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GROWTH OF LETTUCE (*Lactuca sativa*) AT MINE TAILINGS FROM SHIHEUNG/KOREA, AND THE EFFECT OF ADDED ORGANIC COMPLEXANTS ON METAL MOBILITIES

WZROST SAŁATY (*Lactuca sativa*) NA HAŁDACH KOPALNI W POBLIŻU SHIHEUNG (KOREA) ORAZ WPŁYW DODATKU ORGANICZNEGO CZYNNIKA KOMPLEKSUJĄCEGO NA MOBILNOŚĆ METALI

Abstract: In a series of pot experiments, lettuce seedlings (*Lactuca sativa*) were grown at tailings left from the abandoned mine at Shiheung/Korea, to test possible remediation strategies of the site. The test substrate contained far too much As, Cd, Cu, Pb, and Zn for agricultural use, available K and Mg were sufficient, but organic carbon, P, and N were low. About 1/3 to 1/2 of the original sulphidic material was already weathered to yield carbonates and hydroxides. At the base of the experimental pots, moderately contaminated soil was put to monitor downward migration. NaCl, as well as complexants EDTA, NTA, citrate, and malate were added together with the irrigation water to influence plant uptake. Addition of 20 % compost to the tailings, as well as plants growth in an artificially Pb/Cd-contaminated farmland soil, were run within further experimental series. Bulk soils, root soils, and base soils were sampled before the experiment, and after 5, 10 and 15 days of plant growth. All solids were submitted to a Tessier-like sequential leaching procedure, to yield exchangeables, carbonaceous, Mn-oxide, Fe-oxide, sulphidic, and residual fractions, and the resulting extracts finally were analyzed by ICP-OES and AAS methods.

The effect of plant growth on speciation shifts in root and bulk soils as well the effects of added complexants thereof is discussed. Exchangeable amounts were significantly higher in root soils than in bulk soils, but Fe-oxide bound fractions were lower. Complexant addition increased exchangeable contaminants in bulk soil, but decreased them in root soil. Washout led to increase of exchangeables in base soils, particularly after 15 days. Citrate caused the most effective washouts. Complexant addition also tended to shift contaminants from the carbonaceous to the exchangeable fraction. Concentrations in carbonaceous (weak acid-releasable) fractions from the mine tailings passed a flat maximum versus time of plant growth. During plant growth, the total amount of the Mn-oxide fraction increased, and the Fe-oxide fraction decreased. The uptake of Pb and Cd from the tailings was less than from artificially polluted farmland soils, like predicted from the mobility of these contaminants in sequential leaching. EDTA, citrate and compost addition increased

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most elemental concentrations in lettuce leaves with respect to the untreated samples, whereas malate and NaCl were largely ineffective.

Keywords: soil sequential leaching, mobility changes during plant growth, mine tailings, complexing agents addition, *Lactuca sativa*

In Korea, tailings deposited from past mining activities, have led to environmental concerns with respect to groundwater pollution, dust formation, and agricultural production [1–3]. In order to establish a suitable and natural soil life again, phytoremediation with general accumulator plants has to be considered, which is a soft, cheap, but long-term strategy. Green plants should grow at contaminated sites, accumulate the toxicants in harvestable parts, and finally have to be ashed as hazardous waste after harvest. But tailings of abandoned mine sites are very low in nutrients, which are essential for the growth of green plants, like nitrogen, phosphorus and magnesium. A major disadvantage of phytoextraction might be the long-term scale needed to achieve levels tolerable for consumption. Therefore, within this study, it was investigated how to accelerate the extractions and to reduce the number of necessary extractions.

Mine tailings may contain parts of the original sulphidic ores, as well as weathered layers thereof, and also silicates and carbonates. During phytoextraction, mobile phases should get rapidly exhausted, but dissolution of sulphidic residues inside the tailings as well as washout to the groundwater should be rather avoided. Second, the transport inside the green plant from the roots to the harvestable leaves, should be high.

Root exudates are mainly low molecular organic compounds with complexation capabilities, which feed the microbial biomass at the rhizosphere [4], and also increase phosphate as well as metal availability by dissolution of carbonates and pedogenic oxides. Experiments were needed to test, whether complexant addition from outside would accelerate the soil to plant transfer by dissolution of carbonates and pedogenic oxides (without touching the residual sulphides), or if complexants may even mask free metal ions versus uptake. Formation of soluble complexes may increase the transport inside the green plants towards the leaves, provided there is an uptake of the complexants, but also increase the transport down to the groundwater. Sequential leaching of the solid substrates sampled during plant growth should indicate, if plant growth and addition of complexants induce speciation changes in the test substrate.

EDTA and NTA were chosen as hardly degradable synthetic chelating agents. Contrary to these, citric and malic acid were chosen as natural chelators, readily available to soil bacteria and the rhizosphere, which might increase soil biomass. Though arsenic does not yield such complexes, its availability is expected to be influenced by dissolution of pedogenic oxides, where it has got adsorbed after weathering of the original minerals.

Within a series of pot experiments, lettuce seedlings (*Lactuca sativa* L.) were selected to grow at material sampled from tailings at the abandoned mine site at Shiheung/Korea, because lettuce seedlings are known as universal accumulator plants [5], and to withstand a wide variety of nutrient and toxicant levels. Almost all plants survived for 15 days, but these crops, however, contained toxic levels of Pb and Cd, and their consumption has to be prohibited. The metal transfer to the plant leaves was less than from a farmland soil soaked with Pb-Cd solution. Fertilization with compost was beneficial with respect to N and Mg supply, whereas addition of complexants like EDTA, citric acid or malic acid induced even more depletion in these essential nutrient elements in the plant tissues. Beneath an N-source, compost also adds a variety of natural microorganisms to the initially dead substrate. The effect of EDTA and NTA as an N-source, however, seemed marginal.

In order to monitor possible speciation changes of metals, As and P from plant growth and complexant addition, bulk soils, root soils, and base soils were sampled initially, and after 5, 10, and 15 days. They were submitted to a Tessier type sequential leaching procedure [6], to yield exchangeables, carbonaceous, Mn-oxides, Fe-oxides, sulphides, and residuals.

Material and methods

The test substrates were collected from the Shiheung Cu-Pb-Zn abandoned mine tailings. Artificially polluted soils were made by mixing the soil with Cd and Pb solution in a ratio 9:1, shaken for 2 hours, and washed 3 times with deionised water. In order to check whether mobile metals were discharges into non-polluted soils, non-polluted soils were put at the bottom of each pot. Each 40 cm^3 of chelating agent solution (EDTA, NTA, citric acid, malic acid) were added into the pots every three days. On the other days, 40 cm^3 of distilled water was added. Eluates from the pots were returned again. In order to evaluate nutrient effects, a series of tailings mixed with 20 % of compost was run.

Seedlings were grown in a greenhouse equipped with supplementary lighting for 14 hours per day, at 24–28 ^{o}C. 10 day-old seedlings were transplanted in sets of three into soil equivalent to 1 kg of dry mass, placed in 15 plastic pots of 15 cm diameter. Transplanted seedlings were well watered for 3 day before chelating agent application. After 5, 10 and 15 days, the plants were harvested.

For *aqua regia* digestion of soil samples, 2.00 g of solid sample were weighed into long-necked glass vessels, 21 cm^3 conc. HCl and 7 cm^3 conc. HNO_3 were added, and a reflux condenser was put on top. After some hours break, the sample was heated for 1/2 hour to 60^{o} and for 1 1/2 hours to 140^{o}, filtered, and made up to 100 cm^3.

For sequential leaching, 1.00 g solid sample was weighed into centrifuge vessels, shaken for 2 hours with 20 cm^3 1 M magnesium chloride, centrifuged and filtered to yield the exchangeable fraction. After washing with water, subsequent extractions with 20 cm^3 0.16 M acetic acid overnight, 20 cm^3 0.5 M hydroxylamine pH 2 overnight, and 20 cm^3 0.2 M oxalate buffer pH 3 overnight, were done. Fraction V was obtained after two times oxidation with 3 cm^3 H_2O_2 and 2 cm^3 dil. HNO_3 (35 cm^3 in 1 dm^3) till almost evaporation in a heating block, and final uptake into 20 cm^3 0.16 M acetic acid (from step II). The residue was treated with *aqua regia* like the original sample (modified after [6]).

Reagent solutions for sequential leaching:

– 0.5 M magnesium chloride = 101.5 g $MgCl_2 \cdot 6H_2O$ in 1 dm^3 H_2O, adjusted to pH 7 with approximately 7 cm^3 1 + 1 NH_3;

– 0.16 M acetic acid = 9.1 cm^3 conc. acetic acid in 1 dm^3;
– 0.5 M hydroxylamine pH 2 = 31.75 g $NH_2OH \cdot HCl$ + 3.1 cm^3 HNO_3 in 1 dm^3;
– 0.2 M oxalate buffer pH 3 = 12.6 g oxalic acid + 14.2 g ammonium oxalate in 1 dm^3;
– diluted HNO_3 = 35 cm^3 conc. HNO_3 in 1 dm^3.

In order to simulate the effect of leachate spills of soluble salts on farmland soils, a sample was artificially polluted with Cd-solution (100 mg/kg Cd) and Pb-solution (30000 mg/kg Pb). The soil was shaken with the pollutant solution for 2 hours, and finally washed 3 times with deionised water.

Bulk soil, root soil, and base soil, as well as plant roots and plant leaves have been separately analysed by ICP-OES multielement spectrometry. The elements Al, As, Ba, Be, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, Sr, V and Zn were read in fractions II to VI of sequential leaching, as well as in the *aqua regia* soil extracts. For the exchangeable fraction of sequential leaching (fraction I), neutralized magnesium chloride was preferred over ammonium nitrate(V), ammonium acetate or others. The investigated substrate was poor in nitrogen, not far away from the seaside, and the pH of this extract would be close to natural conditions. In ICP-OES, however, the high Mg levels encountered in the exchangeable fraction, yield some spectral interferences (mainly increased background noise). Therefore, the exchangeable fraction was only read for Cd, Cr, Cu, Fe, Mn, Na, P, and also S. Exchangeable Pb was determined by flame-AAS. Exchangeable sulphur (presumably sulphate) is a reasonable parameter, whereas sulphur losses might occur in other fractions and in the *aqua regia* digestion. In addition, in the fractions III and IV, Ge, Sn and Sb were read, which are not mobile in fractions I and II due to their chemical properties. But Ge is volatile in the *aqua regia* digestion, and no total element contents could be obtained from this procedure. In spite of high arsenic, the Ge, Sn and Sb data were quite low and at the limits of detection, therefore they were not considered for further evaluations.

In the nitric(V)/chloric(VII) acid digest of lettuce plants and lettuce roots, the elements Al, As, Ba, Be, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, Sr, V, and Zn were determined.

Results and discussion

Main phases of the substrate

Tailings from an abandoned mine site at Shiheung/Korea, near Seoul capital city, were analysed by *aqua regia* extraction, as well as by sequential leaching. The farmland soil sampled nearby, was much higher in K than the tailings, and slightly enriched in Ba, Cr, Ni and V with respect to usual farmland soils, but extraordinary low in Ca and P. It was polluted with soluble Pb and Cd salts in the laboratory, in order to show possible contrasts in mobility and soil to plant transfer. High Pb, Zn, Cd, Cu and As met in the tailings are known to be hazardous to soil life and to agricultural use (Table 1). The pH of the substrate has been classified as weakly acid, but the sulphides in the mine tailings have a great potential of acidification, when they get oxidized. With respect to intended agricultural use, the action levels for As, Cd, Cu, Pb, and Zn, are superseded, whereas in case of Ba, Be, Co, Cr, Cu, Mo, Ni, and V, no action is needed. The action level is the concentration which is sufficient to provoke toxic effects.

Table 1

Composition ranges of the mine tailing test substrate and the artificially polluted farmland soil found in *aqua regia*, with respect to target and action levels for agricultural use, proposed by Eikmann and Kloke 1993 [8]

	Test substrates	Polluted farmland soil	Target of remediation	Need of action
pH	5.5–6.9	5.7	—	—
Loss on ignition	1.08–2.90 %	3.42 %	—	—
Organic carbon	0.09–0.93 %	1.17 %	—	—
Main elements:				
Al	1.80–2.31 %	4.62 %	—	—
Ca	3.67–4.83 %	0.13 %	—	—
Fe	5.46–7.39 %	4.78 %	—	—
K	0.066–0.084 %	1.16 %	—	—
Mg	0.63–0.81 %	1.08 %	—	—
Mn	0.104–0.132 %	0.042 %	—	—
Na	0.008–0.014 %	0.015 %	—	—
P	0.060–0.083 %	0.051 %	—	—
Hazardous to soil life:				
As	31–423 mg/kg	4.4 mg/kg	40	50
Cd	97–164	22.9	2	5
Cu	2678–4334	67	50	200
Pb	2.09–2.93 %	1.01 %	0.05 %	0.10 %
Zn	1.17–2.41 %	0.041 %	0.030 %	0.060 %
Others, ambient levels:				
Ba	22–51 mg/kg	200 mg/kg	300	1500
Be	0.85–1.07	2.1	2	5
Co	32–39	14	200	1000
Cr	33–45	158	200	500
Li	7–9	28	—	—
Mo	< 0.2	0.8	20	100
Ni	21–38	51	100	200
Sr	58–73	10	—	—
V	22–32	56	100	400

Sequential leaching roughly helps to assign the amounts bound to carbonates, hydroxides, sulphides, organics and the almost insoluble silicate matrix (Table 2).

The test substrate contains some rests of sulphidic minerals, which are partially weathered to yield carbonates and oxides. About 2 thirds was material insoluble in *aqua regia*, or at least not found. In *aqua regia*, among main elements, silicon and titanium were not determined.

Table 2

Main elements found in *aqua regia*, expressed as main phases due to sequential leaching

	Main phases due to sequential leaching	
Al	0.43–0.59 % $Al(OH)_3$	3.49–3.93 % Al_2O_3
Ca	5.99–8.96 % $CaCO_3$	1.72–1.83 % CaO
Cu	0.58–0.65 % CuS	
Fe	2.85–3.40 % $Fe(OH)_3$	9.75– 10.60 % FeS_2
K	0.08–0.10 % K_2O	
Mg	0.17–0.60 % $MgCO_3$	1.03–1.16 % MgO
Mn	0.06–0.07 % MnOOH	0.09–0.13 % MnO
P	0.14–0.19 % P_2O_5	
Pb	1.06–1.94 % $PbCO_3$	1.97–3.83 % PbS
Zn	1.26–2.34 % $ZnCO_3$	0.59–1.42 % ZnS
Insoluble	62–65.4 %	

The range of main elements given in Tables 1 and 2 has been taken from 5 subsamples, used before the start of the pot experiments. Some inhomogeneity with respect to the oxidation of the subsamples was noticed. It is well known, however, that ZnS and CdS are much easier oxidizable than PbS and CuS.

Fractions I+II+III have been assigned to carbonate minerals $CaCO_3$, $MgCO_3$, $PbCO_3$, and $ZnCO_3$. Fractions III+IV contain $Fe(OH)_3$, $Al(OH)_3$ and MnOOH, which are hardly seen in X-ray diffraction because they are partly amorphous. As organic carbon is very low, fractions V and VI may be attributed to be sulphides. In fraction VI, some silicates also begin to dissolve, but as K and Na are low, silicate dissolution may be limited. In case of Al, Ca, Mg, and Mn, the rest has been termed as “oxides” in the geochemical traditional way, which may be in reality also organics, phosphates or silicates.

The sulphide bound metals, mainly Pb, Zn and Cu, are not immediately available, but impose a great risk of metal intoxication and acidification after aeration.

Similar results had been found in the tailings at a historical Pb mining site in Northern England, appreciable Pb and Zn was found residual, and major parts of Zn was bound to the Mn/Fe-oxides, whereas Cd was highly exchangeable (7–52 %) [9]. In Pb-Zn mine tailings from the “old lead belt” in Missouri/USA, sequential extraction showed that major parts were still bound to the residual and oxidizable fractions [10].

Predicted effects of complexants upon main phases

Among the main components of the mine tailings, Fe is supposed to form the strongest complexes. Addition of complexants should first of all dissolve the pedogenic oxides and liberate all the traces bound therein.

In Table 3, solubilities of assumed main phases present in the test substrate in presence of EDTA, citrate and oxalate, have been calculated. EDTA and citrate have been added during the pot experiments, and oxalate has been used in sequential leaching. For calculations, total soluble sulphide was estimated as 0.1 mol/dm^3.

The added complexants, as well as steps I–IV in sequential leaching, mainly attack the weathered phases, like $Fe(OH)_3$, $PbCO_3$, $ZnCO_3$ and $PbSO_4$, whereas the original sulphidic ore minerals are left untouched. (Data for FeS_2 were not available).

Table 3

Calculations from solubility products and complex formation constants

pH = 5–7	EDTA	Citrate	Oxalate	Remarks
$Fe(OH)_3$	Soluble	Soluble	Soluble pH < 6–7	
Fe_2S_3	Insoluble	Soluble pH < 6	Insoluble	
$PbSO_4$	Soluble	Partially soluble	Oxalate precipitation	Soluble in $MgCl_2$-extract
$PbCO_3$	Soluble	Insoluble	Oxalate precipitation	
PbS	Insoluble	Insoluble	Insoluble	
CdS	Insoluble	—	—	
CuS	Insoluble	—	—	
ZnS	Partially soluble	—	—	
MnS	Soluble	—	Oxalate precipitation	

Exchangeable fraction

The exchangeable fraction is regarded to be immediately plant mobile and plant available. Readily soluble as well as amounts adsorbed upon various surfaces (humics, clay minerals) as well as the soluble contents of living cells are found here. The sum of the equivalents exchangeable versus neutral $BaCl_2$-solution is termed the cation exchange capacity, which is known as a common parameter to characterize soils (Table 4).

Table 4

Sum of exchanged equivalents (meq/kg) versus Mg^{2+}

	Soil + Cd/Pb	Tailing	Tailing + compost	Soil + Cd/Pb	Tailing	Tailing + compost
	meq(Ca-Cd-Cr-Cu-Fe-Mn-Pb-Zn)/kg			meq (Cd-Cu-Fe-Zn)/kg		
Before plant growth	72	42	69	1.87	3.90	8.09
Bulk soil, 5 days	77	34	54	1.52	3.69	3.97
Bulk soil, 10 days	66	34	47	1.52	4.53	4.07
Bulk soil, 15 days	81	38	57	3.27	4.70	6.10
Base soil, 5 days	124	212	226	0.12	0.63	0.52
Base soil, 10 days	122	221	161	0.12	0.54	0.76
Base soil, 15 days	276	366	407	10.2	29.6	61.2
Root soil, 5 days	180	769	784	34.1	212	211
Root soil, 10 days	183	785	159*	33.0	206	9.6*
Root soil, 15 days	185	514	509	26.0	146	139

* Flyer, mainly from fixation of Zn.

Table 4 contains the calculated sum of milli-equivalents exchanged versus Mg in the first step of sequential leaching (Na and K were not determined in this extract). From this, the cation exchange capacity seems rather low in the mere tailings, but approached usual values in base soils, and extraordinary high values in the root soils of the tailings. Differences in exchanged cations were less for the artificially contaminated farmland soil than for the tailings.

Transport of exchangeables to the base soil is remarkably seen after 15 days (see below). Within Tables 5 and 6, the effect of added complexants is illustrated from differences in exchanged milliequivalents with respect to the untreated tailings. Complexants and compost increased exchangeable cations from bulk soil (Ca-Cd--Cr-Cu-Fe-Mn-Pb-Zn) in all cases. In the base soil, however, a decrease of exchangeable equivalents was noted for EDTA and NTA, whereas it was variable for the other cases. In the root soil, all exchangeables decreased with respect to the untreated sample.

Table 5

Milliequivalents (Ca-Cd-Cr-Cu-Fe-Mn-Pb-Zn) exchanged versus Mg^{2+} in fraction I

	Compost	EDTA	NTA	Citrate	Malate	NaCl	Citrate + NaCl
Before plant growth	26.7	–0.3	–13.9	–5.1		32.3	
Bulk soil, 5 days	20.3	19.2	14.8	15.6	13.2	32.1	23.0
Bulk soil, 10 days	12.5	12.8	11.8	7.3	7.5	15.3	16.6
Bulk soil, 15 days	19.8	29.3	17.7	16.6	0.4	33.9	25.8
Base soil, 5 days	14.7	–45.8	–80.1	–68.1	–69.3	–49.5	–69.2
Base soil, 10 days	–60.1	–87.5	–163.2	118.2	95.2		131.6
Base soil, 15 days	40.7	–24.8	–78.7	172.0	–73.4	93.3	14.8
Root soil, 5 days	14.5	–2.0	–624	12.0	4.5	48.1	41.7
Root soil, 10 days	–626	–276	–278	–285	–279	–280	–269
Root soil, 15 days	–5.0	–17.0	–25.8	–20.9	41.4	18.9	66.0

Table 6

Milliequivalents Cd-Cu-Fe-Zn exchanged versus Mg^{2+} in fraction I

	Compost	EDTA	NTA	Citrate	Malate	NaCl	Citrate + NaCl
Before plant growth	4.19	–0.21	–1.02	–0.68		3.94	
Bulk soil, 5 days	0.28	3.24	3.75	2.90	0.38	1.04	0.91
Bulk soil, 10 days	–0.45	3.18	4.19	0.65	–0.93	–0.39	–0.30
Bulk soil, 15 days	1.40	9.24	8.20	5.50	–0.67	3.88	3.35
Base soil, 5 days	–0.11	6.42	1.58	1.24	–0.07	0.24	–0.31
Base soil, 10 days	0.23	9.02	0.63	47.8	16.6		26.8
Base soil, 15 days	31.6	14.2	–7.9	44.9	–10.0	34.2	11.9
Root soil, 5 days	–0.12	–39.4	–195	–49.9	–54.4	–35.6	–35.6
Root soil, 10 days	–196	–79.5	–86.2	–87.9	–78.7	–80.6	–78.5
Root soil, 15 days	–6.28	–18.7	–23.0	–31.0	0.81	–21.9	–8.3

If just Cd-Cu-Fe-Zn are considered (main component = Zn), which give significantly more stable complexes with any of the added complexants than Mg, a similar increase of exchangeables was noted for EDTA, NTA, and citric acid from the bulk samples, whereas conditions varied for the rest. In the base soil, exchangeable Cd-Cu-Fe-Zn increased in most cases, but strongly decreased in the root soil. The difference between the data in Tables 5 and 6 is mainly due to Ca+Pb.

The added chelators are supposed to be released into the exchangeable fraction with $MgCl_2$, unless they have been degraded. Anglesite ($PbSO_4$), which might have been possibly formed from PbS within the tailings, is also sufficiently soluble to appear in the exchangeable fraction. Decrease of exchangeables in the root soil diminishes the difference between bulk and root soil. Differences due to different degradation rates of the added chelators during plant growth were not apparent.

From the artificially polluted farmland soil, which had been prepared by soaking with Cd- or Pb-solution, release of exchangeable Ca, Cu, Zn and S was higher from bulk soil, but lower from root and base soil, with respect to the mine tailings (Table 4).

Carbonaceous fraction

In the second step of sequential leaching, weak acid is applied to dissolve carbonates and the mobile fractions of those cations, which are likely to yield hydroxides in step one, or which are exchangeable against H^+. Usually, buffer pH 5 is applied. In the Korean mine tailings, however, Ca is low, and slightly more acid conditions (0,16M acetic acid) were chosen to ensure possible dissolution of $PbCO_3$ (cerussite and dolomite).

Within the artificially polluted farmland soil, released matter in fraction II was lower in Ca-Cd-Cu-K-Mn-P-S-Sr-Zn, but higher in Mg. In both cases, the release versus time curves passed a flat maximum. The release of As-Be-Cr-Li-Mo-V into fraction II was negligible. A look at the proportions of released matter in the exchangeable over the carbonaceous fraction will show possible shifts of mobilities due to the addition of complexants.

In the artificially polluted soil, exchangeable Cd, Pb, Mn and Cr were significantly higher than for the carbonaceous fraction In the tailings, exchangeable Cd and Cr was higher, and exchangeable Ca, Cu, Fe, Mn, Pb, and Zn was lower than for the carbonaceous fraction. As predicted, addition of complexants resulted in shifts from the carbonaceous to the exchangable fraction. EDTA was the most effective, mobilizing Ca, Cd, Cu, Mn, and Zn. NTA mobilized Cd-Cu-Zn, citric acid mobilized Cd and Zn, and malic acid Cu and Mn.

During plant growth in the untreated tailings, the equivalents of weak-acid-mobile heavy metals (Pb-Cd-Zn), alkaline earths (Ca-Mg-Sr), nutrients (K+P), and Fe+Mn all pass a maximum, possibly at maximum growth rate. The Cd-Pb-treated soil showed the same effects at a lower level (Table 8). The proportion of concentrations of root soil over bulk soil released from the mine tailings during plant growth, however, steadily increased for a lot of elements (contaminants Cd-Pb-Zn, mains Al-Ca-K-Mn-S. traces Co-Li-Sr). Addition of complexants interrupted these trends, except for Cd. Na, which had been added together with the complexants, got steadily enriched in the root soil during plant growth.

Table 7

Results for the second fraction of sequential leaching

	Tailings before		Polluted farmland soil	
	Min.	Max	Min.	Max
Al	0.5	3.1	2.6	7.1
Ba	3.4	13.3	5.8	11.8
Ca	8467	9086	84	701
Cd	13.2	26.1	0.41	1.31
Co	0.97	1.64	<	0.69
Cu	48.4	94.7	0.17	4.07
Fe	1.21	5.81	0.60	2.11
K	35.1	62.4	<	22.6
Mg	292	625	1467	1977
Mn	81.9	95.3	3.5	19.9
Na	16.1	29.9	6.1	26.0
Ni	0.67	0.89	<	0.43
P	2.92	5.02	<	1.22
Pb	1000	2041	1152	3893
S	32.3	48.9	<	3.9
Sr	5.90	6.83	0.15	0.75
Zn	2368	3611	19.8	81.9

Table 8

Milliequivalents released in the carbonaceous fraction during plant growth
(equivalents of physiologically similar elements have been added for simplification)

Concentration [meq]	Mine tailing				Cd-Pb-pretreated soil			
	before	5 days	10 days	15 days	before	5 days	10 days	15 days
Ca + Mg + Sr	475	1081	1156	285	165	194	169	126
Fe + Mn	3.64	8.56	8.63	3.56	0.63	1.29	1.13	0.44
Pb + Zn + Cd	121	258	186	66	22.8	39.3	34.2	11.7
$K_2O + P_2O_5$	59.2	92.0	98.8	0.44	30.0	29.0	26.9	<

Addition of compost immobilised Cu-Cd-P-Sr-Zn in bulk soil, and Al-Ba-Ca-Mn in root soil. More Cd-Pb-Zn was mobilised from base soil, and more Zn from root soil. Maybe, the nutrient input increased biomass, which is not fully attacked in this step, but also increased weathering of the base soil.

Fractions III and IV – the "pedogenic oxides"

In the weathering of primary minerals, Fe-Al-Mn hydroxides are formed, which scavenge a lot of other ions from the soil solution. Sequential leaching with acidified hydroxylamine (fraction III) and oxalate buffer (fraction IV) should discriminate a Mn-rich phase and an Fe-rich phase (reviewed eg [11]). The load of these "pedogenic oxides" is usually homogenous, whereas the amount of pedogenics may vary within a soil profile. In soils, they may be also formed from degradation of humics, or oxidation of soluble Fe^{2+}. They get dissolved by complexants, like root exudates (see Table 3).

40 cm^3 of 20 mM chelating agent in aqueous solutions have been added every 3 days to 1 kg of soil in each pot, starting on the 4th day; this means 3.2 mmole. Al and Fe form the strongest complexes with all chelators added. Added complexants are much less than all pedogenic oxides, present in the test substrates. The pedogenic oxides shifted from fraction III to fraction IV.

Growing roots release considerable amounts of organic carbon into the rhizosphere. This leads to the reduction of MnO_2 by organic acids, reduction/chelation of Fe^{3+} by phenolics, or phosphate mobilization from Al/Fe-phosphates by citric acid [12].

During plant growth, the pedogenic oxides increased in fraction III, and decreased in fraction IV, the sum remained surprisingly constant. For the Mn-oxides (fraction III), EDTA, NTA and citrate increased this effect, whereas Pb^{2+} pretreatment and compost retarded it. For the Fe-oxides (fraction IV), all followed this trend, except NaCl and malate. In the base soils, pedogenic oxides may be formed from washout and precipitation of divalent iron; there were the same trends, but less pronounced (Tables 9 and 10).

In root soils, decrease of pedogenics in fraction IV occurred in all cases. Fraction III increased again, except for compost treatment, and for the Cd-Pb treated farmland soil. Similar effects were noted at other mine tailing sites [13].

In the Mn-rich hydroxide fraction (fraction III), at the untreated tailings, enrichment for the root soils occurred just for Cd-Co-Cu-V, whereas the root soil contained less Al-As-B-Ni-Zn. But almost all additions (all except citrate) caused enrichment of Al and As in the root soil.

Within this fraction, Cu got the most strongly enriched element the in root soil, but this enrichment was lowered during plant growth from compost and NaCl addition. At the artificially polluted soil sample, root soil got also depleted in Al-As-Cu-Ni-Pb. When the action of various additives is compared with conditions at the untreated tailings,

EDTA and NTA addition lowered the differences of already initially depleted Al-Cr-Cu between root and bulk soil, but they increased the differences for Cd-Mn-Ni-Zn. NTA and citrate addition increased contaminant Cd in the root soils; and EDTA treatment lowered Co-Mn-Fe-Ni-Zn in root soil. The excess of Mn-oxide bound V in root soil was further increased by NaCl addition.

In the Fe-rich hydroxide fraction (fraction IV) of root soils from the untreated tailings, less As-B-Fe-Ni-Sb-Sn-Zn was found, but no element got enriched with respect to the corresponding bulk samples. A lot of similar root soil depletions were noted for various treatments also. Compared with the conditions met in the untreated tailings, EDTA even enlarged the root soil depletions for Cd-Co-Mn-Pb, NTA for Co-Mo-Pb, malate for Co-Cu-Mn, and NaCl for Zn.

Table 9

Pedogenic oxides in bulk soils during plant growth; sum of milli-equivalents Fe+Al+Mn

Hydroxyl- -amine, pH = 2	Untreated tailings	Tailing + EDTA	Tailing + NTA	Tailing + citrate	Tailing + malate	Tailing + NaCl	Tailing + citrate + NaCl	Tailing + compost	Cd/Pb-treated soil
Before	161	139	164	158		241		185	219
5 days	253	203	194	190	199	308	300	227	285
10 days	200	363	366	285	389	429	554	195	253
15 days	565	573	502	478	142	198	160	543	657
Oxalate pH = 3									
Before	1036	1008	1023	1024		899		1012	915
5 days	724	932	754	814	1006	667	599	678	576
10 days	620	429	384	400	438	347	374	596	583
15 days	360	406	423	500	1016	1018	1012	402	448

Table 10

Pedogenic oxides in base soils during plant growth; sum of milli-equivalents Fe+Al+Mn

Hydroxyl- -amine pH = 2	Untreated tailings	Tailing + EDTA	Tailing + NTA	Tailing + citrate	Tailing + malate	Tailing + NaCl	Tailing + citrate + NaCl	Tailing + compost	Cd/Pb-treated soil
Before	161	139	164	158		241		185	219
5 days	173	230	283	228	257	199	249	196	264
10 days	99	223	309	238	376		329	208	213
15 days	271	352	347	479	368	347	366	365	369
Oxalate pH = 3									
Before	1036	1008	1023	1024	×	899	×	1012	915
5 days	560	505	481	510	565	439	485	562	472
10 days	449	553	1031	214	263	×	323	603	432
15 days	295	366	359	524	307	362	296	364	221

Table 11

Pedogenic oxides in root soils during plant growth; sum of milli-equivalents Fe+Al+Mn

Hydroxyl-amine pH = 2	Untreated tailings	Tailing + EDTA	Tailing + NTA	Tailing + citrate	Tailing + malate	Tailing + NaCl	Tailing + citrate + NaCl	Tailing + compost	Cd/Pb treated soil
Before	161	139	164	158		241		185	219
5 days	321	290	295	266	307	331	337	383	72
10 days	362	426	393	371	402	429	408	184	77
15 days	367	386	399	393	421	451	414	370	116
Oxalate pH = 3									
Before	1036	1008	1023	1024	×	899	×	1012	915
5 days	533	611	668	667	558	600	617	574	609
10 days	479	456	561	372	357	344	351	527	531
15 days	354	441	421	451	414	434	436	380	559

The oxidizable fraction

After extraction of the pedogenic oxides, oxidation of the samples was performed to destruct organics and sulphides, and the resulting solubles were extracted like in fraction II. As the organic carbon content of the test substrates was very low, mainly sulphides were found here, just from the root soils some rests of the rhizosphere might have contributed. This was a main fraction for the contaminants met in the tailings, and represented unweathered parts. With respect to the sum of leached amounts, median Cu was 43.9 %, median Cd was 25.4 % median Pb was 23.7 %, median Zn was 19.2 % and median Mn was 10.2 % found here. Just Fe and As were low, with a median of 0.21 % As and 0.24 % of totally leached, because they were got mainly as residuals.

In the root soils from the untreated tailings, less As-Ca-Cd-Cu-Fe-Mg-Mn-Pb-Sr-Zn than in the bulk soils was found, but none got enriched. From the artificially contaminated soil sample, depletion of Cu-P-Pb, as well as enrichment of Zn was noted.

Addition of compost and complexants to the tailings mainly increased the proportion of root over bulk soil in this organic/sulphidic fraction, thus decreasing the differences between root and bulk soil. Each case was different, and the significance of the effects should be proven in more experiments. For example, Cd and Zn in the root soils got enriched from compost, NTA, citrate, malate, and NaCl, Cu got enriched from EDTA, NTA, malate, and NaCl, and Pb just from compost and citrate. K-Li-Mg-P-S were increased in the root soil by compost-NTA-citrate-malate addition during plant growth.

Mobile sulphur

Aerobic bacterial oxidation of sulphides present in the tailings may lead to elution of sulphate, mobilization of metals and significant acidification of the drainage waters. Soil micro-organisms steadily mineralize organically-bound sulphur to yield sulphate, which is either readily consumed by higher plants, or rapidly washed out [14]. Sulphur (presumably sulphate) found in the exchangeable fraction, has been found within a range expectable for arable soils (Table 12). Arable soils (of Central Europe) contain 200–800 mg/kg of total sulphur, of which 60–90 % are organically-bound. The addition of compost was no significant source of mobile S.

Addition of NaCl, and NaCl + citric acid did not change the level of exchangeable sulphur, whereas the complexants lowered exchangeable sulphate to about half. The artificially polluted sample yielded drastically less sulphate. Root soil was slightly higher, and washout to base soil was not seen in the soluble fraction. Plant growth and respective sulphate uptake were of negligible effect. Reasons may be changes of sulphatase activities, or uptake by soil microbes.

Within the subsequently eluted “carbonaceous fraction”, the sulphur eluted from the tailings before plant growth, was in about the same range than the exchangeable. During plant growth, “carbonaceous” sulphur passed a strong maximum after 5–10 days, then went down to zero, and might approach the original values again. Complexants seemed to accelerate this wave, whereas compost addition had no significant effect on sulphur mobility. In the root soils, exchangeable sulphur was higher than in the corresponding

Table 12

Effect of various treatments on exchangeable sulphur

	Untreated tailings	Tailing + EDTA	Tailing + NTA	Tailing + citrate	Tailing + malate	Tailing + NaCl	Tailing + citrate + NaCl	Tailing + compost	Cd/Pb treated soil
Before	51.5	27.6	22.7	24.6		86.1		55.1	3.8
Soil:									
5 days	44.3	26.7	25.8	18.5	24.4	69.0	59.1	50.6	4.8
10 days	33.7	20.6	26.6	19.0	18.8	49.1	52.9	40.2	4.3
15 days	43.7	30.6	24.7	15.2	22.7	49.7	50.7	51.3	7.0
Base soil:									
5 days	53.8	28.2	21.3	25.6	24.1	44.4	26.4	56.6	4.0
10 days	22.2	19.5	42.8					68.1	3.8
Root soil:									
5 days	67.2	40.1	33.0	35.0	34.5	82.0	82.2	72.2	6.5
10 days	54.1							66.6	6.0

bulk soils, whereas for the "carbonaceous" fraction, this was reverse; the sum of both fractions, however, was always smaller for the root soils, indicating plant uptake.

Potassium

According to the Austrian guidelines for correct fertilization [15], supply of mobile K for medium soils within 113–212 mg/kg is classified as sufficient. Potassium in the weak-acid leachable fraction at the start of the experiments was about 40 mg/kg, but usually exchangeable K is more than weak-acid-leachable. Though exchangeable K had not been measured, it can be concluded from fraction II and the differences between *aqua regia* and the sum of fractions II+V+VI that the K supply in the mine tailings is sufficient, and it is high in the root and base soils. The compost addition did not increase mobile K. The base soil was generally higher, and the root soil was generally lower in mobile K.

Effects on the soil-plant transfer

The primary goal of this experimental series was to increase the soil to plant transfer of contaminant metals [7]. Chelates increase metal solubility in soil, overcome the diffusional limitation of metal in the rhizosphere, and facilitate translocation from roots to shoots. Inside the plant, formation of metal complexes may facilitate the transport to the shoots, and change toxicity effects.

In the lettuce leaves grown at the artificially contaminated farmland soil, Pb, Cd, and surprisingly Ba, were significantly higher than from the tailings (Table 13). This matches the predictions of sequential leaching, in which added Cd was largely recovered as exchangeable, and added Pb mainly as exchangeable + carbonaceous. On the other hand, high levels in the tailings caused higher Cu and Zn in lettuce leaves from the mine soils. Decreasing K, Mg, and P during plant growth might indicate the toxic effects of the metals.

Table 13

Selected element contents of lettuce leaves grown at mine tailings and at the artificially contaminated farmland soil

Contents [mg/kg]	After 5 days at tailings	After 10 days at tailings	After 15 days at tailings	After 5 days at contaminated soil	After 10 days at contaminated soil	After 15 days at contaminated soil
Cd	19.8	24.3	19.0	67.8	70.1	36.5
Pb	48.5	57.2	37.4	1323	2411	805
Ba	9.6	11.1	5.3	50.9	50.8	32.3
Cu	12.9	20.8	12.8	8.1	9.3	8.8
Zn	284	465	309	76	64	111
% K	2.51	3.50	3.19	2.60	1.63	1.86
% Na	0.284	0.467	0.433	0.231	0.104	0.154
% Mg	0.145	0.181	0.139	0.159	0.095	0.097
% P	0.178	0.221	0.236	0.225	0.215	0.150

Table 14

Concentrations in lettuce leaves grown at treated mine tailings over those grown at untreated mine tailings, after 5 days, 10 days, and 15 days of growth

	EDTA			NTA		Citrate			Malate			NaCl			NaCl + Citrate		
	after 5d	after 10d	after 15d	after 5d	after 10d	after 5d	after 10d	after 15d	after 5d	after 10d	after 15d	after 5d	after 10d	after 15d	after 5d	after 10d	after 15d
Al	1.47	12.8	0.86	0.66	5.41	2.20	1.54	1.13	1.41	1.05	1.50	1.25	0.70	0.70	1.62	1.21	0.92
Ba	0.90	1.74	0.90	0.21	1.41	1.08	0.92	1.21	0.91	0.89	1.27	1.42	0.48	1.06	1.37	1.02	1.84
Ca	1.13	1.26	1.07	0.30	0.65	1.07	0.79	0.81	1.13	0.57	0.81	1.04	0.64	0.83	1.19	0.81	0.96
Cd	3.35	2.42	5.38	0.23	0.57	0.87	1.20	0.88	0.82	0.48	1.15	0.93	1.26	1.83	1.05	1.29	1.99
Co	2.65	16.1	5.17	0.78	5.11	3.26	1.97	2.18	1.50	1.25	1.83	0.98	0.75	1.14	1.29	1.27	1.49
Cr	1.07	3.16	0.43	0.37	1.25	1.45	0.58	0.74	0.97	0.41	0.78	1.59	0.33	0.49	1.29	0.86	0.45
Cu	7.39	22.2	7.54	2.25	6.79	4.19	1.74	1.34	1.32	0.73	1.37	1.16	0.65	0.81	2.41	1.11	1.04
Fe	1.38	19.9	0.71	0.70	6.14	2.25	1.83	1.09	1.01	1.11	1.48	1.31	0.72	0.71	1.61	1.16	0.73
K	1.34	0.71	0.82	0.46	0.46	1.03	0.98	0.69	1.04	0.51	0.74	1.35	0.76	0.90	1.26	0.72	0.80
Li	1.10	7.52	1.03	0.45	3.12	1.31	1.17	0.89	1.13	0.63	1.01	1.17	0.67	0.71	1.32	0.92	0.77
Mg	1.56	1.67	1.57	0.37	0.74	1.25	1.12	1.19	1.13	0.56	0.79	1.53	0.82	1.24	1.43	1.12	1.25
Mn	6.02	5.18	9.41	0.61	1.64	4.09	3.51	3.83	2.90	1.17	2.19	2.96	1.92	2.35	3.18	3.28	3.40
Mo	1.23	1.68	0.73	0.14	1.21	0.33	0.66	0.67	0.62	0.35	0.35	0.50	0.58	0.64	0.73	0.49	0.28
Na	5.29	3.01	4.74	2.38	1.73	1.92	3.03	2.05	1.35	0.55	0.72	3.78	2.33	3.09	2.75	1.85	2.25
Ni	1.14	6.54	1.12	0.92	3.04	2.26	1.32	1.15	1.60	0.84	0.80	1.16	0.59	0.76	1.04	0.98	0.59
P	1.12	0.82	0.65	0.63	0.74	1.00	0.88	0.61	1.09	0.67	0.66	1.10	0.66	0.60	0.86	0.74	0.51
Pb	13.6	48.2	14.2	3.97	19.0	3.91	3.33	1.99	2.23	1.58	2.84	1.14	0.93	1.01	1.58	2.92	1.13
Sr	1.18	1.44	1.12	0.31	0.88	1.20	1.00	1.02	1.15	0.62	0.84	1.14	0.62	0.91	1.12	0.84	0.96
Zn	5.29	9.82	6.94	1.52	2.50	3.73	1.68	1.84	2.53	1.17	1.48	1.18	0.75	1.05	1.50	1.16	1.37

When the concentrations encountered in the lettuce plant leaves grown at the mere tailings, are divided over the respective concentrations met in treated tailings, values 1 mean an increased extraction effect (Table 14). From this, EDTA and compost addition were the most effective. EDTA increased Cd-Co-Cu-Mn-Pb-Zn concentrations in the leaves, but also Al-Li-Mg-Na-Ni-Sr. Citrate acted like EDTA, but is more moderate. NTA worked just for Cu-Na-Pb-Zn, and malate addition caused just moderate increase of Al-Co-Mn-Pb-Zn. Addition of NaCl had +/– no effect, except for higher Na and Mn levels. When NaCl and citrate were applied simultaneously, the action of citrate was just lowered. Compost admixture with the test substrate increased almost all elements, except Ca-Mo-P, which are essential for plant growth, possibly due to a competition effect with introduced soil bacteria.

Transfer to the base soil

Without the addition of complexants, the washout of metal cations (Cu, Pb, Cr) from columns of bare non-contaminated soil to deeper layers was marginal, even if addition of $CuSO_4$ to wineyard soils had occurred some years before. Similarly, added soluble Mo, V, and Se got rapidly fixed within upper layers [16]. To the contrary, complexing agents effectively washed out heavy metal contaminations from soils polluted with soluble salts, within 20 pore volumes in 10–15 hours [17]. Equilibrium conditions can be estimated from constants given in table 15, but there is no equilibrium during plant growth at all. Citrate, tartrate and oxalate were applied as a model for plant root exudates, and EDTA and DTPA as artificial and hardly-degradable substances. All were effective in removing contaminations of soluble Cd, Pb, Cr, and Cu, but the amount depended on the soil type. EDTA and DTPA leached 4–5 times more macronutrients, such as Ca, Mg, and Fe [17]. Mobility in the soil column has to be considered with respect to groundwater protection. Transfer from bulk soil to base soil may indicate washout reactions. In particular, objections exist against hardly degradable man-made complexants. EDTA is readily leachable, and 50–60 % of added EDTA could be recovered in aqueous soil extracts [18].

Complexants like root exudates, or added chelators, are supposed to increase metal mobility by dissolution of Fe-oxides and various sulphides. In order to monitor downward migration during the experiment, each pot contained a layer of base soil, which was submitted to *aqua regia* digestion and sequential leaching also. This base soil was contaminated with Cd, Cu, Zn, and above all, with Pb, and in addition to the tailings, also with Cr and Ni. Below the lettuce plants grown on untreated tailings, most of the fractions of main and contaminant elements steadily declined, like Mn-Cd-Pb-Zn in fractions II, III, and IV (carbonaceous, Mn-hydroxides, Fe-hydroxides), Fe-Cu in fraction IV (Fe-hydroxides), as well as Fe-Pb in fraction VI (residuals). High amounts of the main contaminants Cd-Cu-Pb-Zn, as well as Fe and Mn had moved to the exchangeable fraction after 15 days, which indicates a load readily adsorbed from the soil solution.

Table 15

Equilibrium constants (pK) and solubility products (pLp) used for calculations Ox = oxalate; Pb forms its complex with H-citrate

	EDTA	Citrates	Oxalates	Sulphides	Others
H^+	2.0/2.67/6.16/10.26	3.13/4.76/6.40	1.27/4.27	7.04/14.92	—
Fe^{3+}	25.1	25	25	Fe_2S_3: 88	$Fe(OH)_3$: 37.4
Mn	14.04		14.96	MnS: 15.2	
Ca	10.69		8.4	—	$CaCO_3$: 8.32
Pb	18.04	PbH: 6.5	PbOx: 9.32	PbS: 29.0	$PbCO_3$: 12.82 $PbSO_4$: 7.74
Cu	18.8		CuOx: 7.64	CuS: 37.4	
Zn	16.5		ZnOx: 7.56	ZnS: 23.0	$ZnCO_3$: 10.2
Cd	16.46		CdOx: 7.04	CdS: 28.0	

Remark: from this, the solubility of $PbSO_4$ would be 28 mg Pb/dm^3. Extraction in the exchangeable fraction was performed for 1 g in 20 cm^3, which means 0.56 mg Pb, in 1 g means 560 mg/kg. Maximum 90 mg/kg have been found.

Both EDTA and compost addition during plant growth slightly increased exchangeable soil fractions, and lowered the decrease of the moderately mobiles. NaCl addition increased the washout of contaminants, but not of Fe and Mn. The most significant effects, however were noted from citrate addition – almost all fractions of contaminant Cd-Cu-Pb-Zn steadily increased during plant growth. This means a high downward flux. Citrate strongly complexed Fe, which seems to be mobilized from the test substrates, and reprecipitated in the base soils, whereas EDTA and NTA also react with Ca and Mg. On the other hand, citrate addition had low effects on the plant soil transfer.

EDTA was shown to be slowly biodegraded to CO_2 in soils and sediments under aerobic conditions [19], but there is probably no degradation of added EDTA and NTA during this experiment. Degradation of citrate and malate is possibly done by soil bacteria, but it is at least slow enough to increase transport to the base soils. In metal polluted substrates, respiration and enzyme activities are significantly lowered, but activities involved in the N-, P- and S-cycling are much more involved than those in the C-cycle [20].

Conclusions

Lettuce plants grown at mine tailings from Shiheung/Korea were capable to transfer a lot of contaminant elements to their harvestable leaves. When EDTA or citrate solutions, or compost, were added, both the trace element concentration in the lettuce leaves as well as downward migration was increased. Whereas EDTA caused the strongest effects for the plant tissues, citrate was the most effective to promote downward migration, and adsorption in deeper soil layers.

Speciation changes due to the action of complexants and plant growth can be monitored by a Tessier-type sequential leaching procedure. Exchangeable sulphur was rather constant, but weak acid mobile ("carbonaceous") sulphur at first increased, and then was depleted at the probable maximum growth rate of the roots. In the root soils, increase of Mn-oxide bound fractions (hydroxylamine leachable) occurred, and simultaneous decrease of Fe-oxide bound fractions (oxalate leachable). Intoxications from complexants might be indicated by wiping away the differences between root and bulk soils, whereas intense consumption enlarges these differences.

Beneath beneficial effects in nutrient supply [7] to promote plant growth and microbial activities, compost addition favoured the transfer of contaminant metals to the harvestable parts of the lettuce plants. Thus, fixation of contaminants could not be achieved, but phytoremediation with lettuce seedlings in presence of compost would be promising. Simultaneous addition of compost and complexants might be tried within further experiments.

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WZROST SAŁATY (*Lactuca sativa*) NA HAŁDACH KOPALNI W POBLIŻU SHIHEUNG (KOREA) ORAZ WPŁYW DODATKU ORGANICZNEGO CZYNNIKA KOMPLEKSUJĄCEGO NA MOBILNOŚĆ METALI

Abstrakt: Przedmiotem badań były sadzonki sałaty (*Lactuca sativa*), uprawiane na hałdach opuszczonej kopalni w pobliżu Shiheung (Korea) w celu oceny możliwości ich wykorzystania w rekultywacji terenu. Badane podłoże zawierało zbyt dużo As, Cd, Cu, Pb i Zn, aby mogło być wykorzystane do celów rolniczych. Zawartości K i Mg były wystarczające, ale stężenie węgla organicznego P i N było małe. Około 1/3 do 1/2 wcześniej istniejących związków siarczkowych uległo zwietrzeniu oraz przekształceniu w węglany i wodorotlenki. W celu śledzenia migracji zanieczyszczeń w pojemnikach umieszczono średnio zanieczyszczoną glebę. Do wody, którą podlewano rośliny, dodawano NaCl, jak również kompleksotwórcze EDTA, NTA, cytryniany i jabłczany. Badano wzrost roślin na glebie zawierającej 20 % kompostu z odpadów oraz sztucznie zanieczyszczonej Pb / Cd. Próbki gleby, w której rosły rośliny, próbki gleby pobranej z obszarów przy korzeniu oraz próbki głębszych warstw gleby, były badane przed i po 5, 10 15 dniach wzrostu roślin. Wszystkie próbki gleby były poddane ekstrakcji zgodnie z procedurą sekwencyjną Tessiera. Pozwoliło to na wyodrębnienie frakcji wymiennej, węglanowej, tlenku Mn, tlenku Fe, siarczkowej oraz pozostałych frakcji. Roztwory po ekstrakcji analizowano za pomocą ICP-OES i metody AAS.

Omówiono wpływ zmian specjacyjnych w warstwie korzeniowej i w całej glebie na wzrost roślin, a także wpływ związków kompleksujących. Frakcje wymienne były znacznie większe w warstwie korzeniowej niż w całej glebie, ale frakcje związane tlenków Fe były mniejsze. Dodanie związków kompleksujących spowodowało wzrost zanieczyszczeń w wymiennej frakcji gleby, ale ich zmniejszenie w warstwie korzeniowej. Wymywanie doprowadziło do wzrostu frakcji wymiennej w głębszych warstwach gleby, w szczególności po 15 dniach. Cytrynian był najskuteczniejszy w procesie wymywania. Ponadto związki kompleksujące powodowały tendencję do przenoszenia zanieczyszczeń z frakcji węglowych do frakcji wymiennej. Zależność stężenia we frakcjach węglanowych (uwalnianych przez słabe kwasy) od czasu wzrostu rośliny wykazywała płaskie maksimum. Podczas wzrostu roślin łączna liczba frakcji tlenków Mn rosła, a frakcja tlenków Fe malała. Pobieranie Pb i Cd z odpadów był mniejszy niż z gleb zanieczyszczonych sztucznie, co było zgodne z informacjami o ruchliwości tych zanieczyszczeń otrzymanymi doświadczalnie. Dodanie EDTA, cytrynianu i kompostu powodowało wzrost stężenia większości pierwiastków w liściach w porównaniu do próbek kontrolnych. Dodanie jabłczanów i NaCl przeważnie było mało skuteczne.

Słowa kluczowe: ługowanie gleby, zmiany mobilności podczas wzrostu roślin, odpady kopalniane, dodawanie związków kompleksujących, *Lactuca sativa*

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INTEGRAL ASSESSMENT OF THE TRANSBOUNDARY MESTA RIVER TROPHIC POLLUTION IN BULGARIA

KOMPLEKSOWA OCENA ZANIECZYSZCZENIA TROFICZNEGO TRANSGRANICZNEJ RZEKI MESTY W BUŁGARII

Abstract: A conceptual model for integrated river pollution assessment is proposed. Integral assessment of the trophic pollution of the transboundary Mesta River has been made for the initial (background) and final (reference) point of the Bulgarian section by means of some basic indicators. A time period characterizing the economic activity in the country during the transition to market economy has been considered for evaluation of the indicators.

The dynamics of the indicators: BOD_5, nitrates(V), nitrates(III), integral index for the trophic pollution level, average annual water amount and biotic index has been analyzed. The integral index is based on the organic and nutrients loading suspended and dissolved substances. Long-term trends seasonal patterns and data set structures are studied by the use of statistical analysis.

A trend towards reduction of the indicator values has been identified for background and reference point during the considered period (1989–1995). Only the nitrate(V) content in the background point exhibits a trend towards increasing the values due to natural processes in the catchment. On the basis of the retrospective analysis the conclusion can be drawn that the water quality needs the normative requirements of the river category in Bulgaria.

Keywords: quality assessment, water pollution, trend, integral indices

The European Framework Water Directive [1] requires an integrated approach to the water quality management that includes integration of water use and preserving of ecosystems, of quantity and quality of surface and groundwater, of the measures intended for management (action plan) on the basis of the physical and functional harmony of catchment elements.

The main objective of the integrated management is the sustainable utilization of water resources on the basis of the economic and social goals and the ecological requirements at water basin level.

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The European Water Directive demands "good quality" as a target for all water bodies within the range of the water basin. In the case of surface waters the "good status" is defined on the basis of physicochemical, hydrobiological and hydromorphological characteristics. The water quality indicators are parameters characterizing the type of pollution and its impact on the functioning of river ecosystems and water quality formation.

The basic water quality indicators are the indices for organic and nutrient pollution, the indices for pollution with harmful substances as the toxicity index, heavy metal content, petroproduct content, etc., the indices for the level of pollution, which are used for evaluating the effectiveness of water quality management. Some basic indicators have been applied for the assessment of the trophic pollution of the Mesta River.

The transboundary Mesta River flows in the western part of Bulgaria (Fig. 1).

The catchment area of the Bulgarian part of the river is 2768 km^2 with an average altitude of 1318 m. It is situated in a mountainous region, which is characterized by high

Fig. 1. Catchment area of Mesta River in Bulgarian section

degree of afforestations and 93 settlements with 135 000 inhabitants. The forests comprise 50 % of the catchment area [2]. Above the benchmark of 1100 m there is no anthropogenic activity in the Mesta River catchment that could exert negative impact on water quality, which is formed under natural conditions. Below the benchmark of 1100 m the water of the Mesta River is polluted by household and industrial wastewater from the settlements, agriculture, soil erosion and other sources.

The objective of the present work is to identify the tendencies in the dynamics of the basic indicators of organic and nutrient pollution (trophic pollution) in the beginning (background point) and in the end (reference point) of the Bulgarian section of the Mesta River.

The indicators are evaluated for a period, characterizing the economic activity in the country after the transition from the so-called totalitarian to market economy.

The dynamics of the indicators reflects the degree of the anthropogenic impact in the Mesta River catchment in the Bulgarian section.

Materials and methods

The present work considers the basic indicators for organic and nutrient pollution of the Mesta River water as BOD_5, nitrates and the integral index for the level of organic and nutrient pollution [3], the average annual water amount and the biotic index. The biological quality of the Mesta River at the background and the reference point has been determined on the basis of the biotic index information. The biological quality of water in Bulgaria is determined according to an Irish and a Bulgarian biotic index [4, 5].

A system of integral ecological indices has been worked out to assess the degree of pollution of running waters (Ip), sediments (Is) and rivers (Ir) [6].

The system of integrated indices for the level of river pollution is based on the ecological integrity of river flow [1]. Ecological integrity can only be achieved if physical, chemical and biological integrity simultaneously occur.

The integral index for the level of trophic pollution is based on the indices MES, BOD, COD, TOC, N_{tot}, P_{tot}, NO_3^-, NO_2^-, PO_4^{3-} and O_2 [7–9]. To assess the level of trophic pollution for a certain time period, it is necessary to determine the ratios between the actually measured values of the individual variables (C_i) and standard values ($C_{i,0}$) for a particular water body, or:

$$\gamma_I = C_i/C_{i,0} \tag{1}$$

The integral index for the level of trophic pollution for a certain period of time should be determined as the arithmetic mean of all measured values of individual parameters, or:

$$I_t = \frac{I}{n}\sum_{j=1}^{n}\sum_{i=1}^{m}\frac{C_{i,j}}{C_{i,0}} \tag{2}$$

m – number of indices,
n – number of determination for indicator parameters [6, 10, 11].

It is important for the targets of the water quality management system to follow the mullti-annual dynamics of the average annual and maximal values of the mentioned indices. The assessment of water quality is based on information from three typical points – Yakoruda in the upper course, Momina Kula in the middle and Hadzhidimovo in the lower course of the river at a distance of 23 km from the border with Greece.

The water quality indicators are parameters characterizing the type of pollution and its impact on the functioning of river ecosystems and water quality formation.

Information from National System for Water Monitoring for a period 1990–2003 is analyzed. The information from the monitoring is standardized by help of Z-transformation.

The information about BOD_5 and the nitrates for determining the integral parameter of trophic pollution for the period 1989–2004 is analyzed. The tendency in the indicator dynamics is represented by a linear function of the type: $y = ax + b$.

The Statistica 5.5 software and Origin 6 has been applied for identifying the trend [12, 13]. The trend is used for evaluation of water quality in accordance with the requirements of the European Framework Water Directive.

Results and discussion

A) Background point Yakoruda

The multi-annual fluctuations of the average annual and maximal values of the BOD_5 indicator in the Yakoruda point for the respective years are shown in Fig. 2 and Fig. 3.

The trend in the dynamics of this indicator is represented by linear functions with high correlation coefficients $r = –0.74$ and $r = –0.63$, respectively.

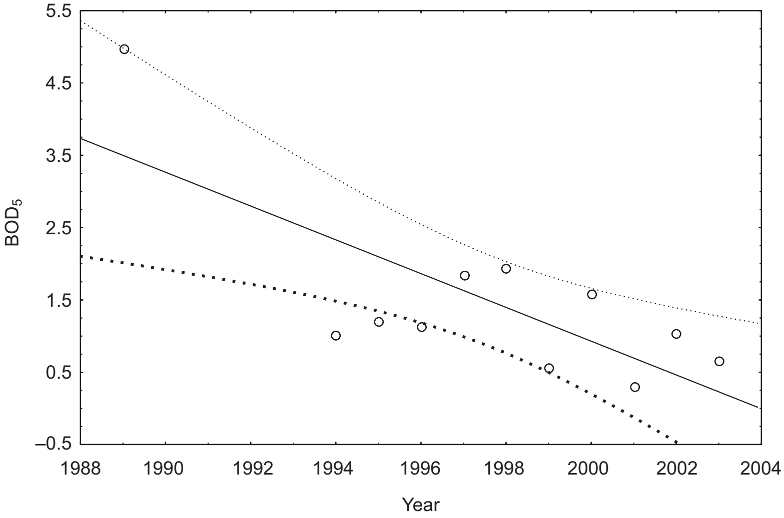

Fig. 2. Dynamics of the long-term values of the indicator – BOD_5 for the Mesta River at the Yakoruda point

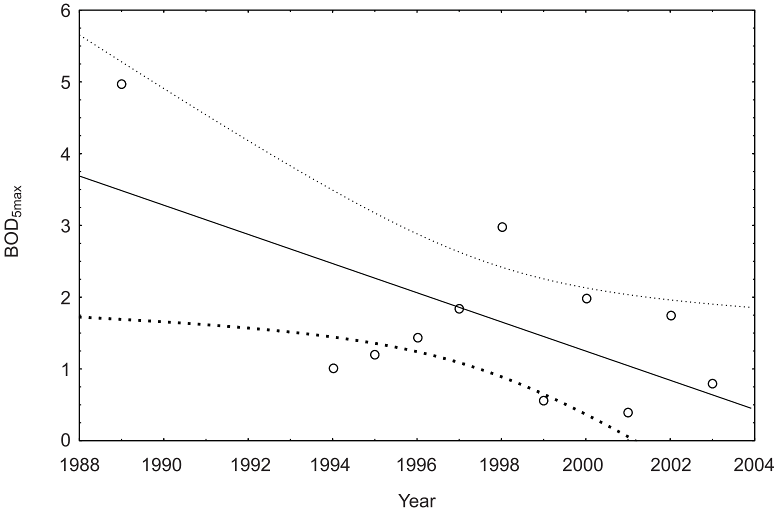

Fig. 3. Dynamics of the long-term values of the indicator – BOD_{5max} for the Mesta River at the Yakoruda point

A stable tendency towards decreasing the indicator values is observed for the considered period. The trend reflects the reduced organic pollution due to the decline of the economic activity in the region.

The multiannual fluctuations of the average annual and maximal values of the nitrates indicator and the trend for the considered period are shown in Fig. 4 and Fig. 5.

The trend is represented by linear functions with correlation coefficients r = –0.69 and r = –0.74, respectively. A tendency towards increasing the indicator values has been

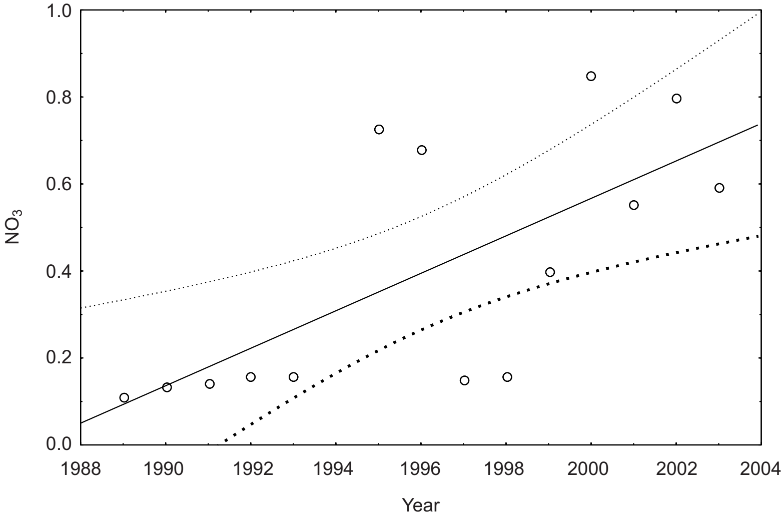

Fig. 4. Dynamics of the long-term values of the indicator – NO_3 for the Mesta River at the Yakoruda point

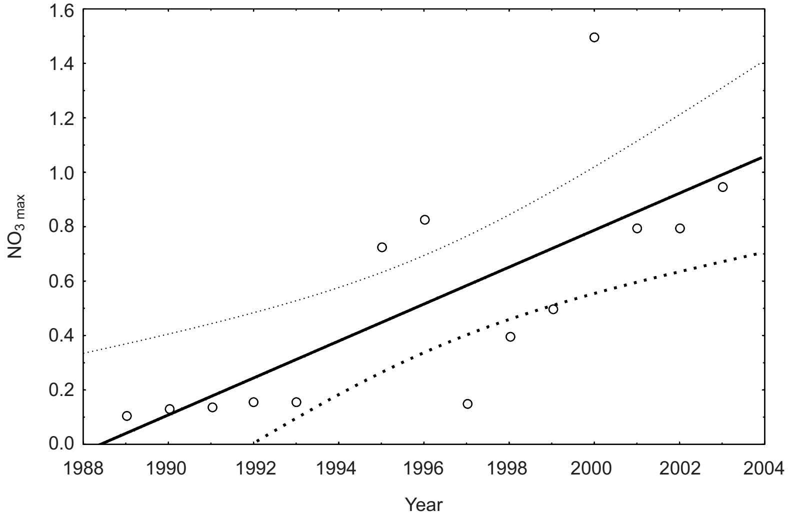

Fig. 5. Dynamics of the long-term values of the indicator – NO_{3max} for the Mesta River at the Yakoruda point

established, which do not exceed the admissible norm. The nutrient pollution of the Mesta River water at the background point of Yakoruda is connected with natural processes occurring in the adjacent to the point catchment area.

The multi-annual fluctuations of the average annual values of the integral indicator for water trophic pollution and the trend for the considered period are shown in Fig. 6.

The data in Fig. 6 exhibit a slightly expressed tendency towards increasing of the I_t parameter values, which are significantly lower than unity.

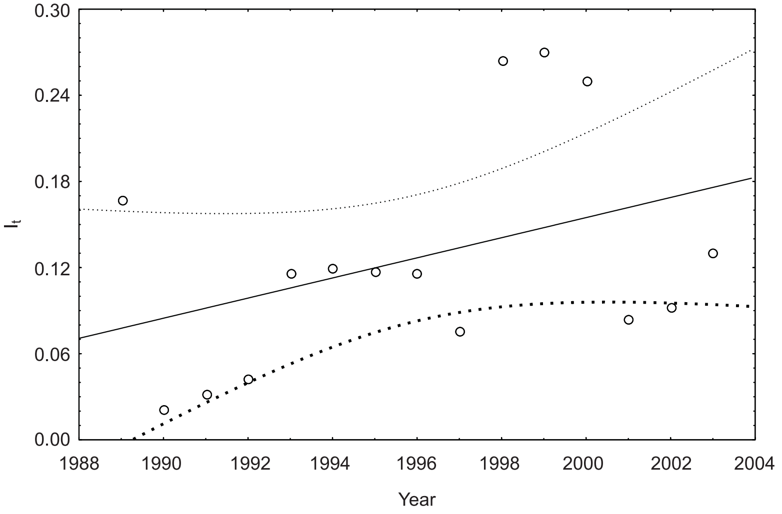

Fig. 6. Dynamics of the long-term values of the indicator – I_t for the Mesta River at the Yakoruda point

The analyzed information about the BOD_5 and nitrates indicators, as well as about the I_t parameter for the Yakoruda point provide the grounds to point out that the values of all trophic pollution indicators do not exceed the admissible norms ($\gamma_i < 1$).

The biotic index at the Yakoruda point for the period 1990–2004 shows values BI = 4.5, which is an indication of very good to excellent biological quality.

B) Reference point Momina Kula

In order to evaluate the distribution of pollution of the Mesta River from point and diffuse sources in the catchment area (Razlog, Bansko), a retrospective analysis was carried out of the information about the indicators I_t, phosphates, oxygen, nitrates and BOD_5 at the Momina Kula reference point.

The multi-annual fluctuations of the average annual and maximal values of the BOD_5 indicator for the respective years and the trend are shown in Fig. 7.

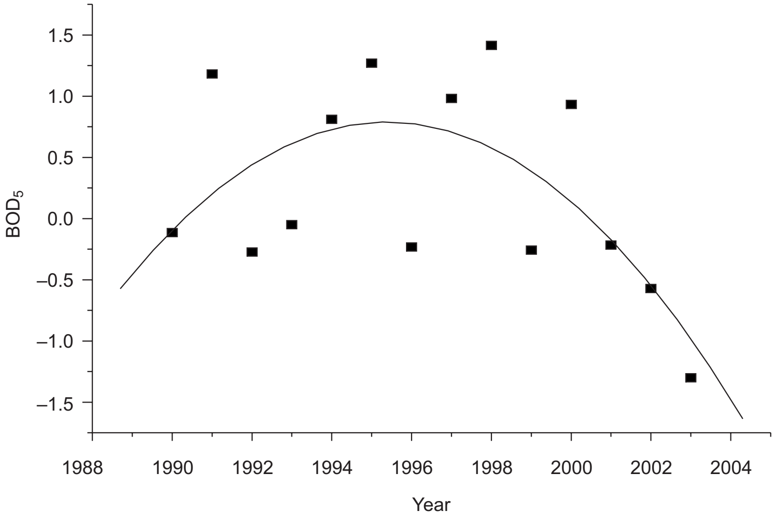

Fig. 7. Dynamics of the long-term values of the indicator – BOD_5 for the Mesta River at the Momina Kula point

The trend of the BOD_5 indicator is represented by a polynomial from second degree function with correlation coefficients – r = 0.42. A stable tendency towards decreasing the indicator values is observed for the considered period.

The multi-annual fluctuations of the average annual and maximal values of the nitrates indicator and the trend are shown in Fig. 8.

The linear function describing the trend in the dynamics of the average annual values exhibits a low correlation coefficient – r = 0.11. A slightly expressed tendency towards increasing the values of this indicator is observed for the considered period.

The multi-annual fluctuations of the average annual of the phosphates indicator and the trend for the considered period are shown in Fig. 9.

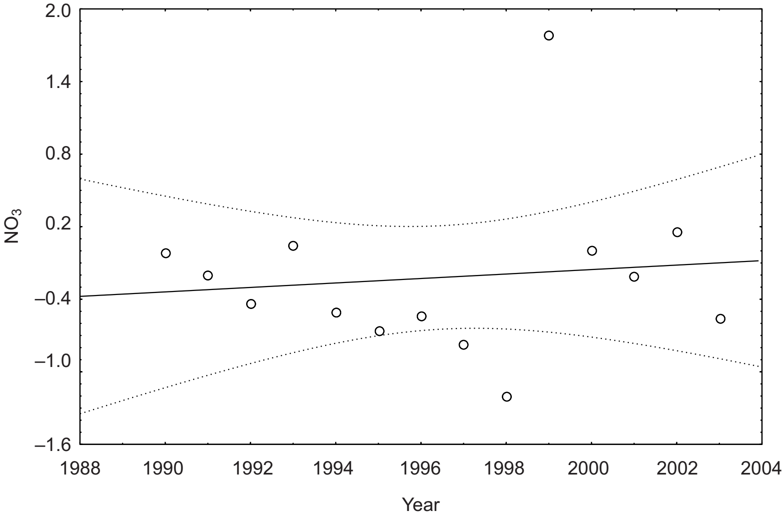

Fig. 8. Dynamics of the long-term values of the indicator – nitrates for the Mesta River at the Momina Kula point

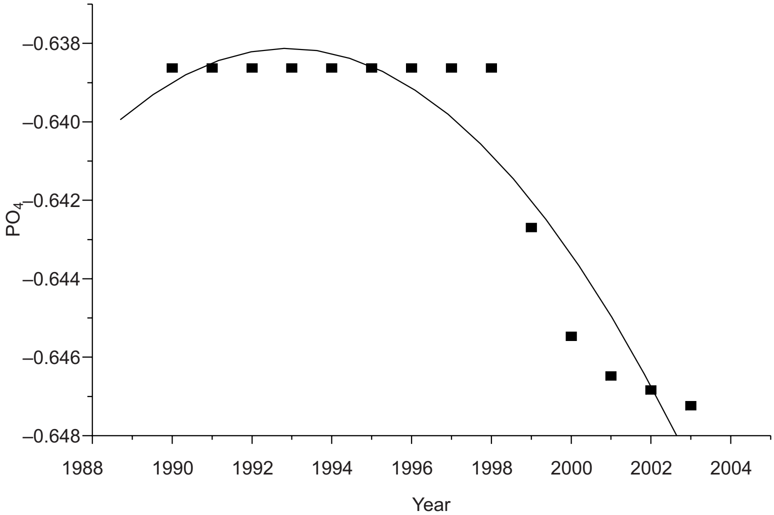

Fig. 9. Dynamics of the long-term values of the indicator – phosphates for the Mesta River at the Momina Kula point

The trend of the phosphates indicator is represented by a polynomial from second degree function with correlation coefficients r = 0.90. A stable trend towards decreasing the indicator values has been established. It can be used also a model for the phosphate dynamics.

The multi-annual fluctuations of the average annual of the oxygen indicator and the trend for the considered period are shown in Fig. 10.

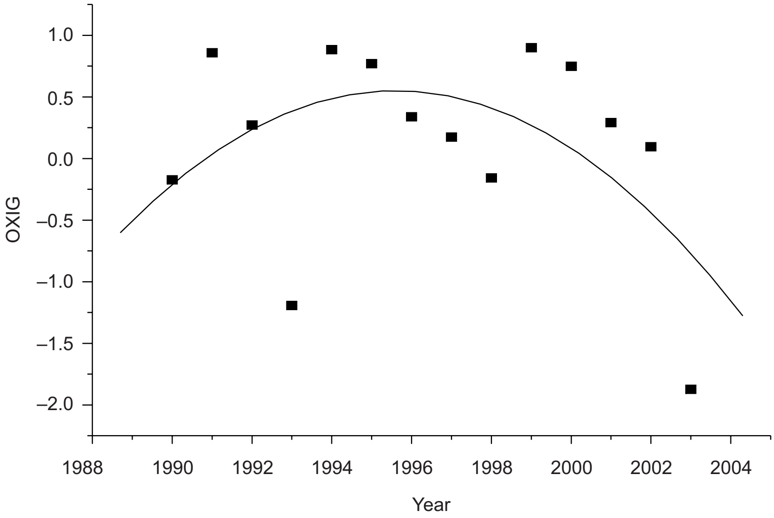

Fig. 10. Dynamics of the long-term values of the indicator – oxygen for the Mesta River at the Momina Kula point

The trend of the oxygen indicator is represented by a polynomial from second degree function with correlation coefficients r = 0.25. A significant tendency towards decreasing the indicator values has been established

The integral index of trophic pollution in this point (Fig. 11) displays a tendency towards decreasing the values for the regarded period. The correlation coefficient of a polynomial from second degree function describing the trend is r = –0.73.

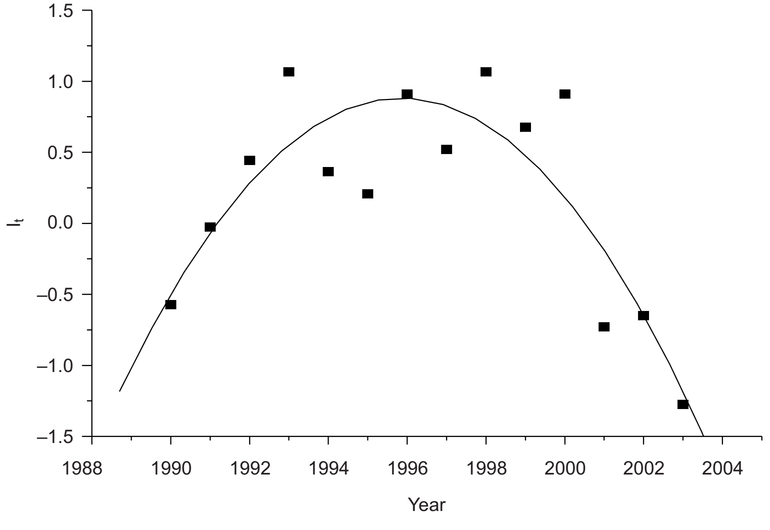

Fig. 11. Dynamics of the long-term values of the integral indicator for the Mesta River at the Momina Kula point

The trend in the dynamics of the maximal values exhibits stabilization of the results within certain range and do not exceed the admissible norm.

The analyzed information about the parameters BOD_5 and nitrates, phosphates, oxygen and for the integral index I_t, provides the grounds to point out that the values of all the trophic pollution indicators do not exceed the admissible norms ($\gamma_i < 1$) at this point.

The identified tendency towards reduction of the organic and nutrient pollution reflects the changes in the anthropogenic impact of the settlements and the industry in the considered region for the investigated period.

C) Reference point Hadzhidimovo

In order to evaluate the distribution of pollution of the Mesta River from point and diffuse sources in the catchment area, a retrospective analysis was carried out of the information about the indicators BOD_5 and nitrates at the Hadzhidimovo reference point.

The multi-annual fluctuations of the average annual and maximal values of the BOD_5 indicator for the respective years and the trend are shown in Fig. 12 and Fig. 13.

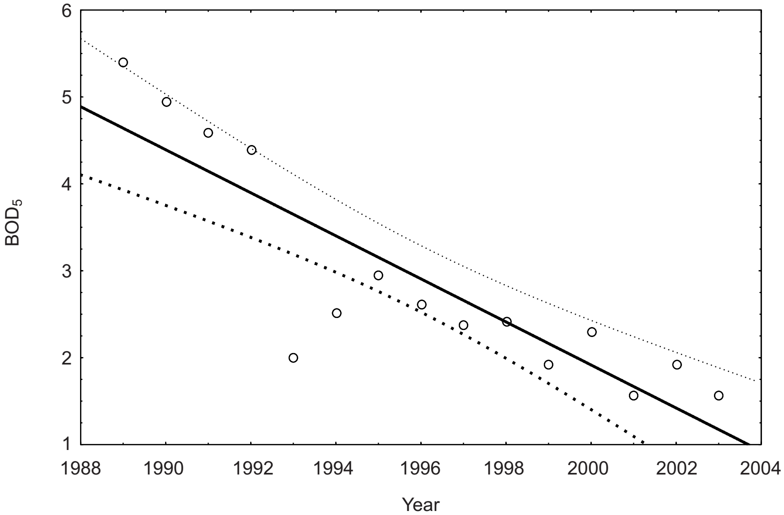

Fig. 12. Dynamics of the long-term values of the indicator – BOD_5 for the Mesta River at the Hadzhidimovo point

The trend of the BOD_5 and nitrates indicators is represented by linear functions with high correlation coefficients $r = -0.87$ and $r = -0.72$, respectively. A stable tendency towards decreasing the indicator values is observed for the considered period.

The reduced organic pollution in the reference point close to the border is related with the changes in the economic activity in the catchment area of the Bulgarian section of the river.

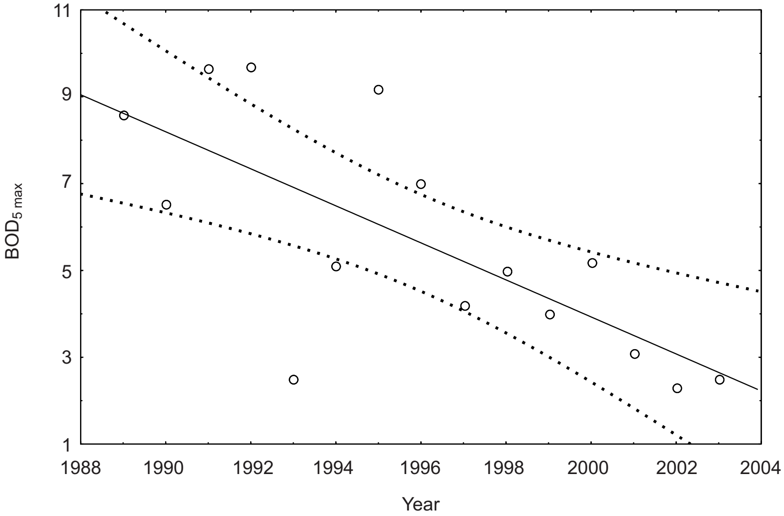

Fig. 13. Dynamics of the long-term values of the indicator – $BOD_{5\ max}$ for the Mesta River at the Hadzhidimovo point

The multi-annual fluctuations of the average annual and maximal values of the nitrates indicator and the trend are shown in Fig. 14 and Fig. 15.

The linear function describing the trend in the dynamics of the average annual values exhibits a low correlation coefficient – $r = -0.27$. A slightly expressed tendency towards decreasing the values of this indicator is observed for the considered period.

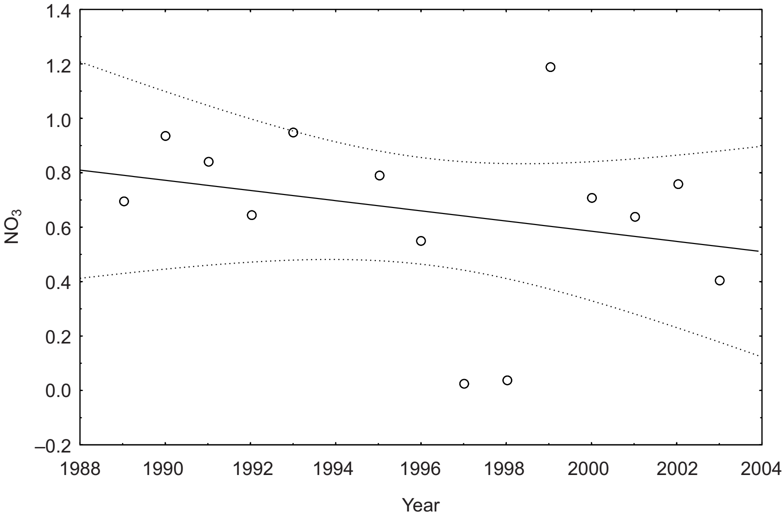

Fig. 14. Dynamics of the long-term values of the indicator – NO_3 for the Mesta River at the Hadzhidimovo point

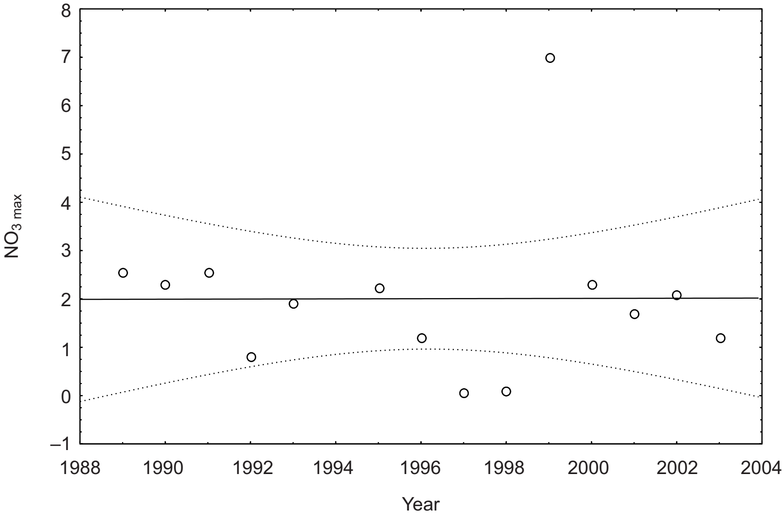

Fig. 15. Dynamics of the long-term values of the indicator – $NO_{3\ max}$ for the Mesta River at the Hadzhidimovo point

The trend in the dynamics of the maximal values exhibits stabilization of the results within a certain range and do not exceed the admissible norm.

The integral index of trophic pollution in this point (Fig. 16) displays a tendency towards decreasing the values for the regarded period. The correlation coefficient of the linear function describing the trend is r = –0.48.

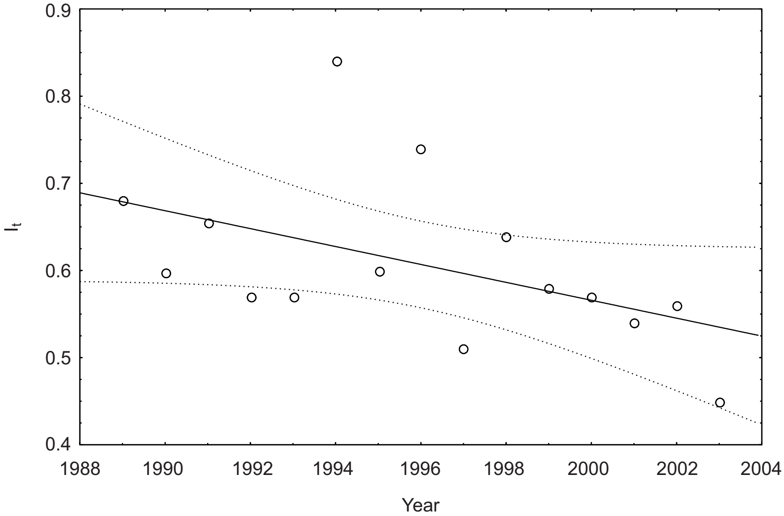

Fig. 16. Dynamics of the long-term values of the indicator – I_t for the Mesta River at the Hadzhidimovo point

The analyzed information about the parameters BOD_5 and nitrates, and for the integral index I_t, provides the grounds to point out that the values of all the trophic pollution indicators do not exceed the admissible norms ($\gamma_i < 1$) at the Hadzhidimovo point.

In accordance with the European Framework Water Directive of EU, a standard comparative assessment has been made of the values of the considered indicators at both points. For this purpose, the dynamics of the ratio between the average annual values of BOD_5 and nitrates at the Hadzhidimovo (C_i) and Yakoruda (C_0) points has been investigated for the considered period.

The dynamics of the C_i / $C_{i,0}$ ratio for the BOD_5 indicator is shown in Fig. 17.

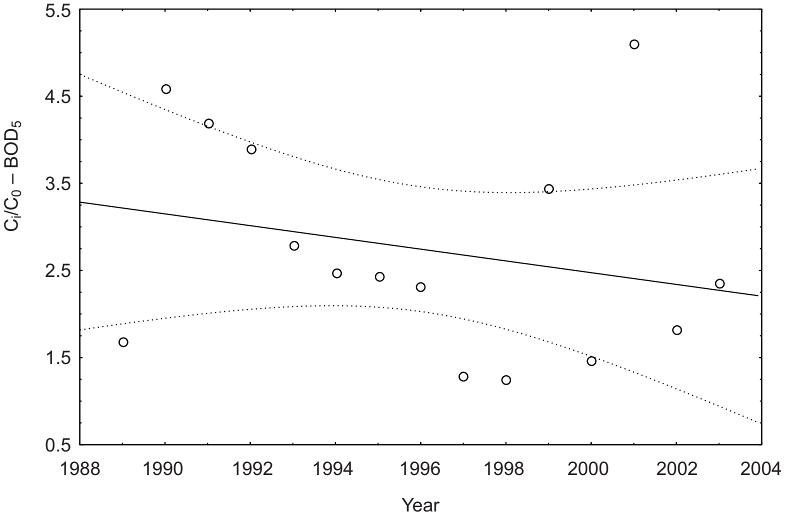

Fig. 17. Dynamics of the long-term values of the indicator – C_i/C_0 BOD_5 for the Mesta River at the Hadzhidimovo/Yakoruda points

A slightly expressed trend towards decreasing the values of this ratio is observed. The correlation coefficient of the linear function describing the trend is r = –0.24. The results show that the organic pollution of the river at the reference point exhibits a trend towards decreasing, ie the anthropogenic organic loading is becoming lower.

The multiannual fluctuations of the values of the ratio between the nitrates content in Hadzhidimovo and Yakoruda are shown in Fig. 18.

The linear function describing the trend has a correlation coefficient r = –0.82, ie the trend is stable. The data reveal a stable trend towards decreasing of the C_i / $C_{i,0}$ values for the considered period.

The identified tendency towards reduction of the organic and nutrient pollution reflects the changes in the anthropogenic impact of the settlements and the industry in the considered region for the investigated period.

The biotic index at the reference point of Hadzhidimovo for the period 1990–2004 exhibits values of 3.0–3.5, which is an indication of very good, acceptable biological

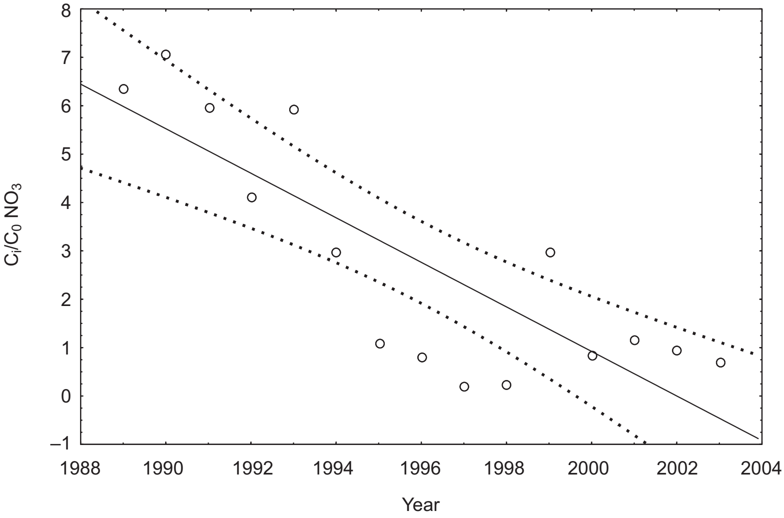

Fig. 18. Dynamics of the long-term values of the indicator – C_i/C_0 NO_3 for the Mesta River at the Hadzhidimovo/Yakoruda points

quality. As a result of the tendency towards reduced organic and nutrient pollution of the Mesta River water at the Hadzhidimovo point, improvement of the biological water quality may be expected at this point.

River runoff represents a basic indicator for surface water quality formation [14–17]. The dynamics of the average annual values of the river runoff for a period longer than 30 years is an important parameter for the formation of the qualitative and quantitative surface water characteristics [18, 19]. A retrospective analysis of the average annual water amount fluctuations in the considered points has been made in the present work. The dynamics of the average annual water amounts in Yakoruda and Hadzhidimovo is shown in Fig. 19 and Fig. 20.

The linear function describing the trend in Yakoruda has a correlation coefficient r = – 0.74, and in Hadzhidimovo – r = –0.70. A stable trend towards decreasing the average annual runoff values is observed for the considered period in both points. The investigations carried out identify a stable tendency in the dynamics of the river runoff formation processes in the catchment area of the Bulgarian section of the Mesta River.

On the basis of the retrospective analysis of the water quality indicators, the conclusion may be drawn that a tendency towards decreasing their values has been identified at the background and at the reference point for the studied period. Only the nitrates content at the background point exhibits the tendency of increasing values, obviously originating from natural processes in the catchment.

This positive result for the Mesta River water quality is due to the strongly reduced economic activity of the population in the catchment area after 1989. The recovery of the economic activity is an inevitable necessity for the development of the region and hence measures have to be envisaged for preserving water quality on both regional and transboundary level.

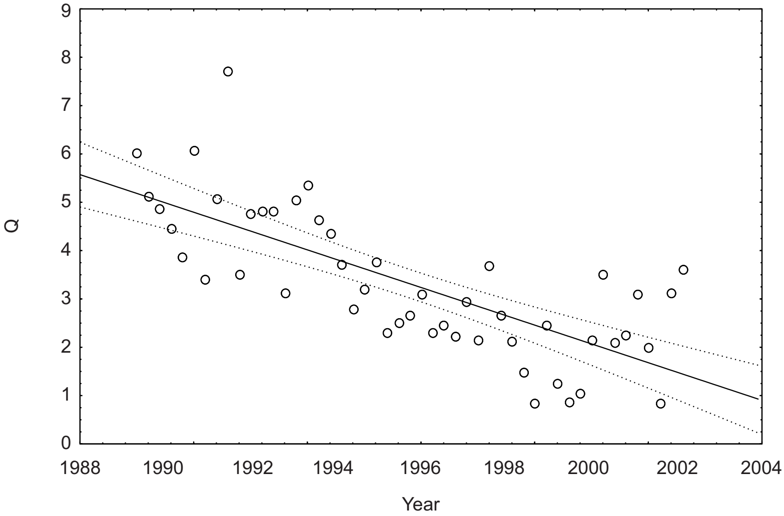

Fig. 19. Dynamics of the long-term values of the indicator – Q for the Mesta River at the Yakoruda point

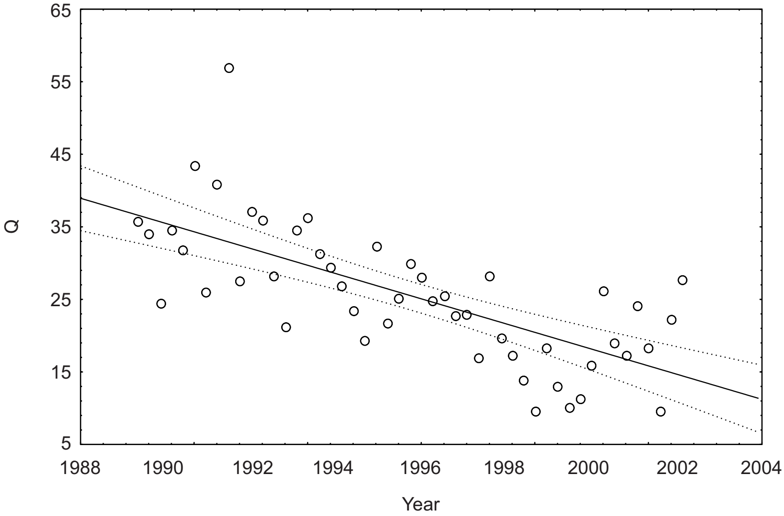

Fig. 20. Dynamics of the long-term values of the indicator – Q for the Mesta River at the Hadzhidimovo point

Conclusions

On the basis of the analysis of the nutrient pollution of the Mesta River at the three typical points, the conclusion can be drawn that the capacity of the self-purification processes of the river has not been disturbed and the water quality meets the normative requirements for the river category in Bulgaria.

The values of the water quality indicators at the background point show the influence of natural conditions on the water quality formation in the region.The chosen water quality indicators reflect adequately the anthropogenic impact intensity in the catchment for the considered period.The integral index for the level of trophic water pollution is an instrument of river water quality management. The trend in the dynamics of this index gives an opportunity for evaluating the efficiency of the water management strategy.

The organic and nutrient pollution of the Mesta River in the Bulgarian section does not result in any negative trends to aggravating the river water quality. The water trophic pollution meets the requirements of the Bulgarian standards. The water quality of the Mesta River in the end of the Bulgarian section is improved according to the investigated indicators for the studied period, which reflects the decline in the economic activity after the changes in 1990.

Acknowledgement

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KOMPLEKSOWA OCENA ZANIECZYSZCZENIA TROFICZNEGO TRANSGRANICZNEJ RZEKI MESTY W BUŁGARII

Abstrakt: Zaproponowano koncepcję modelu zintegrowanej oceny zanieczyszczenia rzeki. Dokonano całościowej oceny troficznego zanieczyszczenia transgranicznej rzeki Mesty na podstawie wartości podstawowych wskaźników zmierzonych w początkowym (tło) i końcowym (referencyjnym) bułgarskim odcinku rzeki. Dokonując ocen wskaźników, uwzględniono zmiany gospodarcze zachodzące w kraju, w tym przejście do gospodarki rynkowej. Analizowano wartości takich wskaźników, jak: BZT_5, stężenie azotanów(V), stężenie azotanów(III), całkowity wskaźnik zanieczyszczeń troficznych, średni roczny przepływ wody i wartości indeksu biotycznego. Całkowity wskaźnik uwzględnia ładunek organicznych i zawieszonych składników pokarmowych oraz substancje rozpuszczone. Dokonano analizy statystycznej długoterminowych trendów sezonowych i struktury rozpatrywanych zbiorów danych. W badanym okresie (1989–1995) zaobserwowano tendencję w kierunku zmniejszenia się wartości wskaźników w punkcie tła i punkcie odniesienia. Tylko azotany(V) w punkcie tła w zlewni wykazują tendencję do wzrostu wartości w wyniku naturalnych procesów. Na podstawie analizy retrospektywnej można stwierdzić, że jakość wody mieści się w normatywnych wymogach dla rzek w Bułgarii.

Słowa kluczowe: oceny jakości, zanieczyszczenia wód, tendencja, wskaźniki całkowity

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CONTENT OF SOME MACRO- AND MICROELEMENTS IN A SOIL TOPOSEQUENCE IN THE LANDSCAPE OF ICE-DAMMED LAKES IN SEPOPOL LOWLAND

ZAWARTOŚĆ WYBRANYCH MAKRO- I MIKROELEMENTÓW W TOPOSEKWENCJI GLEB KRAJOBRAZU RÓWNIN ZASTOISKOWYCH NIZINY SĘPOPOLSKIEJ

Abstract: The study was carried out using soil catena method in Sepopol Lowland, representing the zone of ice-dammed lakes of young glacial landscape. The sequence of soils in the catena was as follows: black earth, proper deluvial soil, humous deluvial soil, mucky soil, strongly and slightly silted peat-muck soils. Soil texture, soil reaction, total content of carbon and nitrogen as well as total amounts of Ca, Mg, K, P, Na, Fe, Mn, Zn and Cu were analysed in the mentioned soils.

The reaction of investigated soils ranged from slightly acid in deluvial soils to neutral in peat-muck soils. In the studied toposequence of soils, the amounts of total carbon and nitrogen increased towards the depression.

Variation of the amounts of analysed elements in the catena depended on the location of soil in a relief. Quantitatively, the content of elements in eroded black earths was in the following sequence: Fe > Ca > Mg > K > P > Na > Mn > Zn > Cu. Deluvial soils contained more potassium than magnesium, and peat-muck soils contained more calcium than iron.

Arable horizons of proper deluvial soils, which were located in the middle of the slope, contained the lowest amounts of macro- and microelements in relation to both deeper layers of the soil profile and parent material of eroded soils. The highest accumulation of macro- and microelements was reported in the soils located at the bottom of the slope. Humous deluvial soils were distinguished by the highest content of K and Fe, mucky soils by the highest content of Mg, Cu, Zn, Mn, and the peat-muck – Ca, P and Na. Humous deluvial soils and upper-silted organic soils are the barriers which prevent the expansion of biogens from agricultural areas to wetlands.

Keywords: landscape of ice-dammed lakes, macro- and microelements, toposeqence, black earths, deluvial soils, mucky soils, strongly and slightly silted peat-muck soils

In young glacial area of north-eastern Poland, three zones of landscape, with different morphogenesis of the land, types of soil formations and character of soil cover,

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were distinguished: northern – of ice-dammed lake origin, middle – of morainic hills and southern – of outwash plains [1]. The zone of ice-dammed lake plains includes Sepopol Lowland, formed as a result of deglaciation phase of Pomeranian Vistula Glaciation, during which clay and loam of clay facies were accumulated [2, 3]. Brown soils and black earths were formed from these formations. They have high quality and are intensively used for agricultural purposes [4]. In meltwater basins, which had been supplied with waters flowing on impermeable soil formations (flowing type of hydrological supply), hydrogenic soils were formed [4]. Human activity led to the diversification of soil cover and the formation of soil toposequences comprising eroded and deluvial soils along the slope as well as upper-silted organic soils (mucky soils and peat-muck soils with various degree of silting) in land depressions. The soils in the catenas are interrelated by leaching, translocation and accumulation of soil material. These processes affect the carbon cycle and the cycles of mineral elements in young glacial landscape [5–7]. Owing to high organic matter content and location in a relief, hydrogenic soils play a significant role in the circulation of elements [8, 9].

The distribution of elements in the zone of morainic hills of Mazurian Lakeland was presented in a previous work [10]. In this zone, pro-ecological management is promoted [4].

The aim of the study was to investigate the distribution of some selected macro- and microelemnts in a catena and within the profiles of soils used as agricultural lands in the zone of ice-dammed lakes landscape of north-eastern Poland. Such studied are very important as excessive accumulation of elements may occur locally, which was noted in organic soils of midmoraine depressions in the zone of morainic hills [11–13].

Materials and methods

For the research, Reszel site with coordinates 54°03′16.4″ N, 21°04′22.0″ E was chosen. It is located in southern part of Sepopol Lowland. The transect was made from the top of the slope towards the depression and 12 soil profiles were described. The slope gradient was up to 7.1 % and the soil sequence was as follows: black earths on the top, proper deluvial soils in the middle of the slope, humous deluvial soils in lower parts and at the bottom of the slope, and mucky soils as well as slightly and strongly silted peat-muck soils in the depression.

Deluvial soils occupied the section of approximately 120 m and the thickness of deluvial deposits amounted to 118 cm. In the middle and lower parts of the slope, deluvial deposits were lying on mineral materials, whereas in the depression on organic formations. In mucky soils, under deluvial mineral-organic deposit of thickness of 27–28 cm, a 17–19 cm layer of muck occurred. Beneath, moderately decomposed reed peats underlain by the detritus-calcareous gyttja lying on clay gyttja occurred. In the studied transect, the investigated soils occupied a 35-meter section and turned into strongly silted peat-muck soils formed from reed peats underlain by clay-calcareous gyttja at a depth of 93 cm. Underneath, at a depth of 180 cm, clay gyttja occurred. Muck was strongly silted (50.2–54.0 % of ash) (Table 5) and had thickness of 32 cm. Slightly silted peat-muck soils were formed from deep (200 cm) reed peats lying on detritus-calcareous gyttja which was underlain by clay gyttja at a depth of 580 cm.

The soils samples were collected from selected genetic horizons and the following laboratory analyses were carried out: soil reaction in H_2O and KCl potentiometrically, organic carbon by Tiurin method, total nitrogen by Kjeldahl method, ash content after heating the soil at a temperature of 550 ^{o}C.

Total content of Ca, Mg, K, P, Na, Fe, Mn, Zn and Cu was analysed after mineralization of soil samples in the mixture of $HClO_4$ and HNO_3. Calcium, potassium and sodium was measured photometrically using Jenway flame photometer, phosphorous was measured calorimetrically using Specol EK 1 spectrocolorimeter and magnesium was measured using AAS 1 Zeiss Jena analyzer. Total content of Fe, Mn, Zn and Cu was measured by applying AAS techniques using 30 Zeiss Jena analyser.

Statistical analyses (mean, standard deviation, correlation coefficients) were conducted using Statistica 8.0.

Results and discussion

Black earths were formed from loam, which was lying on heavy clay (Table 1). The amounts of silt fraction in Ap horizon ranged from 46 % to 47 %, and the amounts of clay from 20 % to 25 %. The content of silt and clay in deluvial soils increased down the slope (Table 1). Shallow humous deluvial soils located in the depression contained 4–5 % more silt fraction and 8–16 % more clay than proper deluvial soils located in the middle part of the slope. Humus horizons of proper deluvial soils had loam texture whereas of humous deluvial soils at the bottom of the slope – clay loam texture.

The reaction of examined soils ranged from slightly acidic to neutral (Table 2). The lowest values of pH were noted in proper and humous deluvial soils (pH_{KCl} 5.9–6.6) and the highest in peat-muck soils (pH_{KCl} 6.8–7.1). In the studied soil toposequence, the amounts of carbon and nitrogen increased towards the centre of depression (Table 2). Humous deluvial soils contained on average 2.5-fold more carbon in relation to eroded black earths. Black earths, deluvial and mucky soils had narrow C:N ratio (9.97–11.65) (Table 2). In peat-muck soils this ratio was wider and amounted to 17.6 in slightly silted mucks.

Quantitatively, the content of studied elements in eroded black earths (Ae horizon), can be ordered as follows: Fe > Ca > Mg > K > P > Na > Mn > Zn > Cu (Table 3). In proper deluvial soils (Adw horizon) and in humous deluvial soils (Adp horizon) potassium prevailed over the magnesium, whereas in peat-muck soils (Mtsz and Mtz horizons) there was more calcium than iron.

Mean amounts of calcium were lower in proper deluvial soils (8.40 $g \cdot kg^{-1}$) and eroded black earths (11.10 $g \cdot kg^{-1}$), and increased towards the centre of depression simultaneously with organic matter content, which was proved by positive statistically significant correlation coefficients between the amount of Ca and organic matter (Table 4). Similar relationships were found by Bieniek [11] as well as by Smolczynski and Orzechowski [10] in the catenas in the zone of morainic hills of young glacial

Table 1

Particle-size analysis of soils at Reszel catena

Profile No. and location	Genetic horizon*	Depth [cm]	Percentage of mineral particles [mm]									Soil formation
			> 2.0	2.0–1.0	1.0–0.5	0.5–0.25	0.25–0.1	0.1–0.05	0.05–0.02	0.02–0.002	< 0.002	PTG 2008 [23]
Proper black earth*												
1. Top of the slope	Ap	0–25	0	0	6	9	5	14	14	32	20	loam
	Aa	25–38	0	0	3	10	6	13	15	32	21	loam
	C	38–150	0	0	0	3	5	4	2	25	61	heavy clay
Shallow proper deluvial soil												
2. Middle part of the slope	Ap	0–24	0	0	3	14	4	13	14	32	20	loam
	Aa	24-48	0	0	2	11	6	13	14	29	25	loam
	C	48–150	0	0	2	5	2	12	17	37	25	silt loam
Medium deep proper deluvial soil												
3. Middle part of the slope	Ap	0–26	0	1	6	13	6	15	13	34	12	loam
	A2	26–48	0	0	5	10	4	16	10	31	24	loam
	A3	48–85	0	0	6	13	7	11	10	26	27	loam

Table 1 contd.

Profile No. and location	Genetic horizon*	Depth [cm]	Percentage of mineral particles [mm]									Soil formation
			> 2.0	2.0–1.0	1.0–0.5	0.5–0.25	0.25–0.1	0.1–0.05	0.05–0.02	0.02–0.002	< 0.002	PTG 2008 [23]
Deep humous deluvial soil												
4. Lower part of the slope	Ap	0–25	0	0	3	9	7	11	19	27	25	loam
	A2a	25–65	0	0	4	9	7	14	14	32	20	loam
	A3a	65–100	0	0	2	8	6	13	13	27	31	clay loam
	A4a	100–118	0	0	2	6	6	12	14	29	31	clay loam
	C	118–150	0	0	3	5	7	19	25	23	18	loam
Medium deep humous deluvial soil, on peat												
5. Lower part of the slope	Ap	0–26	0	0	3	5	7	14	16	33	22	loam
	Aa	26–64	0	0	3	6	4	8	19	20	40	clay loam
Shallow humous deluvial soil, on peat												
6. Bottom of the slope	Ap	0–26	0	0	2	3	5	11	18	33	28	clay loam
	A2	26–48	0	0	2	4	6	10	16	30	32	clay loam

* – according to the Systematics of Polish soils [22].

Table 2

Soil reaction, content of carbon and nitrogen in the soils studied

Horizon	pH			Humus	N	C:N
	in H_2O	in KCl	[$g \cdot kg^{-1}$]			
Ae	7.2–7.3	6.5–6.7	20.1* 16.2–24.0**	34.65 27.9–41.4	1.72 1.69–1.75	11.65 9.6–13.7
Adw	6.7–7.4	6.0–6.6	23.03 19.8–28.4	39.65 34.1–41.5	2.30 2.04–2.74	10.15 7.2–12.9
Adp	6.8–7.1	5.9–6.6	51.42 30.8–86.2	88.65 53.1–148.6	4.67 2.75–7.65	11.15 10.1–13.5
AO	7.0–7.9	6.4–6.9	90.57 76.4–104.1	8.13 15.4–19.6	9.15 7.2–10.54	9.97 9.1–10.6
Mtsz	7.1–7.2	6.8–6.9	215.2 199.4–231.0	47.90 46.0–49.8	17.17 16.93–17.4	12.55 11.5–13.6
Mtz	7.4–7.4	7.0–7.1	263.4 261.2–265.6	51.55 51.0–52.1	14.99 14.96–15.02	17.6 17.4–17.8

Explanations: Ae – humus horizon of eroded black earths, Adw – humus horizon of proper deluvial soils; Adp – humus horizon of humous deluvial soils, AO – humus horizon of mucky soils, Mtsz – strongly silted muck, Mtz – slightly silted muck.
* – mean; ** – oscillation range.

Table 3

Mean total amounts of elements in surface horizons of the soils studied

Horizon	Feature	Ca	Mg	K	Na	P	Fe	Cu	Zn	Mn
		[g · kg^{-1}]						[mg · kg^{-1}]		
I Ae*	X	11.1	6.3	0.95	0.15	0.50	28.15	15.20	45.30	508.20
	S	2.19	0.00	0.07	0.07	0.14	0.78	0.85	2.55	27.44
	CV	19.73	0.0	7.37	46.67	28.00	2.77	5.59	5.63	5.40
II Adw	X	8.40	5.50	7.00	0.15	1.12	23.98	21.74	56.36	380.22
	S	3.70	1.16	1.62	0.04	0.35	3.51	6.22	15.07	151.0
	CV	44.05	21.09	23.14	26.67	31.25	14.64	28.61	26.74	39.71
III Adp	X	12.69	6.18	8.71	0.20	1.15	29.46	22.25	56.33	441.48
	S	6.51	0.67	2.72	0.02	1.01	3.02	4.30	10.34	65.56
	CV	51.30	10.84	31.23	10.00	87.82	10.25	19.33	18.36	14.85
IV AO	X	13.83	6.56	6.01	0.37	1.60	26.78	25.97	68.71	578.83
	S	2.54	0.65	1.76	0.08	0.28	1.39	3.19	7.66	86.58
	CV	18.36	9.91	29.28	21.62	17.50	5.19	12.28	11.15	14.96
V Mtsz	X	63.60	4.55	2.65	0.41	2.40	12.75	16.50	34.30	508.00
	S	34.22	0.78	2.05	0.05	0.84	1.63	2.83	9.48	39.60
	CV	53.81	17.14	77.36	12.20	35.00	12.78	17.15	27.64	7.80
VI Mtz	X	75.90	4.40	2.85	0.38	2.50	14.60	18.35	32.15	577.50
	S	39.74	0.00	2.05	0.16	0.42	1.56	3.46	0.49	10.61
	CV	52.36	0.00	71.93	42.11	16.80	10.68	18.86	1.52	1.84
Statistically significant differences $\alpha = 0.05$		IV < V	IV > V	I < II I < III III > IV	II < III III < IV	IV < V	II < III IV > V	IV > V	III < IV IV > V	III < IV

* Explanations as below Table 2, X – mean, S – standard deviation, CV – coefficient of variance.

landscape. During erosion, calcium is transformed into soluble forms and it is translocated with erosional waters. Chudecki and Niedzwiecki [14] described this process as chemical erosion. In the examined catena, the content of iron was inversely related to calcium and reached the highest amounts in humous deluvial soils and black earths, and the lowest in peat-muck soils (Table 3). The amount of this element was positively correlated with the amount of mineral fraction with diameter of 0.05–0.002 mm and < 0.002 mm (Table 4). Mean amounts of phosphorous increased from 0.50 g $\cdot$ kg^{-1} in eroded black earths to 2.50 g $\cdot$ kg^{-1} in slightly silted peat muck soils simultaneously with organic matter content (Table 3). This relationship was confirmed by the positive statistically significant value of correlation coefficient (Table 4).

Humous deluvial soils had the highest content of magnesium (Table 3). In mucky soils the content of this element was statistically significantly lower. Humus horizons (AO) of mucky soils had the highest amount of potassium. However, the differences in relation to surface horizons in humous deluvial soils (Adp), proper deluvial soils (Adw) and black earths (Ae) were insignificant. Potassium and magnesium were positively correlated with mineral fraction of diameter of 0.05–0.002 mm as well as clay fraction (< 0.002 mm) (Table 4). According to the study carried out by Chodak et al [15], magnesium is translocated with the soil particles during erosion, whereas potassium and phosphorus are translocated also in a form of solution.

The content of zinc, copper and manganese in the soils of studied catena reached the highest value in AO horizons of mucky soils (Table 3). In humous deluvial soils the contents of zinc and manganese were statistically significantly lower. The contents of copper did not show significant differences between Adw and Adp horizons of deluvial soils as well as Ae horizons of black earths (Table 3). In the light of threshold limit values [16] the contents of copper and zinc did not exceed their natural level.

The soils of the studied toposequence, in comparison with similar soil units in the catenas in the zone of morainic hills of young glacial landscape [10] had higher amounts of Ca, Mg, K and Cu. The contents of other elements in the soils of both landscape zones were similar.

Taking into consideration bulk density of studied soil formation, the contents of analysed elements were calculated in relation to the volume of dry soil and the results are presented in Table 5. Excluding calcium and phosphorous, the lowest contents of studied elements were found in peat-muck soils. The contents of Mg, K, Fe, Cu and Zn increased successively in mucky soils and humous deluvial soils, but the differences were statistically insignificant. The variations of the contents of these elements among humous deluvial soils, proper deluvial soils and black earths were small and generally insignificant.

In order to present the profile distribution of the studied elements, the ratio of the amounts of elements in surface layer (5–10 cm) to the amount at a depth of 30–40 cm was calculated. According to the data presented in Table 6, the highest values of enrichment coefficients, excluding calcium, were found in slightly and strongly silted peat-muck soils and mucky soils. It indicates considerable accumulation of the examined elements in surface horizons of the soil profiles. It may be a result of simultaneous sedimentation of deluvial deposits and macro- and microelements on soil

Table 4

Correlation coefficients between the content of organic matter, mineral particles and total amounts of elements in the soils studied

Properties	Ca	Mg	K	P	Na	Fe	Mn	Zn	Cu
	$[g \cdot kg^{-1}]$						$[g \cdot kg^{-1}]$		
< 0.002 m	0.567*	0.895*	0.800*	0.206	0.295	0.780*	0.613*	0.235	0.230
0.05–0.002 mm	0.469*	0.872*	0.666*	0.248	0.338	0.632*	0.839*	0.108	0.031
Organic matter	0.669*	0.260	0.230	0.588*	–0.206	0.286	–0.185	0.109	0.659*

* – significance level at $\alpha = 0.05$.

Table 5

Mean total amounts of elements in surface horizons of soils in relation to dm^3 of dry soil

Genetic horizon	Feature	Ca	Mg	K	Na	P	Fe	Cu	Zn	Mn
		[g · kg^{-1}]						[g · kg^{-1}]		
I Ae	X S CV	16.39 3.71 22.64	9.32 0.27 2.90	1.41 0.14 9.92	0.22 0.10 45.45	0.74 0.23 31.08	41.65 2.34 5.62	22.46 0.61 2.72	66.94 1.84 2.75	752.21 62.14 8.26
II Adw	X S CV	9.64 2.42 25.10	6.79 2.31 34.02	8.69 3.25 37.40	0.20 0.10 50.00	1.31 0.21 16.03	30.57 11.82 38.67	25.93 6.68 25.76	66.97 14.41 21.52	523.86 291.47 55.64
III Adp	X S CV	15.77 8.23 52.19	7.76 1.17 15.08	11.06 3.79 34.27	0.25 0.04 16.00	1.35 0.94 69.63	37.08 5.78 15.59	27.50 2.52 9.16	71.15 15.80 22.21	555.23 103.73 18.68
IV AO	X S CV	10.72 2.53 23.60	5.14 1.37 26.65	4.71 1.66 35.24	0.29 0.09 31.03	1.21 0.14 11.57	20.85 4.34 20.82	19.91 2.21 11.10	52.71 5.82 11.04	450.98 115.96 25.71
V Mtsz	X S CV	33.15 16.69 50.35	2.41 0.51 21.16	1.42 1.14 80.28	0.21 0.03 14.29	1.26 0.40 31.75	6.74 1.13 16.77	8.66 1.14 13.16	18.18 5.72 31.46	267.30 10.09 3.77
VI Mtz	X S CV	38.48 16.03 41.66	2.30 0.28 12.17	1.56 1.25 80.13	0.20 0.11 55.00	1.30 0.06 4.62	7.69 1.73 22.50	9.50 0.66 6.95	16.82 1.76 10.46	301.99 30.79 10.20
Statistically significant differences $\alpha = 0.05$		I > II IV<V	III > IV IV>V	I < II I < III III > IV IV>V		I < II	III > IV IV > V	I < III III > IV IV > V	III > IV IV > V	I > III

Explanations as below Table 1 and 2.

Table 6

Enrichment or impoverishment coefficients of total amounts of elements in surface horizons (5–10 cm) in relation to the layer at a depth of 30–40 cm

Soil unit*	Ca	Mg	K	Na	P	Fe	Cu	Zn	Mn
Black earths (eroded)	0.75^{-}	1.00	0.90	2.00^{+}	0.67^{--}	0.96	1.08	1.08	0.93
Proper deluvial soils	$\underline{0.82}^{-}$ 0.74–0.91	$\underline{0.83}^{-}$ 0.69–0.98	$\underline{0.81}^{-}$ 0.61–1.01	$\underline{0.92}$ 0.85–1.0	$\underline{1.17}$ 1.00–1.33	$\underline{0.87}$ 0.78–0.96	$\underline{0.89}$ 0.69–1.10	$\underline{0.94}$ 0.79–1.09	$\underline{1.07}$ 1.02–1.11
Humous deluvial soils	$\underline{1.30}^{+}$ 1.09–1.40	$\underline{1.12}$ 1.05–1.18	$\underline{1.16}$ 1.08–1.22	$\underline{1.00}$ 1.00–1.00	$\underline{1.56}^{+}$ 0.80–2.20	$\underline{0.89}$ 0.83–0.94	$\underline{0.90}$ 0.85–0.96	$\underline{0.93}$ 0.83–1.09	$\underline{1.25}^{+}$ 1.07–1.44
Mucky soils	$\underline{0.88}$ 0.51–1.07	$\underline{1.33}^{+}$ 1.04–1.66	$\underline{1.81}^{+}$ 1.10–2.44	$\underline{1.37}^{+}$ 1.00–1.96	$\underline{0.75}^{=}$ 0.45–1.00	$\underline{1.28}^{+}$ 0.92–1.75	$\underline{1.46}^{+}$ 1.09–2.15	$\underline{1.94}^{+}$ 1.36–3.10	$\underline{1.49}^{+}$ 1.31–1.67
Strongly silted peat-muck soils	1.10	3.00^{++}	4.10^{++}	1.47^{+}	2.25^{++}	4.66^{++}	6.04^{+++}	7.19^{+++}	3.47^{++}
Slightly silted peat-muck soils	0.71^{-}	2.32^{+}	4.30^{++}	1.53^{+}	1.38^{+}	4.59^{++}	4.18^{++}	3.61^{++}	2.45^{++}

Explanations: 1.20–2.00 – evident enrichment (+); 2.01–5.00 – strong enrichment (++); > 5.00 very strong enrichment (+++); 0.85–0.71 – evident impoverishment (–); 0.70–0.51 – strong impoverishment (– –); < 0.50 very strong impoverishment (– – –).

* – according to the Systematics of Polish soils [22].

Table 7

Enrichment or impoverishment coefficients of total amounts of elements in surface Ap horizons (5–10 cm) in relation to the parent material of eroded black earth

Profile No.	Soil unit*	Ca	Mg	K	Na	P	Fe	Cu	Zn	Mn
1	Black earths (eroded)	0.44^{---}	0.39^{---}	0.08^{---}	1.00	0.67^{--}	0.66^{--}	0.70^{--}	0.62^{--}	0.99
2	Proper deluvial soils	0.34^{---}	0.35^{---}	0.72^{-}	1.00	1.50^{+}	0.62^{--}	0.94	0.66^{--}	1.12
3	Proper deluvial soils	0.23^{---}	0.23^{---}	0.41^{---}	0.55^{--}	2.00^{+}	0.43^{---}	0.66^{--}	0.61^{--}	0.70^{--}
4	Humous deluvial soils	0.22^{---}	0.37^{---}	0.62^{--}	1.05	1.33^{+}	0.63^{--}	0.85^{-}	0.69^{--}	0.96
5	Humous deluvial soils	0.95	0.42^{---}	1.04	1.00	1.67^{+}	0.68^{--}	0.88	0.77^{-}	1.12
6	Humous deluvial soils	0.99	0.44^{---}	1.06	1.00	1.83^{+}	0.69^{--}	0.90	0.79^{-}	1.03

Explanations: 1.20–2.00 – evident enrichment (+); 2.01–5.00 – strong enrichment (++); > 5.00 very strong enrichment (+++); 0.85–0.71 – evident impoverishment (–); 0.70–0.51 – strong impoverishment (– –); < 0.50 very strong impoverishment (– – –).

* – according to the Systematics of Polish soils [22].

surface. Bieniek and Piascik [17] stressed, that the chemical composition of organic soils in land depressions depends largely on the geomorphological form of the surrounding area. Moreover, fluctuations of groundwater level as well as oxidation and reduction processes play important roles in these soils. Iron, manganese and phosphorous in hydrogenic soils is precipitated from rising groundwaters in the aeration zone of the soil profile [18]. When the soil moisture content is high, increased solubility of some elements, for example manganese, may take place [19]. The lowest values (less than unity) of calculated coefficients for Mg, K, Na, Fe, Cu and Zn were noted in proper deluvial soils, and for P and Mn in black earths (Table 6). Impoverishment of arable horizons of these soils, due to its location on the slope, is a result of both surface flowing processes and migration of colloidal clay together with macro- and microelements deeper into the soil profile. Bieniek [11] stressed, that the eluviation of clay fraction contributes to loosening of humus horizons of deluvial soils, which facilitates infiltration of rainwaters.

During translocation and accumulation deluvial deposits are re-sorted and the granular size of deluvial soils is the derivative of the texture of eroded soils. Therefore the content of studied elements in humus horizons (5–10 cm) were also related to the amounts in the parent material of eroded black earths (Table 7). The highest values of these coefficients were found in humous deluvial soils. However, only for Ca, K, P, Mn and Na these values were approximate or higher than unity. In relation to parent material of eroded soils, proper deluvial soils (profile 3) were most impoverished in macro- and microelements (excluding phosphorous) (Table 7). Uggla et al [20] described these soils as ‘washed deluvium’ and declerd they contained less nutrients than eroded soils on the top of the slope. Lower content of studied elements (excluding phosphorous) in arable horizons as compared with parent material of eroded soil indicates that agricultural activity did not contribute to excessive accumulation of elements in the environment.

The described distribution of elements in the studied catena revealed that the location of soils in a relief is an important factor which differentiates the content of macro- and microelements. Relief, next to the soil type and soil texture, considerably influences the concentration of biogens in groundwaters [21]. The research proved that humous deluvial soils and mucky soils located at the bottom of the slope and containing substantial amounts of organic matter are a place of accumulation of majority of macro- and microelements in the landscape of ice-dammed lakes. They are the first barrier protecting hydrogenic sites in land depressions against the infiltration of biogens from surrounding slopes, on which the soils in this zone of young glacial landscape are intensively used for agricultural purposes.

Conclusions

1. In the studied toposequence of black earths, proper and humous deluvial soils, mucky soils as well as strongly and slightly silted peat-muck soils, the content of examined elements depended on the location of soils in a relief.

2. Most abundant in macro- and microelements were the soils at the bottom of the slope. Humous deluvial soils had the highest contents of K and Fe, mucky soils – Mg, Cu, Zn, Mn, and peat-muck soils – Ca, P and Na.

3. Arable horizons of proper deluvial soils located on the slope were impoverished in macro- and microelements as compared with deeper horizons of the soil profile and parent material of eroded soils. Considerable enrichment of surface layers in the studied elements (excluding calcium) was noted in mucky soils and silted peat-muck soils.

4. In the zone of ice-dammed lakes of young glacial landscape, humous deluvial soils and mucky soils surrounding wetlands protect them against the infiltration of biogens from agricultural areas.

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ZAWARTOŚĆ WYBRANYCH MAKRO- I MIKROELEMENTÓW W TOPOSEKWENCJI GLEB KRAJOBRAZU RÓWNIN ZASTOISKOWYCH NIZINY SĘPOPOLSKIEJ

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Abstrakt: Badania przeprowadzono metodą katen glebowych na Nizinie Sępopolskiej reprezentującej strefę zastoiskową krajobrazu młodoglacjalnego. Sekwencja gleb w katenie przedstawiała się następująco: czarne ziemie, gleby deluwialne właściwe, gleby deluwialne próchniczne, gleby namurszowe, gleby torfowo-murszowe silnie i słabo zamulone. W badanych glebach oznaczono: uziarnienie, odczyn pH, zawartość węgla i azotu ogólnego oraz całkowitą zawartość Ca, Mg, K, P i Na, Fe, Mn, Zn i Cu.

Odczyn badanych gleb kształtował się od lekko kwaśnego w glebach deluwialnych do obojętnego w glebach torfowo-murszowych. W analizowanej toposekwencji gleb w kierunku centrum zagłębienia zwiększała się zawartość węgla i azotu.

Zróżnicowanie zawartości oznaczonych pierwiastków w glebach badanej toposekwencji było uzależnione od ich położenia w reliefie. Szereg ilościowy oznaczonych pierwiastków w czarnych ziemiach erodowanych przedstawiał następująco: Fe > Ca > Mg > K > P > Na > Mn > Zn > Cu. W glebach deluwialnych więcej było potasu niż magnezu, a w glebach torfowo-murszowych więcej jest wapnia niż żelaza.

Poziomy uprawne, zlokalizowanych w środkowej części stoku, gleb deluwialnych właściwych wykazywały największe zubożenie makro- i mikroelementów w odniesieniu zarówno do głębszych warstw profilu, jak i do skały macierzystej gleb erodowanych. Największą kumulację makro- i mikroelementów wykazywały gleby usytuowane u podnóża stoku. Gleby deluwialne próchniczne wyróżniały się największą zawartością K i Fe, gleby namurszowe – Mg, Cu, Zn i Mn, a gleby torfowo-murszowe – Ca, P i Na. Gleby deluwialne próchniczne oraz odgórnie namulone gleby organiczne stanowią bariery, zapobiegające rozprzestrzenianiu się pierwiastków biogennych z terenów rolniczych do siedlisk mokradłowych.

Słowa kluczowe: krajobraz zastoiskowy, makro- i mikroelementy, toposekwencja gleb, czarne ziemie, gleby deluwialne, gleby namurszowe, silnie i słabo zamulone gleby torfowo-murszowe

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SEASONAL VARIATION IN THE CONCENTRATIONS AND LOADS OF MINERAL NITROGEN COMPOUNDS IN ATMOSPHERIC PRECIPITATION IN THE VICINITY OF OLSZTYN (NE POLAND)

SEZONOWA ZMIENNOŚĆ STĘŻEŃ I ŁADUNKÓW MINERALNYCH ZWIĄZKÓW AZOTU W OPADACH ATMOSFERYCZNYCH W OKOLICACH OLSZTYNA

Abstract: The present study was conducted during the hydrological years 1993–2008 at the meteorological station in Tomaszkowo near Olsztyn (NE Poland). The study involved the determination of daily precipitation levels, water sampling after each precipitation event, and the measurement of pH, electrolytic conductivity (EC), the concentrations and loads of mineral nitrogen compounds: $N\text{-}NO_2$, $N\text{-}NO_3$, $N\text{-}NH_4$. It was found that the 1993–2008 period was characterized by the average amount of precipitation in the vicinity of the city of Olsztyn. Three years were classified as wet, three years were dry and one year was very dry. The majority of the annual precipitation (77 % on average) occurred in the summer half-year. Summer was the wettest season (37 % of the total annual precipitation on average), and winter was the driest season (17 %). The pH of precipitation water ranged from 3.8 to 8.12 (pH 5.6 on average). Rainfall was usually slightly acidic (72 %) to acidic (21 %). The total nitrogen load in precipitation was determined by precipitation levels and the concentrations of mineral nitrogen compounds in precipitation. As regards nitrogen concentrations and loads, the predominant compound was $N\text{-}NH_4$, while $N\text{-}NO_2$ was present in low quantities. In the vicinity of Olsztyn, the input of mineral nitrogen via precipitation per ha, per year ranged from 5.34 to 9.74 kg, including 5.17 $kg \cdot ha^{-1}$ $N\text{-}NH_4$, 2.27 $kg \cdot ha^{-1}$ $N\text{-}NO_3$ and 0.05 $kg \cdot ha^{-1}$ $N\text{-}NO_2$ on average. The highest mineral nitrogen load was noted in wet years (8.87 $kg \cdot ha^{-1}$ on average), while the lowest – in years with normal precipitation (4.80 $kg \cdot ha^{-1}$ on average). Most nitrogen was deposited from the atmosphere in precipitation over the growing season, which supported its accumulation in plants and soil, thus reducing groundwater and surface water pollution.

Keywords: precipitation water, nitrogen, atmospheric deposition

In recent years the natural process of soil acidification has intensified due to improper agricultural practices and the application of high mineral fertilizer rates. Another important reason is anthropogenic atmospheric pollution with sulfur compounds and nitrogen oxides, which are the principal cause of acid rain [1]. Due to

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natural air pollution, the pH of rain is at a level of 5.65. Atmospheric precipitation whose pH is lower is referred to as acid rain. The pH of precipitation is determined by the content of sulfuric acid and nitric acid in ambient air in 60–70 % and 30–40 %, respectively [2]. Soil acidification has harmful effects on the environment as it induces and accelerates many processes that lead to soil impoverishment caused by the loss of basic calcium, magnesium and potassium ions, increased release of aluminum and manganese ions toxic to plants, as well as increased mobility and availability of other heavy metals [1]. Atmospheric precipitation is a significant source of non-point pollution in soil and surface water. In contrast to mineral fertilizers, precipitation water contains dissolved components which facilitates their migration in the environment. During intensive precipitation nitrogen quickly migrates to groundwater with surface and subsurface runoff [3, 4]. The input of mineral nitrogen load to the catchment varies in time and space. It is higher in areas exposed to strong human pressure and in regions characterized by high levels of precipitation. Atmospheric deposition is a valuable source of nitrogen in farmlands, where it ensures a grain yield of 2 $Mg \cdot ha^{-1}$. However, in natural areas (nature reserves) high nitrogen loads may contribute to the rapid growth of nitrophilous plants and the disappearance of plants characteristic of nutrient-poor habitats [5]. The runoff from agricultural catchments is affected primarily by the intensity and distribution of atmospheric precipitation, land use type, the properties and type of soils, vegetation cover, the growth stage of vegetation as well as soil reclamation and drainage systems [6, 7].

The objective of this study was to estimate the concentrations and loads of mineral nitrogen compounds in precipitation water, deposited on the land surface, and to determine their potential effect on the natural environment in the Olsztyn Lakeland.

Materials and methods

The study was conducted during the hydrological years 1993–2008 at the meteorological and hydrological station in Tomaszkowo near Olsztyn, in the Olsztyn Lakeland, in the western part of the Masurian Lakeland – the most westward macroregion among Eastern Baltic Lakelands. The Olsztyn Lakeland covers an area of approximately 3820 km^2. The local landscape was shaped in the Poznan and Pomeranian phases of the Vistulian glaciation during the last glacial period. The axis of symmetry of seven concentric arcs of end moraines is the Lyna River flowing south to north. Olsztyn, the main city of the province of Warmia and Mazury, is situated on the Lyna River, in the central part of the region. The climate of the Masurian Lakeland is cooler than in the neighboring macroregions. It is affected by topographic features and the proximity of numerous water bodies, forests and peatlands. Brown soils used as arable land and grassland dominate in this Lakeland [8].

The study involved the determination of daily precipitation levels – with a Hellmann rain gauge, water sampling after each precipitation event – with a precipitation collector design based on a model [7], the measurement of pH – with a potentiometer, electrolytic conductivity (EC) – with a conductometer, the concentrations of mineral nitrogen compounds: nitrate(III) nitrogen ($N\text{-}NO_2$) – colorimetrically, using sulfanilic acid, nitrate(V) nitrogen ($N\text{-}NO_3$) – using disulfophenolic acid, ammonium nitrogen ($N\text{-}NH_4$) – colorimetrically, using Nessler's reagent. The loads of $N\text{-}NH_4$, $N\text{-}NO_3$ and $N\text{-}NO_2$ were calculated as the product of their concentrations and the volume of each precipitation event in a given month, expressed as $kg \cdot ha^{-1}$. As the loads of $N\text{-}NO_2$ were very low (below $0.01\ kg \cdot ha^{-1}$), their values are not listed in the table, but they were used for calculating the total mineral nitrogen load ($N\text{-}NH_4 + N\text{-}NO_3 + N\text{-}NO_2$). The results are presented for individual months, years, half-years (winter half-year: November – April and summer half-year: May – October) and seasons (fall: September – November, winter: December – February, spring: March – May and summer: June – August). The obtained precipitation data were compared with the long-term average of 1951–2000 (615.9 mm), and the analyzed years, periods (half-years) and seasons were classified according to the system proposed by Kaczorowska [9] as: extremely wet (above 155 % of the long-term average), very wet (136–155 %), wet (111–135 %), normal (90–110 %), dry (75–89 %), very dry (51–74 %) and extremely dry (below 50 % of the long-term average).

Results and discussion

During the hydrological years 1993–2008, the annual precipitation totals in the vicinity of Olsztyn ranged from 408.2 to 827.2 mm (Table 1).

Table 1

Annual precipitation totals and mean air temperatures in the vicinity of Olsztyn in the years 1993–2008

Year	Precipitation		Temperature [°C]
	[mm]	Kind of year	
1993	679.0	normal	7.8
1994	649.3	normal	8.6
1995	716.9	wet	8.2
1996	408.2	very dry	5.8
1997	556.1	normal	7.3
1998	550.0	dry	7.3
1999	671.3	normal	8.2
2000	558.2	normal	8.6
2001	653.6	normal	7.4
2002	640.1	normal	8.4
2003	477.8	dry	7.5
2004	696.3	wet	7.3
2005	460.5	dry	8.3
2006	645.0	normal	7.9
2007	827.5	wet	8.5
2008	561.5	normal	8.9
Mean	609.5	normal	7.9
1951–2000	615.9	—	7.1

In comparison with the long-term average of 1951–2000, according to the classification proposed by Kaczorowska [8], three years were found to be wet, nine years were normal, three years were dry and one year was very dry. Over the experimental period, the mean annual air temperature ranged from 5.8 to 8.9 °C (7.9 °C on average), and it was by 0.8 °C higher than the long-term average of 1951–2000.

In the years 1993–2008, monthly precipitation totals varied widely in time (Fig. 1). Extremely low (3.1 mm – December) and high (188.4 mm – July) values were noted in 1997. However, during the 16-year period February was the driest month (26.3 mm on average), and July was the wettest month (86.0 mm on average). As regards precipitation amounts, months with normal precipitation (39 %) and dry months (19 %) dominated over this period. The hydrological year 1996 was found to be driest (annual precipitation total of 408.2 mm) and coolest (mean temperature of 5.8 °C), the year 2007 was wettest (827.2 mm) and the year 2008 was hottest (8.9 °C).

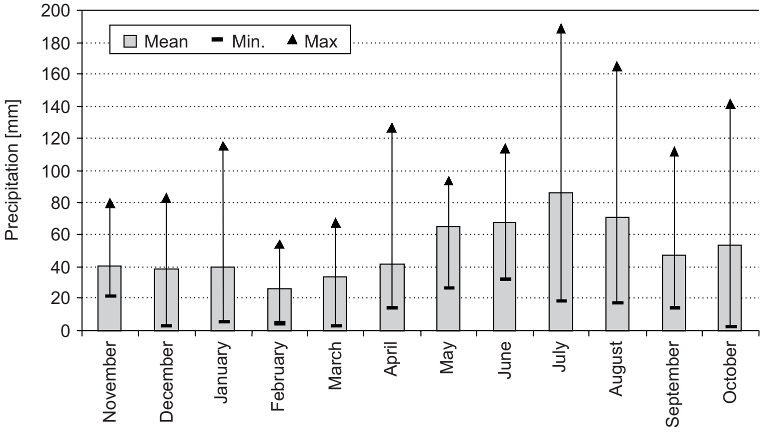

Fig. 1. Dynamics of monthly precipitation totals in the vicinity of Olsztyn in the years 1993–2008

Compared with the analogous periods in 1951–2000, the winter half-years were characterized by lower amounts of precipitation, by 10.5 mm on average. The lowest precipitation total was recorded in the winter half-year of 1996 – by 126.7 mm lower than the long-term average, while the highest precipitation total was noted in the winter half-year of 1994 – by 104.6 mm higher than the long-term average (Table 2).

In the summer half-years of the experimental period mean precipitation was by 4.1 mm higher than the long-term average, varying from 121.3 mm below to 125.8 mm above this average. As regards the seasons, mean precipitation during the experimental period was lower compared with the long-term average, by 17.4 mm in the fall, by 3.3 mm in the summer and by 2.8 mm in the winter. Higher precipitation levels, by 17.0 mm on average, were observed only in the spring. The winter and summer half-years as well as all seasons were warmer (from 0.3 °C in the fall to 1.0 °C in the winter) in the experimental period, as compared with the analogous periods in 1951–2000. The values of calculated means and medians revealed different trends with

Table 2

Seasonal variation in precipitation totals and mean air temperatures in the vicinity of Olsztyn in the years 1993–2008

Season	Precipitation [mm]				Temperature [°C]			
	Min.–Max	Median	V (%)	Average	Min.–Max	Median	SD	Average
Half year								
Winter	103.6–334.9	215.9	30	219.8	–1.9–3.2	1.1	1.2	1.3
Summer	264.3–511.4	409.6	17	389.7	13.4–15.5	14.5	0.7	14.5
Season of year								
Autumn	67.0–243.3	137.9	32	140.4	5.4–11.2	7.8	1.4	7.9
Winter	35.0–184.3	116.9	38	104.4	–6.7–1.0	–1.2	1.8	–1.3
Spring	80.6–232.7	134.1	29	140.1	5.6–9.2	7.6	1.2	7.4
Summer	99.0–329.8	237.5	28	224.6	16.1–19.0	17.7	1.0	17.5

SD – Standard deviation; V – coefficient of variation; Min.–Max – the lowest and the highest monthly values.

respect to both precipitation totals and air temperatures in the summer and winter half-years. The amounts of precipitation were characterized by considerably greater variation in the winter half-years (V = 30 %) than in the summer half-years (V = 17 %). However, in the winter half-years of the analyzed 16-year period wet and dry periods remained in a relative equilibrium, whereas in the summer half-years wet periods dominated over dry periods. With regard to seasons, winter months showed the greatest variation (V = 38 %) in rainfall amount. Similarly as in the summer and spring, humid periods dominated also in the winter, and dry periods were more frequent only in the fall.

During the experimental period, the pH of precipitation water ranged from 3.8 to 8.12 (pH 5.6 on average) and electrolytic conductivity (EC) ranged from 7 to 103 $\mu S \cdot cm^{-1}$ (29 $\mu S \cdot cm^{-1}$ on average) (Table 3).

Rainfall was usually slightly acidic (72 %) to acidic (21 %), with a low proportion of slightly basic (5 %) and neutral (approx. 2 %) samples. As a result, precipitation water was slightly acidic (pH 5.3 to pH 6.0 on average) and only in 2004 – acidic (pH 4.9 on average). The amount of precipitation had no significant effect on the pH and EC of precipitation water. However, a trend was noted towards lower pH values in wetter years and greater variation in EC in drier years, in comparison with the mean values throughout the entire experimental period. Statistically significant correlations between precipitation and pH levels (Table 4) were observed only in one dry year (1998 – r = 0.67) and one wet year (2004 – r = –0.68), while a significant correlation between precipitation and EC was reported in one of the normal years (1999 – r = –0.69). In the winter half-years, the pH of precipitation water was somewhat lower (pH 5.5 on average, ranging from 4.9 to 6.0) and less variable than in the summer half-years (pH 5.7 on average, ranging from 4.4 to 6.3).

Table 3

Variation in the pH and electrolytic conductivity of precipitation water
in the vicinity of Olsztyn in the years 1993–2008

Year	Reaction pH				EC [$\mu S \cdot cm^{-2}$]			
	Min.–Max	Median	SD	Average	Min.–Max	Median	V (%)	Average
1993	4.6–6.7	5.7	0.6	5.8	19–64	29	40	32
1994	5.4–6.6	5.7	0.4	5.9	15–50	26	35	29
1995	5.3–6.7	5.6	0.5	5.7	17–50	27	36	31
1996	5.0–6.9	6.0	0.5	5.9	20–65	33	38	33
1997	5.0–6.5	5.8	0.5	5.7	13–51	36	34	33
1998	4.7–6.6	5.9	0.6	5.8	13–67	33	48	37
1999	4.6–8.1	5.8	0.9	6.0	17–66	32	41	33
2000	4.1–6.2	5.6	0.6	5.5	3–61	29	48	29
2001	3.8–7.3	5.3	1.1	5.5	7–72	32	45	35
2002	4.6–6.1	5.5	0.5	5.4	14–64	32	35	34
2003	4.0–6.8	5.4	0.9	5.3	20–63	36	34	39
2004	3.9–6.2	4.8	0.9	4.9	10–49	29	37	30
2005	4.2–7.5	5.1	1.0	5.4	20–103	38	55	41
2006	3.9–7.0	5.5	0.9	5.5	18–48	31	30	33
2007	4.2–7.7	5.0	1.0	5.4	19–75	36	42	37
2008	4.0–7.5	6.0	1.3	5.9	18–62	31	45	34
Average				5.6				29
Half year								
Winter	4.9–6.0	5.5	0.3	5.5	26–43	35	14	35
Summer	4.4–6.3	5.8	0.4	5.7	21–41	32	14	33
Season of year								
Autumn	4.9–7.0	5.6	0.5	5.6	18–39	27	24	28
Winter	4.6–6.1	5.2	0.5	5.3	20–42	34	17	34
Spring	5.4–7.3	5.7	0.5	5.8	31–49	36	14	38
Summer	4.0–6.6	5.8	0.6	5.7	23–50	36	18	35

SD – Standard deviation; V – coefficient of variation; Min.–Max – the lowest and the highest monthly values.

The values and the range of variation of EC in particular years of the study (V from 30 to 55 %) are indicative of relatively high differences in the concentrations of substances in atmospheric precipitation, resulting from various intensity of pollutant emissions throughout the year. The above variation was affected by the amount and distribution of annual precipitation, although this effect was statistically non-significant. The relatively low variation in EC values in the analyzed half-years (V = 14 %) and seasons (V = 14–24 %) points to a low level of air pollution dynamics in the vicinity of Olsztyn.

Variable weather conditions during the experimental period contributed to high differences in the concentrations and loads of mineral nitrogen compounds in precipitation water in the vicinity of Olsztyn, which were strongly affected by the

Table 4

Correlations between the concentrations and loads of mineral nitrogen compounds in atmospheric precipitation at $N = 192$ and $p < 0.05$

Variable	Precipitation	Temperature	Reaction pH	EC	Concentration N-NH$_4$	Concentration N-NO$_3$	Concentration N-NO$_2$	Load N-NH$_4$	Load N-NO$_3$	Load N-NO$_2$
Temperature	0.44*									
Reaction pH	–0.01	0.22*								
EC	–0.07	–0.03	0.17*							
Concentration N-NH$_4$	–0.14	–0.01	0.09	0.07						
Concentration N-NO$_3$	–0.25*	–0.21*	0.08	0.17*	0.59*					
Concentration N-NO$_2$	–0.06	0.08	0.12	–0.02	0.01	–0.01				
Load N-NH$_4$	0.48*	0.33*	–0.02	–0.01	0.63*	0.10	–0.03			
Load N-NO$_3$	0.60*	0.22*	0.03	0.10	0.18	0.47*	–0.02	0.44*		
Load N-NO$_2$	0.33*	0.22*	0.08	–0.02	–0.06	–0.09	0.87*	0.16*	0.23*	
Load N mineral	0.58*	0.34*	–0.01	0.02	0.58*	0.22*	–0.02	0.97*	0.66*	0.21*

* – essential correlations; EC – electrolytic conductivity.

distribution and intensity of precipitation in particular years. A comparison of mean values obtained in the studied 16 years showed that $N\text{-}NH_4$ dominated in precipitation water ($0.924 \text{ mg} \cdot \text{dm}^{-3}$ on average) – its concentration was over twofold higher than the concentration of $N\text{-}NO_3$ ($0.42 \text{ mg} \cdot \text{dm}^{-3}$ on average). $N\text{-}NO_2$ concentrations were very low ($0.009 \text{ mg} \cdot \text{dm}^{-3}$), compared with the other compounds (Table 5). The mean monthly concentrations of $N\text{-}NH_4$ ranged from 0.036 to $6.195 \text{ mg} \cdot \text{dm}^{-3}$, of $N\text{-}NO_3$ – from 0.005 to $1.889 \text{ mg} \cdot \text{dm}^{-3}$, and of $N\text{-}NO_2$ – from 0.001 to $0.036 \text{ mg} \cdot \text{dm}^{-3}$. Among the analyzed nitrogen forms, $N\text{-}NH_4$ concentrations were characterized by the greatest annual variation, which reached the highest level (V = 138 %) in precipitation water in the dry year 2005. This was probably related to the low amount of precipitation which therefore contained a low concentration of ammonia absorbed from ambient air. However, as shown by the coefficients of variation in the concentrations of the investigated nitrogen forms during the 16-year experimental period, $N\text{-}NO_3$ concentrations were most variable (V from 42 to 97 %).

The concentrations of mineral nitrogen in precipitation water tended to decrease along with an increase in the amount of precipitation, as indicated by the negative values of correlation coefficients. However, due to high variation in precipitation totals and air pollution levels over the 1993–2008 period, a significant correlation ($r = -0.25$) was found only between the amount of precipitation and $N\text{-}NO_3$ concentrations. The concentrations of $N\text{-}NH_4$ (by 13 % on average) and $N\text{-}NO_3$ (by 34 % on average) were higher in the winter half-years, marked by lower precipitation totals, than in the summer half-years.

The highest concentrations of mineral nitrogen compounds in precipitation water were noted in the spring ($1.397 \text{ mg} \cdot \text{dm}^{-3}$ $N\text{-}NH_4$, $0.673 \text{ mg} \cdot \text{dm}^{-3}$ $N\text{-}NO_3$ and $0.010 \text{ mg} \cdot \text{dm}^{-3}$ $N\text{-}NO_2$ on average). The high concentrations of ammonia nitrogen and nitrate(V) nitrogen in atmospheric precipitation over spring months could result from a higher level of air pollution with ammonia from organic fertilizers (slurry and manure) applied at that time in grassland and arable land. Another source of nitrogen compounds in precipitation water could be soil processes leading to the release of ammonia formed during organic matter decomposition.

The input of mineral nitrogen via precipitation per ha of catchment area ranged from 5.34 to 9.74 kg, including 3.48–8.09 kg $N\text{-}NH_4$, 1.42–3.88 kg $N\text{-}NO_3$ and 0.03–0.08 kg $N\text{-}NO_2$ (Table 6). These quantities do not pose a threat to contamination in agricultural land where nitrogen from atmospheric deposition may contribute to soil enrichment, in particular under conditions of low fertilization rates [5].

The amount of mineral nitrogen deposited by precipitation was determined by precipitation totals and by nitrogen concentrations in precipitation water, although the concentrations of $N\text{-}NH_4$ and $N\text{-}NO_3$ decreased in precipitation water along with an increase in its amount. The variation in the load of total mineral nitrogen and its forms was very low, compared with the variation in mineral nitrogen concentrations. Similarly as in the case of nitrogen concentrations, the greatest variation in the atmospheric deposition of $N\text{-}NH_4$ was noted in the dry year 2005 (V = 18 %), and of $N\text{-}NO_3$ – in 2006 (V = 11 %). The highest mineral nitrogen load ($9.74 \text{ kg} \cdot \text{ha}^{-1}$) was recorded in the wet year 1995, and the lowest ($5.34 \text{ kg} \cdot \text{ha}^{-1}$) – in the normal year 2001.

Table 5

Variation in the concentrations of mineral nitrogen compounds [mg · dm^{-3}] in precipitation water in the vicinity of Olsztyn in the years 1993–2008

Year	N-NH$_4$				N-NO$_3$				N-NO$_2$			
	Min.–Max	Median	V (%)	Average	Min.–Max	Median	V (%)	Average	Min.–Max	Median	V (%)	Average
1993	0.48–1.13	0.71	28	0.74	0.05–0.84	0.37	58	0.38	0.002–0.016	0.008	46	0.008
1994	0.49–1.27	0.79	28	0.79	0.10–0.57	0.36	44	0.31	0.004–0.014	0.009	35	0.009
1995	0.48–1.48	0.93	36	0.93	0.04–0.50	0.35	50	0.31	0.002–0.012	0.006	49	0.006
1996	0.68–2.95	1.95	58	1.85	0.23–1.90	0.64	77	0.79	0.002–0.016	0.007	53	0.008
1997	0.46–1.51	0.90	38	0.93	0.09–0.93	0.45	60	0.46	0.002–0.018	0.010	51	0.009
1998	0.21–1.33	0.64	43	0.70	0.05–0.86	0.31	69	0.38	0.002–0.013	0.006	55	0.007
1999	0.21–1.52	0.65	56	0.75	0.12–0.67	0.42	42	0.39	0.001–0.018	0.006	61	0.007
2000	0.17–1.71	0.54	63	0.63	0.07–1.38	0.29	87	0.41	0.001–0.011	0.005	53	0.005
2001	0.04–2.03	0.42	92	0.61	0.03–0.80	0.35	69	0.33	0.002–0.009	0.003	51	0.004
2002	0.16–1.50	0.62	58	0.69	0.15–1.12	0.40	67	0.44	0.004–0.036	0.009	79	0.013
2003	0.25–2.69	0.82	77	0.97	0.19–1.14	0.31	70	0.48	0.007–0.018	0.009	31	0.011
2004	0.14–1.20	0.75	50	0.72	0.01–0.90	0.17	97	0.29	0.003–0.078	0.007	57	0.013
2005	0.09–6.20	0.50	138	1.24	0.13–1.05	0.24	78	0.35	0.003–0.016	0.008	41	0.009
2006	0.10–4.58	0.58	116	1.28	0.02–1.26	0.30	96	0.47	0.004–0.033	0.010	67	0.014
2007	0.10–2.35	0.60	80	0.78	0.25–1.15	0.43	53	0.52	0.004–0.018	0.010	41	0.011
2008	0.22–2.95	0.66	86	1.17	0.22–1.02	0.41	52	0.48	0.006–0.019	0.012	31	0.012
Average				0.92				0.42				0.009

Table 5 contd.

Year	$N-NH_4$				$N-NO_3$				$N-NO_2$			
	Min.–Max	Median	V (%)	Average	Min.–Max	Median	V (%)	Average	Min.–Max	Median	V (%)	Average
Half year												
Winter	0.56–2.87	0.84	57	0.99	0.191.32	0.51	49	0.51	0.005–0.016	0.009	38	0.009
Summer	0.32–1.52	0.82	34	0.86	0.22–0.45	0.34	24	0.34	0.003–0.016	0.009	39	0.009
Season of year												
Autumn	0.18–1.56	0.50	58	0.59	0.10–0.64	0.28	53	0.32	0.003–0.013	0.008	55	0.008
Winter	0.34–2.90	0.64	81	0.75	0.11–1.39	0.34	71	0.41	0.003–0.016	0.007	57	0.007
Spring	0.82–2.91	1.18	42	1.40	0.34–1.19	0.64	35	0.67	0.005–0.021	0.009	48	0.010
Summer	0.40–2.71	0.83	58	0.95	0.17–0.45	0.30	30	0.30	0.004–0.018	0.008	40	0.009

V – coefficient of variation; Min.–Max – the lowest and the highest monthly concentration.

Table 6

Variation in the monthly and total annual loads of mineral nitrogen forms [mg · dm^{-3}] in precipitation water in the vicinity of Olsztyn in the years 1993–2008

Year	$N-NH_4$				$N-NO_3$				N mineral			
	Monthly loads			Sum total	Monthly loads			Sum total	Monthly loads			Sum total
	Min.–Max	Median	V (%)		Min.–Max	Median	V (%)		Min.–Max	Median	V (%)	
1993	0.07–083	0.44	5	5.10	0.04–0.54	0.15	6	2.29	0.11–1.33	0.57	5	7.44
1994	0.09–0.93	0.36	5	5.27	0.04–0.39	0.13	6	2.06	0.12–1.31	0.50	5	7.39
1995	0.10–1.66	0.44	7	7.25	0.01–0.54	0.15	7	2.45	0.12–2.21	0.57	7	9.74
1996	0.10–094	0.40	4	5.49	0.05–0.44	0.15	5	2.13	0.15–1.38	0.59	5	7.65
1997	0.01–1.69	0.33	9	5.67	0.01–0.49	0.15	7	2.26	0.03–2.20	0.47	8	7.99
1998	0.11–0.48	0.30	4	3.54	0.03–0.37	0.14	5	1.87	0.14–0.86	0.47	4	5.45
1999	0.05–1.15	0.31	7	4.76	0.06–0.53	0.16	6	2.33	0.12–1.55	0.47	6	7.14
2000	0.01–0.92	0.21	7	3.73	0.01–0.33	0.12	6	1.86	0.02–1.17	0.36	6	5.62
2001	0.01–0.83	0.19	8	3.48	0.01–0.33	0.15	6	1.83	0.05–1.17	0.34	7	5.34
2002	0.08–1.04	0.30	7	4.14	0.04–0.54	0.13	7	2.76	0.17–1.57	0.47	6	6.98
2003	0.01–0.81	0.29	7	4.53	0.01–0.34	0.16	6	1.99	0.03–1.08	0.50	6	6.57
2004	0.06–0.96	0.39	6	5.22	0.00–0.67	0.11	9	2.10	0.14–1.64	0.51	6	7.39
2005	0.02–5.20	0.24	18	8.09	0.03–0.31	0.10	6	1.42	0.05–5.32	0.37	15	9.57
2006	0.03–1.35	0.28	8	5.42	0.01–1.13	0.11	11	2.78	0.04–1.82	0.39	8	8.28
2007	0.06–1.04	0.36	6	5.51	0.08–0.83	0.24	6	3.88	0.26–1.89	0.58	6	9.47
2008	0.08–1.29	0.41	7	5.59	0.07–0.32	0.20	3	2.33	0.16–1.44	0.70	5	7.99
Average				5.17				2.27				7.50

Table 6 contd.

Year	$N\text{-}NH_4$				$N\text{-}NO_3$				N mineral			
	Monthly loads			Sum total	Monthly loads			Sum total	Monthly loads			Sum total
	Min.–Max	Median	V (%)		Min.–Max	Median	V (%)		Min.–Max	Median	V (%)	
Half year												
Winter	1.13–1.45	1.71	28	1.86	0.51–1.68	0.91	31	0.96	1.94–4.42	2.62	25	2.84
Summer	1.45–6.36	3.14	37	3.31	0.60–2.20	1.27	37	1.31	2.41–7.40	4.68	29	4.65
Season of year												
Autumn	0.16–1.78	0.68	61	0.78	0.13–1.19	0.36	70	0.41	0.34–2.41	1.18	55	1.20
Winter	0.17–1.73	0.55	58	0.73	0.14–0.92	0.32	59	0.38	0.39–2.56	0.89	55	1.12
Spring	1.23–2.05	1.59	16	1.61	0.47–1.50	0.76	30	0.79	1.82–3.34	2.32	19	2.41
Summer	0.82–5.90	1.90	61	2.06	0.32–1.37	0.59	47	0.69	1.21–6.24	2.73	48	2.77

V – coefficient of variation; Min.–Max – the lowest and the highest monthly loads.

With regard to the amount of atmospheric precipitation, the experimental period was divided into very dry, dry, normal and wet years. The highest load of total mineral nitrogen and nitrogen forms was noted in wet years (8.87 kg · ha^{-1} on average), while the lowest load of total mineral nitrogen (7.13 kg · ha^{-1} on average) as well as N-NH$_4$ (4.80 kg · ha^{-1} on average) – in years with normal precipitation. The lowest deposition levels of N-NO$_3$ (1.76 kg · ha^{-1} on average) and N-NO$_2$ (0.03 kg · ha^{-1} on average) was observed in dry and very dry years, respectively. As regards the loads of total mineral nitrogen and its main component, N-NH$_4$, the years of the studied period may be listed in order of decreasing values, as follows: wet > very dry > dry > normal. This confirms the observation that the amount of atmospheric deposition of mineral nitrogen was determined primarily by the concentrations and loads of N-NH$_4$, which were on average twofold higher than those of N-NO$_3$.

The proportions of mineral nitrogen forms in precipitation water varied widely, both within (seasonal variation) and between the analyzed years. In the normal year 2002 and in the wet year 2007, the levels of N-NH$_4$ and N-NO$_3$ were similar, but in the dry year 2005 the contribution of N-NH$_4$ was six-fold higher. Particularly high disproportions between the share of N-NH$_4$ and N-NO$_3$ were noted in 2005, when the amount of N-NH$_4$ was 11-fold higher in the summer half-year, and 19-fold higher over summer months. The periodic variation (V = 25 % in the winter half-years, V = 29 % in the summer half-years) and seasonal variation (V = 19 % in the spring, V = 55 % in the fall and winter) in mineral nitrogen loads was affected mostly by precipitation amounts and the concentrations of nitrogen forms in precipitation water (Table 6). As the amount of precipitation was higher in the summer half-years than in the winter half-years, the atmospheric deposition of mineral nitrogen compounds was also substantially higher (by 64 %) in the summer half-years, and the predominant compound was N-NH$_4$ whose share was by 78 % higher in this period.

Mineral nitrogen loads were twofold higher in the summer and spring than in the fall and winter. The highest loads of N-NH$_4$ and N-NO$_2$ were reported in the summer, and of N-NO$_3$ – in the spring. It is highly desirable that the major part of the annual mineral nitrogen load is deposited in the summer half-year, i.e. during the growing season, as it supports the bioaccumulation of nitrogen by plants and the sorption of ammonium ions in the soil. On the other hand, the fact that the summer load of nitrogen, in particular N-NH$_4$, is larger than the spring load is less beneficial because the nutrient requirements of field crops and grassland vegetation are lower in the summer. The important role of vegetation cover in reducing atmospheric pollution is reflected by a significant decrease in the nutrient outflow in the summer half-year. The pollutant adsorption capacity of vegetation, in particular in forest areas, is reduced in wet years [10].

Conclusions

1. In the vicinity of Olsztyn, the 1993–2008 period was dominated by years with average precipitation levels (9 years), three years were found to be wet, three years were dry and one year was very dry. The majority of the annual precipitation (77 % on average) occurred in the summer half-year. Summer was the wettest season (37 % of the

total annual precipitation on average), followed by the spring and fall (23 % each), and winter was the driest season (17 %).

2. The pH of precipitation water ranged from acid ($pH = 3.8$) to slightly basic ($pH = 8.12$). The average pH determined for the years 1993–2008 was 5.6. Rainfall was usually slightly acidic (72 %) to acidic (21 %), with a low proportion of slightly basic (5 %) and neutral (approx. 2 %) samples.

3. The amount of nitrogen atmospheric deposition was determined by precipitation levels and the concentrations of mineral nitrogen compounds in precipitation. $N\text{-}NH_4$ dominated in precipitation water with respect to both concentrations ($0.92\ mg \cdot dm^{-3}$ on average) and loads ($5.17\ kg \cdot ha^{-1}$ on average). The concentrations and loads of $N\text{-}NH_4$ were twofold higher than those of $N\text{-}NO_3$, while $N\text{-}NO_2$ was present in low quantities (annual deposition below $0.01\ kg \cdot ha^{-1}$).

4. In the vicinity of Olsztyn, the input of mineral nitrogen via precipitation per ha per year ranged from 5.34 to 9.74 kg, including $5.17\ kg \cdot ha^{-1}$ $N\text{-}NH_4$, $2.27\ kg \cdot ha^{-1}$ $N\text{-}NO_3$ and $0.05\ kg \cdot ha^{-1}$ $N\text{-}NO_2$ on average. The highest mineral nitrogen load was noted in wet years ($8.87\ kg \cdot ha^{-1}$ on average), while the lowest – in years with normal precipitation ($4.80\ kg \cdot ha^{-1}$ on average).

5. Most nitrogen was deposited from the atmosphere in precipitation over the growing season, which supported its accumulation in plants and soil, thus reducing groundwater and surface water pollution.

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SEZONOWA ZMIENNOŚĆ STĘŻEŃ I ŁADUNKÓW MINERALNYCH ZWIĄZKÓW AZOTU W OPADACH ATMOSFERYCZNYCH W OKOLICACH OLSZTYNA

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Abstrakt: Badania prowadzono w latach hydrologicznych 1993–2008 w stacji meteorologicznej w Tomaszkowie koło Olsztyna. Obejmowały one: pomiary dobowych ilości opadów atmosferycznych, pobrania wód po każdym opadzie atmosferycznym, pH, EC oraz stężeń i ładunków mineralnych związków azotu: $N\text{-}NO_2$, $N\text{-}NO_3$, $N\text{-}NH_4$. Badania wykazały, że w latach 1993–2008 w okolicach Olsztyna dominowały lata o przeciętnych ilościach opadów atmosferycznych, a także wystąpiły 3 lata wilgotne, 3 suche oraz 1 rok bardzo suchy. Przeważająca część (średnio 77 %) opadu rocznego występowała w półroczu letnim. Spośród pór roku najwilgotniejsze było lato (średnio 37 % opadu rocznego), a najsuchsza była zima z ilością opadów na poziomie 17 % sumy rocznej. Wody opadowe miały odczyn pH 3,8 do pH 8,12), średnio pH 5,6. Wśród nich dominował odczyn słabo kwaśny (72 %) i kwaśny (21 %). Ładunek azotu wnoszonego wraz z opadami zależał od ich ilości i stężenia w nich mineralnych związków azotu. Pod względem stężenia i wielkości ładunku dominował $N\text{-}NH_4$, a $N\text{-}NO_2$ występował w marginalnych ilościach. Na 1 ha w okolicach Olsztyna wraz z opadami atmosferycznymi rocznie trafiało od 5,34 do 9,74 kg azotu mineralnego, w tym średnio: 5,17 kg $\cdot$ ha^{-1} $N\text{-}NH_4$, 2,27 kg $\cdot$ ha^{-1} $N\text{-}NO_3$ i 0,05 kg $\cdot$ ha^{-1} $N\text{-}NO_2$. Największy ładunek azotu mineralnego wystąpił w latach wilgotnych (średnio 8,87 kg $\cdot$ ha^{-1}), a najmniejszy (średnio 4,80 kg $\cdot$ ha^{-1}) w latach normalnych. Przeważająca część depozycji atmosferycznej azotu występowała w okresie wegetacji, co umożliwiało jego akumulację w roślinach i glebie, a w efekcie ograniczało zanieczyszczenie nim wód gruntowych i powierzchniowych.

Słowa kluczowe: wody opadowe, azot, depozycja atmosferyczna

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ADSORPTION, DESORPTION AND DEGRADATION OF CARBENDAZIM IN THE LOESS-LIKE SOIL

ADSORPCJA, DESORPCJA I DEGRADACJA KARBENDAZYMU W GLEBIE LESSOWATEJ

Abstract: Studies on the adsorption, desorption, reaction kinetic and degradation of carbendazim in samples from Ap, Eg, Bt and $2C_{Ca}$ horizons of gleyed lessivé soil formed from loess-like formations (Gleyic Luvisol) were carried out. Degradation experiments were performed at 5 and 25 ^{o}C. Degradation data fitted very well to the exponential form of the three-half kinetic model and, to a much lesser extent, to the first-order kinetic model. This was probably due to the changes in microbial activity of biomass during the degradation process. Therefore, the zero-order kinetic equation was also used. The *DT50* values from first-order equation at 25 ^{o}C were 12.3, 20.2, 76.6 and 183.1 days for the Ap, Eg, Bt and $2C_{Ca}$ horizon, respectively. The activation energies E_a for all soil horizons were in the range of 52–67 kJ · mol^{-1}. The adsorption process proceeded very quickly, within 2 hours equilibrium was achieved, and after maximum 8 hours in the case of desorption. Kinetics data fitted well to the two-site kinetic model. The achieved Freundlich isotherms were of L-type. The estimated K_d^{ads} values were the largest in samples from Bt horizon – 32.89 and the smallest in samples from $2C_{Ca}$ horizon – 0.656. The same was in the case of desorption: 34.99 for Bt and 1.105 for $2C_{Ca}$ horizon. The results suggest that clay minerals play an important role in sorption-desorption processes of this compound in the soil. The calculated Groundwater Ubiquity Score (GUS) index assessed carbendazim on the border between non-leaching and transient compounds.

Keywords: carbendazim, adsorption, desorption, kinetics, degradation, loess-like soil, GUS index

Carbendazim (methyl-1H-benzimidazol-2-ylcarbamate) is a systematic fungicide that controls a wide range of pathogens of cereals, vegetables, fruits, grapes and ornamental plants [1] and is used at a large scale in agriculture throughout Europe [2]. In soils with acid reaction this basic compound accepts a proton and may be adsorbed as a conjugated acid on organic matter and mineral clays by ion exchange [3]. Optimal adsorption occurs near the pH numerically equal to the pK_a of the conjugate acid, that is 4.53 [4], but an enhanced protonation [3] may occur which can shift the maximum adsorption even to 2.5–3.5 pH units above the pK_a of the fungicide [5, 6]. Also hydrogen bonds and van der Waals forces are probable mechanisms of retention of this

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compound [1, 3]. Organic matter is probably the most important fraction related to carbendazim adsorption in soils [1]; values of K_{oc} are mostly in the range of 400–2800 [6, 7]. The compound is slowly degraded with half-lives in the range of 3–15 weeks in the topsoils with natural moisture [2, 8] and about 8–15 weeks in anaerobic conditions [8]. 2-Aminobenzimidazole is its primary degradation product in soils [9].

Despite of relatively strong sorption carbendazim was found in groundwater at concentrations exceeding the maximum allowable concentration for drinking water in the European Union [10]. Such translocation is probably connected with the macropore transport. Therefore, detailed adsorption/desorption and degradation studies on this compound in various soils are necessary to assess the potential risk of water contamination [6]. Almost all investigations concerning this fungicide were concentrated on topsoils, whereas, successful modelling of its translocation to the groundwater requires realistic sorption-desorption and degradation data on the whole soil profile.

The aim of the presented study was to provide detailed data concerning adsorption, desorption and kinetics of these processes, as well as degradation data in horizons of typical Polish loess-like soil, and also preliminary estimation of the leaching potential of carbendazim in this soil on the base of GUS index. The cover of the soil taken into investigations is only 80 cm deep, and because of this it may have a larger potential for substance translocation down the profile and groundwater contamination than the typical loess soil.

Material and methods

Studies were carried out using the samples taken up from the Ap, Eg, Bt and $2C_{Ca}$ horizons of a gleyed lessivé soil formed from loess-like formations (Gleyic Luvisol) from Olszanka (22°43′00″N, 51°02′05″E) located in the central part of the Gielczew Elevation of Lublin Upland. Soils from this area are described in details in literature [11–13]. The loess and loess-like soils belong to the best Polish arable soils and cover about 7 % of their total area. The loess-like formations contain slightly more sand and clay than typical deep loesses. The underlying materials of the examined soil are Upper Cretaceous marls [12]. Soil sampling was performed in different horizons taking care that no contamination by other soil layers occurred. The depth of samples was chosen taking into account changes in microbial biomass of soil. The main characteristic of the soil is given in Table 1.

The soil samples for incubation experiments were dried to 40 % of their water holding capacity (WHC), passed through 2 mm sieve, averaged and stored in the dark at 4 °C. The period between collecting from the field and the beginning of the experiments was no longer than 1 month. Substrate-induced respiration (SIR) was used for determination of soil microbial biomass (C_{mic}) [14]. The soil samples for kinetic and adsorption/desorption experiments were air-dried, sieved using a 2 mm mesh diameter sieve and averaged.

Incubation experiments were performed according to the OECD guideline [15]. Before the start of the experiments the soil samples stored at 4 °C were preincubated for a 5 day period in the dark at 25 °C. Then, 50 g dry mass samples of soil were placed in

Table 1

Summary of the physical, chemical and biological characteristics of the soil

Soil horizon	Ap	Eg	Bt	$2C_{Ca}$
Depth [cm]	5–15	25–35	50–60	80–90
Sand [%]	27	19	27	39
Silt [%]	68	71	49	48
Clay [%]	5	10	24	13
pH ($CaCl_2$)	5.9	5.9	6.0	7.3
C_{org}	1.06	0.26	0.24	0.11
WHC [%]	38.3	33.6	65.5	34.0
C_{mic} [$\mu g \cdot g^{-1}$]	273.9	111.9	3.85	1.85
Mineral. comp.*	S > I > K	S > I ≥ K	S >> I	S >> C

* Mineralogical composition determined by X-ray diffraction (S – dioctahedral smectite + chlorate(III), I – illite, K – kaolinite, C – calcite).

incubation flasks and spiked with carbendazim solution to obtain concentration of 5 $mg \cdot kg^{-1}$ dry mass. Analytical pure carbendazim (certified purity 99.1 %) purchased from Sigma-Aldrich was used for the experiments. At the end, the water content was adjusted with the sterile redistilled water to 40 % of WHC of each soil horizon. The last operation was repeated weekly. On 0, 4, 9, 16, 30, 60, 90, 120 day for the Ap and Eg and 0, 2, 5, 11, 33, 68, 100 and 131 day for the Bt and $2C_{Ca}$ the 5 g dry mass samples were taken from the incubation flasks to polypropylene tubes. Then, 10 cm^3 of methanol was injected and the tubes were agitated on a rotary shaker for 1 h and centrifuged (10 min, 4000 rpm) to separate the liquid phase for analyses. The recoveries were in the range of 76–93 %. All degradation experiments were performed in duplicate at 5 and 25 ^oC.

The batch kinetic and adsorption/desorption experiments were performed according to the OECD guideline [16] at 22 ± 1 ^oC. Selection of an appropriate soil/solution ratio (1:5) was made in the preliminary study.

In the kinetic adsorption experiments tubes with 2 g of air-dried soil and 9.0 cm^3 of 0.01 $mol \cdot dm^{-3}$ $CaCl_2$ were equilibrated overnight and 1 cm^3 of 50.0 $\mu g \cdot cm^{-3}$ carbendazim in 0.01 $mol \cdot dm^{-3}$ $CaCl_2$ was added. Afterwards, the tubes were agitated on a rotary shaker for 2, 4, 6, 8, 24 or 48 h, centrifuged (10 min, 4000 rpm) and the liquid phase was sampled for analyses. In the kinetic desorption experiments the tubes with 2 g of soil and 9 cm^3 of 0.01 $mol \cdot dm^{-3}$ $CaCl_2$ were equilibrated overnight, then 1 cm^3 portions of 50.0 $\mu g \cdot cm^{-3}$ carbendazim in 0.01 $mol \cdot dm^{-3}$ $CaCl_2$ were dosed and tubes were shaken for 24 h, centrifuged and the aqueous phase was recovered as much as possible and analyzed. The volume of solution removed was replaced by an equal volume of 0.01 $mol \cdot dm^{-3}$ $CaCl_2$, agitated for 2, 4, 6, 8, 24 or 48 h and centrifuged to separate the liquid phase for analyses. All kinetic experiments, including the controls (9 cm^3 of 0.01 $mol \cdot dm^{-3}$ $CaCl_2$ and 1 cm^3 of 50.0 $\mu g \cdot cm^{-3}$ carbendazim in 0.01 $mol \cdot dm^{-3}$ $CaCl_2$) and blanks (2 g of soil and 10 cm^3 of 0.01 $mol \cdot dm^{-3}$ $CaCl_2$) were performed in duplicate.

Five concentrations of carbendazim (70.0, 50.0, 38.0, 26.0 and 10 μg $\cdot$ cm^{-3} for Ap, Eg and Bt samples and 50.0, 38.0, 26.0 10.0 and 5.0 μg $\cdot$ cm^{-3} for 2C$_{\mathrm{Ca}}$ samples) in 0.01 mol $\cdot$ dm^{-3} $CaCl_2$ were used for adsorption/desorption experiments. The 2 g soil samples were placed in 50 cm^3 polypropylene tubes and equilibrated with 9 cm^3 of 0.01 mol $\cdot$ dm^{-3} $CaCl_2$ overnight (12 h). Afterwards, 1 cm^3 of the appropriate carbendazim solution was added. The tubes were agitated for 24 h to achieve equilibrium and centrifuged. The aqueous phase was recovered as completely as possible and analyzed. In the step performed to examine the desorption process, the volume of solution removed was replaced by an equal volume of 0.01 mol $\cdot$ dm^{-3} $CaCl_2$. The new mixture was agitated for 24 h, and then centrifuged to separate the liquid phase for analyses. The amounts of adsorbed/desorbed carbendazim as a function of time were calculated from the difference between the initial and the final substance concentrations in solution [16]. All adsorption/desorption experiments were performed in triplicate.

A 20 mm^3 portion of solution was injected into a WellChrom (Knauer, Berlin, Germany) HPLC equipped with two K-500 pumps, a K-2500 UV-VIS detector and a Hypersil Gold C_{18} column (100 × 3.0 mm i.d., 3 μm particle size, Thermo Electron Corporation, Runcorn, United Kingdom) preceded by a Hypersil Gold C_{18} guard column (10 × 3.0 mm i.d., 3 μm particle size). The mobile phase was 26:74 (v/v) acetonitrile/citrate buffer (pH 6.5). The flow rate of the mobile phase was 0.7 cm^3 $\cdot$ min^{-1}, the run time was 6 min per sample and the detection wavelength $\lambda = 223$ nm. The detection limit was 0.5 μg $\cdot$ dm^{-3} and reproducibility of the results with the relative standard deviation lower than 5 %.

The zero-order kinetic model $C_t = C_0 - \mathrm{k}_0 \cdot t$ (C_0 and C_t [mg $\cdot$ kg^{-1}] – masses of compound in the soil at the beginning of the experiment and at time t [d], k_0 – zero-order degradation rate coefficient), first-order kinetic model $C_t = C_0 \exp(-k \cdot t)$ (k – first-order degradation rate coefficient) and three-half kinetic exponential model [17] $C_t = C_0 \cdot \exp(-k_1 \cdot t - E_0/\mu(\exp(\mu \cdot t) - 1))$ (k_1 – initial rate constant, μ – growth rate constant and E_0 – starting cell concentration) was used for estimation of parameters from incubation experiments. In the last equation assumption $k_1 = 0$ was made. For calculating activation energy E_a [kJ $\cdot$ mol^{-1}] the transformed form of Arrhenius equation was used: $E_a = R \ln(DT50_1/DT50_2)/(1/T_1 - 1/T_2)$ ($DT50_1$ and $DT50_2$ – 50 % dissipation times at temperature T_1 and T_2 (K), R [kJ $\cdot$ K^{-1} $\cdot$ mol^{-1}] – gas constant); the details are described in [18].

The Freundlich equation $S^{ads/des} = K_F^{ads/des}\, C^{1/n}$ ($S^{ads/des}$ [μg $\cdot$ cm^{-3}] – amount of adsorbed/remain after desorption solute per unit mass of soil, $K_F^{ads/des}$ and $1/n$ – empirical constants) was used to fit the adsorption/desorption data and the two-site model $S_t^{ads} = S_1^{ads} \cdot (1 - \exp(-k_1 \cdot t)) + S_2^{ads} \cdot (1 - \exp(-k_2 \cdot t))$ and $S_t^{des} = S_e^{des} + S_1^{des} \exp(-k_1 \cdot t) + S_2^{des} \exp(-k_2 \cdot t)$ ($S_1^{ads/des}$ and $S_2^{ads/des}$ [μg $\cdot$ g^{-1}] – amounts of solute adsorbed/remain after desorption at time t to the sites with the first order adsorption/desorption rate constants k_1 and k_2, respectively) to fit the results from kinetics experiments. At adsorption/desorption equilibrium $S_e^{ads} = S_1^{ads} + S_2^{ads}$ and $S_e^{des} = S_e^{ads} - S_1^{des} - S_2^{des}$. The details concerning estimations with using of above equations were described in other papers [19, 20].

Parameters of the above equations were estimated by nonlinear regression (Marquardt method, Statgraphics, Manguistic).

Results and discussion

The data from incubation experiments are plotted in Fig. 1. The rate of degradation decreased with the increase of soil depth and with the decrease of temperature. It should be noted that degradation process was only a little slower in the soil samples from Eg horizon in comparison with the respectable samples from Ap horizon despite of large differences with C_{mic} content (Table 1). In the samples from lower horizons, degradation was much slower; clearly the slowest in the $2C_{Ca}$ horizon, although, small C_{mic} content in Bt and $2C_{Ca}$ horizons suggest that both horizons should be in practice biologically inactive. Fitting the first-order kinetic equation to the experimental results gave generally good results ($r^2 > 0.7$), with the exception of samples from Bt and $2C_{Ca}$ horizons at 5 °C (Table 2). But r^2 values are generally smaller when degradation is slow [21] despite of low *RSS* values. Values of E_a calculated for particular soil horizons were in the range of 52.0–67.0 kJ · mol^{-1}. It is probable that larger values of activation energy in the Bt and $2C_{Ca}$ horizons are only the result of difficulties with exact estimation of *DT50* of the slowly degraded compound.

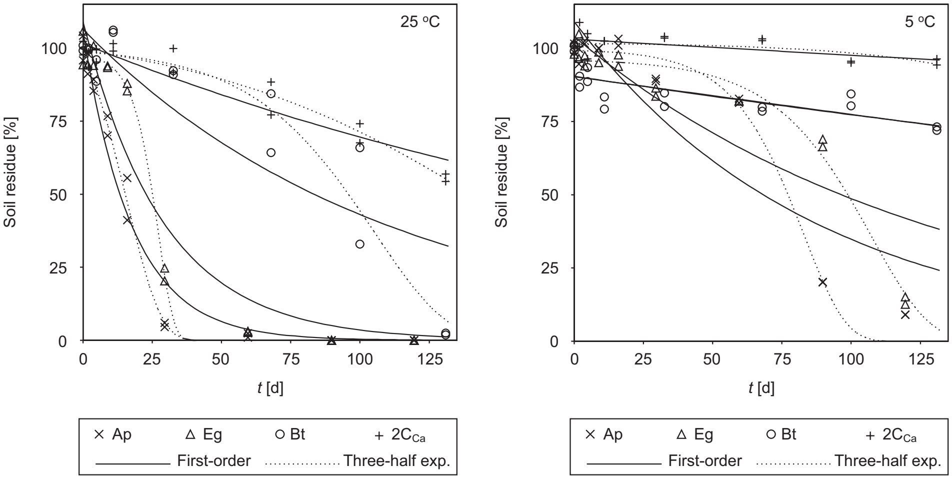

Fig. 1. Fit of first-order and three-half kinetic models to data of carbendazim incubation experiments in soil horizons at 25 °C and 5 °C

The results of fitting, using the exponential form of the three-half kinetic model are presented in Table 3 and as dash lines in Fig. 1. The values of r^2 in this Table are greater in comparison with the appropriate values in Table 2, and *RSS* values are lower. This suggests that changes in biomass influence the degradation process of carbendazim. The presence of the lag-phase in the Eg and Bt horizons at 25 °C and in Ap and Eg horizons at 5 °C also confirm this (Fig. 1).

Table 2

Degradation rate estimates and their standard errors estimated with using the first-order kinetic equation

Horizon/Temp		k $[d^{-1}]$	$DT50$ [d]	r^2	RSS	E_a $[kJ \cdot mol^{-1}]$
Ap	25 °C	0.0561 ± 0.0058	12.3 ± 1.3	0.970	1.29	54.9
	5 °C	0.0114 ± 0.0020	60.7 ± 10.4	0.825	4.52	
E_g	25 °C	0.0343 ± 0.0057	20.2 ± 3.3	0.922	4.80	52.0
	5 °C	0.0076 ± 0.0014	91.4 ± 15.7	0.779	5.53	
Bt	25 °C	0.0090 ± 0.0019	76.6 ± 15.3	0.777	7.61	60.4
	5 °C	0.0016 ± 0.0004	441.9 ± 105.5	0.569	0.88	
$2C_{Ca}$	25 °C	0.0038 ± 0.0004	183.1 ± 18.9	0.892	0.88	67.0
	5 °C	0.0005 ± 0.0002	1303.7 ± 449.8	0.381	0.33	

$DT50 = \ln 2/k$ – time required for 50 % dissipation of initial concentration.

Table 3

Degradation rate estimates using the simplified exponential form ($k_1 = 0$) of the three-half kinetic model

Soil horizon		E_0 $[d^{-1}]$	μ $[d^{-1}]$	r^2	RSS
Ap	25 °C	0.0223	0.0821	0.993	0.29
	5 °C	0.0003	0.0661	0.988	0.43
E_g	25 °C	0.0013	0.1801	0.996	0.26
	5 °C	0.0003	0.0481	0.965	0.89
Bt	25 °C	0.0007	0.0382	0.938	2.14
	5 °C	0.0014	0.0014	0.563	0.89
$2C_{Ca}$	25 °C	0.0014	0.0152	0.947	0.43
	5 °C	$7.7\ 10^{-5}$	0.0241	0.441	0.31

Since most of the examined degradation curves did not show the real first-order kinetic behavior and that caused difficulties with the estimation of $DT50$ and E_a values, the zero-order equation was used. Values of r^2 and RSS indicated that this equation gave a better fit of the experimental data than the first-order equation, however worse than the three-half kinetic model. Also the values of E_a from Table 4 are scattered to a lesser extent than those from Table 2. It seems that in the cases in which changes in microbial biomass influence the degradation process, the zero-order kinetic equation may give more reliable results than the first-order one. Values of r in comparison C_{mic} with $DT50$ at 25 °C and 5 °C calculated from zero-order equation were –0.770 and –0.702, respectively, whereas, for the first order equation –0.738 and –0.695, which also confirms the above thesis.

The values of $DT50$ for carbendazim in this study are lower or are in the lower range of the results obtained by other authors [2, 8, 22]. Degradation potential of the examined soil was high and an adaptation phase, which in the case of this compound is usually relevant to this process [9, 23], was relatively short. The values of E_a correspond well with mean activation energy 54.1 $kJ \cdot mol^{-1}$ calculated by Walker et al [24] on the basis of 114 observations of various pesticides (but without carbendazim) and proposed as a default value for EU.

Table 4

Degradation rate estimates and their standard errors using the zero-order kinetic equation. $DT50 = C_0/2k_0$

Horizon/Temp	k_0 [$\text{mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$]	$DT50$ [d]	r^2	RSS	E_a [$\text{kJ} \cdot \text{mol}^{-1}$]
Ap 25 °C	0.1184 ± 0.0047	15.6 ± 0.5	0.985	0.27	50.2
5 °C	0.0279 ± 0.0022	66.7 ± 4.2	0.909	2.34	
Eg 25 °C	0.1017 ± 0.0144	21.8 ± 2.5	0.833	2.57	50.0
5 °C	0.0265 ± 0.0028	87.9 ± 7.7	0.849	3.77	
Bt 25 °C	0.0289 ± 0.0030	79.6 ± 6.6	0.865	4.58	51.4
5 °C	0.0057 ± 0.0013	353.3 ± 78.3	0.562	0.89	
$2C_{Ca}$ 25 °C	0.0145 ± 0.0012	159.3 ± 11.3	0.916	0.69	62.0
5 °C	0.0024 ± 0.0008	962.8 ± 318.0	0.385	0.33	

The data on kinetics of adsorption/desorption in soil are presented in Fig. 2. The course of these processes in all soil horizons was similar. In a less than 2 hours time soil adsorbed an appropriate portion of carbendazim and equilibrium was obtained. Such fast adsorption kinetics was also observed by Dios Cancela et al [1]. Carbendazim desorption was also very fast at first, then slowed down rapidly, the stage of slow desorption started and equilibrium was obtained in a time of about 8 hours. As it was in the case of previous studies [20], kinetics data fitted well to the two-site kinetic model. Values of r^2 for adsorption/desorption for Ap, Eg, Bt and $2C_{Ca}$ horizons were 0.978/0.872, 0.985/0.962, 0.999/0.872 and 0.728/0.802, respectively. On the basis of these results it may be assumed that both adsorption and desorption of carbendazim

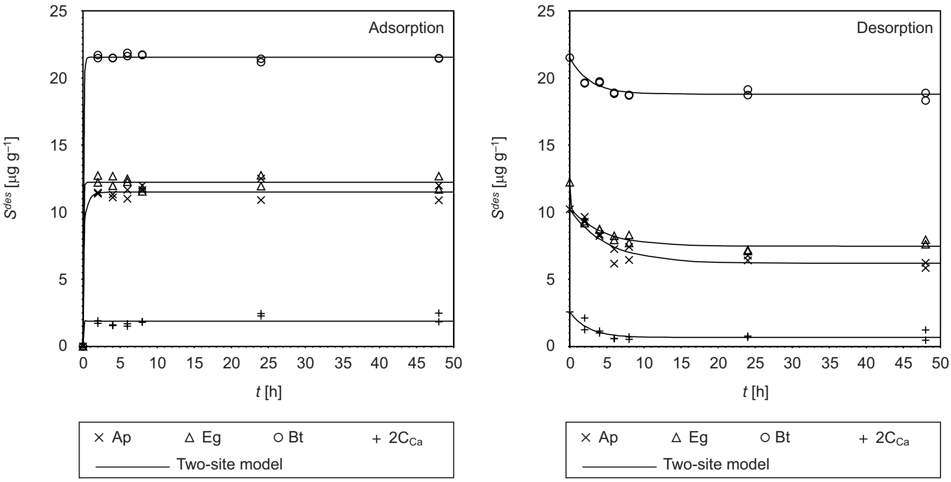

Fig. 2. Carbendazim adsorption and desorption in soil horizons as a function of reaction time

proceeded quickly and 24 hours of equilibration time was long enough to obtain adsorption and desorption isotherms. It also can be concluded that the experiments with these processes were not influenced by degradation of carbendazim.

After desorption, the amounts of non-desorbed carbendazim were high in comparison with the previously adsorbed amounts; in the Ap and Eg horizons 64.3 and 63.5 %, extremely high in Bt horizon – 87.3 % and only 27.1 % in $2C_{Ca}$ horizon. This suggests existence of strong bonds between the fungicide and the soil constituents (especially in the Bt horizon) in fraction not capable of desorption and weak, probably physical forces, in desorbed fraction.

The adsorption and desorption isotherms are shown in Fig. 3. The estimated values of parameters of Freundlich equation and also K_d values are given in Table 5. The obtained isotherms are of L-type ($1/n < 1$) which is usually attributed to the sorption of high polar or monofunctional ionic substances presenting very strong intermolecular interactions into organic and mineral matrices [25, 26].

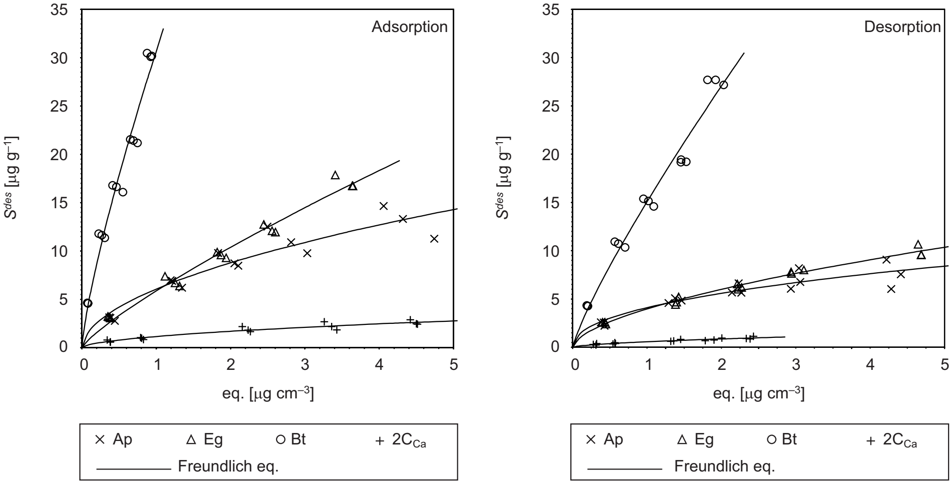

Fig. 3. Adsorption and desorption isotherms of carbendazim in soil horizons

The smallest K_F^{ads} value was calculated for the samples from the $2C_{Ca}$ horizon (Table 5) larger for Ap and Eg horizons and the largest for the samples from Bt horizon. The same was observed for desorption isotherms; the smallest K_F^{des} value was in the samples from the $2C_{Ca}$ horizon and the largest again in the samples from Bt horizon. The obtained K_F^{des} and K_d^{des} values were higher than the corresponding K_F^{ads} and K_d^{ads} values. Such commonly found in sorption experiments hysteresis [3, 25] may be caused by eg slow kinetic of desorption, diffusion-limited transport within the particles and, which is supposed to be the most probable in the case of carbendazim, strong or irreversible binding of this compound to soil particles [6, 7].

Comparison values of C_{org} from Table 1 with the respectable values of K_F^{ads} and K_d^{ads} did not give good correlation (–0.159 and –0.207). Enough good positive values of r were obtained in comparison values of these parameters with total clay content (0.842

Table 5

Adsorption/desorption parameters and their standard errors using the Freundlich equation

Soil horizon	Adsorption					Desorption				
	K_F^{ads}	$1/n$	r^2	K_d^{ads}	r^2	K_F^{des}	$1/n$	r^2	K_d^{des}	r^2
Ap	6.059 ± 0.481	0.534 ± 0.069	0.877	4.289 ± 0.263	0.726	6.168 ± 0.224	0.448 ± 0.065	0.848	6.541 ± 0.428	0.584
Eg	5.875 ± 0.301	0.821 ± 0.049	0.975	5.043 ± 0.172	0.917	6.856 ± 0.099	0.595 ± 0.027	0.984	6.995 ± 0.324	0.807
Bt	30.68 ± 1.03	0.785 ± 0.060	0.963	32.89 ± 1.63	0.848	32.66 ± 1.23	0.844 ± 0,057	0.970	34.99 ± 1.32	0.909
$2C_{Ca}$	1.155 ± 0.091	0.547 ± 0.064	0.907	0.656 ± 0.043	0.684	0.982 ± 0.042	0.592 ± 0.068	0.902	1.105 ± 0.060	0.728

and 0.869). It can be concluded from this that clay minerals play an important role in sorption of carbendazim in the examined soil, especially in the deeper soil horizons. The predominant clay minerals of the soil are complexes of dioctahedral smectites and chlorate(III) (Table 1) with significant accumulation in Bt horizon (30 % higher than in Ap horizon). The confirmation of this is the highest WHC of Bt horizon. The samples from $2C_{Ca}$ horizon composed of cretaceous marls have much higher pH value and because of this its clay minerals have different sorption properties than minerals of upper horizons. Therefore, r values for comparison values of K_F^{ads} and K_d^{ads} with clay content only for Ap, Eg and Bt were higher (0.966 and 0.973). Montmorillonite, the principal clay mineral of smectite group, has a very high selectivity for carbendazim, and illite has a very low one [1]. The largest adsorption of carbendazim on montmorillonite at pH 5–6 is usually explained by an enhanced protonation of its neutral molecules at the clay-solution interface and adsorption by cation exchange [1]. This can explain high adsorption in Bt horizon.

The results of the experiments of Dios Cancela et al [1] showed that organic matter might be the most important soil fraction related to carbendazim adsorption in soils. The optimum pH for maximum adsorption of basic molecules on this soil constituent is usually near the pK_a value of the conjugate acid [3]. This may explain why in the presented study comparison between C_{org} and MCPA sorption parameters did not give positive results. The role of organic matter in sorption of pesticides in soils is very often underestimated [27] and the data presented in this paper also suggest this thesis.

The GUS index ($GUS = (4 - \log K_{oc}) \log(DT50)$) asses the leaching potential of pesticides and the possibility of finding these compounds in the groundwater [28]. The values of this index calculated for the topsoil on the basis of $DT50$ values estimated from the zero- and first-order kinetic equations and recalculated to 20 °C are 1.85 and 1.73, respectively. This classifies carbendazim in the examined loess-like soil on the border between non-leaching and transient compound ($1.8 < GUS < 2.8$ transient compound; $GUS < 1.8$ no leacher). Taking into account very high adsorption and low desorption in the Bt horizon it seems to be improbable that it could be found in groundwater of the soil taken into investigations at concentrations exceeding the maximum allowable concentration.

Conclusions

1. A relatively fast degradation of carbendazim occurred in the Ap horizon of the soil and lower in the deeper horizons. The E_a values were in the range of the mean default value for EU. Degradation data fitted very well to the exponential form of the three-half kinetic model and, to a much lesser extent to the first-order kinetic model. This was probably due to the changes in microbial activity of biomass during the degradation process. In the cases in which the lag-phase was observed, the zero-order kinetic equation was more useful for estimation of degradation parameters than the first-order equation.

2. Carbendazim was quickly and strongly adsorbed and only partly capable of desorption. The highest adsorption and the lowest desorption was observed in the Bt

horizon. On the basis of high correlation values between adsorption coefficients and amount of clay it can be concluded that clay minerals played an important role in adsorption of this compound in the examined soil, especially in the deeper soil horizons.

3. The GUS index assessed the behavior of carbendazim on the border between non-leaching and transient compound. Taking into account very high adsorption and low desorption in the Bt horizon it seems to be improbable that carbendazim could be a leacher in the soil taken into investigations.

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ADSORPCJA, DESORPCJA I DEGRADACJA KARBENDAZYMU W GLEBIE LESSOWATEJ

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Abstrakt: Badania adsorpcji, desorpcji, kinetyki reakcji i degradacji karbendazymu przeprowadzono w próbkach z poziomu Ap, Eg, Bt i $2C_{Ca}$ gleby płowej opadowo-glejowej wytworzonej z utworów lessowatych (Gleyic Luvisol). Doświadczenia z degradacją przeprowadzono dla temp. 5 i 25 °C. Dane z degradacji były bardzo dobrze opisywane wykładniczą postacią modelu trzy i półrzędowej kinetyki i w dużo mniejszym stopniu przez model kinetyki pierwszorzędowej. Było to prawdopodobnie spowodowane zmianami mikrobiologicznej aktywności biomasy w czasie procesu degradacji. Dlatego zastosowano również równanie kinetyki rzędu zerowego. Wartości *DT50* z równania pierwszego rzędu dla 25 °C dla poziomu Ap, Eg, Bt and $2C_{Ca}$ wyniosły odpowiednio 12,3, 20,2, 76,6 i 183,1 dni. Energie aktywacji E_a dla wszystkich poziomów gleby mieściły się w zakresie 52–67 $\text{kJ} \cdot \text{mol}^{-1}$. Proces adsorpcji przebiegał bardzo szybko i w czasie 2 godz. ustalała się równowaga, a w przypadku desorpcji po maksymalnie 8 godz. Dane kinetyczne były dobrze opisywane za pomocą modelu kinetyki dwucentrowej. Otrzymano izotermy Freundlicha typu L. Największe wartości K_d^{ads} otrzymano dla próbek z poziomu Bt – 32,89, najmniejsze dla próbek z poziomu $2C_{Ca}$ – 0,656. Tak samo było w przypadku desorpcji: 34,99 dla poziomu Bt i 1,105 dla $2C_{Ca}$. Wyniki wskazują, że minerały ilaste odgrywają ważną rolę w procesach sorpcyjno-desorpcyjnych tego związku w glebie. Wyznaczona wartość indeksu GUS wskazuje, że karbendazym jest na granicy pomiędzy związkami niepodatnymi na wymywanie i przejściowymi.

Słowa kluczowe: karbendazym, adsorpcja, desorpcja, kinetyka, degradacja, gleba lessowata, indeks GUS

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ANALYSIS OF FLAVONOIDS CONTENT IN ALFALFA

ANALIZA ZAWARTOŚCI FLAWONOIDÓW W LUCERNIE

Abstract: Flavonoid glycosides constitute important group of plant secondary metabolites. This class of natural products play significant role in different physiological processes. Liquid chromatography (HPLC) was used to determine the flavonoid profiles and their concentration in aerial parts of three alfalfa (*Medicago sativa* L.) cultivars. It was shown that flavonoids of alfalfa are glycosides of four flavone aglycones: apigenin, luteolin, tricin and chrysoeriol. All flavonoid glycosides possesed glucuronid acid in sugar chain. Some of them were acylated with ferulic, coumaric or sinapic acids. It was shown that dominant flavonoids of alfalfa were the flavones: tricin and apigenin glycosides (65–72 % of total). The concentration of luteolin and chrysoeriol glycosides did not exceed 30 % of the total. The dominant flavonoid in Radius and Sapko cultivars was glucoside of tricin, in Sitel cultivar glycoside of apigenin. Thus, tricin and apigenin glycosides were the major flavones found in alfalfa aerial parts.

Keywords: *Medicago sativa*, flavonoids, herbivore, *Acyrthosiphon pisum*

Alfalfa (*Medicago sativa* L. (Fabaceae)) is the world's oldest and most important livestock feeding crop [1]. Alfalfa has great potential as food and/ or fodder. High nutritional quality of alfalfa has been determined by the high content of good quality protein and carbohydrates [2, 3]. Alfalfas are one of sources of chlorophyll, vitamines, some digesting enzymes and β-carotene [4]. However, alfalfa contains different classes of secondary metabolites, showing biological activities, but they are not yet fully characterized. The best recognized secondary metabolite groups are carotenoids and saponins [5, 6]. In alfalfa the phenolic composition, including flavonoids is poorly understood [7].

Flavonoids are the group of secondary metabolites that can be found in most of the plant families. Most flavonoids in plant cells are present as glycosides. Flavonoid glycosides constitute a structurally diverse group of plant secondary metabolites [8].

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The flavonoids are usually divided into many subclasses: flavones, isoflavones, flavanones, anthocyanins, aurones and others. These natural products play a very important role in plant development and physiology, especially during their interactions with other living organisms [9]. Flavonoid glycosides and free aglycones are involved in pathogenic and symbiotic interactions with microorganisms [10, 11]. They also act as UV protectants in plant cells [12], pigment sources for flower colouring compounds [13, 14] and they play important roles in interactions with insects [15]. This class of compounds also affect the human and animal health because of their significance in the diet, which is ascribed to their antioxidant and UV properties [16–19], estrogenic action [20] and a wide spectrum of antimicrobial and pharmacological activities [21, 22].

Recent work on alfalfa flavonoids revealed that they consist of apigenin, luteolin, tricin and chrysoeriol glycosides and the only sugar unit found in sugar chains is glucuronic acid. It was ducumented that a number of flavones are acylated with caffeic, ferulic or sinapic acids [23–25]. Very little is, however, known about the concentration of flavones in alfalfa cultivars. Due to the importance of the flavonoids and their glycosides too and in living organisms, the identification and determination the level of such compounds occuring in plant tissue or other biological systems play an important role in many areas of science, particularly in plant science. Thus, the aim of the present study was to analyse the flavonoid profiles and to determine their concentration in green aerial parts of three alfalfa cultivars which are common crops in Poland.

Material and methods

Alfalfa (*Medicago sativa* L.) (Fabaceae), Radius, Sapko and Sitel, was used in the experiments. Cultivars of alfalfa, Radius and Sapko, were obtained from Institute of Plant Breeding and Acclimatisation (IHAR), Radzikow/ Blonie, near Warsaw. Cultivar Sitel was bought in Horticultural Plant Breeding, Seed Production and Nursery in Ozarow Mazowiecki, Poland. Seeds of the studied cultivars were germinated in a climatic chamber kept at 21 ± 1 ^{o}C, L16:D8 photoperiod, and 70 % r.h. The plants were grown in 7 × 7 × 9 cm plastic pots in a standardised soil mixture structure, one plant per pot. The plants were regularly watered and no extra fertiliser was added. The six months old plants of alfalfa were used in the experiments.

Aerial parts of the 6-month-old plants were harvested, freeze-dried, ground, and kept in a desiccator in darkness until analysed. Each extracts were obtained using the ASE 200 Accelerated Solvent Extractor (Dionex Corporation, Sunnyvale, USA) for 20 minutes with 70 % methanol. The extracts were concentrated at 40 C on a rotary evaporator until the methanol was removed and then loaded on C_{18} cartridges (Waters, Poland) preconditioned with water. The flavonoids were then successively washed from the cartridges with water and 40 % methanol. Eluates were evaporated to the dryness, redissolved in 1 cm^3 of 40 % MeOH and used for HPLC determination.

The high performance liquid chromatographic (HPLC) analysis was performed on a Waters Alliance liquid chromatograph (Waters, Milford, MA) equipped with a model 616 pump, a model 600s controller, and a model 996 photodiode array detector. The

Millenium Chromatography Manager was used to monitor chromatographic parameters and to process the data. The alfalfa samples were applied to a Eurospher PD 82 column and eluted at 1 cm^3 min^{-1} with a linear gradient of 1 % phosphoric acid in water : 40 % acetonitrile in 1 % H_3PO_4 (65:35 %), increasing to 0:100 % over 60 min. Chromatograms were registered and integrated at λ = 350 nm. Standards of glycosides, purchased from the Biochemical Laboratory Institute of Soil Science and Plant Cultivation (Pulawy, Poland), were used for calibration curve preparation.

Results and discussion

Flavonoid glycosides are present in the plants. They have great diversity, which suggest their function in plant – herbivores association (oviposition, feeding stimulants, toxicity and inhibitory effect for insects) [16]. Because Fabaceae are important crops it seems crucial to characterize its flavonoid composition and concentration.

The HPLC flavonoid profiles of studied cultivars are similar and contain eighteen individual compounds (Fig. 1). These compounds showed absorption spectra characteristic for apigenin (six glycosides), luteolin (three glycosides), chrysoeriol (one glycoside) and tricin (eight glycosides) derivatives [23–25].

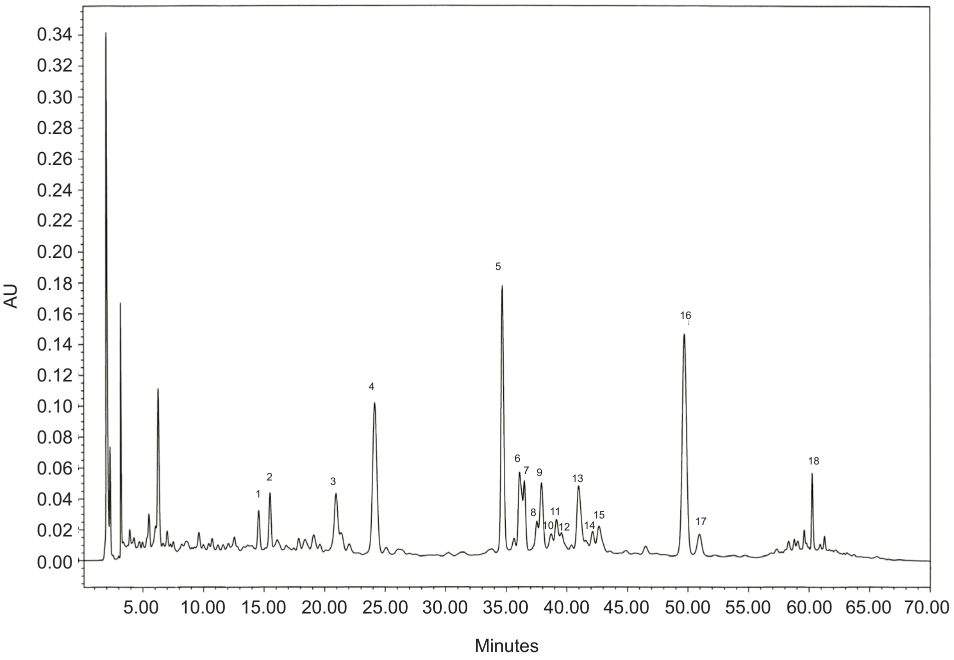
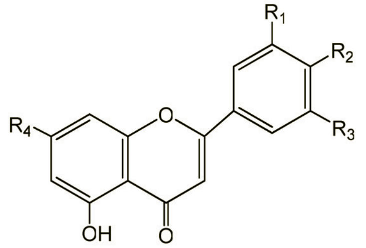


Fig. 1. Identified flavone aglycones: (1) apigenin; (2) luteolin; (3) luteolin; (4) luteolin – acylated; (5) apigenin – acylated; (6) chrysoeriol; (7) apigenin – acylated; (8) apigenin – acylated; (9) apigenin; (10) tricin; (11) tricin – acylated; (12) tricin – acylated; (13) apigenin – acylated; (14) tricin; (15) tricin; (16) tricin; (17) tricin; (18) tricin

The structures of ten individual flavones were elucidated with MS method (Fig. 2). These compounds have been previously separated from alfalfa aerial parts and their structures were confirmed by UV, MS and NMR spectroscopy [23–25]. All flavonoid glycosides possesed glucuronid acid in sugar chain. Some of them were acylated with ferulic, coumaric or sinapic acids. Compounds 4, 5, 7, 12 and 13 were acylated with ferulic, compound 8 with coumaric and compound 11 with sinapic acids.



Compound	R_1	R_2	R_3	R_4
1	-H	-OGluA	-H	-OGluA(2→1)GluA
4	-OH	-OGluA	-H	-OGluA(2→1)GluA-2-*O*-Feruloyl
5	-H	-OGluA	-H	-OGluA(2→1)GluA-2-*O*-Feruloyl
7	-H	-OH	-H	-OGluA(2→1)GluA-[GluA(1→3)]-2-*O*-Feruloyl
8	-H	-OH	-H	-OGluA(2→1)GluA-[GluA(1→3)]-2-*O*-*p*-Coumaroyl
9	-H	-OH	-H	-OGluA
10	$-OCH_3$	-OH	$-OCH_3$	-OGluA
11	$-OCH_3$	-OH	$-OCH_3$	-OGluA(2→1)GluA-2-*O*-Sinapoyl
12	$-OCH_3$	-OH	$-OCH_3$	-OGluA(2→1)GluA-2-*O*-Feruloyl
13	-H	-OGluA(2→1)GluA-2-*O*-Feruloyl	-H	-OH

Fig. 2. Chemical formula of analyzed alfalfa flavones

Alfalfa cultivars demonstrated differences in concentration of the individual flavone glycosides (ANOVA, $p < 0.001$) (Fig. 3). The major flavones found in alfalfa aerial parts were tricin and apigenin glycosides (66–72 % of total). The flavonoids

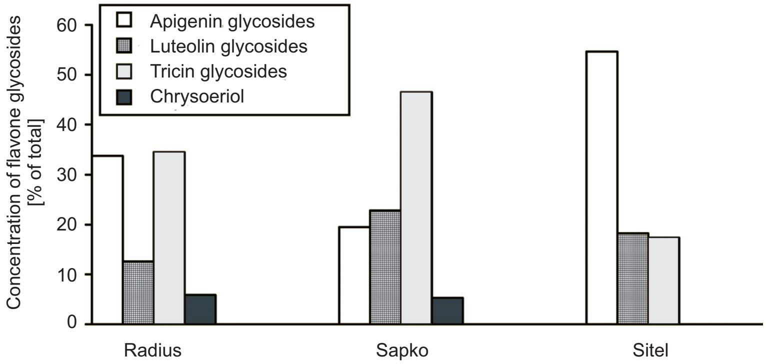


Fig. 3. Content of flavone glycosides from aerial parts of alfalfa cultivars

concentration within alfalfa aerial parts was rather high as compared with other plant sources [18, 26]. For Radius cultivar the tricin glycosides were the dominant and made up 34.5 % of total flavonoids. Their concentration was very similar to the sum of apigenin glycosides (33.8 % of total). Luteolin and chrysoeriol made up 12.6 and 5.9 % of total, respectively. For Sapko cultivar the tricin glycosides were the dominant, too and made up 46.5 % of total. Apigenin, chrysoeriol and luteolin made up 19.5, 5.3 and 22.8 % of total, respectively. For Sitel cultivar the apigenin glycosides were the dominant (54.6 % of total). Concentration of tricin, chrysoeriol and luteolin did not exceed 36 % of total. Thus, tricin and apigenin glycosides were the major flavones found in alfalfa aerial parts. Stochmal and Oleszek [26] made similar observations.

Flavonoid analyses revealed a substantial individual variation (ANOVA, $p < 0.001$). It was shown that compounds number 5 and 16 were the dominant apigenin and tricin glycosides of alfalfa cultivars (Fig. 4). Stochmal and Oleszek [26] showed that in all tested varieties the dominant flavonoid was tricin glycosides.

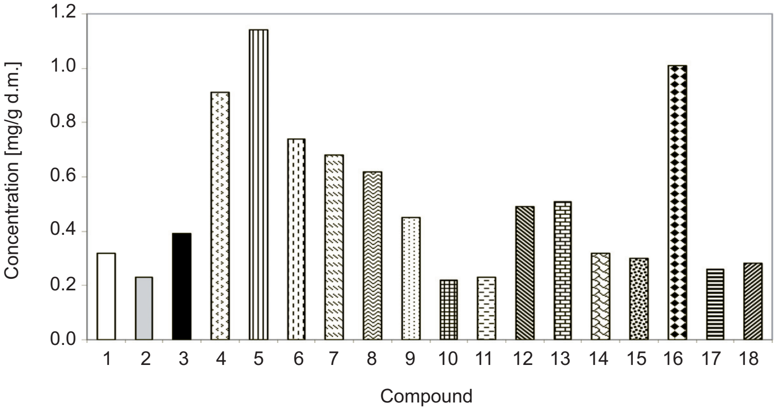


Fig. 4. The concentration of individual flavones from aerial parts of alfalfa cultivars (average for three studied alfalfa cultivars)

Alfalfa flavones are a mixture of acylated and nonacylated forms. The ratio of acylated to nonacylated forms in the plants are important, because the concentration of these compounds probably may reflect the influence of environmental conditions [27]. Considering this fact, accumulation of acylated and nonacylated forms in different cultivars were also analyzed. The average total concentration of acylated flavones in studied alfalfa cultivars ranged between 3.77–5.58 mg/g d.m. There was no significant difference in the concentration of acylated and nonacylated flavones between studied cultivars. The ratio of acylated to nonacylated flavones for studied alfalfa cultivars was similar, too (0.89–1.14).

Conclusions

1. It was shown that the HPLC flavonoid profiles of studied cultivars are similar.

2. Flavonoids of alfalfa are glycosides of four flavone aglycones: apigenin, luteolin, tricin and chrysoeriol.

3. Dominant flavonoids of studied alfalfa were the flavones: tricin and apigenin glycosides (65–72 % of total). The concentration of luteolin and chrysoeriol glycosides did not exceed 30 % of the total.

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ANALIZA ZAWARTOŚCI FLAWONOIDÓW W LUCERNIE

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Abstrakt: Wobec doniesień literaturowych ukazujących różne aspekty interakcji roślina–owad celowe wydaje się poszukiwanie roślinnych substancji chemicznych biorących udział w obronie roślin przed negatywnym wpływem biotycznych czynników środowiskowych. Przykładowo, flawonoidy oddziałują jako deterenty pokarmowe, inhibitory trawienia, a także bezpośrednie toksykanty w stosunku do owadów. Lucerna z uwagi na bogactwo struktur flawonów jest dobrym modelem do prowadzenia prac związanych z tym zagadnieniem. Wiedza na temat występowania flawonoidów w lucernie siewnej, będącej jedną z głównych roślin pastewnych, nie jest pełna, dlatego celem przeprowadzonych badań było zidentyfikowanie i porównanie zawartości flawonoidów w powszechnie użytkowanych odmianach lucerny siewnej (*Medicago sativa* L.).

Badaniom poddano trzy odmiany lucerny: Radius, Sapko i Sitel. Ekstarkcję flawonoidów przeprowadzono z 6-miesięcznych roślin za pomocą metanolu. Rozdział i identyfikację flawonoidów wykonano metodą wysokosprawnej chromatografii cieczowej (HPLC) sprzężonej ze spektrometrią mas.

Stwierdzono, że flawonoidy badanych odmian lucerny to glikozydy czterech aglikonów flawonów: apigeniny, luteoliny, trycyny i chryzoeriolu. W łańcuchu cukrowym wszystkich glikozydów występował kwas glukuronowy, a niektóre z nich były acylowane kwasem ferulowym, kumarowym bądź synapinowym. Wykazano, że dominującymi związkami były pochodne trycyny i apigeniny. Łączna zawartość glikozydów apigeniny i trycyny wahała sie w granicach 65 do 72 % sumy flawonów badanych lucern. Zawartość glikozydów luteoliny i chryzoeriolu nie przekraczała 30 % sumy flawonów. Dla odmian Radius i Sapko związkiem dominującym był glikozyd trycyny, którego maksymalne stężenie (2.49 mg/ g s.m.) odnotowano w tkankach odmiany Radius. W przypadku odmiany Sitel w największych ilościach występował glikozyd apigeniny (1.31 mg/ g s.m.).

Słowa kluczowe: *Medicago sativa*, flawonoid, owady rośliożerne, *Acyrthosiphon pisum*

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EFFECT OF THE CONTROLLED FERTILIZATION ON THE MINERAL COMPONENTS OF CHOSEN VARIETIES OF ONION (*Allium cepa* L.) PART I. MACROELEMENTS

WPŁYW KONTROLOWANEGO NAWOŻENIA NA ZAWARTOŚĆ SKŁADNIKÓW POKARMOWYCH W WYBRANYCH ODMIANACH CEBULI (*Allium cepa* L.) CZ. I. MAKROSKŁADNIKI

Abstract: The main aims of conducted studies was the estimation of the controlled fertilization effect in case of 21 chosen cultivars of onion (*Allium cepa* L.) and the bulb storage in controlled conditions on their nutritive value based on the macroelement content: nitrogen, phosphorus, potassium, calcium, magnesium and sulphur. During the total period of vegetative experiments, controlled fertilization was applied based on cyclic soil analyses recommended in the Integrated Production of Vegetables. A significant effect was found to be exerted by the cultivars on the content of dry matter and macroelements in the onion bulbs. At the same time, no significant effect was found to be exerted by the storage on the content of dry matter as well as on potassium and calcium content in the onions. On the other hand, the storage significantly decreased content of nitrogen, phosphorus and sulphur with a simultaneous increase of magnesium content. Because of a high content in the onion dry matter of (average in d.m.) phosphorus (0.43 % P), calcium (1.97 % Ca) and magnesium (0.26 % Mg), onion is a valuable source of these elements in the human diet. The controlled fertilization based on regular chemical soil analyses is useful to obtain good quality crops in horticulture production.

Keywords: onion, controlled fertilization, macroelements, cultivar-induced differentiation, storage, nutritive value

Because of dietetic reasons, fresh vegetables should be frequently applied in human diets. The low content of dry matter causes that usually vegetables are characterized by a low caloric value. However, they are a very rich source of biologically active compounds. Vegetables are the basic sources of vitamins: C, A, B_1, B_2, B_6 and of such compounds as terpenoids, flavonoids, tannins and quinones [1]. Thanks to a high content of cellulose, they exert a favorable effect on the digestive duct facilitating food

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digestion [2]. Furthermore, vegetables are a valuable source of macro- and microelements. Among factors modifying the nutritive value of vegetables, one can mention, among others, the method of their cultivation [3], the applied fertilizers, both the mineral and the organic ones [4–7], the cultivar and the applied substrate [8, 9].

One of the most frequently consumed vegetables in Poland is onion which occupies in the consumption structure the 5th place (11 %), following immediately after tomatoes (17 %), cabbage (14 %), cucumbers (14 %) and carrot (13 %) [10]. On the seed-market, there are dozens of onion cultivars. Therefore, the essential question is the number of the nutritive components contained in the bulbs of the particular onion cultivars.

The main aims of conducted studies was the estimation of the controlled fertilization effect in case of 21 chosen cultivars of onion (*Allium cepa* L.) and the storage of onions in controlled conditions on their nutritive value based on the macroelement content: nitrogen, phosphorus, potassium, calcium, magnesium and sulphur on their macroelement, which extends both our knowledge in the field of human nutrition and in the field of onion cultivation.

Material and methods

The studies was carried out in 2008–2009 years in a private horticultural farm growing a collection of 83 onion cultivars, while the laboratory studies were carried out in the Department of Horticultural Plant Nutrition in the University of Life Sciences in Poznan. Twenty-one onion cultivars (*Allium cepa* L.) included: ‘Bennito’ (Seminis), ‘Mission’, ‘Napoleon’, ‘Stamford’ (Syngenta), ‘Eureka’, ‘Wolska’ (Vilmorin), ‘Slawa Ozarowa’, ‘Sochaczewska’, ‘Wiktoria Skierniewic’ (PNOS Ozarow Mazowiecki), ‘Kutnowska’ (PlantiCo Zielonki), ‘Cymes’, ‘Topolska’ (Polan), ‘Grabowska’ (Doruchowski, Zuranski), ‘Lawica’, ‘Wojka’, ‘Wola’ (Spojnia Nochowo), ‘Marbella’, ‘Sherpa’ (Frimeko), ‘Tandem’ (MoravoSeed), ‘Aurora’, ‘Consuelo’ (ISI Sementi).

Seed sowing was carried out in the period from the 15th to the 20th of April. During the whole period of studies, controlled fertilization was applied based on cyclical soil analyses carried out three times in the period of vegetation: before seed sowing, in the middle of the vegetation season and in the moment of onion leaves breaking down (end of vegetation). The soil before seeds sowing characterized following of average chemical composition [mg $\cdot$ dm^{-3}]: N-NH$_4$ 12.0, N-NO$_3$ 0.0, P-PO$_4$ 49.0, K 75.1, Ca 720.0, Mg 44.1, S-SO$_4$ 0.1, Cl 13.0, Fe 30.6, Mn 4.1, Zn 5.0, Cu 0.1, pH 7.80, EC 0.20 mS $\cdot$ cm^{-1}. The content of nutritive components was maintained based on the cyclic chemical soil analyses on the standard level [mg $\cdot$ dm^{-3}]: N 90.0, P 70.0, K 225.0, Mg 90.0, Fe 50.0, Mn 15.0, Zn 25.0, Cu 5.0, pH in H_2O 6.0–6.5, EC < 0.6 mS $\cdot$ cm^{-1} [11–13 modified]. It was used fertilizers in following doses (per ha): 180 kg of nitrate ammonium (34 % N), 300 kg of double phosphate (40 % P_2O_5), 200 kg of potassium sulphure (50 % K_2O), 150 kg of magnesium sulphure (16 % MgO), 30 kg manganese sulphure (32.3 % Mn), 30 kg zinc sulphure (22 % Zn), 40 kg copper sulphure (25.6 % Cu), 2 kg of borax (11.3 % B).

Experiment was established in a belt-and-row design. On one field patch, 5 rows were localized, where seeds were sown by a single-seed drill Monosem (seed density was 100 seeds · m^2). Experimental plot for the given cultivar was represented by a field patch of 1.35 × 100 m divided into two blocks. All agrotechnical treatments were carried out according to the actual recommendations for onion cultivation. Yielding and plant health were optimal throughout the whole period of studies.

Soil samples were taken for analyses using Egner's sampling stick; 18–20 individual samples from the arable layer (0–20 cm) were mixed and the mixture constituted a representative mixed sample (0.4–0.5 dm^3). For chemical analyses, 20 cm^3 of soil in the state of actual moisture were taken using the Drews' instrument which permits to obtain the same sample density as it is in the given field patch [11]. The volumetric sample was transferred to a glass bulb and then, in order to extract macroelements and chlorides, active carbon and 200 cm^3 of extraction solution (acetic acid – 0.03 M CH_3COOH) were added in 1:10 proportion of soil to extraction solution. The suspension was shaken for 30 minutes in rotational agitator, and then, it was filtered. In order to determine microelements, a successive soil sample was taken, it was flooded with Lindsay's solution containing in 10 dm^3: 50 g of EDTA (ethylenediaminetetraacetic acid), 90 cm^3 of 25 % NH_4OH solution, 40 g of citric acid, 20 g of $Ca(CH_3COO)_2 \cdot 2H_2O$ [11, 14] and then, the same procedure was carried out as in case of the extraction with acetic acid. Determination of components was performed using the following methods: nitrate nitrogen, ammonia nitrogen – by microdistillation method (according to Bremner in Starck's modification), phosphorus – colorimetrically using the phosphovanadomolybdic complex, potassium and calcium – by flame photometry, chlorides – nephelometrically with $AgNO_3$, sulphate sulphur ($S\text{-}SO_4$) –nephelometrically with $BaCl_2$, magnesium, iron, manganese, zinc and copper – by atomic absorption spectrometry (AAS) on Carl Zeiss Jena AAS apparatus. Furthermore, the following determinations were made: salinity (in EC units) – conductometrically at soil : water proportion = 1 : 2 (v/v).

After the termination of vegetation, representative mixed samples of plant material were taken (consisting of 18–20 healthy onions typical of the given cultivar), without any symptoms of mechanical damages or diseases. The collected plant material was divided into 2 parts. One half of the bulbs were peeled, sliced, dried at 45–50 ^{o}C and homogenized. The other half of the collected plant material was transferred for a period of 60 days to a refrigerator, where the temperature was maintained at 6 ^{o}C and 50 % of air humidity. After 60 days, the material was prepared according to the procedure – mentioned earlier and homogenized.

In order to determine the general forms of nitrogen, phosphorus, potassium, calcium and magnesium, the homogenized plant material was mineralized in concentrated sulphuric acid and sulphur was mineralized in a mixture of nitric(V) and chloric(VII) acids (3:1 v/v) [14]. After mineralization of plant material, the following determinations were made: total nitrogen – by distillation method acc. to Kjeldahl in Parnas-Wagner apparatus, phosphorus –colorimetrically with ammonium molybdate (acc. to Schillak), potassium, calcium and magnesium – by AAS method (on Carl Zeiss Jena AAS-3 apparatus), sulphur – nephelometrically with $BaCl_2$.

Analysis of variance was carried out and the effects of cultivars and of the storage method exerted on the content of macroelements in the onion bulbs were studied.

Coefficients of variance (CV) of nutritive components content were determined both before and after the storage of onion bulbs. Statistical analyses were carried out with the use of the Duncan's Test at the significance level of $\alpha = 0.05$.

Results and discussion

Effect of the cultivar on the contents of dry matter and macroelements

In our studies, a significant influence was found to be exerted by the cultivar on the content of dry matter in onion bulbs (Table 1).

Table 1

Effect of cultivar and of the storage on the content of dry matter [%], nitrogen [% N in d.m.], phosphorus [% P in d.m.] and on the coefficient of variation

Variety (A)	[% of dry matter]			N			P		
	T1(B)	T2(B)	Mean	T1(B)	T2(B)	Mean	T1(B)	T2(B)	Mean
'Aurora'	8.10	8.00	8.05	2.08	1.58	1.83	0.40	0.35	0.38
'Bennito'	9.20	8.83	9.02	2.59	2.19	2.39	0.80	0.45	0.63
'Consuelo'	8.40	7.10	7.75	1.71	1.73	1.72	0.40	0.35	0.38
'Cymes'	12.13	11.40	11.77	2.62	1.84	2.23	0.64	0.33	0.49
'Eureka'	10.87	10.20	10.54	2.58	2.45	2.52	0.47	0.34	0.41
'Grabowska'	8.73	8.66	8.70	2.30	2.73	2.52	0.39	0.40	0.40
'Kutnowska'	12.40	12.06	12.23	1.91	1.74	1.83	0.41	0.33	0.37
'Lawica'	11.56	10.00	10.78	2.20	2.03	2.12	0.40	0.39	0.40
'Marbella'	10.10	9.50	9.80	2.46	1.80	2.13	0.45	0.46	0.46
'Mission'	10.70	9.30	10.00	2.63	2.55	2.59	0.50	0.38	0.44
'Napoleon'	9.37	9.10	9.24	2.26	2.64	2.45	0.40	0.36	0.38
'Sherpa'	10.50	10.40	10.45	2.45	2.18	2.32	0.43	0.40	0.42
'Slawa Ozarowa'	9.80	9.50	9.65	2.23	2.18	2.21	0.35	0.37	0.36
'Sochaczewska'	11.83	11.80	11.82	2.23	2.50	2.37	0.43	0.36	0.40
'Stamford'	10.40	10.20	10.30	2.63	2.25	2.44	0.63	0.40	0.52
'Tandem'	9.50	8.80	9.15	1.78	1.88	1.83	0.38	0.35	0.37
'Topolska'	13.00	12.00	12.50	2.28	2.50	2.39	0.40	0.35	0.38
'Wiktoria Skierniewicka'	9.36	9.20	9.28	2.20	1.83	2.02	0.93	0.39	0.66
'Wojka'	11.00	10.70	10.85	2.46	2.19	2.33	0.42	0.37	0.40
'Wola'	10.60	9.20	9.90	2.20	2.06	2.13	0.47	0.38	0.43
'Wolska'	11.73	11.60	11.67	2.00	2.40	2.20	0.35	0.36	0.36
Mean	10.44	9.88	10.16	2.28	2.15	2.22	0.48	0.37	0.43
CV [%]	12.60	13.30	13.10	11.70	15.10	13.60	31.20	12.60	28.60
LSD for A	0.52			0.12			0.10		
LSD for B	n.s.			0.11			0.05		
LSD for A×B	0.70			0.13			0.07		

T1 – directly after harvest; T2 – after 60 days of storage; n.s. – no significant.

A higher dry matter content in the particular cultivars, usually predisposes them to a longer storage, while a smaller dry matter content qualifies them rather for fresh consumption. The smallest dry matter content included cultivars: ‘Consuelo’ (7.75 %) and ‘Aurora’ (8.05 %), while the highest dry matter content was shown by ‘Kutnowska’ (12.23 %) and ‘Topolska’ (12.50 %) (Table 1). The mean coefficient of variation (CV) for dry matter content (from the studied cultivars) was comparatively low (CV 13.1 %).

Similarly as in case of dry matter, also a significant variation was found in the contents of macroelements (nitrogen, phosphorus, potassium, calcium, magnesium and sulphur) in the onion bulbs. The determined nitrogen values ranged from 1.72 % N (‘Consuelo’) to 2.59 % N d.m. (‘Mission’) (Table 1). The determined low nitrogen coefficient of variation indicates that the content of this component (N) shows a comparatively small mean value (CV 13.6 %). Cultivars with the smallest content of phosphorus included: ‘Slawa Ozarowa’ (0.36 % P), ‘Tandem’ and ‘Kutnowska’ (each contained 0.37 % P). Significantly the highest content of P characterized the cultivar ‘Wiktoria Skierniewicka’ (0.66 % P). Calculated mean coefficient of variation for phosphorus was comparatively high (CV 28.6 %) which indicates high differences between the cultivars. The determined values of potassium ranged from 1.62 % K (in case of ‘Wola’ cultivar) to 2.55 % K (‘Bennito’ cultivar) (Table 2).

Table 2

Effect of cultivar and of the storage on the content of potassium [% K in d.m.] and calcium [% Ca in d.m.] and on the coefficient of variation

Variety (A)	K			Ca		
	T1(B)	T2(B)	Mean	T1(B)	T2(B)	Mean
‘Aurora’	1.95	2.00	1.95	2.00	1.95	2.00
‘Bennito’	2.55	2.22	2.55	2.22	2.55	2.22
‘Consuelo’	2.13	2.12	2.13	2.12	2.13	2.12
‘Cymes’	1.84	2.12	1.84	2.12	1.84	2.12
‘Eureka’	2.13	1.84	2.13	1.84	2.13	1.84
‘Grabowska’	1.75	2.05	1.75	2.05	1.75	2.05
‘Kutnowska’	1.80	1.76	1.80	1.76	1.80	1.76
‘Lawica’	1.71	2.06	1.71	2.06	1.71	2.06
‘Marbella’	2.24	2.03	2.24	2.03	2.24	2.03
‘Mission’	1.96	1.88	1.96	1.88	1.96	1.88
‘Napoleon’	2.23	2.08	2.23	2.08	2.23	2.08
‘Sherpa’	1.95	2.12	1.95	2.12	1.95	2.12
‘Slawa Ozarowa’	2.10	2.04	2.10	2.04	2.10	2.04
‘Sochaczewska’	1.93	2.25	1.93	2.25	1.93	2.25
‘Stamford’	2.16	1.79	2.16	1.79	2.16	1.79
‘Tandem’	1.63	1.63	1.63	1.63	1.63	1.63
‘Topolska’	1.74	2.02	1.74	2.02	1.74	2.02
‘Wiktoria Skierniewicka’	1.93	1.98	1.93	1.98	1.93	1.98
‘Wojka’	1.82	2.14	1.82	2.14	1.82	2.14

Table 2 contd.

Variety (A)	K			Ca		
	T1(B)	T2(B)	Mean	T1(B)	T2(B)	Mean
'Wola'	1.62	1.88	1.62	1.88	1.62	1.88
'Wolska'	1.88	1.90	1.88	1.90	1.88	1.90
Mean	1.95	2.00	1.97	2.00	1.95	1.97
CV [%]	12.10	8.60	10.50	31.00	31.50	31.40
LSD for A	0.11			0.02		
LSD for B	n.s.			n.s.		
LSD for A×B	0.17			0.03		

Descriptions as in Table 1.

Potassium coefficient of variation was comparatively low and showed 10.5 %. In case of calcium, its content was significantly the lowest (1.63 % Ca) in 'Tandem' cultivar, while the highest value was shown in 'Sochaczewska' cultivar (2.25 % Ca) (Table 2). The determined coefficient of variation for calcium was high amounting to 31.4 %. Similarly as in case of calcium, the cultivar with the lowest content of magnesium was 'Tandem' (0.19 % Mg) (Table 3).

Table 3

Effect of cultivar and of storage on the content of magnesium [% Mg in d.m.]
and sulphur [% S in d.m.] and on the coefficient of variation

Variety (A)	Mg			S		
	T1(B)	T2(B)	Mean	T1(B)	T2(B)	Mean
'Aurora'	0.19	0.31	0.25	0.37	0.41	0.39
'Bennito'	0.26	0.22	0.24	0.45	0.53	0.49
'Consuelo'	0.21	0.39	0.30	0.38	0.43	0.41
'Cymes'	0.20	0.41	0.31	0.52	0.40	0.46
'Eureka'	0.25	0.41	0.33	0.95	0.43	0.69
'Grabowska'	0.23	0.30	0.27	0.75	1.12	0.93
'Kutnowska'	0.21	0.32	0.27	0.62	0.43	0.53
'Lawica'	0.20	0.24	0.22	0.83	1.01	0.92
'Marbella'	0.25	0.25	0.25	0.85	0.40	0.62
'Mission'	0.23	0.27	0.25	0.64	0.40	0.52
'Napoleon'	0.23	0.25	0.24	0.85	0.40	0.63
'Sherpa'	0.26	0.28	0.27	0.59	0.45	0.52
'Slawa Ozarowa'	0.22	0.26	0.24	0.48	0.42	0.45
'Sochaczewska'	0.22	0.31	0.27	0.81	0.44	0.63
'Stamford'	0.26	0.25	0.25	0.69	0.42	0.56
'Tandem'	0.16	0.21	0.19	0.45	0.43	0.44
'Topolska'	0.20	0.35	0.28	0.69	0.55	0.62
'Wiktoria Skierniewicka'	0.22	0.22	0.22	1.00	0.48	0.74

Table 3 contd.

Variety (A)	Mg			S		
	T1(B)	T2(B)	Mean	T1(B)	T2(B)	Mean
‘Wojka’	0.22	0.25	0.24	0.74	0.88	0.81
‘Wola’	0.20	0.36	0.28	0.49	0.75	0.62
‘Wolska’	0.21	0.28	0.25	0.51	0.42	0.46
Mean	0.22	0.29	0.26	0.65	0.53	0.59
CV [%]	13.80	21.20	23.70	28.80	40.30	35.40
LSD for A	0.07			0.12		
LSD for B	0.03			0.07		
LSD for A×B	0.03			0.11		

Descriptions as in Table 1.

The highest content of Mg was shown by ‘Eureka’ cultivar (0.33 % Mg). In reference to magnesium, the cultivars were characterized by a high variability (CV 23.7 %). A significant differentiation was found between the cultivars in the content of sulphur. The least content of sulphur was shown by ‘Aurora’ and ‘Consuelo’ (0.39 and 0.41 % S, respectively), while the highest S content was shown by ‘Lawica’ and ‘Grabowska’ cultivars (0.92 and 0.93 % S, respectively). Sulphur showed the highest variation among the studied nutritive components (CV 35.4 %).

Effect of onion storage on the content of dry matter and macroelements

No significant influence of onion storage in controlled conditions (temperature 6 ^{o}C, air humidity 50 %) was found to be exerted on their content of dry matter or on their content of potassium and calcium. The determined coefficients of variation (CV) for the contents of dry matter, both before and after storage, were similar (12.6 and 13.3 %, respectively). Together with the storage time, the content of nitrogen decreased significantly (from 2.28 to 2.15 % N d.m.), but the nitrogen content variation increased (from 11.7 to 15.1 %). Also the phosphorus content significantly decreased from 0.48 to 0.37 % P d.m. with a simultaneous decrease of the coefficient of variation from 31.2 to 12.6 %. Similarly as in case of nitrogen and phosphorus, also the sulphur content decreased (from 0.65 % to 0.53 % S). In turn, there was a significant increase of the mean content of magnesium (from 0.22 to 0.29 % Mg d.m.).

Nutritive value of onion in comparison with other vegetable species

Results of chemical analyses of onion in reference to the content of macroelements were compared with earlier studies referring to this species [3] and with other vegetable species: common white cabbage (leaves) [4], eggplant (fruits) [15], sweet pepper (fruits) [6], spinach (leaves) [5], tomato (fruits) [8] and potatoes (bulbs) [7]. Onion as species (the average from the studied cultivars) is characterized by a content of dry matter similar to the majority of the studied vegetables (Table 4). Distinctly a smaller dry matter content was shown only by tomato fruits (6.52–6.94 % d.m.). Regarding nitrogen content, spinach contained distinctly less dry matter (0.65–0.77 % N), while potato contained the highest N content (15.4–17.3 % N d.m.). Among the comparable vegetable species, immediately after potato, onion contained the greatest amount of phosphorus (0.37–0.48 % P d.m.). In reference to potassium, onion was the last one among all studied vegetable species. Onion is a rich source of calcium (1.95–2.00 % Ca d.m.), although earlier studies showed distinctly lesser amounts of this element [3]. Magnesium content determined in the present studies (0.22–0.29 % Mg d.m.) was distinctly higher than that in the mentioned earlier studies [3]. Higher contents of magnesium were shown by spinach (1.02–1.10 % Mg d.m.) and potato (0.5–0.9 % Mg d.m.). There were no data referring to sulphur content in the comparable vegetable species.

Table 4

Contents of dry matter [% of d.m.] and macroelements [% in d.m.] in the edible part of selected vegetable species (according to different authors)

Species	Nutrient [% in d.m.]						
	Dry matter [%]	N	P	K	Ca	Mg	S
Onion – own studies	9.88–10.44	2.15–2.28	0.37–0.48	1.95–2.00	1.95–2.00	0.22–0.29	0.53–0.65
Onion	9.95–11.19	1.91–2.57	0.32–0.33	1.33–1.72	0.08–0.18	0.04–0.06	—
White cabbage	8.96–9.14	2.67–2.87	0.25–0.29	2.22–2.31	0.64–0.72	0.14–0.15	—
Eggplant	—	1.99–2.10	0.26–0.28	2.41–3.15	0.13–0.16	0.10–0.13	—
Sweet pepper	10.15–11.59	2.44–2.86	0.19–0.22	2.60–2.95	—	0.06–0.13	—
Tomato	6.52–6.94	1.91–2.66	—	3.49–4.12	0.12–0.16	0.13–0.16	—
Potato	—	15.4–17.3	2.5–3.1	14.1–16.2	0.7–1.2	0.5–0.9	—

Generally, human body demand for mineral elements is differentiated depending on age, body weight, physiological status (pregnancy, breast feeding) and sex (among others for magnesium, iron, zinc and selenium). Calcium and phosphorus are usually analysed together because the correct mineralization of human bones depends on the contents and proportions of these elements in the diet, as well as on the content of vitamin D. On the other hand, magnesium is mainly necessary for the regular functioning of the nervous and muscular systems [16]. Taking into consideration the nutritive value determined on the basis of nutritive components content in dry matter, one can conclude that onion is a valuable source of phosphorus, calcium and magnesium in the human diet. The controlled fertilization based on regular chemical soil analyses is useful to obtain good quality onion bulbs in horticulture production.

Conclusions

1. The factor which significantly modifies the content of dry matter and of macroelements in case of onion is the cultivar.

2. No differentiating effect was found to be exerted by the storage on the content of dry matter, as well as on the potassium and calcium contents in onion bulbs. However, storage significantly decreased the contents of nitrogen, phosphorus and sulphur – with a simultaneous increase of magnesium content.

3. Comparing the nutritive value of onion defined on the basis of the nutritive components contents with other vegetable species, one can conclude that onion – next to its specific taste values – is a valuable source of phosphorus, calcium and magnesium in the human diets.

4. The controlled fertilization based on regular chemical soil analyses is useful to obtain good quality crops in horticulture production. The consideration of chemical soil analyses in the fertilization programs may reduction and protection soil against possible contamination with excessive mineral fertilizers.

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WPŁYW KONTROLOWANEGO NAWOŻENIA NA ZAWARTOŚĆ SKŁADNIKÓW POKARMOWYCH W WYBRANYCH ODMIANACH CEBULI (*Allium cepa* L.)

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Abstrakt: Głównymi celami przeprowadzonych badań była ocena wpływu kontrolowanego nawożenia w przypadku 21 wybranych odmiany cebuli (*Allium cepa* L.) oraz przechowywania cebuli w kontrolowanych warunkach na ich wartość odżywczą określoną na podstawie zawartości makroskładników: azotu, fosforu, potasu, wapnia, magnezu i siarki. Przez cały okres doświadczeń wegetacyjnych stosowano, polecane w Integrowanej Produkcji Warzyw, kontrolowane nawożenie poprzez cykliczne analizy gleby. Wykazano wyraźny wpływ odmiany na zawartość suchej masy i wspomnianych makroelementów w główkach cebuli. Równocześnie nie stwierdzono różnicującego wpływu przechowywania na zawartość suchej masy, a także potasu i wapnia w cebulach. Przechowywanie natomiast znacznie zmniejszało zawartość azotu, fosforu i siarki – przy równoczesnym znacznym wzroście zawartości magnezu. Ponadto cebula jest cennym źródłem zawartości (średnio w s.m.) fosforu (średnio 0,43 % P), wapnia (1,97 % Ca) oraz magnezu (0,26 % Mg) w diecie człowieka. Kontrolowane nawożenie korzystające z cyklicznych analiz chemicznych gleby jest użyteczne, by otrzymać dobrej jakości plony w produkcji ogrodniczej.

Słowa kluczowe: cebula, kontrolowane nawożenie, makroelementy, zróżnicowanie odmianowe, przechowywanie, wartość odżywcza

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USE OF MASS SPECTROMETRY FOR OIL SPILL IDENTIFICATION

ZASTOSOWANIE CHROMATOGRAFII GAZOWEJ DO IDENTYFIKACJI ROZLEWÓW OLEJOWYCH

Abstract: A quick and simple method of separating oil products into the aliphatic and aromatic fractions are presented. The aliphatic fraction was analysed, using the GC/MS technique.

Results of analyses of the predominant in oil group of biomarkers, ie the *n*-alkanes and two compounds from the isoprenoid group: pristane and phytane, are presented. Ratios of the content of these compounds (Pr/F), Pr/n-C_{17} and F/n-C_{18} are characteristic for a given oil, and this is used to identify the oil products.

Keywords: oil spills, GC/MS, biomarkers

Oil pollution of sea areas is caused mainly by oil spilled in result of damages of tankers, operation of drilling rigs and of leakage from tanks in oil terminals. As a rule these are megaspills, resulting in significant ecological damage. The large environmental danger connected with oil spills in sea areas, and the global range of their effects, are reflected in numerous publications [1–6]. Special attention is given to the chemical, physical-chemical and biological influence of oil hydrocarbons on the water ecosystem [7–11].

Proper assessment of the level of danger, and selection of appropriate methods of remediation, is possible only if the materials introduced into water and soil are well investigated prior to taking action. Because of this, analysis of oil products is the subject of many investigations, aiming at more effective methods of their determination.

Due to the complex composition and differentiation of properties of the oil products, analysis of environmental pollution by these products is an especially difficult task. Additional difficulties result from changes in composition of the oil products caused by environmental conditions and by matrix properties [12–13].

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Especially useful for oil product analysis is the gas chromatography with mass sensitive detector (GC/MS) technique [14–18]. Identification of hydrocarbons is largely facilitated by the similarity of fragmentation of compounds belonging to the same homologous series. Paraffin hydrocarbons form only a few molecular ions, especially in macromolecular compounds. This group of hydrocarbons is characterised by the series of ions distanced by 14 Da (-CH_2-), which occur for values m/z 15, 29, 43, 71, 85, 99 etc. Of the naphthene hydrocarbons, the most easily identified are compounds belonging to alkylcyclohexanes, in which the main ion is m/z 83. In mass spectra of aromatic and alkylaromatic hydrocarbons, the peak of the molecular ion is well visible. Table 1 presents a list of characteristic ions, used for identification of the main groups of hydrocarbons, in that of the specific biomarkers (isoprenoid hydrocarbons: pristane and phytane, triterpanes, steranes) occurring in oil and oil products [14, 15, 17, 19, 20].

Table 1

Characteristic ions (m/z) useful for identifying oil hydrocarbons

Class of compounds	Characteristic ions (m/z)
n-alkanes	113, 183
n-alkenes	57, 71, 85, 99
Isoprenoids (pristane, phytane)	55, 97
Acylcyclohexanes	82, 83
Benzene, alcylbenzene	78, 91, 105, 106, 119, 120
PAH	molecular ions, eg 178 for anthracene and phenanthrene, 202 for pirene, 228 for benzopirenes
Diterpenoids	109, 123, 163, 191
Hopanes	177, 191
Steranes, diasteranes	217, 218, 231, 259

Oil products are multicomponent mixtures of chemical compounds, hydrocarbons mainly, containing also small amounts of heteroorganic or other compounds. A characteristic feature of the composition of oil and oil products is the presence of hydrocarbons belonging to various groups of compounds (paraffin, cycloparaffin, aromatic hydrocarbons, differing by molecular mass, degree of ring condensation and substitution). They can occur in the form of homologous series, eg: *n*-alkanes, alkylcyclohexanes, alkylbenzenes, alkyltoluenes and others.

In non-polar capillary columns, non-polar compounds elute in accordance with the value of temperature of boiling. Chromatograms with characteristic profiles are obtained, the so-called “fingerprints”. In turn, selective ion monitoring (SIM) and extraction of selected ions from chromatograms by TIC (*Total Ion Current*) method allow obtaining additional fingerprints, specific for the given oil products.

Analysis of selected fragments of ions, and identification of biomarkers, characteristic to given oils and oil products, is especially useful for identifying sources of pollution and for monitoring the natural environmental degradation of oil pollutants and/or the effectiveness of adopted methods of remediation. Biodegradation of hydrocarbons depends, among others, on their structure; *n*-alkanes are biodegraded much easier than isoprenoid hydrocarbons, while the least susceptible to biodegradation are hopanes. Therefore, the chromatographic profile of a product differs from the initial chromatographic profile of the pollutant. At least it will have no low-molecular *n*-alkanes [14, 15, 17, 19–21].

The objective of the investigations, carried out by the Department of Protection of Environment of the Maritime Institute, was to evaluate the possibilities of using gas chromatography and mass spectrometry for identifying spilled oil substances and for determining the degree and magnitude of marine environment pollution in result of the spill.

Material and methods

Analysis of selected biomarkers was carried out for:

- diesel oil,
- light heating oil,
- multiseason oil for Diesel engines Elf TURBO 15W40 Diesel,
- heavy heating oil,
- crude oil.

Additionally, for the first four products, the content of biomarkers was determined after three weeks, during which the investigated products were left in open vessels in order to test, in static conditions, for changes in the composition of these products (mainly in effect of evaporation). The diameter of the vessels was 10 cm, thickness of oil layer was 2 cm, mean air humidity – 45 %, mean temperature – 22 ^{o}C. Group separation of hydrocarbons was carried out on silica gel type 100 (70–230 mesh), which, directly before use, was conditioned for 12 hours at 160 ^{o}C.

Determination of asphaltene content

Asphaltene content was determined in samples of crude oil and heavy heating oil. From each sample 0.5 g of oil was taken and dissolved in 10 cm^3 of hexane. Each subsample was filtered through a 0.22 μm teflon filter. The filtrate was collected into a 25 cm^3 measuring flask, leaving it for further analysis. Asphaltenes collected on the filter were washed with dichloromethane into a tared vessel, and weighed after removing the dissolvent. Results are presented in Table 2.

Table 2

Gravimetrically determined asphaltene content in crude oil and heavy heating oil

Samples	Mean asphaltene content in sample [%]	Standard deviation SD
Crude oil	2.22	0.03
Heavy heating oil	6.85	0.06

Group separation of reference mixture

A 1 cm diameter glass column was filled with 2 g of silica gel and conditioned with 20 cm^3 of hexane. Next, 1 cm^3 of the reference mixture was put on the silica gel. The mixture contained 20 aliphatic hydrocarbons (18 *n*-alkanes, pristane and phytane) of 2 $\mu g/cm^3$ concentration and 16 polycyclic aromatic hydrocarbons (PAH) of 1 $\mu g/cm^3$ concentration, and was eluted using:

– 10 cm^3 of hexane (fraction 1),
– 10 cm^3 of hexane – toluen mixture of volumetric ratio 1:1 (fraction 2), and
– 10 cm^3 of toluene (fraction 3).

The obtained fractions were vaporized in a nitrogen jet to 1 cm^3 and compared with the standard containing *n*-alkanes from C_8 to C_{40}, pristane, phytane and PAH. In fraction 1 only aliphatic hydrocarbons were found, in fraction 2 – PAH, while in fraction 3 none of the above compounds were present. Determined by these investigations recoveries and standard deviations are shown in Table 3.

Table 3

Values of recoveries of aliphatic hydrocarbons after group separation on silica gel

Compound	Recovery [%]	Standard deviation SD
	Fraction 1	
C-8	95.50	0.42
C-10	96.95	0.35
C-12	96.95	0.21
C14	98.00	0.42
C-16	97.85	0.92
C-17	97.95	0.64
Pristane	98.45	0.64
C-18	99.80	0.42
Phytane	105.5	0.71
C-20	107.2	1.13
C-22	107.6	1.20
C-24	107.4	1.56
C-26	106.0	2.05
C-28	106.1	1.98
C-30	105.0	2.83
C-32	103.9	0.07
C-34	104.5	1.34
C-36	103.5	1.41
C-38	103.1	0.57
C-40	101.9	1.56

Group separation of crude oil and selected oil products

20 mg of the sample, dissolved in hexane, was put on the columns with silica gel. The columns were eluted with 10 cm^3 of hexane to collect aliphatic hydrocarbons

(fraction 1), and next 10 cm^3 of the hexane/toluene mixture – collected in this way fraction 2 contained PAHs. Group separation of crude oil and heavy heating oil was carried out for samples, from which previously were removed asphaltenes. Fraction 1 was tested for the presence of biomarkers (*n*-alkanes, pristane and phytane).

Chromatographic analysis

Biomarker analysis was carried out using a gas chromatograph coupled with a Hewlett-Packard mass spectrometer. The chromatograph was provided with a HP-5 column with dimensions 25 m × 0.25 mm and film thickness 0.25 μm. The separation was performed in the following temperature program: 40 ^oC (2 min), 5 ^oC/min up to 320 ^oC (10 min). Feeder temperature was 300 ^oC. The spectra were recorded in Total Ion Current (TIC) mode in the range m/z 30–600, and in Selective Ion Monitoring (SIM) mode for m/z 71 and 85.

Results and discussion

In the tested oil products (except diesel oil), *n*-alkanes were the predominant compounds forming each of the chromatographic profiles. The highest concentration of normal-chained aliphatic hydrocarbons was found in light heating oil, then in crude oil, diesel oil and lowest in heavy heating oil.

The differences in distribution of *n*-alkanes in the tested oil products and in crude oil are shown graphically in Figs. 1a–d and 2.

In oil products, for which tests of environmental influence (mainly evaporation) on the biomarker content were carried out, the loss of mass was:

- 5.0 % for diesel oil;
- 7.1 % for light heating oil;
- 0.5 % for multiseason diesel oil;
- 4.2 % for heavy heating oil.

The low loss of mass of multiseason diesel oil proves that it is composed mainly of hydrocarbons with higher temperatures of boiling.

Comparison of initial biomarker content with the content after three weeks, during which volatile components were allowed free evaporation, showed that in all products disappeared the three first *n*-alkanes (C_8-C_{10}), concentration of C_{11} decreased by about 50 %, and of C_{12} by about 15 %. There was only a slight change in the concentration of *n*-alkanes with higher numbers of carbon atoms.

The tested products differed by chromatographic profiles. The highest similarity was observed for diesel oil and light heating oil – the range of determined *n*-alkanes was: from C_8 to C_{28}, with a maximum at C_{13}-C_{17}. These products differed visibly only by the size of the non-separated surface (in analytes, which contain a large number of isomers with similar temperatures of boiling, but at very low concentrations, a large part of the compounds is non-separated – the non-separated surface causes a lifting of the basic line of the chromatogram, and in case of heavy products – a characteristic hump in the curve).

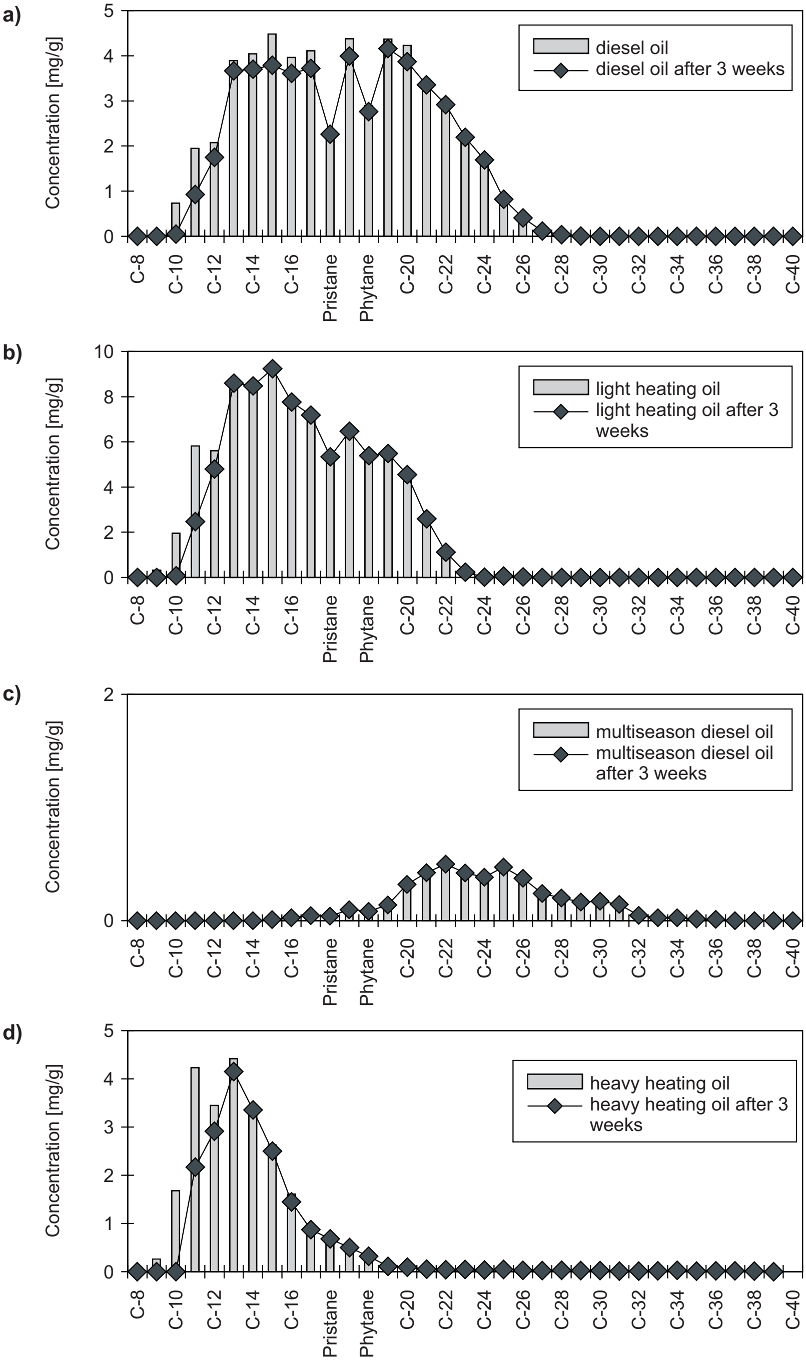

Fig. 1. Concentration of *n*-alkanes in: a) diesel oil, b) light heating oil, c) multiseason diesel oil, d) heavy heating oil

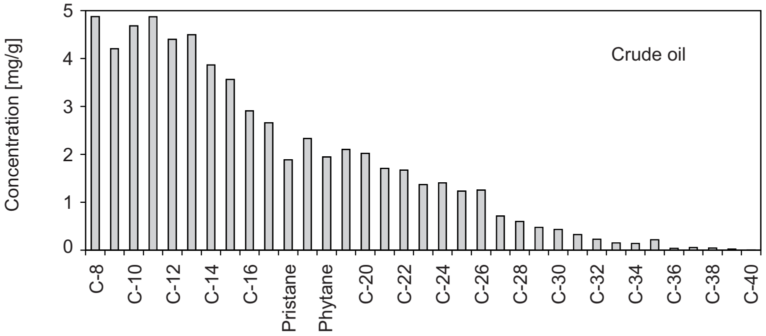

Fig. 2. Concentration of *n*-alkanes in crude oil

Heavy heating oil was characterised by a "wider" composition of hydrocarbons than the two earlier mentioned products (from C_8 to C_{39}), and the point at which the highest concentrations of *n*-alkanes where found was shifted towards lighter hydrocarbons (C_{11}-C_{15}). The non-separated surface was < 50 %.

A characteristic chromatogram was obtained for multiseason diesel oil – over 90 % of non-separated surface (which significantly hindered identification and quantitative determination), resulted in a characteristic hump in the C_{23} to C_{36} range, which, together with the relatively low content of *n*-alkanes, proves that iso- and cycloparaffin hydrocarbons are present in the oil [5, 17]. Since the compounds occur at very low concentrations, they are not recorded in the chromatogram. Besides, none of the first four *n*-alkanes was found in the tested multiseason diesel oil and concentrations of the next seven were below 0.1 mg/g.

The chromatographic profile of crude oil was different from chromatograms obtained for oil products. All determined *n*-alkanes are present, with a maximum at C_8-C_{13} [2].

For distinguishing the investigated oil products, the most often used in oil geochemistry indexes were used [5, 8, 10]:

– Carbon Preference Index (preference of odd *n*-alkanes over even *n*-alkanes):

$$\mathrm{CPI}_{(\mathrm{Total})} = \frac{(C_{17} + C_{19} + \ldots + C_{27} + C_{29}) + (C_{19} + C_{21} + \ldots + C_{29} + C_{31})}{2\,(C_{18} + C_{20} + \ldots + C_{28} + C_{30})},$$

$$C_{(17-23)} = \frac{(C_{17} + C_{19} + C_{21}) + (C_{19} + C_{21} + C_{23})}{2\,(C_{18} + C_{20} + C_{22})},$$

– pristane/phytane ratio (Pr/F) – sedimentation environment index:
Pr/F – sedimentation environment,
< 1 – anaerobic – marine sediments,
1–3 – aerobic – marine sediments,
> 3 – land sediments – carbons;

- *n*-heptadecane to pristane ratio (*n*-C_{17}/Pr) and *n*-octadecane to phytane ratio (*n*-C_{18}/F), allows to assess the degree of alteration of the maternal organic substance of the oils and to evaluated the influence of biodegradation.

The distribution of isoprenoids changes with the degree of alteration of organic substance, however not to such a degree as *n*-alkane distribution. Phytane is produced more quickly than pristine, and in effect the Pr/F ratio decreases. Pr/*n*-C_{17} values below 0.5 are typical for marine organic matter, while values over 1.0 are characteristic for the land environment [8, 10]. However, the magnitude of this change is rather small. During generation of hydrocarbons, the concentration of isoprenoids increases much slower than the concentration of *n*-alkanes, therefore the Pr/*n*-C_{17} and F/*n*-C_{18} ratios decrease with growing degree of alteration. Common (comprehensive) interpretation of the *n*-alkane and isoprenoid distributions allows both to evaluate the degree of alteration and the influence of biodegradation.

The described above geochemical indexes were determined for the investigated oil products. Results are shown in Tables 4 and 5.

Table 4

Values of geochemical indexes calculated for the investigated products

Geochemical indexes	Diesel oil	Light heating oil	Multiseason diesel oil	Heavy heating oil	Crude oil
P/F	0.813	0,.977	0.459	2.183	0.967
P/C-17	0.573	0.734	0.867	0.769	0.708
F/C-18	0.661	0.829	0.85	0.635	0.835
CPI total	—	—	0.918	1.003	0.935
CPI 17-23	0.944	0.964	0.845	1.006	0.967

Table 5

Values of geochemical indexes calculated for the investigated oil products
after three weeks of interaction with environment

Geochemical indexes	Diesel oil	Light heating oil	Multiseason diesel oil	Heavy heating oil
P/F	0.819	0.990	0.488	2.125
P/C-17	0.608	0.742	0.889	0.782
F/C-18	0.692	0.834	0.845	0.64
CPI total	—	—	0.955	0.983
CPI 17-23	0.972	0.971	0.868	0.973

It was found that crude oil, diesel oil, light heating oil, multiseason diesel oil and heavy heating oil differ from each other by values of the geochemical indexes. However these indexes can be used for identifying oil spills only to a limited extent – obtaining a chromatographic profile, determination of the *n*-alkane, pristane and phytane concentrations can be only one of the stages of investigations leading to the recognition of spilled oil products.

Values in Tables 3 and 4 differ from each other only slightly for the same oil products (within the margin of error of quantitative determination), which means that they potentially could be used as markers of spilled oil products. However, it should be remembered that in the experimental conditions the factors influencing the tested materials were relatively stable and limited to:

- temperature,
- solar radiation (to a limited extent),
- air humidity.

In natural conditions the number of factors is much larger – movement of air, waves, interaction with water and soil, disintegration by microorganisms and many others.

Conclusion

A quick and simple method of separating oil products into the aliphatic and aromatic fractions is presented. The obtained aliphatic fraction was analysed using the GC/MS technique.

The presented investigations of *n*-alkane, phytane and pristane content and the obtained chromatographic profiles of oil products constitute the first stage of works, which aim at utilising gas chromatography coupled with mass spectrometry for identifying spilled oil products. However, in order to attain unequivocal identification of the origin of oil substances in the environment, it is necessary to extend the investigations to other compounds characteristic to oil substances – PAHs, benzothiophenes, and for petrols – to volatile and medium volatile aromatic hydrocarbons.

Characterisation and identification of oil products, which have changed significantly due to the influence of atmospheric factors, will be possible with the use of steranes and hopanes, which, similarly to *n*-alkanes, belong to the biomarker group. An especially important index in this respect is the ratio of hopane C_{29} to C_{30}. This ratio remains practically unchanged for very long time spans.

The laboratory of the Department of Protection of Environment plans a systematic extension of its library of oil product spectra, and of the spectra of products of degradation, in order to facilitate quick identification of oil spills.

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ZASTOSOWANIE CHROMATOGRAFII GAZOWEJ DO IDENTYFIKACJI ROZLEWÓW OLEJOWYCH

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Abstrakt: Przedstawiono szybką i prostą metodę rozdziału produktów naftowych na frakcję alifatyczną i aromatyczną. Otrzymaną frakcję alifatyczną badano przy zastosowaniu techniki GC/MS.

W pracy przedstawiono wyniki analiz dominującej w ropie naftowej grupy biomarkerów – n-alkanów oraz dwóch związków z grupy izoprenoidów: pristanu i fitanu. Stosunki ilości tych związków (Pr/F) oraz Pr/n-C17 i F/n-C18 są charakterystyczne dla określonej ropy, co wykorzystywane jest przy identyfikacji produktów naftowych.

Słowa kluczowe: rozlewy olejowe, GC/MS, biomarkery

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REMOVAL OF PHYTOESTROGENS FROM WATER SOLUTIONS USING TUBULAR NANOFILTRATION MEMBRANES

USUWANIE FITOESTROGENÓW Z ROZTWORÓW WODNYCH ZA POMOCĄ RUROWYCH MEMBRAN NANOFILTRACYJNYCH

Abstract: The research focused on the effectiveness of phytoestrogens (daidzein and coumestrol) removal from different water solutions using a tubular nanofiltration membrane (AFC-30). The micropollutants (concentration of 5 μg/dm^3) were added to different matrices (deionized and tap water – with and without humic acid). The retention coefficient of phytoestrogens and volumetric permeate flux were determined during membrane filtration. The membrane was characterized in terms of the removal of salts representing mono- and divalent ions ie NaCl and $MgSO_4$. The highest retention coefficient was found for coumestrol during the filtration of tap water with humic acid added, however, the volumetric permeate flux for this filtration was the lowest. The removal of inorganic and organic matter (measured as electric conductivity of water and UV absorbance at $\lambda = 254$ nm) from the waters was high. Thanks to those properties, nanofiltration can be used to remove simultaneously organic micropollutants, excessive hardness and precursors of disinfection by-products formation, and also partially desalt water.

Keywords: nanofiltration, water matrix, phytoestrogens, water treatment

The compounds of estrogenic activity identified in the aqueous environment include xenoestrogens, mycoestrogens and plant hormones, the so-called phytoestrogens [1]. In the human body, phytoestrogens act like estrogens and their strength ranges from 1/500 to 1/1000 of that typical of 17 β-estradiol [2]. In surface waters, the most frequently identified phytoestrogens include biochanin A, daidzein, genistein and coumestrol [3–7]. The level of phytoestrogens concentrations assayed in the river water in Australia, Switzerland, Germany and Italy fell within 1–12 ng/dm^3 [3–6], although daidzein and genistein assayed in Japan displayed 43 μg/dm^3 and 143 μg/dm^3 respectively [7].

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Pressure-driven membrane operations, notably reverse osmosis (RO) and nanofiltration (NF), are regarded as possible technique for removing organic micropollutants from water [8]. The first investigations date back to the 1990s and dealt with the removal of pesticides by nanofiltration, which led to the launch of several installations operating on a pilot and industrial scale [9]. However, the identification of new compounds in waters makes us continue the research to acquire a better knowledge of membrane operations with respect to organic micropollutants removal.

This works was aimed at assessing the efficiency of phytoestrogens (daidzein and coumestrol) removal by nanofiltration. The filtration was carried out on waters of different matrices containing standard solutions of micropollutants, using a tubular nanofiltration membrane (AFC-30). The membrane was also characterized in terms of organic and inorganic matter removal.

Materials and methods

Nanofiltration was conducted on a TMI 14 installation manufactured by J.A.M. INOX Produkt equipped with a laboratory testing module operating in the cross-flow mode. The tubular AFC-30 membrane employed was produced by ITI PCI Membranes (Poland). Table 1 shows its characteristics. The research took the following steps:

– initial filtration that covered the conditioning of the membrane, using deionized water at a transmembrane pressure of 1.0–2.0 MPa for 3 hours (with and without 1 g/dm^3 NaCl and $MgSO_4$ solutions added),

– 5-hour specific filtration of model water made of deionized and tap water (with and without 10 mgC/dm^3 humic acid added) and 5 mg/dm^3 micropollutants standard solutions.

Table 1

Characteristics of membrane (manufacturer data)

Membrane type	Material	Max pH range	Max pressure [MPa]	Max temp. [^{o}C]	Removal of $CaCl_2$ [%]
AFC-30	composite (active layer-polyamide)	1.5–9.5	6.0	60	75

The effectiveness of the filtration was assessed by determining the volumetric and relative permeate fluxes (J_w – for deionized water, J_v – for the water with micropollutants standard solutions added (1) and α – relative permeability of membrane (2), Table 2). The concentrations of the micropollutants were assayed in the treated water (feed) and that purified with the membrane techniques (permeate), which then formed the basis for calculating their retention coefficients R (3).

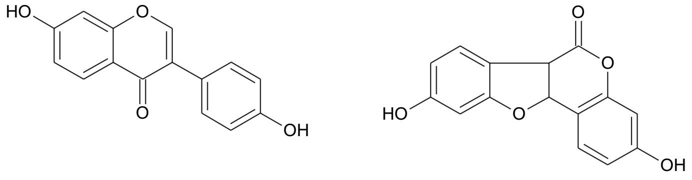
Table 2

Equations used to evaluate membrane properties and removal efficiencies

Parameter	Equation
Volumetric permeate flux, J_v (J_w)/(m^3 $m^{-2} \cdot s^{-1}$)	$J_v(J_w) = \frac{V}{F \cdot t}$ (1)
Relative permeability of membrane, α	$\alpha = \frac{J_v}{J_w}$ (2)
Retention coefficient, R/%	$R = \left(1 - \frac{C_p}{C_f}\right) \cdot 100$ (3)

V – volume [dm^3], F – membrane area [m^2], t – filtration time [s], C – concentrations [$\mu g\ dm^{-3}$], f – feed, p – permeate.

The standard solutions of the phytoestrogens and humic acid were supplied by Sigma-Aldrich (Poznan, Poland). Two phytoestrogenic compounds ie daidzein and coumestrol representing isoflavones and coumestans were chosen for the tests, Fig. 1. Some selected physico-chemical properties of the micropollutnats are given in Table 3. The phytoestrogens were separated from a water sample (200 cm^3), using the solid phase extraction SPE while GC-MS helped carry out quantitative analyses. The detailed methodology of phytoestrogens determination was described in [10].



daidzein (Daid)

coumestrol (Coum)

Fig. 1. Chemical structures of phytoestrogens

Table 3

Physico-chemical properties of phytoestrogens

Compounds	Molecular mass [g/mol]	Water solubility [mg/dm^3]	log K_{ow}[1]
Daid	254.24	568.4	2.55
Coum	268.22	281.0	1.57

[1] log K_{ow} values calculated from computer program named "SRC K_{ow} WIN".

Results and discussion

The permeability of the AFC-30 membrane increases with an increasing trans-membrane pressure and the dependence is of linear nature, Fig. 2. The filtration of NaCl

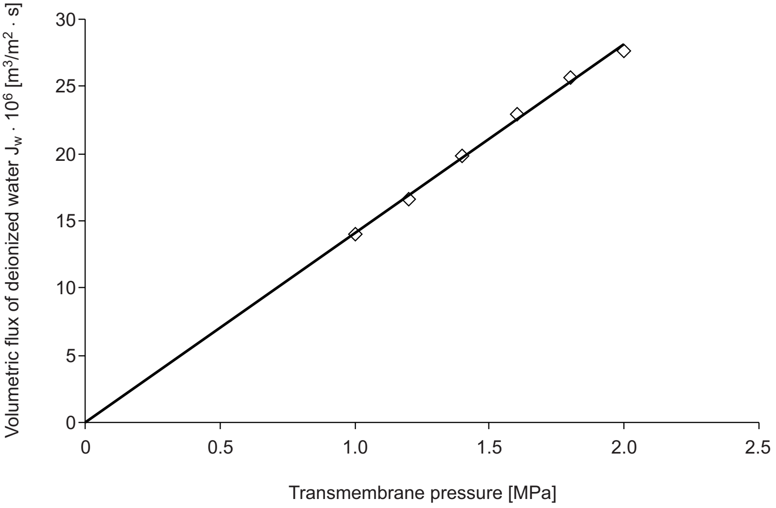


Fig. 2. Relationship between volumetric flux of deionized water and transmembrane pressure

and $MgSO_4$ representing mono- and divalent ions revealed a decrease in the permeability of the membrane during its initial stage, Fig. 3. The relative volume permeate flux was lower than 1, being 0.98 and 0.96 for NaCl and $MgSO_4$ respectively, Table 4. The nanofiltration membrane was very efficient in removing divalent ions (R_{Mg} > 86 %), however, it did not display such efficiency for monovalent ions (R_{Cl} > 58 %). Thus, the AFC-30 membrane can be used to remove the excessive hardness of waters and partially desalt water.

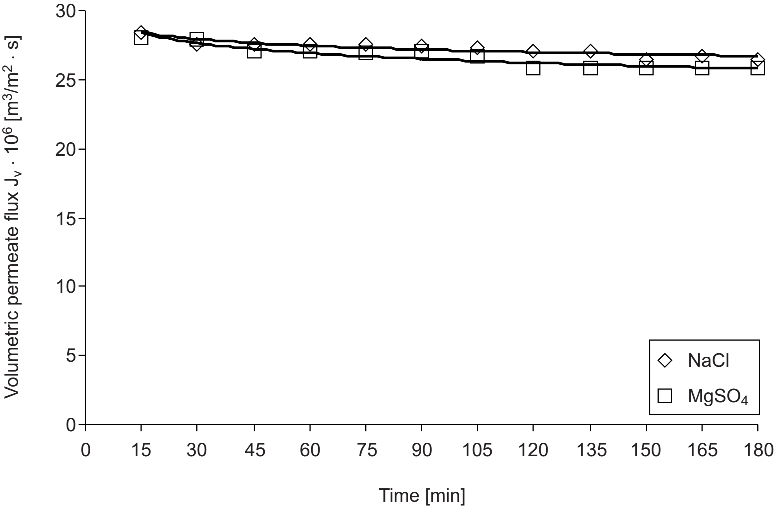


Fig. 3. Volumetric permeate flux on time during filtrations of salt solutions (transmembrane pressure 2.0 MPa, temperature 20 °C and velocity 3.4 m/s)

Table 4

Volumetric permeate flux, relative permeability of the membrane and retention coefficient of NaCl and $MgSO_4$

Salt	Volumetric flux of deionized water, $J_w \cdot 10^6$ $[m^3/m^2 \cdot s]$	Relative permeability of the membrane, α [-]	Salt retention [%]
NaCl	27.6	0.98	58.2
$MgSO_4$		0.96	86.2

The filtration of the model solutions revealed the lowest volume permeate fluxes J_v for tap water with an addition of humic acid and standard solutions of phytoestrogens, Fig. 4A. However, the coefficients of phytoestrogens retention were the highest with reference to the comparative filtration of deionized and tap waters (without humic acid added) containing standard solutions of micropollutants, Fig. 4B and Table 5. A similar trend was shown in paper [11] which investigated the effect of organic matter on the removal of xenoestrogens in the dead-end mode. The increase in the removal of estrogenic micropollutants was caused by the formation of natural organic matter--estrogenic micropollutants complexes and membrane fouling. Coumestrol, characterized by a higher molecular mass (Table 3), turned out to be removed more efficiently than the comparatively tested daidzein. Moreover, the compound displays lower water solubility in water.

Table 5

Retention coefficients of phytoestrogens, membrane transport properties and removal of selected physico-chemical parameters of the waters

Compound	Water solutions		
	Deionized water + phytoestrogens	Tap water + phytoestrogens	Tap water + HA + phytoestrogens
	Retention coefficient [%]		
Daid	70.9	71.0	80.3
Coum	69.9	71.4	92.0
Membrane transport properties	Volume		
Relative permeability of the membrane, α [-]	0.98	0.91	0.78
Physico-chemical parameters	Removal [%]		
Coductivity[1] [μS/cm]	—	66.0	57.5
Absorbance in UV (λ = 254 nm)[2] $[cm^{-1}]$	—	92.9	96.3

[1] Conductivity in tap water: 782 μS/cm (without HA) and 998 μS/cm (with HA); [2] Tap water absorbance: 0.028 cm^{-1} (without HA) and 0.321 cm^{-1} (with HA).

The membrane also enables an effective removal of organic and inorganic matter from water, depending on the water matrix, Table 5. The conductivity of the model

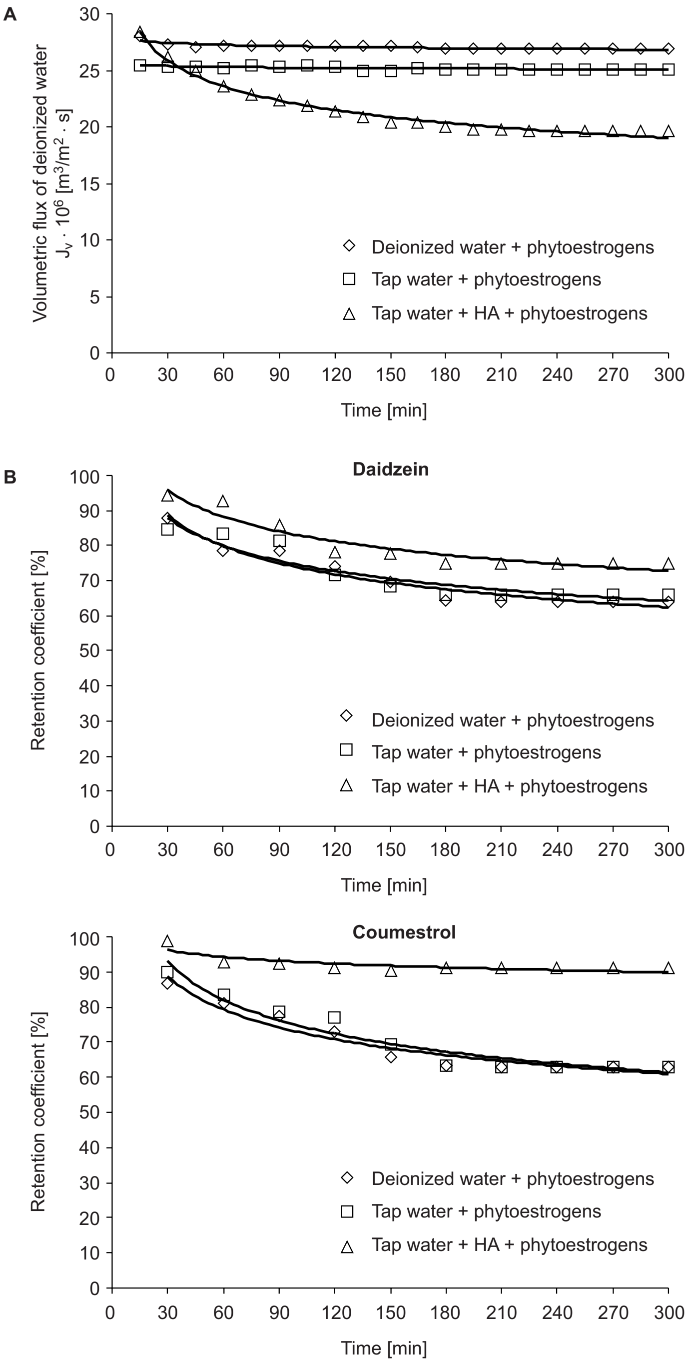


Fig. 4. Volumetric permeate flux (A) and retention coefficient of phytoestrogens (B) during filtrations of water solutions

solution made of tap water with and without an addition of 10 mgC/dm^3 humic acid was 782 μS/cm and 998 μS/cm, respectively. As to its removal by nanofiltration, it reached 66 % and 57.5 % for tap water without and with humic acid added, respectively. The removal of organic matter for both waters tested was high and exceeded 92 %.

Conclusions

1. The removal of phytoestrogens from water solutions by the tubular nanofiltration membrane depends on a water matrix and type of a compound. The highest retention was found for coumestrol (R = 92 %) during filtration of tap water with humic acid added. However, the volumetric permeate flux for that type of filtration was the lowest.
2. The AFC-30 nanofiltration membrane sufficiently removes mono- and divalent ions, which makes it feasible to use it in the removal of excessive hardness and partial desalination of waters.
3. The high removal of organic matter by nanofiltration enables a considerable reduction in the formation of by-products in water disinfection during the technological process of its treatment.

Acknowledgement

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USUWANIE FITOESTROGENÓW Z ROZTWORÓW WODNYCH ZA POMOCĄ RUROWYCH MEMBRAN NANOFILTRACYJNYCH

Wydział Inżynierii Środowiska i Energetyki
Politechnika Śląska

Abstrakt: Badania dotyczyły efektywności usuwania dwóch fitoestrogenów (daidzeina i kumestrol) z różnych roztworów wodnych przy użyciu rurowej membrany nanofiltracyjnej (AFC-30). Mikrozanieczyszczenia dodawano do różnych matryc (woda dejonizowana i woda wodociągowa – bez i z dodatkiem kwasu humusowego) w stężeniu 5 μg/dm^3. W trakcie filtracji membranowej wyznaczano współczynniki retencji fitoestrogenów oraz objętościowy strumień permeatu. Membranę scharakteryzowano pod kątem usuwania soli reprezentujących jony jedno- i dwuwartościowych, mianowicie NaCl i $MgSO_4$. Największy współczynnik retencji uzyskano dla kumestrolu podczas filtracji wody wodociągowej z dodatkiem kwasu humusowego, ale objętościowy strumień permeatu dla tej filtracji był najmniejszy. Usunięcie substancji nieorganicznej i organicznej (mierzone jako przewodność elektryczna właściwa wody i absorbancja UV dla λ = 254 nm) z badanych wód było duże. Z uwagi na te własności proces nanofiltracji może być stosowany do równoczesnego usuwania mikrozanieczyszczeń organicznych, nadmiernej twardości, prekursorów tworzenia ubocznych produktów dezynfekcji oraz do częściowego odsalania wody.

Słowa kluczowe: nanofiltracja, matryca wody, fitoestrogeny, oczyszczanie wody

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APPLICATION OF LASER BIOTECHNOLOGY FOR MORE EFFICIENT PHYTOREMEDIATION OF BIOGENIC ELEMENTS

ZASTOSOWANIE BIOTECHNOLOGII LASEROWEJ DO ZWIĘKSZENIA FITOREMEDIACJI PIERWIASTKÓW BIOGENNYCH

Abstract: Some species of aquatic plants have capability to remove biogenic elements from contaminated water and therefore are used for water treatment. Some hydrophytes (like eg duckweed, yellow iris, reed-mace, common reed) are used in small household hydrobotanical treatment plants.

The aim of experiment was to increase the efficiency of hydrobotanical sewage treatment plant by the stimulation of the duckweed (*Lemna minor*) and yellow iris (*Iris pseudoacorus*) with the argon laser and two laser diodes. The groups of experimental plants were exposed to different parameters of laser stimulation. The results of experiments showed that photostimulation by laser light significantly speeds up cell divisions and causes a significant growth of biomass stimulating quicker and more efficient uptake of biogenic elements contained in sewage. The beneficial influence of laser biotechnology was extension of the vegetation season of plants.

Keywords: laser stimulation, phytoremediation, hydrophytes, biogenic elements, eutrophication, hydrobotanical treatment plant

Eutrophication is an increase of the natural fertility in water reservoirs caused by the inflow of biogenic compounds. The main sources of water pollution are sewage effluents, waste dumps, septic tanks and agricultural flows. Significant quantity of nutrients disturb the naturally balance in water ecosystems and accelerate the primary production. The effective solution for improving water quality is to use in small household hydrobotanical treatment plants, based on hydrophytes' ability to bioremediation of biogenic elements from water.

Cleanup technologies based on phytoremediation process are cheap, effective, comfortable in use and friendly to the environment. The efficiency of purification depends on vegetation season, plants sensitivity on a large range of pollutants

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concentration and slow biomass increase. Attempts of increase phytoremediation efficiency rely mostly on genetic engineering, agrotechnical measures and use of nontoxic chelation compounds (EC Regulation No. 16702007 of 19th February 2007).

The application of the laser stimulation in environmental biotechnology to the optimization of natural processes (eg to sewage treatment, soil reclamation and sewage sludge management) was introduced by Dobrowolski in the last decades of 20th century [1]. Effects of photostimulation by monochromatic, coherent and polarized light on biological material can increase the base for the activation of biological mechanisms.

The laser biostimulation of plants increase their ecological resistance on unfavorable environmental factors: low temperatures, a short growth season and environmental pollution. [2–5]. Specific parameters of laser stimulation (the type of diode, wave length, time and power of irradiation) of hydrophytes may prolong their growth season and increase the biomass production in experimental groups. A very interesting issue is a biostimulation effect on phytoremediation abilities. Laser biotechnology is also promising for more efficient bioremediation of trace elements and biogenic from contaminated soil and sewage [1, 6, 7].

Material and methods

The purpose of research was to increase the efficiency of hydrobotanical method of sewage treatment by the photostimulation of some species of hydrophytes.

Duckweed (*Lemna minor*) and additionally yellow iris (*Iris pseudoacorus*) were chosen as an experimental material. These plant species are used in hydrobotanical and municipal sewage treatment plants. The experimental samples were exposed to variable parameters of laser stimulation: the type of diode, wave length, time and power of irradiation (four parameters optimization). Each of experimental plant groups were irradiated with argon laser and laser diodes. These coherent light sources emit light wave corresponding to blue and red colours.

Following light sources were used in the experiment:

– red light emitting laser diode with a wavelength of 660 nanometers and output power of 20 mW,

– blue light emitting laser diode with a wavelength of 473 nanometers and output power of 20 mW, produced by Changchun New Industries Optoelectronics Tech Co.,

– argon gas laser with a wavelength of 514 nanometers (celadon) and output power of 20 mW, type ILA-120, produced by Carl Zeiss Jena (Ar).

Parameters of laser stimulation were optimized from the point of plants biomass increase. The experimental samples were exposed to variable parameters of laser stimulation: the type of diode, wavelength, time and power of irradiation. It was twenty centimeters distance between coherent light source and irradiated plants. Parameters of stimulation were chosen individually for each plant species. Incidence angle of radiation beam was perpendicular. Parameters of stimulation were chosen in laboratory conditions and after them experiment was continued in natural conditions. One part of experimental groups was left without exposure on laser light as control groups. All groups were planted in ponds filled with partially treated sewage that was previously subjected to mechanical treatment process. Initially, all plants in each group were in similar condition. Total number of duckweed in each group was the same. Rootstocks of yellow iris in groups were selected according to their weight.

During cultivation, after each vegetation season, observations in equal time intervals were made on growth rate, health condition and changes in the number of plants in experimental groups.

For measurement of biomass increase microscope Nikon Eclipse e6000 with experimental setting to visualization and picture analyze, digital video camera Nikon DXM 1200, digital camera Nikon Coolpix 995 and image analysis software Aphelion, version 3.0 were use. Biomass of plants was also measured with a laboratory scale.

Biogenic elements (N, K) content in the dry plant biomass was measured by atomic absorption spectrometry method (AAS) after each of the growth seasons.

Results and discussion

The research objective was finding optimal laser stimulation parameters fitting selected hydrophytes species that could be use in hydrobotanical treatment plants. The results of experiments can make a base to create optimal parameters of the preparation of plant material for sewage treatment process. The laser stimulation caused faster growth of selected plants species in experimental groups.

Optimal parameters of laser stimulation (the type of coherent light source, radiation power, time and irradiation method) of duckweed (*Lemna minor*) were obtained for the experimental group irradiated with laser diode with the following parameters: wavelength of light emitting: $\lambda = 660$ nm, power of radiation 20 mW and irradiation time: 3 times for 3 seconds (Fig. 1). The biggest increase of biomass, in comparison with control group, was observed in this experimental group (300 %) already after at the end

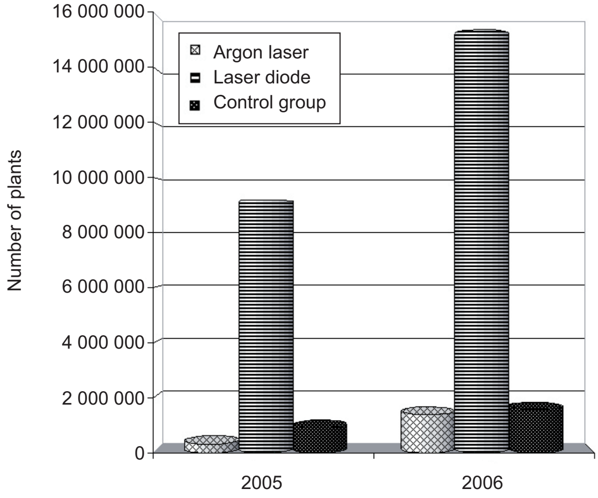

Fig. 1. Comparison of duckweed biomass increase in experimental groups, years 2005–2006

of the first growth season. This effect lasted during subsequent vegetation seasons without repetition of laser stimulation because generative propagation of duckweed causes biostimulation effects in filial plants.

The group of experimental plants irradiated with laser diode was also characterized by better survival rate and resistance to unfavorable environmental conditions such as temperature decrease. This group was also characterized by a small number of plants with chlorosis symptoms.

It was observed that there were considerable differences between plants from experimental groups planted in similar environmental conditions. The duckweed after irradiation with argon laser emits light at λ = 514 nm (during 3 times for seconds) have the bigger surface of leafs recounted per one plant.

The increase of nitrogen and phosphorus concentration in dry duckweed biomass after chemical analysis in experimental groups irradiated with argon laser was found (twice in comparison with control groups) and laser diode (λ = 660 nm) (Figs. 2, 3).

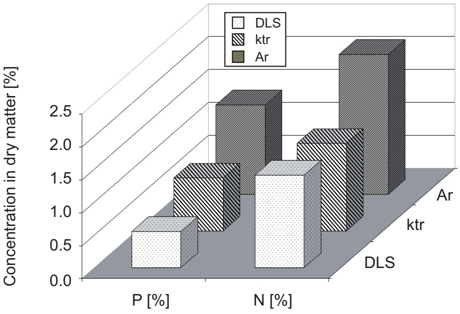

Fig. 2. Comparison of biogenic elements concentration in dry mass of duckweed (*Lemna minor*) in experimental groups in 2006 (ktr – control group, Ar – plants irradiated with an argon laser λ = 514 nm., DLS – plants irradiated with a laser diode λ = 660 nm)

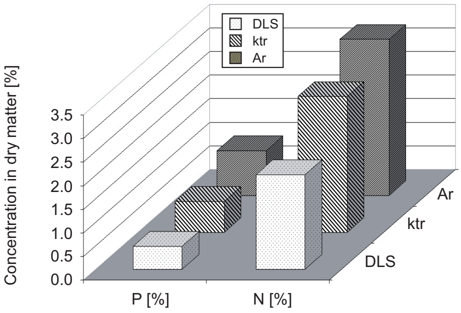

Fig. 3. Comparison of biogenic elements concentration in dry mass of duckweed (*Lemna minor*) in experimental groups in 2007 (ktr – control group, Ar – plants irradiated with an argon laser λ = 514 nm., DLS – plants irradiated with a laser diode λ = 660 nm)

Each of hydrophytes species has a different uptake ability of biogenic elements from contaminated water. The duckweed (*Lemna minor*) has specific ability to nutrients remediation. Nitrogen and phosphorus content in duckweed biomass are not a linear function of these elements concentration in water, but confirm cumulative properties of the plant. The ability of the duckweed to store high nutrient levels in tissues is called the "luxury uptake" [6].

The largest biomass increase of the yellow iris (*Iris pseudoacorus*) was in experimental group which was exposed to argon laser (wavelength λ = 514 nm) for 3 times for 30 seconds (Fig. 4). Yellow iris is not universally used in sewage treatment process, but it may become a valuable element of garden sewage treatment plant for its aesthetic values. The laser stimulation of iris rootstocks caused a significant increase of leaf biomass, number of flowers and plants regeneration after autumn cutting.

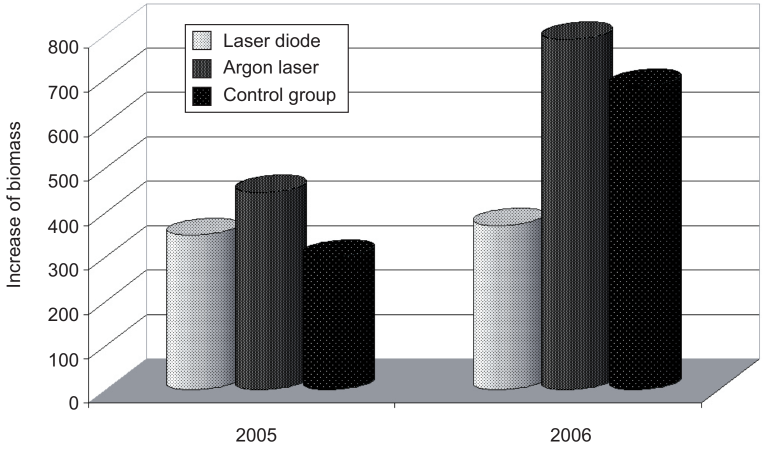

Fig. 4. Comparison of yellow iris biomass increase in experimental groups, years 2005–2006

Differences of biogenic elements concentration in the yellow iris dry biomass from experimental samples were not observed.

It has to be asserted that a choice of adequate hydrophyte species characterized by luxury uptake enables to remove biogenic elements from sewage in a quantity proportional to the size of duckweed biomass in a sewage pond. Optimization of laser stimulation parameters should carry on increase biogenic elements uptake, not only by biomass increase but also by nutrients concentration in dry mass of plants. Removing of nutrients from sewage is a very important part of sewage treatment process because surplus of biogenic compounds may cause surface waters eutrophication [5, 6, 11].

The results of the research showed that stimulation by coherent, monochromatic and polarized light significantly speeds up cell divisions and causes a significant growth of plants.

Application of laser stimulation enables to optimize natural biological processes and use them in environmental biotechnology without changes in plants genotype, but by optimal phenotype expression. The biggest differences in stimulation effects were observed between experimental groups planted in a polluted environment [7].

Conclusions

It was observed that photostimulation by laser light caused a significant growth of biomass and increase plants ability to the remediation of pollutants.

This thesis showed:

– statistically important increase of irradiated plant biomass in comparison with control group,

– increased plants resistance to environmental pollutions and unfavorable environmental factors: low temperatures, short growth season and environmental pollution,

– increased concentration of biogenic elements in plants tissues and slowed down eutrophication process,

– irradiation of plants with a coherent light caused reduction of trace elements concentration (Pb, Zn, Ni, Cd) in duckweed tissues and protected plants against phytotoxic effects [8, 12],

– biostimulation effects in duckweed groups kept during subsequent vegetation seasons without the repetition of irradiation.

It was stated additionally that biostimulation effects after irradiation with argon laser (λ = 514 nm) and blue laser diode (λ = 473nm) were very similar. These light sources had a similar physical characteristic, but they were significantly different in costs of capital expenditure and exploitation. Application of a laser diode in laser biotechnology was more economical.

Acknowledgements

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ZASTOSOWANIE BIOTECHNOLOGII LASEROWEJ DO ZWIĘKSZENIA FITOREMEDIACJI PIERWIASTKÓW BIOGENNYCH

Wydział Geodezji Górniczej i Inżynierii Środowiska
Akademia Górniczo-Hutnicza im. S. Staszica w Krakowie

Abstrakt: Fitoremediacja to metoda, w której do oczyszczania m.in. wód powierzchniowych, gruntowych oraz gleb wykorzystuje się zdolności różnych gatunków roślin do usuwania, stabilizowania i unieszkodliwiania zanieczyszczeń. Pewne gatunki hydrofitów są wykorzystywane powszechnie w hydrobotanicznych oczyszczalniach ścieków.

Celem przeprowadzonych doświadczeń była próba zwiększenia skuteczności hydrofitowej oczyszczalni ścieków poprzez stymulację rzęsy drobnej (*Lemna minor*) i kosaćca żółtego (*Iris pseudoacorus*) przy użyciu diod laserowych oraz lasera argonowego. Grupy roślin doświadczalnych poddano ekspozycji na działanie światła spójnego, stosując różne algorytmy naświetlania. Uzyskane wyniki potwierdziły statystycznie wpływ światła spójnego na przyspieszenie podziału komórek, co prowadziło do zwiększenia przyrostu biomasy oraz szybszego wychwytu pierwiastków biogennych. Dodatkowo zaobserwowano wydłużenie wegetacji roślin w grupach doświadczalnych.

Słowa kluczowe: stymulacja laserowa, fitoremadiacja, hydrofity, pierwiastki biogenne, eutrofizacja, roślinne oczyszczalnie ścieków

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COMPARATIVE STUDY OF THE SEED EXTRACTS OF *Apiaceae* PLANTS IN ULTRASONIC CONDITIONS

BADANIA PORÓWNAWCZE EKSTRAKTÓW Z NASION ROŚLIN Z RODZINY *Apiaceae* W WARUNKACH ULTRADŹWIĘKOWYCH

Abstract: There are many bioactive compounds with known effects on microorganisms (fungi, bacteria, viruses) in plants of the *Apiaceae* family. The properties of selected extracts obtained with classic method, and their antifungal activities were described in previous works. In this work, the results of a study concerning the properties of the following seed extracts are described: parsley (*Petroselinum crispum*), common hogweed (*Heracleum sphondylium*), hogweed Sosnowsky (*Heracleum Sosnowsky*), ground-elder (*Aegopodium podagraria*), dill (*Anethum graveolens*), lowage (*Levisticum officinale*) and cow parsley (*Anthriscus sylvestris*). These extracts were obtained with the use of ethyl acetate as an extractant, at room temperature in ultrasonic bath. The analysis of the extracts was carried out on GC-MS an Agilent 6890N gas chromatograph. Antioxidant content in the extracts was measured with two different methods: with the use of ascorbic acid or linoleic acid in the first method and *N,N*-dimethyl-*p*-phenylenediamine (DMPD) in the second method. The obtained results showed that the extracts obtained with the proposed method contain more native agents so their biological activity should be higher.

Keywords: *Apiaceae*, seeds extracts, antioxidant activity, DMPD

There are many bioactive compounds with known effects on microorganisms (fungi, bacteria, viruses) in plants of the *Apiaceae* family. The properties of selected extracts obtained with classic method, and their antifungal activity (to *Fusarium culmorum, Botrytis cinerea* and *Alternaria alternata*) were described in previous works [1]. In this work, the results of a study concerning the properties of the following seed extracts are described: parsley (*Petroselinum crispum*), common hogweed (*Heracleum sphon-*

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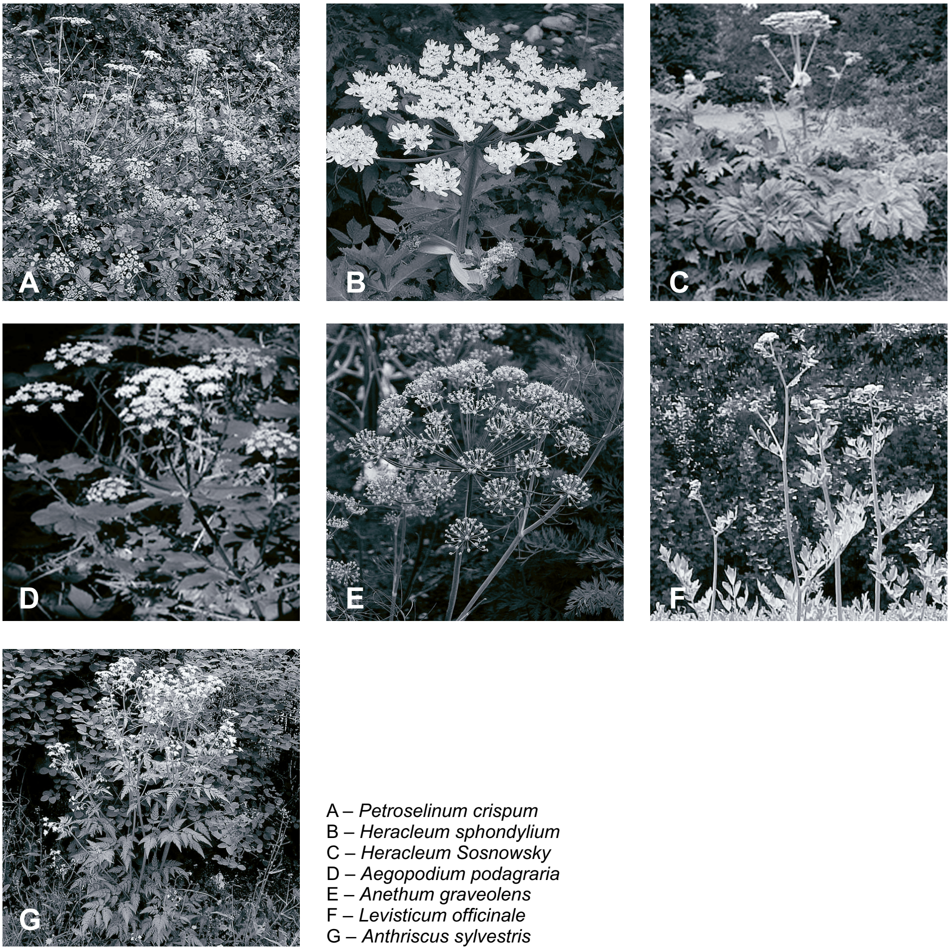
dylium), hogweed Sosnowsky (*Heracleum Sosnowsky*), ground-elder (*Aegopodium podagraria*), dill (*Anethum graveolens*), lowage (*Levisticum officinale*) and cow parsley (*Anthriscus sylvestris*).

Materials and methods

All the chemicals used were of the analytical grade. The *N,N*-dimethyl-*p*-phenylenediamine dihydrochloride (DMPD), ascorbic acid, linoleic acid, pyrogallol and all analytical grade solvents, were purchased from Sigma Aldrich. Iron(II) chloride was purchased from Fluka Chemical Co.

The dry and ripe seeds were collected in summer 2008. Plant material was ground to powder in order to obtain extracts.



A – *Petroselinum crispum*
B – *Heracleum sphondylium*
C – *Heracleum Sosnowsky*
D – *Aegopodium podagraria*
E – *Anethum graveolens*
F – *Levisticum officinale*
G – *Anthriscus sylvestris*

Fig. 1. The photos of plants used during researches

Ethyl acetate extracts were obtained by extracting the plant material: 24 hours at room temperature (maceration); 30 hours in Soxhlet apparatus; half an hour at room temperature in ultrasonic bath (35 kHz). The solvent was removed in a vacuum evaporation. The yield of extractions is showed in Table 1.

GC-MS analyses of extracts were performed with the use an Agilent 6890N series gas chromatograph equipped with an Agilent 5975C inert XL MSD (Agilent Technologies). A vaporization injector in the split mode (1:50) at 270 ^{o}C, with a HPMS5 capillary column was used. The oven temperature was programmed from 50 ^{o}C to 140 ^{o}C and the speed of temperature increase was 10 ^{o}C/min. At 140 ^{o}C the analysis was performed isothermally after 2 min; then the temperature was increased to 250 ^{o}C with the same speed of temperature increase as previously. At 250 ^{o}C the analysis was performed isothermally after 15 min.

High purity helium was used as the carrier gas. The identity of each compound was determined by means of a comparison of its spectral data with the data from the Wiley library spectral bank (G1035B; Rev D.02.00; Agilent Technologies).

Antioxidants content in the extracts was measured with two different methods with the use of ascorbic acid/linoleic acid in the first method [2] and *N,N*-dimethyl-*p*-phenylenediamine (DMPD) in the second method [3]. The total phenolate compounds content in the extracts was determined by a colorimetric assay, using the Folin-Ciocalteu reagent and pyrogallol as a standard [4].

Results and discussion

The lowest efficiency of the extraction was obtained when the maceration at room temperature was used, according to the expected results. This group of extracts was not used in our following consecutive investigations.

The efficiencies of extractions performed in ultrasonic bath (USBE) were higher in all the cases, than the efficiencies of extractions performed for a long time, at high temperature, in Soxhlet apparatus. The efficiency of the extraction was from 6.3 % for ground elder to 35.6 % for hogweed Sosnowsky (Table 1).

Table 1

The yield of ethyl acetate extraction [%]

Plants	Hot extract (Soxhlet apparatus)	Cold extract (maceration)	Ultrasonic bath
Common hogweed (*Heracleum sphondylium*)	9.6	4.1	26.2
Hogweed Sosnowsky (*Heracleum Sosnowsky*)	10.8	6.9	45.6
Dill (*Anethum graveolens*)	5.0	2.0	15.1
Lowage (*Levisticum officinale*)	9.6	5.4	13.6
Parsley (*Petroselinum crispum*)	8.2	3.2	24.9
Ground-elder (*Aegopodium podagraria*)	1.4	0.4	6.3
Cow parsley (*Antriscus sylvestris*)	30.1	7.6	23.8

In high temperature extraction, some of compounds were decomposed (unsaturated compounds and glycosides, with discharge aglycone with considerably smaller

Table 2

Contents of group of chemical components identified in extracts (GC-MS)

Compounds	Common hogweed		Hogweed S.		Dill		Lowage		Parsley		Ground-elder		Cow parsley	
	SA	UB	SA	UB	SA	UB	SA	UB	SA	UB	SA	UB	SA	UB
n-octyl alcohol	—	3.7	1.4	8.7	—	—	—	—	—	—	—	—	—	—
Other alcohols	—	—	7.7	—	—	—	0.3	—	—	—	—	8.9	2.0	—
Octyl esters	44.9	54.2	14.0	32.6	—	—	—	—	—	—	—	—	—	—
Terpenes	1.1	0.5	—	—	72.0	97.0	46.6	74.1	3.8	14.3	1.0	1.2	56.2	65.1
Furanecumarin and coumarin	9.0	4.3	52.2	20.8	2.1	1.2	20.8	2.2	3.7	—	—	—	16.7	10.2
Phenols	—	—	1.1	0.2	0.9	0.3	—	—	—	—	—	—	2.4	1.8
Unsaturated ketones	0.6	—	—	—	—	—	0.5	—	—	—	—	—	—	—
Unsaturated esters	1.1	4.1	—	—	7.2	1.5	—	—	—	—	—	—	3.3	5.0
Unsaturated fat acids	11.1	21	4.6	4.8	3.6	—	7.8	6.8	—	—	30.8	39.5	—	—
Saturated fat acids	—	—	—	—	—	—	0.5	—	—	—	—	—	—	—
Saturated esters	—	7.7	5.6	25.7	—	—	—	—	—	—	—	—	3.3	5.0
Sitosterol	0.4	—	—	—	—	—	—	—	—	—	—	—	—	—
Hydrocarbons	0.6	—	2.5	—	2.9	—	—	—	—	—	—	—	—	—
Unsaturated aldehydes	—	—	—	—	—	—	1.2	—	—	—	32.6	26.1	3.3	6.7
Sesquiterpenes	—	—	—	—	—	—	0.3	—	4.4	—	—	—	0.5	—
Phthalide	—	—	—	—	—	—	6.0	8.1	—	—	—	—	—	—
Phenylpropene derivative	—	—	—	—	—	—	—	—	77.1	84.7	—	—	—	—
Others	—	—	—	—	—	—	—	—	0.2	—	2.2	—	—	—
Others, not identifying	32.1	4.5	10.9	7.2	11.3	0.0	6.0	8.8	10.8	1.0	33.4	14.3	11.6	2.4

SA – Soxhlet apparatus, UB – Ultrasonic bath.

molecular mass). In comparison with the extracts made in Soxhlet apparatus, the extracts obtained in ultrasonic baths (USBE) did not include at all, or included only a very small quantity of not-cumarin phenols. The corresponding values for cow parsley amount to 2.4 and 1.8 %, for hogweed Sosnowsky – 1.1 and 0.2 %, also less cumarins and furanocumarins (for hogweed Sosnowsky 52.2 and 20.6 %, and for lowage – 20.8 and 2.2 %). At the same time, more terpenes and unsaturated compounds with different chemical nature were found. In the case of extracts from common hogweed and hogweed Sosnowsky – the presence of *n*-octyl alcohol and octyl esters was detected.

Table 3

Antioxidant activity

Plants	Soxhlet apparatus			Ultrasonic bath		
	AECD	DMPD	PyE	AECD	DMPD	PyE
Common hogweed (*Heracleum sphondylium*)	53	2.3	49	87	1.9	31
Hogweed Sosnowsky (*Heracleum Sosnowsky*)	143	3.1	182	198	2.3	123
Dill (*Anethum graveolens*)	116	1.7	35	136	1.6	26
Lowage (*Levisticum officinale*)	44	2.4	103	42	2.2	78
Parsley (*Petroselinum crispum*)	132	2.1	44	157	1.8	23
Ground-elder (*Aegopodium podagraria*)	45	0.6	10	162	0.4	0
Cow parsley (*Antriscus sylvestris*)	46	0.9	92	60	0.4	0

AECD – antioxidant activity measured by the ascorbic acid/linoleic acid assay, in mg/g dry mass of extract; DMPD – antioxidant activity measured by the *N,N*-dimethyl-*p*-phenylenediamine dihydrochloride/iron(II) chloride assay, in milligram Trolox equivalent/g dry mass of extract; PyE – total phenolic compounds, determined as pyrogallol equivalents, in mg per g of dry mass, by Folin-Ciocalteu method.

The received results showed that extracts, obtained with the proposed method, are characterized by more primeval properties; in consequence, they should have a higher biological activity. This effect was especially observed in the extracts of hogweed Sosnowsky and common hogweed's seeds, including more *n*-octyl alcohol and octyl acetate than the plant extracts obtained in Soxhlet apparatus. The research works conducted by the Department of Plant Protection of Agricultural University in Krakow show that the fungistatic activity of this substances is high. This effect is confirmed by the retardation of growth of fungi *Fusarium culmorum*, *Botrytis cinerea*, *Alternaria alternate*, with oil from hogweed Sosnowsky seeds, which includes a lot of *n*-octyl alcohol and octyl ester. A strong inhibition of the tested fungi was also observed in nutrient solution with additives of this substances [5].

The total content of phenols in the obtained extracts and their antioxidative activity were determined, in order to observe the relation between these quantities and the biological activity of plant extracts, which were described in numerous publications [6]. Antioxidant content in the extracts was measured with two methods, based on different mechanisms of chemical process – according to the well-established information of extracts' composition. In DMPD method a transfer of hydrogen atom occurs, characteristic of phenolic compounds. In the case of the use of linoleic acid and ascorbic acid, there occurs a transfer of electron in non-enzymatic formation of peroxides [7, 8].

It was found that the antioxidative capacity, which is determined by the complexion DMPD with Fe^{3+} is decreased. It is connected with the presence of phenols and conform with the results of total content determination. The antioxidative properties of extracts obtained with both methods, studied by means of inhibition of not enzymatic oxidation of linoleic acid give similar results, or even higher, in the case when the USBE method was used. It is connected with a bigger content of terpenes and other unsaturated compounds. According to the experimental works of Jeng-Leun Mau [9], all the compounds of isoprene structure reveal inhibitive effect in relation to peroxidation of linoleic acid. Moreover, terpenes are in synergetic relation to other antioxidants which are present in extracts.

Conclusions

To recapitulate, it should be supposed that the extracts received with the described method, have to be characterized by the same or a higher fungistatic activity. This conclusion will be subject to further investigations.

Acknowledgments

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BADANIA PORÓWNAWCZE EKSTRAKTÓW Z NASION ROŚLIN Z RODZINY *Apiaceae* W WARUNKACH ULTRADŹWIĘKOWYCH

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Abstrakt: W roślinach z rodziny *Apiaceae* występuje wiele substancji znanych ze swej aktywności biologicznej w stosunku do mikroorganizmów (grzybów, bakterii i wirusów). We wcześniejszych badaniach opisano właściwości wybranych ekstraktów otrzymanych metodą klasyczną, ich skład i aktywność w stosunku do grzybów patogennych. W obecnej pracy przedstawiono właściwości ekstraktów otrzymanych z nasion: pietruszki (*Petroselinum crispum*), barszczu zwyczajnego (*Heracleum sphondylium*), barszczu Sosnowskiego (*Heracleum Sosnowsky*), podagrycznika (*Aegopodium podagraria*), kopru ogrodowego

(*Anethum graveolens*), lubczyku (*Levisticum officinale*) i trybuli (*Anthriscus sylvestris*), przez ekstrakcję octanem etylu, w temperaturze pokojowej w łaźni ultradźwiękowej. Analizę otrzymanych ekstraktów prowadzono metodą GC-MS (Agilent 6890N). Właściwości antyutleniające oznaczano dwiema metodami: z udziałem kwasu askorbinowego lub linolowego oraz w obecności *N,N*-dimetyl-*p*-fenylenodiaminy (DMPD). Uzyskane rezultaty pokazały, że ekstrakty otrzymane proponowaną metodą mają bardziej pierwotne właściwości, a co za tym idzie, powinny wykazywać większą aktywność biologiczną.

Słowa kluczowe: *Apiaceae*, ekstrakty z nasion, aktywność antyoksydacyjna, DMPD

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SORPTION FROM MULTICOMPONENT SOLUTIONS ON FREEZE-DRIED CHITOSAN

SORPCJA Z ROZTWORÓW WIELOSKŁADNIKOWYCH NA CHITOZANIE SUSZONYM SUBLIMACYJNIE

Abstract: The process of adsorption in time-variable conditions in a column packed with lyophilized chitosan beads modified with polyvinyl alcohol (CH-PVA), ethylenediaminetetraacetic acid (CH-PVA-EDTA) or 3-chloropropionic acid (CH-PVA-3CPA) was analyzed. Experiments were carried out in a column filled with freeze-dried chitosan beads of diameter $\phi = 2.5$ mm and fed with water ternary solutions of Cu(II), Ni(II) and Zn(II). Sorption efficiency of single ions was analyzed. Additionally, the influence of initial concentration and flow rate was determined. A pseudo-second order equation was used in the description of ion sorption. Sorption equilibrium on freeze-dried chitosan beads with PVA at 25 °C in static conditions was determined. Freundlich equation was recommended in this case.

Keywords: chitosan, lyophilization, sorption equilibrium, heavy metal ions

Chitosan is a natural polymer formed from easily available chitin. Its properties often surpass those of much more expensive synthetic polymers. Chitosan has many valuable features [1–3], including bioactivity (acting on living organisms through inhibition or activation of their life processes), biodegradability (an ability to decompose under the influence of natural environmental factors), biocompatibility and biocomplexing, as well as unique sorption and chelating properties for many heavy metal ions [4–6]. Heavy metals are a special hazard because even in small amounts they can accumulate in living organisms. Thus, from the technological and first of all ecological point of view it is important to remove heavy metals from aqueous solutions. Processes which enable a significant decrease of the concentration of heavy metal ions include chemical precipitation and filtration, coagulation, ion exchange, membrane processes and adsorption [7]. Due to its properties, chitosan often finds application in sorption processes. However, we still search for a chemical formula which would allow us to modify chitosan by adding other chemical compounds, eg polyvinyl alcohol (PVA), ethylenediaminotetraacetic acid (EDTA) or 3-chloropropionic acid (3CPA) that increase its

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sorption abilities and mechanical structure and enhance its durability. A mathematical description of the kinetics of heavy metal ion sorption is also searched for.

The process of adsorption in static conditions and in the column packed with a bed of freeze-dried chitosan beads is discussed in this study. The efficiency of sorption of heavy metal ions, ie copper(II), zinc(II) and nickel(II), from a multicomponent solution was investigated.

Scope of experiments

Formation of chitosan beads

Chitosan beads were made from 90/1000/A1,5 chitosan flakes produced by Heppe GmbH (Germany) with deacetylation degree 90 %. Other reagents were purchased at “Chempur” (Poland). The process of beads formation consisted of several stages. 10 g of chitosan flakes were dissolved in 500 cm^3 acetic acid (2 %). The vessel with chitosan and acetic acid was placed in a water bath at a temperature of 60 ^{o}C for 30 minutes stirring continuously and then it was left for 24 h. 15 g polyvinyl alcohol (PVA) was dissolved in 150 cm^3 distilled water. The solutions of chitosan and PVA were combined and mixed at a temperature of 60 ^{o}C, next a substance modifying chitosan sorption abilities was added (5 g EDTA or 3-chloropropionic acid in 50 cm^3 H_2O). The next stage was drop formation under pressure [7]. Chitosan beads with gel structure of diameter 2.8 to 3.4 mm and moisture content 95.4 % (20.74 kg H_2O/kg d.m.) were formed. Then, the beads were freeze-dried in an ALPHA 2–4 freeze dryer using freezing to –25 ^{o}C and working pressure 0.630 mbar. In this way, beads of diameter ca 2.5 mm, moisture content 5 % and density 175 kg/m^3 characterized by good mechanical properties were obtained. This method was selected because lyophilization has been known as a method for preservation of food, drugs, tissues, etc. which preserves their primary features by dehydration in frozen state (sublimation of ice crystals) under high vacuum.

Photo 1 illustrates appearance of the beads prior to and after drying in a freeze dryer. Photos 2 and 3 show a single chitosan bead and 100-time magnification of its surface which reveals high porosity of the bead after lyophilization.

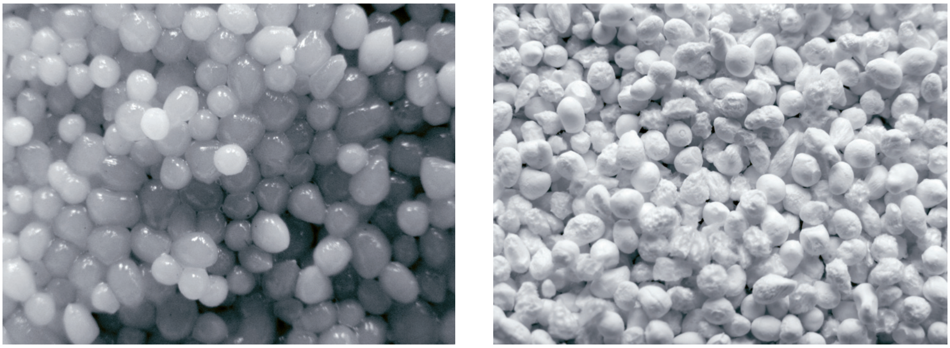

Photo 1. Wet and freeze-dried chitosan beads with PVA

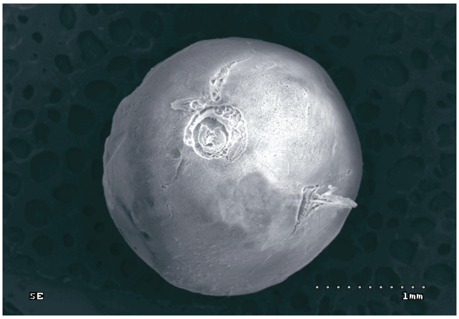

Photo 2. SEM photo of freeze-dried CH-PVA bead

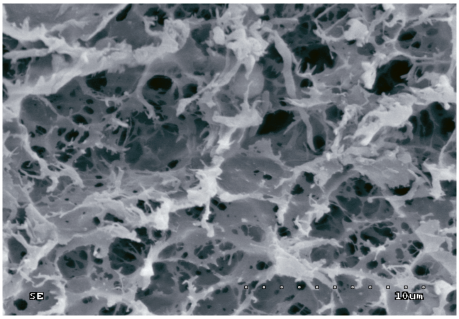

Photo 3. SEM Photo of CH-PVA bead surface

Experimental set-up

Experiments were carried out at a temperature of 25 ^{o}C in two systems: static and dynamic in a column. In the first case in conical flask about 4 g freeze-dried chitosan beads were placed and 200 cm^3 of the mixture of tested salts solutions in the concentration range from 10 to 50 mg/dm^3 were added. Next, they were put into a thermostat (Photo 4) and stirred continuously. A source of particular ions were water solutions of copper(II) ($CuSO_4 \cdot 5H_2O$), nickel(II) ($NiSO_4 \cdot 6H_2O$) and zinc(II) ($ZnSO_4 \cdot 7H_2O$). The experiments were carried out at pH = 5 to 6. Samples for analysis were taken every 30 min until reaching the state of equilibrium.

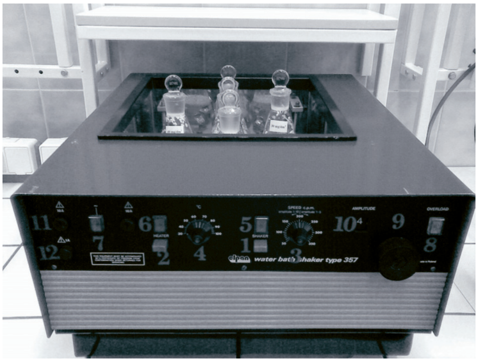

Photo 4. The set-up for determination of heavy metals sorption equilibrium

In the second case the experiments were performed in the column 0.567 dm^3 in volume with feeding solution flow, packed with a sorbent, ie freeze-dried chitosan beads. The whole was placed in a transparent casing equipped with a threaded head and vent valve. The measuring set-up consisted of a metering pump for solution (BL 1.5 model with the maximum delivery 15 dm^3/h) and a vessel with salt solution. The pump took the salt solution from the vessel by means of an aspirator and pumped it to the

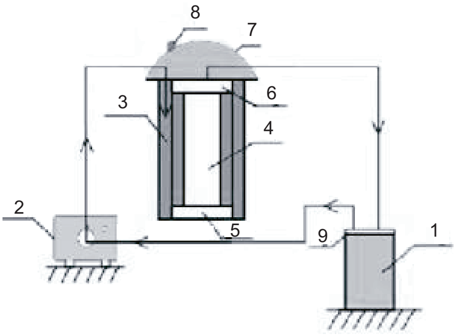

Fig. 1. Schematic diagram of the measuring set-up: 1 – thermostated supply vessel, 2 – metering pump, 3 – column casing, 4 – sorption column, 5 – base, 6 – upper cover, 7 – sealing head, 8 – vent valve, 9 – cover of the supply vessel

column through holes in its lower part from the bottom up to the bed top. After passing through the bed, the solution was fed back to the supply vessel.

Measurements were made for ternary water solutions of salts which contained Cu(II), Ni(II) and Zn(II) ions. The column packing was freeze-dried chitosan mixed with polyvinyl alcohol (CH-PVA) and ethylenediaminotetraacetic acid (EDTA) [9]. Experiments with 3-chloropropionic acid (3CPA) were also carried out. However, despite literature reports [10], they did not bring about expected results.

The measurements were made for initial salt concentrations ranging from 10 to 50 mg/dm^3 and for changeable volumetric flow rate from 1.1 to 5.8 dm^3/h. Each measurement took about 200 min. Determinations were made using a Dionex ICS-1000 ionic chromatograph.

A mathematical description of sorption kinetics

Prediction of sorption kinetics is one of the important topics concerning design of the equipment for carrying out this process. The final rate of adsorption in a packed column depends on constituent processes. The adsorption rate reaches the highest value in the moment when the fluid contacts the adsorbent for the first time. Next, the rate decreases with the process time. In this case, the process of adsorption in a column is controlled by the kinetics of a chemical reaction between chitosan and metal ions. In such a case, the pseudo-first and pseudo-second order, equations are proposed in the literature [11].

In this study the psudo-second order equation (2) is used because this equation is recommended for the metal – biosorbent system and gives good fitting [12, 13].

$$\frac{dq}{dt} = k(q_e - q)^2 \tag{1}$$

Upon integration of equation (1) in the range (t = 0, q = 0) and (t, q), equation (2) is obtained

$$\frac{q}{q_e} = \frac{kq_e t}{1 + kq_e t} \tag{2}$$

After transformation of equation (2), its form suitable for calculation of coefficients k and q_e is obtained

$$\frac{t}{q} = \frac{1}{k \cdot q_e^2} + \frac{t}{q_e} \tag{3}$$

$$q = (C_0 - C_t) \cdot \frac{V}{m} \tag{4}$$

To determine constants K and q_e, a graph of the function in the system of coordinates (t/q, t) should be made basing on results of the measurements. The graph was described by the equation of a straight line using the least squares method.

Based on the results, it was found that equation (2) well described sorption kinetics in all analyzed cases. The correlation coefficients ranged from 0.998 to 1.

Results and discussion

Figures 2 and 3 show a description of sorption equilibrium with the use of Freundlich isotherm – equation (5). For each case constants K and n were determined from equation (6) and correlation coefficient R was calculated.

$$q = KC_e^n \tag{5}$$

After finding the logarithm

$$\log q = \log K + n \log C_e \tag{6}$$

It was observed that in the case of a ternary solution, sorption was the best for Cu(II) ions reaching the value of ca 5 mg/(g dry chitosan). Lower values, about 2.5 mg/g and 2 mg/g, were obtained for Ni(II) and Zn(II) ions, respectively.

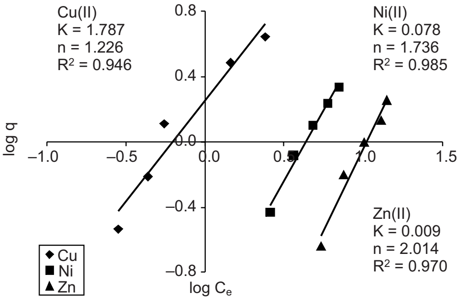

Fig. 2. Examples of constants K and n determined from Freundlich equation

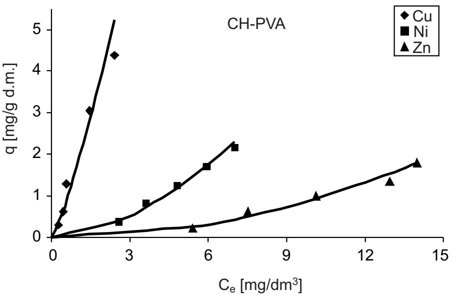

Fig. 3. Description of sorption equilibrium from ternary solution by Freundlich equation

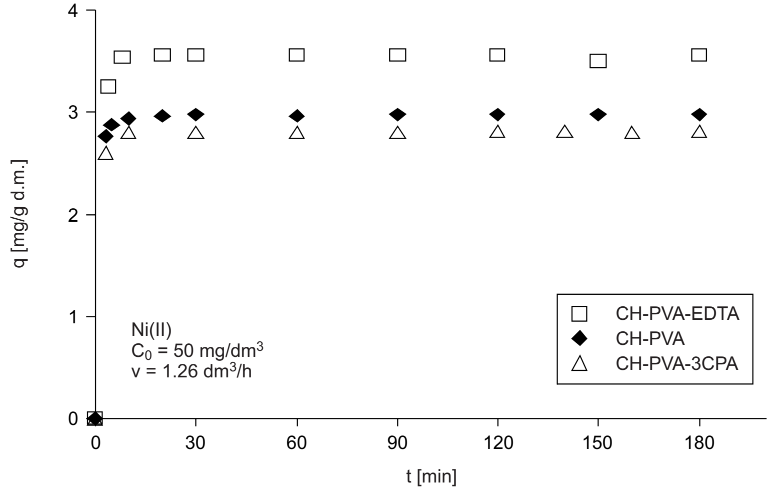

Fig. 4. Effect of additions to chitosan on sorptivity of Cu(II)

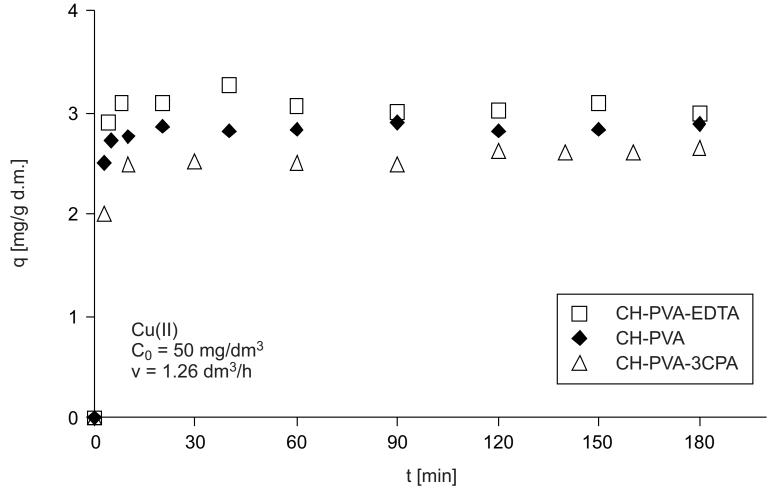

Fig. 5. Effect of additions to chitosan on sorptivity of Ni(II)

Further in the study, metal ion sorption in the column was compared for three types of freeze-dried chitosan: CH-PVA, CH-PVA-EDTA and CH-PVA-3CPA. As follows from Figures 4 and 5, the addition of EDTA causes an increase of sorption abilities of chitosan, while 3-chloropropionic acid, despite of reports presented in the literature, decreases these sorption abilities. Therefore, further studies were carried out for chitosan with the addition of PVA and EDTA, in which the effect of the amount of held-up heavy metal ions depending on the initial concentration of a solution was determined. Figures 6 and 7 show relations which were also confirmed in the case

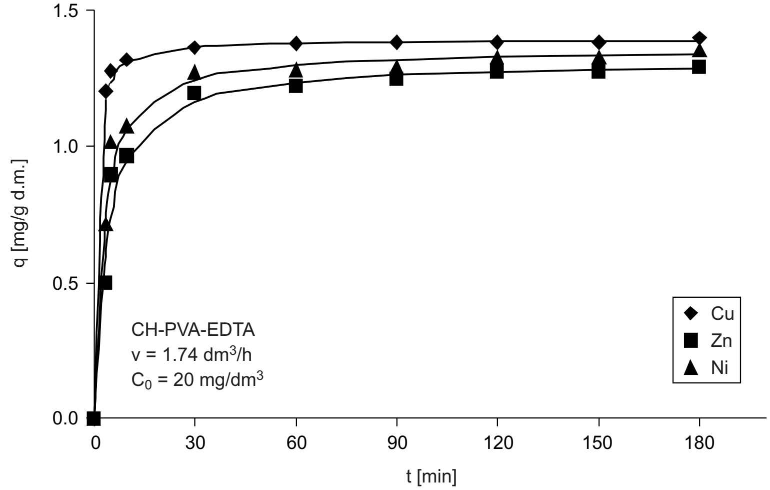

Fig. 6. Sorption isotherm of Cu(II), Ni(II), Zn(II) for C_0 = 20 mg/dm^3

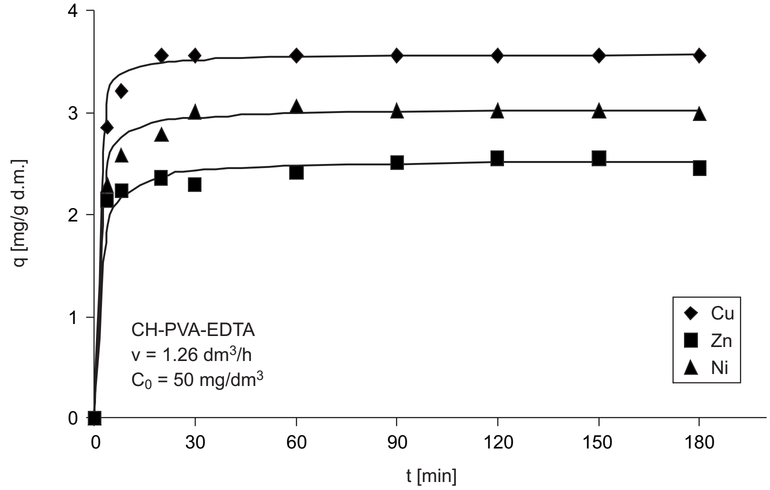

Fig. 7. Sorption isotherm of Cu(II), Ni(II), Zn(II) for C_0 = 50 mg/dm^3

of other concentrations – for higher concentrations bigger values of equilibrium concentrations were obtained. The results confirm some selectivity of chitosan in relation to metal ions expressed by the sequence: Cu(II) > Ni(II) > Zn(II).

In the next part of this work, for example for nickel ions, the effect of volumetric flow rate on the amount of adsorbed ions is presented (Fig. 8). It was found that the rate of liquid stream flow through the bed had no significant influence on the process intensification.

Figure 9 illustrates the effect of the initial concentration of heavy metal complexes on the amount of adsorbed ions of one type, in this case copper. It was also observed for

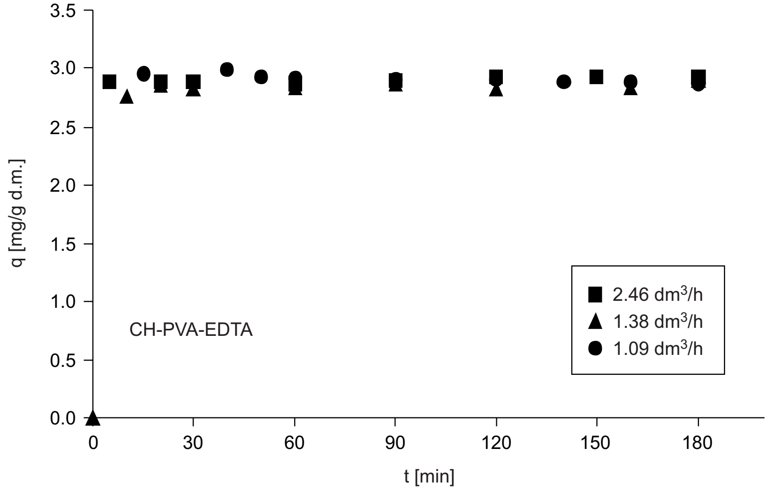

Fig. 8. Effect of volumetric flow rate on sorption properties of Ni(II) ions

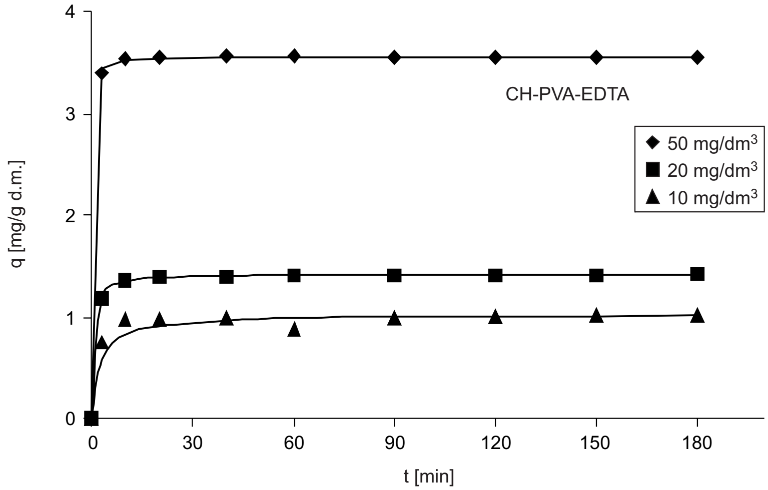

Fig. 9. Kinetics of Cu(II) ion sorption vs initial concentration

other ions that with an increase of the concentration, the load of ions trapped by the sorbent grew.

Conclusions

The following conclusions have been drawn from the experiments:

1. Freeze drying of chitosan leads to formation of stable structures with good mechanical properties and prolonged durability, which is important for storage and distribution of the obtained product. High porosity promotes sorption processes.

2. Freeze-dried chitosan in the form of beads, modified with polyvinyl alcohol and EDTA, is good packing of the column for Ni^{2+}, Zn^{2+} and Cu^{2+} ion sorption from water solutions. The process of sorption controlled by a chemical reaction reaches equilibrium already after about 60 minutes.

3. The best effects of sorption from a ternary mixture were obtained for copper ions, and next for nickel and zinc ions.

4. The degree of metal ion hold-up on a chitosan bed increases with an increase of initial concentration of the solution.

5. No effect of the volumetric flow rate in the column on ion sorption was observed.

6. The sorption kinetics is well described by the pseudo-second order equation, which is confirmed by the statistical estimation, while sorption equilibrium is well characterized by the Freundlich equation.

Symbols

C_e – sorption equilibrium of metal ions [mg/dm^3],
C_0 – initial concentration of the solution [mg/dm^3],
C_t – solution concentration at the column outlet after time t,
k – constant in the second order equation [g/(min · mg)],
K, n – Freundlich constants describing sorption equilibrium,
m – dry mass of chitosan [mg d.m.],
t – time [min],
q – the amount of adsorbed metal ions [mg/g d.m. chitosan],
q_e – equilibrium amount of adsorbed ions [mg/g d.m. chitosan],
V – solution volume [dm^3].

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SORPCJA Z ROZTWORÓW WIELOSKŁADNIKOWYCH NA CHITOZANIE SUSZONYM SUBLIMACYJNIE

Wydział Inżynierii Procesowej i Ochrony Środowiska
Politechnika Łódzka

Abstrakt: Badano proces adsorpcji prowadzony w warunkach zmiennych w czasie na złożu z lofilizowanych kulek chitozanowych modyfikowanych alkoholem poliwinylowym (PVA) z dodatkiem kwasu etylenodiaminotetraoctowego (EDTA) lub kwasu 3-chloropropionowego (3CPA). Proces ten prowadzono w kolumnie wypełnionej kulkami o średnicy ok. ϕ = 2,5 mm dla mieszaniny jonów w roztworach wodnych: miedzi(II) ($CuSO_4 \cdot 5H_2O$), niklu(II) ($NiSO_4 \cdot 6H_2O$) oraz cynku(II) ($ZnSO_4 \cdot 7H_2O$). Analizowano kinetykę sorpcji pojedynczych jonów występujących w roztworze trójskładnikowym. Dodatkowo określano wpływ stężenia początkowego i objętościowego natężenia przepływu. Do opisu badanego procesu zastosowano równanie pseudodrugiego rzędu. Określono równowagę sorpcyjną dla liofilizowanego chitozanu z PVA w 25 °C w warunkach statycznych. W tym przypadku zastosowano równanie Freundlicha.

Słowa kluczowe: chitozan, liofilizacja, równowaga sorpcyjna, jony metali ciężkich

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SULPHUR(IV) OXIDATION CATALYSED BY MANGANESE(II) IONS UNDER CONDITIONS REPRESENTATIVE FOR ATMOSPHERIC WATERS

UTLENIANIE SIARKI(IV) KATALIZOWANE JONAMI MANGANU(II) W WARUNKACH REPREZENTATYWNYCH DLA WÓD ATMOSFERYCZNYCH

Abstract: Results of kinetic studies on the S(IV) oxidation catalysed by Mn(II) ions were presented. Laboratory experiments were carried out at concentrations of reactants and pH of solutions representative for acidified atmospheric waters. The results indicate that at an initial pH of the reaction solution from 3.5 to 5.0 the reaction rate is independent of S(IV) concentration ie, the reaction is zero order with respect to S(IV) concentration ($n = 0$). At an initial pH of 6.0 the reaction order n changes with the reaction time. At the beginning of the process $n \neq 0$, and then it changes into 0. The S(IV) oxidation rate depends on both Mn(II) concentration and initial pH of the solution. Under studied conditions the reaction is fast and it may play a significant role in the total oxidation of S(IV) in the atmosphere in highly polluted areas.

Keywords: atmospheric chemistry, atmospheric waters, S(IV) oxidation, manganese, catalysis

One of the important atmospheric processes leading to the formation of acidic precipitation is the catalytic oxidation of S(IV) species by oxygen in atmospheric water droplets. It has been known that some transition metal ions are able to catalyse this process [1]. The most efficient catalysts are iron(II/III) and manganese(II/III). These metals are common constituents of tropospheric aerosols in heavy polluted urban and industrial areas. The common particles containing trace metals are soil dust, fly ash from power plants, and exhaust from combustion engines and industrial operations.

The only source of these metals in the atmospheric aqueous phase is the dissolution of aerosol particles incorporated in water droplets. Concentrations of Mn(II) dissolved in atmospheric waters in urban and industrial areas range from 10^{-7} to 10^{-6} mol/dm^3 in rain [2], and from 10^{-6} to 10^{-5} mol/dm^3 in fog and cloudwater [1, 3].

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The catalysed S(IV) oxidation is regarded as a significant process in the total oxidation of sulphur dioxide in continental clouds and fogs, in particular in regions where the air is highly polluted. This process is the subject of a number of studies [eg 4–15].

The catalysed S(IV) oxidation is complex in both kinetics and mechanism, which has resulted in large discrepancy in both reported kinetic data and in proposed mechanisms. It is a free radical chain reaction which is so sensitive to the reaction conditions that even a slight change in them can cause a change of the dominant path of the reaction course, and thus lead to diverse results. Thus, despite numerous studies on metal ion catalysed S(IV) oxidation more work in this area is needed. Especially there is a lack of information about this process under conditions of highly polluted air where catalytic S(IV) oxidation may play a significant role in the total S(IV) oxidation.

The purpose of the present work was to study the Mn(II)-catalysed S(IV) oxidation under the conditions representative for acidified atmospheric water in heavily polluted areas.

Materials and methods

All chemicals used in this study were of analytical grade (Merck). Milli-Q water was used for preparation of all solutions. Stock solutions of Mn(II) were prepared from $MnSO_4 \cdot H_2O$. The S(IV) solutions were prepared freshly before each run by dissolving Na_2SO_3 in water which was deoxygenated by bubbling high purity argon through the Milli-Q water for at least 30 min. The initial pH of the solutions was adjusted with H_2SO_4. The source of oxygen for oxidation of S(IV) was synthetic air.

Kinetic experiments were conducted in a 500 cm^3 glass cylindrical reactor with four inlet connectors for: pH electrode, introducing reagents, thermometer and teflon tube for sample sipping. The reactor was filled with 450 cm^3 of the solution acidified to the required pH. The reactor was protected from light and immersed into a thermostat to maintain a constant temperature of 25 ± 1 ^oC. The air was introduced at the bottom of reactor through a ceramal at a rate of 100 ± 2 dm^3/h. Under these conditions the gas and liquid phases were well mixed and the reaction took place in the kinetic regime, ie the global rate of the S(IV) oxidation was limited by the rate of chemical reaction, not by the diffusion.

To start the reaction, the air flow was turned on and just after that the Mn(II) solution was injected into the reactor. At selected time intervals the concentration of S(IV) was measured by UV-VIS (Shimadzu, Model UV-2101 PC) spectrophotometer equipped with Sipper 260 (Model L) – using flow cell method. The sipping time was set to 5 s, and the slit width was set to 2.0 nm. The S(IV) measurements were carried out at wavelengths $\lambda = 203$ nm for the initial pH 3.5 and $\lambda = 205$ nm for the initial pH 4.0, 5.0 and 6.0. The pH measurements were performed by an Orion pH-meter (Model 710A) combined with a glass electrode. The concentration of Mn(II) was determined by AVANTA PM atomic absorption spectrometer of the GBC.

The experiments were performed under the following conditions: $[S(IV)] \approx 1 \cdot 10^{-3}$ mol/dm^3, $1 \cdot 10^{-6} \le [Mn(II)] \le 1 \cdot 10^{-5}$ mol/dm^3, $3.5 \le$ the initial pH ≤ 6.0, T = 25 ^oC.

Results and discussion

Some typical results of the kinetic measurements are shown in Figure 1 as the time dependence of $[S(IV)]_t/[S(IV)]_0$ ratios, where $[S(IV)]_t$ is the concentration of S(IV) at time t, and $[S(IV)]_0$ is the initial concentration of S(IV). Based on the measurement results, the kinetic law parameters for the processes studied were determined.

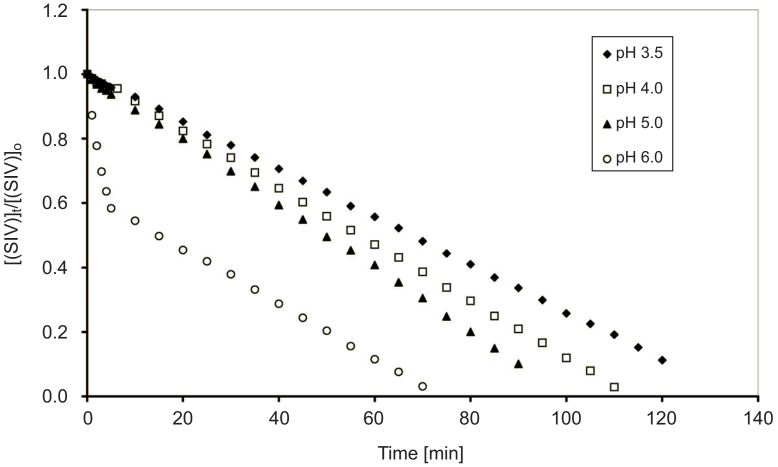

Fig. 1. Effect of initial pH values on the Mn(II)-catalysed S(IV) oxidation. $[S(IV)]_0 = 1 \cdot 10^{-3}$ mol/dm^3, $[Mn(II)] = 5 \cdot 10^{-6}$ mol/dm^3

Since the rate of Mn(II)-catalysed S(IV) oxidation is independent of oxygen concentration [16, 17], the reaction rate has been described by the equation:

$$r = -\frac{d[S(IV)]}{dt} = k_{obs}[S(IV)]^n \tag{1}$$

where k_{obs} is the observed rate constant, and n is the reaction order with respect to S(IV) concentration.

The reaction orders and rate constants determined by the standard integral technique are listed in Tables 1–2. The S(IV) oxidation catalysed by Mn(II) ions is zero order with respect to S(IV) concentration (n = 0) over the entire studied range of Mn(II) concentrations and at initial pH values in the range of 3.5–5.0. At an initial pH of 6.0 the reaction order with respect to S(IV) concentration changes with the reaction time. At the beginning it is different from 0 and then it changes into 0. At $[Mn(II)] \approx 1 \cdot 10^{-6}$ mol/dm^3 n = 3 from 0 to 1800 s, and then it changes into 0. At higher Mn(II) concentrations ($5 \cdot 10^{-6}$ and $1 \cdot 10^{-5}$ mol/dm^3) n = 2 at the beginning of the process and after 300 s it changes into 0, that is, into such order as for lower pH. This change in the reaction order n can be explained by an influence of the initial pH on the S(IV) oxidation rate. The results (Table 1) show that a change in the initial pH from 3.5 to 5.0 gives rather small increase in the oxidation rate whereas the change in the initial pH from 5.0 to 6.0 causes considerably greater increase in the reaction rate. At an initial pH of 6.0 the oxidation proceeds very fast and in consequence pH of the reaction solution decreases rapidly, which causes both a decrease in the oxidation rate and a change in the reaction order.

Table 1

Reaction orders n with respect to S(IV) concentration and observed rate constants k_{obs}

Initial pH	$[Mn] \approx 1 \cdot 10^{-6}$ mol/dm^3		$[Mn] \approx 5 \cdot 10^{-6}$ mol/dm^3		$[Mn] \approx 1 \cdot 10^{-5}$ mol/dm^3	
	n	k_{obs} $(mol/dm^3)^{(1-n)} \cdot s^{-1}$	n	k_{obs} $(mol/dm^3)^{(1-n)} \cdot s^{-1}$	n	k_{obs} $(mol/dm^3)^{(1-n)} \cdot s^{-1}$
3.5	0	$4.023 \cdot 10^{-8}$	0	$1.446 \cdot 10^{-7}$	0	$4.894 \cdot 10^{-7}$
4.0	0	$4.845 \cdot 10^{-8}$	0	$1.739 \cdot 10^{-7}$	0	$5.520 \cdot 10^{-7}$
5.0	0	$5.903 \cdot 10^{-8}$	0	$1.970 \cdot 10^{-7}$	0	$8.263 \cdot 10^{-7}$
6.0	3.0 (0–1800 s)	$6.097 \cdot 10^{2}$ (0–1800 s)	2.0 (0–300 s)	2.403 (0–300 s)	2.0 (0–300 s)	2.703 (0–300 s)
	0 (> 1800 s)	$2.427 \cdot 10^{-8}$ (> 1800 s)	0 (> 300 s)	$1.412 \cdot 10^{-7}$ (> 300 s)	0 (> 300 s)	$3.678 \cdot 10^{-7}$ (> 300 s)

The oxidation rate r is equal to k_{obs} if $n = 0$, and $r = k_{obs}[S(IV)]^n$ if $n \neq 0$. The value of the oxidation rate is dependent on Mn(II) concentration and initial pH of the solution (Table 2).

Table 2

Rates of the Mn(II) catalysed S(IV) oxidation at $[S(IV)] = 1 \cdot 10^{-3}$ mol/dm^3
and at different initial pH values and Mn(II) concentrations

Initial pH	$r\,(mol/dm^3) \cdot s^{-1}$		
	$[Mn] \approx 1 \cdot 10^{-6}$ mol/dm^3	$[Mn] \approx 5 \cdot 10^{-6}$ mol/dm^3	$[Mn] \approx 1 \cdot 10^{-5}$ mol/dm^3
3.5	$4.023 \cdot 10^{-8}$	$1.446 \cdot 10^{-7}$	$4.894 \cdot 10^{-7}$
4.0	$4.845 \cdot 10^{-8}$	$1.739 \cdot 10^{-7}$	$5.520 \cdot 10^{-7}$
5.0	$5.903 \cdot 10^{-8}$	$1.970 \cdot 10^{-7}$	$8.263 \cdot 10^{-7}$
6.0	$6.097 \cdot 10^{-7}$ (0–1800 s)	$2.403 \cdot 10^{-6}$ (0–300 s)	$2.703 \cdot 10^{-6}$ (0–300 s)
	$2.427 \cdot 10^{-8}$ (> 1800 s)	$1.412 \cdot 10^{-7}$ (> 300 s)	$3.678 \cdot 10^{-7}$ (> 300 s)

In the initial pH range from 3.5 to 5.0 the influence of Mn(II) concentration on the reaction rate is larger than the influence of the initial pH. The reaction rate increases about 11–14 times with increasing Mn(II) concentration from $1 \cdot 10^{-6}$ to $1 \cdot 10^{-5}$ mol/dm^3, whereas the change in the initial pH from 3.5 to 5.0 causes only 1.4–1.7 fold increase in the oxidation rate.

At initial pH values in the range of 5.0–6.0 the situation is different. The change in the initial pH from 5.0 to 6.0 leads to significant increase in the reaction rate (from several to a dozen or so times depending on Mn(II) concentration), but after short time (1800 s for Mn(II) concentration $1 \cdot 10^{-6}$ mol/dm^{-3}, and 300 s for higher Mn(II) concentrations) the reaction rate decreases to the level close to that for the initial pH 3.5.

At low pH range (3.5–5.0) the S(IV) oxidation proceeds at constant rate (at a given Mn(II) concentration). At pH 6.0 the course of the reaction is different, in the beginning the S(IV) oxidation rate is high and it is dependent on S(IV) concentration ($n \neq 0$), and then the S(IV) oxidation rate is smaller and it is independent of [S(IV)] ($n = 0$).

Conclusions

The S(IV) oxidation catalysed by Mn(II) ions is zero order with respect to S(IV) concentration ($n = 0$) over the entire studied range of Mn(II) concentrations ($1 \cdot 10^{-6}$–$1 \cdot 10^{-5}$ mol/dm^3 and at initial pH values in the range of 3.5–5.0. At an initial pH of 6.0 the reaction order n changes with the reaction time. At the beginning of the process $n \neq 0$, and then it changes into 0.

The S(IV) oxidation rate depends on both Mn(II) concentration and initial pH of the solution. In the initial pH range from 3.5 to 5.0 the influence of Mn(II) concentration on the reaction rate is larger than the influence of the initial pH. At Mn(II) concentration $1 \cdot 10^{-5}$ mol/dm^3 the reaction proceeds a dozen or so times faster than at Mn(II) concentration $1 \cdot 10^{-6}$ mol/dm^3, whereas a change in the initial pH from 3.5 to 5.0 causes only about 1.5-fold increase in the reaction rate. However, an increase in the initial pH above 5.0 causes a rapid increase in the reaction rate. At the initial pH 6.0 the reaction proceeds very fast. After short time (1800 s for Mn(II) concentration $1 \cdot 10^{-6}$ mol/dm^3, and 300 s for higher Mn(II) concentrations) about 50 % of S(IV) is already oxidized, and then the reaction rate decreases to the level close to that for the initial pH 3.5.

The results obtained indicate that the Mn(II) catalysed S(IV) oxidation may play a significant role in the total oxidation of S(IV) in the atmosphere in highly polluted areas.

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UTLENIANIE SIARKI(IV) KATALIZOWANE JONAMI MANGANU(II) W ROZTWORACH WODNYCH W WARUNKACH REPREZENTATYWNYCH DLA WÓD ATMOSFERYCZNYCH

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Abstrakt: Przedstawiono wyniki badań nad kinetyką utleniania S(IV) katalizowanego jonami Mn(II). Badania laboratoryjne przeprowadzono przy stężeniach reagentów i pH roztworu odpowiadających zakwaszonej wodzie atmosferycznej. Otrzymane wyniki wskazują, że przy początkowym pH roztworu w zakresie od 3,5 do 5,0 szybkość reakcji nie zależy od stężenia S(IV), tj. reakcja jest zerowego rzędu względem stężenia S(IV) ($n = 0$). Przy początkowym pH 6 rząd reakcji n zmienia się z czasem reakcji. Na początku procesu $n \neq 0$, a następnie przyjmuje wartość 0. Szybkość utleniania S(IV) zależy od stężenia Mn(II) i od początkowego pH roztworu. W badanych warunkach reakcja zachodzi szybko i może ona odgrywać znaczącą rolę w całkowitym utlenianiu S(IV) w atmosferze na obszarach silnie zanieczyszczonych.

Słowa kluczowe: chemia atmosfery, wody atmosferyczne, utlenianie S(IV), mangan, kataliza

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MODELLING OF COMPOUND MEASURING WEIR FOR THE TURAWA RESERVOIR

BADANIA MODELOWE PRZELEWU MIERNICZEGO O PRZEKROJU ZŁOŻONYM DLA ZBIORNIKA WODNEGO W TURAWIE

Abstract: The results of experimental research on a physical model of one of the measuring weirs built in the surrounding ditches of the reservoir in Turawa (the Opole province) were presented. The unique nature of this structure – its historical character, there being no original technical documentation and most importantly the highly uncommon shape of its overflow outlet (a combination of several geometric figures, a triangle and two rectangles) – makes the readily-available computational formulae unsuitable. These formulae hold only for standard weirs. Experiments carried out in our lab allowed for a thorough hydraulic description of the structure in the form of tables, formulae and graphs. Field measurements at the actual facility have positively confirmed the results obtained in laboratory.

Keywords: hydro-engineering, measuring weirs, hydrometry

Lower Silesia is well known for its many hydroengineering structures built in the prewar period. In most cases, their technical documentation, design data and usage instructions are missing, having been destroyed or lost during or after the war.

In what concerns the structures and facilities still in use, these documents have now been recovered and updated in inventory works, depending on the facility class and importance. Nonetheless, there are still some hydro-engineering structures (of much lesser importance), whose hydraulic properties remain unknown.

One example of such structures are the prewar measuring weirs on the surrounding ditches of the Turawa reservoir (the Opole province). Generally speaking, their state of preservation is not bad, as repair works were carried out on an ad hoc basis. However, in order for these weirs to be fully functional, their discharge curves are also needed. One of these weirs (Fig. 1) with a compound cross-section (unusual for weir flow

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Fig. 1. Measuring weir in Turawa

measurements) will be investigated in this paper. The cross-section surface of its overflow outlet is a combination of one triangle and two differently sized rectangles.

Integration of the discharge equation for flow through a large, unsubmerged orifice [1–3], well known in academic hydraulics, has led the authors to complex formulae (composition of several functions) of little practical applicability. An extra difficulty lies in the choice of the discharge coefficient μ. Due to the special character of the compound weir, assuming a value based on the literature would have been debatable and inaccurate. In view of the above, hydraulic modelling research was required.

Laboratory research

Research setup

Laboratory research was carried out in the Water Laboratory at Wroclaw University of Environmental and Life Science, Institute of Environmental Engineering. Fig. 2 shows a schematic of the measuring setup including the water supply installation. A model of the studied weir (Figs. 3 and 4) was placed in a straight trapezoidal flume with the following dimensions: 15 m in length, 0.81 m in width at the bottom and side slope 1:2. Depth measurements 1m above the weir ($L > 3H$ required) were done using a point gauge and a piezometer with an accuracy of ±0.1 mm. Total discharge in the laboratory water supply installation was measured using a calibrated certified measuring tank equipped with a gauge glass. For the water scale reading accuracy of ±0.5 mm, the accuracy of volume readings was ±4.5 dm^3. The tank filling time measurements accuracy was ±0.5 s. For measurements of discharge $Q < 50\ dm^3\ s^{-1}$, a circular measuring weir was installed on the flume's inlet. In this case, the reading accuracy of measured discharge was $\pm 0.5\ dm^3\ s^{-1}$.

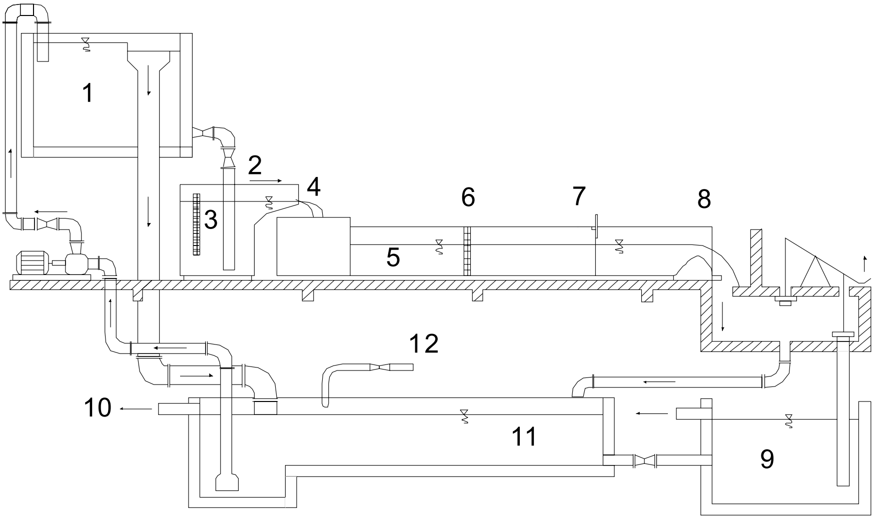

Fig. 2. Schematic view of the measurement set-up: 1 – higher tank, 2 – measuring tank, 3 – gauge, 4 – circular measuring weir, 5 – flume, 6 – piezometer, 7 – gauge, 8 – weir under investigation, 9 – measuring tank, 10 – to sewers, 11 – main tank, 12 – supply pipeline

Model's scale factor

Structure's actual dimensions and the technical capabilities of the laboratory allowed for representing the surrounding ditch and developing the weir model at a scale of 1:2. By analysing the forces involved in the flow of water one finds out that gravity is the most important factor. All other factors (such as viscosity) have little influence and are negligible. In order to convert values from the model to the real structure and the other way round an appropriate Froude's similarity criterion [3, 4] is applied. Denoting the linear dimensions scale (ratio) by:

$$\alpha = \frac{L_N}{L_M} = 2 \tag{1}$$

yields the following scales for other basic quantities:

- time and velocity: $1 : \alpha^{1/2} = 1 : 1.41$
- volume and force: $1 : \alpha^{3} = 1 : 8$
- discharge: $1 : \alpha^{5/2} = 1 : 5.66$

In eq. (1) "M" stands for the size of the model and "N" for that of the real structure. The main parameters of the investigated weir model (Fig. 3) were as follows:

- width of the rectangular part /1/: $b_1 = 810$ mm
- width of the rectangular part /2/: $b_2 = 210$ mm
- width of the triangular part /3/: $b_3 = 210$ mm
- tip opening angle of the triangular part /3/: $\varphi = 160°$

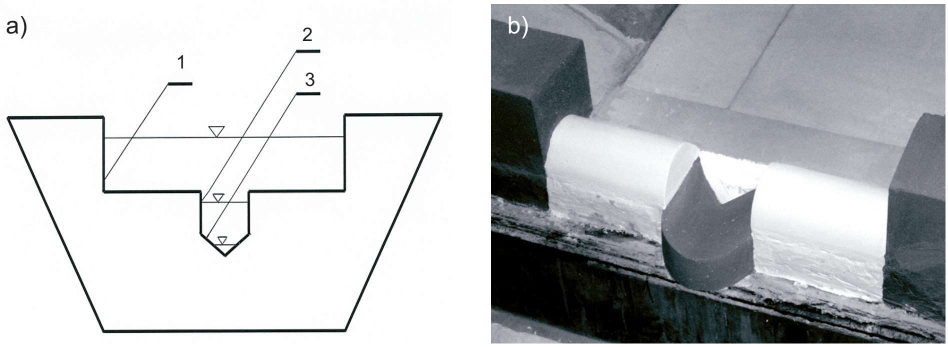

Fig. 3. Laboratory model of the Turawa measuring weir

Fig. 4. Model of the Turawa measuring weir under laboratory investigation

The triangular outlet (V-notch) weir /3/ and the smaller rectangular weir /2/ (Fig. 3) have longitudinal profiles typical for the computational scheme for broad crested weir, where the jump height p = 0. The bigger rectangular outlet /1/ on the other hand is formed by two parts modelled according to the Creager type profile [3]. In this case, crest height for weir /2/ measured from flume's bottom is p = 100 mm.

Methods and scope of research

This paper uses two generally known methods of calibration of hydro-engineering structures: the hydrometric and the hydraulic one. The hydrometric method looks for an empirical relationship Q = f(H) between discharge Q and head H above the weir's crest at the upper end of the structure [1, 5, 6–9].

The hydraulic calibration method was used in order to determine the discharge coefficient μ. The value of μ was calculated indirectly – based on direct measurements of flow parameters for flow defined by the following equation:

$$Q = K\mu F\sqrt{2gH} \tag{2}$$

where: K – constant (eg 2/3 for rectangles and 4/15 for triangles); μ – weir discharge coefficient [-]; F – active cross-section on the weir (F = b H) [m^2]; H – head [m]; b – stream width [m]; g – acceleration due to gravity [m/s^2].

A total of 37 main measurements were carried out, in which the *H* parameter was measured as a function of the initial discharge *Q*. To improve accuracy each measurement of water level and discharge was performed twice. For technical reasons, discharge values measured in the laboratory model had to be limited from above (max value: $Q_{max,L} = 55.0\ dm^3s^{-1}$ with $H_{max,L} = 163.0$ mm): $Q_{max,L} = 55.0\ dm^3\ s^{-1}$ for $H_{max,L} = 163.0$ mm, which translates to the following real-scale values: $Q_{max,N} = 311.1\ dm^3\ s^{-1}$ for $H_{max,N} = 326.0$ mm.

Research results

Weir characteristics

This stage of research aimed to establish a relationship between discharge and the readings of measuring instruments in the laboratory model. These values were recorded as tables, nomograms and formulae. The measurements of Q and H obtained in the lab were scaled up to real-size conditions and are given in Table 1. The following have been calculated: discharge curve Q = f(H) and calibration curve H = f(Q), shown in Fig. 6.

For practical applications it was assumed that one of the formulae Q = f(H) or H = f(Q) would be derived for the entire measuring range of the weir, conditioned by the compound shape of overflow outlet: triangle + small rectangle + big rectangle (Fig. 3). Regression equations were derived for experimental relationships being studied. One of the principal criteria of fitting the mathematical model to measurement data was the highest possible correlation (R > 0.99). It appeared that the following polynomial was optimal:

$$H = f(Q) = 16.93Q^3 - 12.14\ Q^2 + 3.115\ Q + 0.022 \quad \text{with } R^2 = 0.995 \tag{3}$$

and:

$$Q = f(H) = 24.73\ H^3 - 8.82\ H^2 + 1.27\ H - 0.03 \quad \text{with } R^2 = 0.990 \tag{4}$$

where H must be expressed in [m] and Q will be given in [m^3/s].

Discharge coefficient

The discharge coefficient μ for the real-size weir was derived from formula (2). For rectangular cross-sections the formula being used was:

$$\mu = \frac{3Q}{b\sqrt{8gH^{1.5}}} \tag{5}$$

and for the triangular one:

$$\mu = \frac{15Q}{b\sqrt{32gH^{1.5}}} \tag{6}$$

Stream width was assumed to be: $b_3 = 2H\ tg(\varphi/2)$ – for the triangular part of the overflow outlet and for the rectangular outlets $b_2 = 420$ mm and $b_1 = 1620$ mm, respectively. Results of measurements and calculations are given in Table 1.

Table 1

Results of measurements of the relationships Q = f(H) and μ = f(H) for the measuring weir

No.	Model in laboratory		Object in reality			Notes
	Q′ [dm^3/s]	H′ [mm]	1000 · Q [m^3/s]	1000 · H [m]	μ [-]	
1	0.10	10.0	0.57	20.0	0.746	triangle /3/
2	0.15	12.0	0.85	24.0	0.710	
3	0.20	14.0	1.13	28.0	0.644	
4	0.28	16.0	1.60	32.0	0.652	
5	0.80	23.0	4.53	46.0	0.370	triangle /3/ + + rectangle /2/
6	1.60	30.0	9.05	60.0	0.497	
7	1.80	32.0	10.18	64.0	0.507	
8	2.40	37.0	13.58	74.0	0.544	
9	2.60	38.0	14.71	76.0	0.566	
10	3.20	40.0	18.10	80.0	0.645	
11	4.00	42.6	22.63	85.2	0.734	
12	4.40	43.5	24.89	87.0	0.782	
13	5.40	49.0	30.55	98.0	0.803	
14	6.00	54.5	33.94	109.0	0.760	
15	7.60	62.0	42.99	124.0	0.794	
16	8.60	68.0	48.65	136.0	0.782	
17	9.60	78.0	54.31	156.0	0.711	
18	11.20	90.0	63.36	180.0	0.669	
19	12.00	94.0	67.88	188.0	0.671	
20	13.80	99.0	78.07	198.0	0.714	
21	14.80	106.0	83.72	212.0	0.692	
22	17.40	119.0	98.43	238.0	0.429	
23	18.60	120.0	105.22	240.0	0.448	

Table 1 contd.

No.	Model in laboratory		Object in reality			Notes
	Q′ [dm³/s]	H′ [mm]	1000 · Q [m³/s]	1000 · H [m]	μ [-]	
24	19.80	125.5	112.01	251.0	0.424	triangle /3/ + rectangle /2/ + rectangle /1/
25	23.00	126.0	130.11	252.0	0.488	
26	25.00	135.0	141.43	270.0	0.445	
27	28.20	139.5	159.53	279.0	0.463	
28	29.80	140.0	168.58	280.0	0.486	
29	31.80	142.5	179.89	285.0	0.497	
30	35.20	148.5	199.13	297.0	0.499	
31	37.20	149.0	210.44	298.0	0.523	
32	39.40	152.0	222.89	304.0	0.529	
33	43.00	153.0	243.25	306.0	0.569	
34	45.20	153.5	255.70	307.0	0.593	
35	48.00	154.5	271.54	309.0	0.621	
36	50.00	160.0	282.85	320.0	0.597	
37	55.00	163.0	311.14	326.0	0.630	

The discharge coefficient μ for compound weir greatly depends on the shape of its overflow outlet. In the case under study it is a combination of: triangle /3/, small rectangle /2/ and big rectangle /1/. With the increase of discharge each part starts to work hydraulically in the above sequence, and the measurable effect of it is the increase of H (Fig. 3). For the weir under study a relationship $\mu = f(H)$ has been established. Unfortunately, due to its overflow outlet special shape, this relationship is not continuous. Clearly, as shown in Fig. 5, $\mu = f(H)$ has points of discontinuity. It is therefore difficult to give one general practically applicable formula for $\mu = f(H)$ for all the measuring range of the weir. Hence the relationship $\mu = f(H)$ was analysed separately for each part of the outlet. Fig. 5 illustrates it graphically with curves fitted using the least squares method. Their regression equations for the real-size structure are as follows:

– for the triangle (measuring range: $H \le 32.0$ mm):

$$\mu = f(H) = 0.117\ H^{-0.47} \text{ with } R^2 = 0.972 \tag{7}$$

– for the triangle and the smaller rectangle (measuring range: 32.0 mm < H ≤ 240.0 mm):

$$\mu = f(H) = 317.80\ H^3 - 181.30\ H^2 + 30.25\ H - 0.765 \text{ with } R^2 = 0.872 \tag{8}$$

– for the entire cross-section (measuring range: 240.0 mm < H ≤ 326.0 mm):

$$\mu = f(H) = 29.33\ H^2 - 14.25\ H + 2.173\ R^2 = 0.876 \tag{9}$$

In all these equations *H* must be expressed in [m].

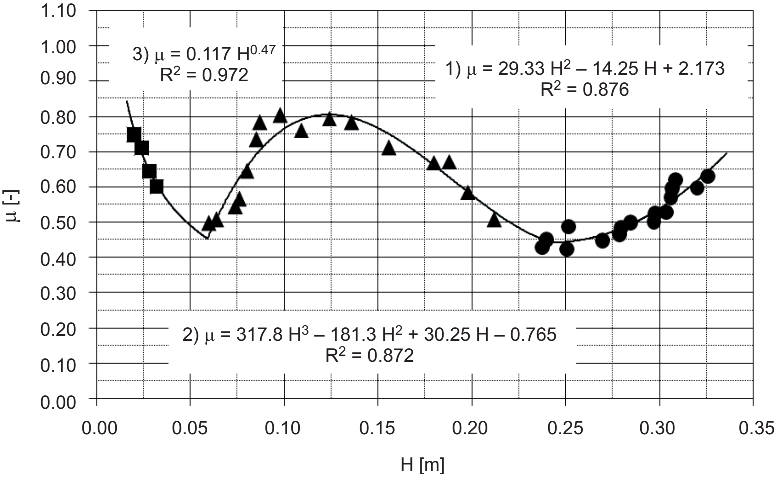

Fig. 5. Discharge coefficient vs head of water for the measuring weir

Verification of results

Laboratory research has allowed for a thorough verification of structure's hydraulic characteristics, given in the form of Table (Table 1), equations (Eqs. 3–9) and plots (Figs. 5 and 6). In order to verify laboratory measurements, field measurements on the actual weir in Turawa were carried out. Two characteristics were measured: discharge Q and head H on the weir. Depth measurements were performed using a portable point gauge (±0.1 mm) and discharge was measured indirectly – by measuring local velocity

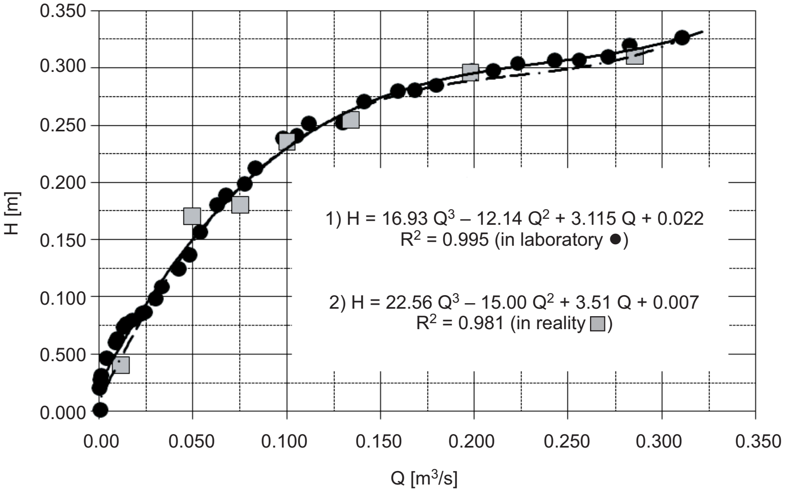

Fig. 6. Head of water vs discharge for the measuring weir under investigation (verification of results)

in multiple points of the channel below the weir. Flow velocity was measured using an electromagnetic flowmeter with ±0.001 m/s accuracy. Small discharge levels were measured volumetrically directly on the weir with a stop-watch (±0.5 s) and a 10 dm^3 measuring vessel calibrated with an accuracy of ±0.1 dm^3. Detailed description of these procedures can be found in the Gluchowska paper [10].

The channel with the weir allowed for a simulation of "artificial flooding" to be carried out with water from a nearby pond. Unfortunately, due to technical constraints, only a few measurement points could be obtained (7 /Q; H/ pairs). These points were marked on the weir calibration curve obtained in the laboratory experiments and scaled up to real-size conditions (Fig. 6, plot /1/).

Regression equation was derived also for field measurement data (Fig. 6, plot /2/). The two calibration curves H = f(Q) plotted in Fig. 6 are in a reasonably good agreement, perfectly satisfactory for practical engineering applications. The average relative error of the formulae (1–2) in Fig. 6, for all the measurements is 4.03 % and the standard deviation amounts to only 0.005. Hence, the modelling results have been positively verified by field measurements.

Conclusions

Hydraulic experimental research on the Turawa weir carried out over a wide parameter range, allowed for a better understanding of the discharge of water flow through a broad unsubmerged orifice having an unusual shape uncommon for engineering practice. This phenomenon is described in literature [1–3]. Our research provides the data required for qualitative and quantitative hydraulic characterization of this type of structures. Data is presented in the form of tables, equations and nomograms.

Noteworthy, the discharge coefficient μ, which is particularly important for discharge calculations, demonstrates considerable variability (Fig. 5, Table 1), even for one type of outlet. Any form of taking average may result in significant errors in determining discharge values. For this reason each section of overflow outlet is described with a separate function $\mu = f(H)$, whose equation was derived experimentally. Practical application of equation (2) for discharge of the weir could therefore be rather difficult.

In engineering applications reading of μ from the nomogram is also one of the options (Fig. 5). Yet another possibility to determine the discharge is to use the computational formula (3) or even directly the calibration table (Table 1).

Unusual, compound weirs must always be considered individually as special cases. Attempts to adapt data available in the literature (calibration curves, formulae, discharge coefficient values etc.) may lead, with considerable probability, to erroneous results. Hence, an optimal approach is to calibrate the structure.

Experimental research (both laboratory and in the field) confirm that the weir on the surrounding ditch of the Turawa reservoir is, despite its untypical overflow outlet, fully operational for control and measurement tasks. Laboratory calibration of the weir provides accurate hydraulic characteristics that have been verified in field measurements. Research has also revealed that the flow over a straight 30 m ($L = 200H_{max}$)

section of the channel above the weir is slow. Real-life flow conditions on the structure are steady, the flow in the upper channel is quasi-uniform (outside the head and depression zones). In the vicinity of the structure there is no moveable rubble that might change water inflow and outflow, nor there are other structures that could negatively affect these conditions.

Research has confirmed that, in general, virtually any type of weir can be calibrated, regardless its profile (broad crown, sharp edge or practical shapes), overflow outlet shape (standard or non-standard geometric figure). It is, however important to ensure hydraulic conditions required for free flowing of water (unsubmerged weirs).

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BADANIA MODELOWE PRZELEWU MIERNICZEGO O PRZEKROJU ZŁOŻONYM DLA ZBIORNIKA WODNEGO W TURAWIE

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Abstrakt: Przedstawiono wyniki badań eksperymentalnych na modelu fizycznym jednego z przelewów mierniczych na rowach opaskowych zbiornika wodnego w Turawie (woj. opolskie). Specyfika badanego obiektu – jego zabytkowy charakter, brak jakiejkolwiek oryginalnej dokumentacji technicznej, a przede wszystkim nietypowy, jak dla przelewów mierniczych, kształt otworu przelewowego, który jest kombinacją kilku figur geometrycznych (trójkąta i prostokątów) – wykluczają możliwość wykorzystania w praktyce dostępnych w literaturze formuł obliczeniowych. Są one właściwe jedynie dla przelewów typowych. Przeprowadzone badania laboratoryjne umożliwiły sporządzenie szczegółowej charakterystyki hydraulicznej obiektu, przedstawionej w postaci tabel, wzorów i wykresów. Pomiary terenowe na obiekcie rzeczywistym pozytywnie zweryfikowały wyniki otrzymane w laboratorium.

Słowa kluczowe: budownictwo wodne, przelewy miernicze, hydrometria

Varia

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- artykułów: w abstraktowanych czasopismach: *Ecological Chemistry and Engineering/Chemia i Inżynieria Ekologiczna* (*Ecol. Chem. Eng.*) ser. A i S oraz niektórych w półroczniku *Chemia – Dydaktyka – Ekologia – Metrologia*.

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

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Prof. dr hab. Maria Wacławek
Przewodnicząca Komitetu Organizacyjnego
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