudności w hierarchizacji ognisk zanieczyszczeń, a w konsekwencji nieefektywne wydatkowanie środków finansowych na oczyszczanie wód podziemnych.

W ostatnich latach dokonano w niektórych krajach europejskich usprawnień procesu zarządzania zasobami zanieczyszczonych wód podziemnych. W pewnej mierze przyczyniła się do tego realizacja międzynarodowego projektu badawczego pt. *Zintegrowana koncepcja remediacji wód podziemnych – INCORE (1999–2003)*, finansowanego z 5. Programu Ramowego Badań, Rozwoju Technologicznego i Prezentacji Unii Europejskiej, w którym obok przedstawicieli instytutów naukowych i lokalnej administracji Niemiec, Włoch, Austrii i Francji uczestniczyli również Polacy – reprezentanci Państwowego Instytutu Geologicznego i Instytutu Ekologii Terenów Uprzemysłowionych.

Przykładem działań zmierzających do szybkiego oraz kompleksowego zastosowania procedur i narzędzi wypracowanych w INCORE, a jednocześnie dalszego ich doskonalenia, jest kolejny projekt pt. *Zarządzanie zasobami wód podziemnych na zanieczyszczonych terenach poprzemysłowych – MAGIC (2005–2008)*. Jego celem jest zastosowanie innowacyjnej metodologii badawczej, wypracowanej w projekcie INCORE, do identyfikacji zanieczyszczeń i doboru metod oczyszczania zanieczyszczonych wód podziemnych na czterech terenach badawczych w trzech krajach Unii Europejskiej, tj:

– w Polsce – na terenie składowiska odpadów poprzemysłowych w Trachach (powiat gliwicki) i na terenie dawnej gazowni w Olsztynie,
– w Czechach – na terenie dawnej koksowni w Vitkovicach k. Ostravy,
– w Niemczech – na terenie dawnej dzielnicy przemysłowej Feuerbach w Stuttgarcie.

Współpartnerami w realizacji projektu MAGIC są:

– Główny Instytut Górnictwa, Katowice, Polska – koordynator projektu;
– Instytut Ekologii Terenów Uprzemysłowionych, Katowice, Polska;
– Urząd Miasta Stuttgart, Niemcy;
– Instytut Zdrowia Publicznego, Ostrava, Republika Czeska;
– Państwowy Instytut Geologiczny, Warszawa, Polska;
– Urząd Miasta Olsztyna, Polska.

Urząd Miasta Olsztyna został zaproszony przez Państwowy Instytut Geologiczny do udziału w projekcie MAGIC jesienią 2004 r. Uzasadnieniem współpracy, niezależnie od wcześniejszych kontaktów, było uwzględnienie w „Programie Ochrony Środowiska m. Olsztyna na lata 2005–2008 z perspektywą do 2011 r." dawnych terenów powojskowych i poprzemysłowych, znajdujących się w granicach miasta i wymagających zbadania stopnia ich zanieczyszczenia i potencjalnego zagrożenia dla środowiska, na potrzeby przyszłej rekultywacji i rewitalizacji, umożliwiającej ponowne, współczesne ich zagospodarowanie.

Spośród znajdujących się w mieście różnych obszarów wymagających przebadania i oczyszczenia/sanacji oraz rewitalizacji – jako teren badawczy dla projektu MAGIC – zaproponowano rejon dawnej gazowni (funkcjonującej w latach 1889–1978, także podczas II wojny), położony w centrum miasta, w zakolu rzeki Łyny.

„Do końca XIX w. Olsztyn był małym, prowincjonalnym miastem – w 1864 r. liczył zaledwie 366 domów mieszkalnych.

Ważne strategiczne położenie miasta doceniono w II połowie XIX w., co przyczyniło się do budowy dróg łączących miasto z bliższymi i dalszymi ośrodkami miejskimi. Komunikację drogową uzupełniono wkrótce kolejową. W 1890 r. w Olsztynie oddano do użytku duży, jak na tamte czasy, węzeł kolejowy, co w niedługim czasie przyczyniło się do rozwoju budownictwa prywatnego i komunalnego, handlu produktami spożywczymi, drewnem i maszynami. W tym okresie kronikarze odnotowali istnienie w mieście 147 lamp naftowych i rosnące zapotrzebowanie na ogólnie dostępną energię.

Zanim jednak w Olsztynie uruchomiono na rzece Łynie pierwszą elektrownię (1907), władze miejskie zainteresowały się najnowszym europejskim wynalazkiem, jakim był gaz. Wiosną 1887 r. rada miasta Olsztyna powołała komisję, która miała zająć się organizacją budowy gazowni. W ramach prac przygotowawczych miejscy radni zwizytowali obiekt funkcjonujący w Poznaniu. Ostatecznie na posiedzeniu komisji w dniu 13 października 1887 r. podjęto uchwałę o budowie gazowni, finansowanej z kasy miejskiej. Na potrzeby tej budowy zakupiono od prywatnego właściciela plac nad Łyną. W budowie konstrukcji obiektów gazowni brała udział murarska firma Toffel, natomiast montaż retort, maszyn, urządzeń i zbiorników wykonywała firma „Schulz & Sackur" z Berlina.

*Uroczyste otwarcie Miejskiej Gazowni w Olsztynie nastąpiło 15 października 1889 r." (*Tomasz Śrutkowski, „Śladami płomienia gazowego", Wyd.El-Set, 1999).

Na przełomie XIX i XX w. produkcja gazu, który oświetlał 206 latarń i 400 mieszkań wynosiła ponad 700 tys. m^3; w 1918 r. miasto otrzymywało do 3,4 mln m^3 gazu.

Gazownia pracowała bezawaryjnie przez cały okres II wojny światowej; zaopatrywała miasto w gaz do lat 70. XX w., gdy do Olsztyna dotarł gaz ziemny.

Budynki i większość instalacji naziemnych starej gazowni rozebrano ostatecznie na przełomie lat 70. i 80. ub. wieku. Na terenie przekazanym władzom miasta przez Zakład Gazowniczy (początek lat 90.) pozostał ostatni zbiornik smół pogazowych oraz całe „bogactwo" podziemnej infrastruktury w postaci fundamentów obiektów gazowni, sieci instalacji technologicznych, itp.

W stosunkowo krótkim czasie po demontażu budynków starej gazowni (1993–1994), z pozostawionego starego zbiornika smół pogazowych zaczęły wyciekać wody zanieczyszczone związkami ropopochodnymi i przedostawać się do pobliskiej rzeki Łyny, stwarzając zagrożenie dla jakości jej wód.

Prace i działania, jakie wtedy podjęto dla ochrony wód rzeki, doprowadziły ostatecznie do opróżnienia zbiornika z zanieczyszczonych wód oraz rozebrania jego części naziemnej. Nieusuniętą pozostałą część podziemną, wypełnioną smołami pogazowymi, które zagęszczono torfem, przykryto folią i przysypano ziemią, tworząc niewielki kurhan, zaopatrzony w 4 kominki, umożliwiające uchodzenie z jego wnętrza substancji gazowych.

Przedstawione wyżej postępowanie zmierzające do likwidacji zagrożenia zanieczyszczenia wód rzeki Łyny wyciekami ze zbiornika starej gazowni w dużym stopniu było spowodowane brakiem środków finansowych, niezbędnych do wydobycia smolistych pozostałości i ich transportu do miejsca unieszkodliwienia.

Projekt MAGIC, realizowany w ramach Programu INTERREG III B CADSES z udziałem dotacji ze środków ERDF w wysokości 75%, wpisuje się w Działanie 4.1. „Wspieranie ochrony środowiska i zarządzanie zasobami na-

turalnymi" Priorytet 4: „Ochrona środowiska, zarządzanie zasobami naturalnymi i ochrona środowiska przed zagrożeniem" tego Programu.

Długoterminowym celem projektu MAGIC jest rewitalizacja terenów zdegradowanych przez przemysł w wyniku eliminacji źródeł zanieczyszczania wód podziemnych.

Bezpośrednim celem projektu jest zastosowanie nowych metod badawczych do identyfikacji źródeł zanieczyszczania, ich oceny i dostosowania do oczyszczania wód podziemnych na czterech terenach badawczych w trzech krajach UE – Czechach, Polsce i w Niemczech.

ZAKRES PROJEKTU MAGIC

Projekt składa się z sześciu pakietów zadaniowych (PZ). Pakiety 1–4 obejmują techniczne działania w obrębie czterech terenów badawczych, gdzie można się spodziewać zanieczyszczonych wód podziemnych. Pakiety 5 i 6 dotyczą wdrażania proponowanej metodologii i procedur zarządzania środowiskiem przez administrację samorządową.

PZ 1 – zbieranie danych, opracowanie informacji o obszarach badawczych oraz planowanie badań;

PZ 2 – badania terenowe i analizy laboratoryjne;

PZ 3 – tworzenie matematycznych modeli przemieszczania się zanieczyszczeń w wodach podziemnych;

PZ 4 – ocena rezultatów badań;

PZ 5 – wdrożenie osiągnięć projektu do procedur administracyjnych;

PZ 6 – zarządzanie projektem i jego koordynacja oraz upowszechnianie rezultatów.

Całkowity budżet projektu wynosi 2 355 000,00 euro; dotacja z ERDF to 1 547 250,00 euro, a udział Partnerów Projektu stanowi 807 750,00 euro.

Internetowy adres projektu:www.magic-cadses.com

Recenzenci artykułów zamieszczonych w Journal of Elementology, vol. 12 no 2 z 2007 roku

Wiesław Barabasz, Magdalena Maj-Żurawska, Wacław Mozolewski, Kazimierz Pasternak, Józefa Wiatr

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Uniwersytet Warmińsko-Mazurski w Olsztynie
pl. Łódzki 2, 10-759 Olsztyn
kaga@uwm.edu.pl

Dr hab. Jadwiga Wierzbowska
Uniwersytet Warmińsko-Mazurski w Olsztynie
ul. Michała Oczapowskiego 8, 10-719 Olsztyn
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University of Warmia and Mazury in Olsztyn
Pl. Łódzki 2, 10-759 Olsztyn, Poland
kaga@uwm.edu.pl

Dr hab. Jadwiga Wierzbowska
University of Warmia and Mazury in Olsztyn
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jawierz@uwm.edu.pl

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