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CARABIDAE (COLEOPTERA) SELECTED NATURAL ENVIRONMENTS IN PUSZCZA BORECKA

CARABIDAE (COLEOPTERA) WYBRANYCH ŚRODOWISK PRZYRODNICZYCH W PUSZCZY BORECKIEJ

Abstract: The paper presents the results of research on Carabidae of a mixed forest site and an oak-hornbeam forest site carried out between 2004 and 2011 within the Integrated Natural Environment Monitoring Base Station in Puszcza Borecka. Carabidae were captured into glycol-filled Barber pitfall traps between May and September every year in five one-month catch cycles. The aim of the study was to characterise assemblage structures of Carabidae in these two habitats with regard to selected abiotic environmental factors. A total of 13,227 individuals of Carabidae representing 31 species were captured. The total yield in the mixed forest was 8,023 individuals of 25 species, compared to 5,204 individuals of 26 species in the oak-hornbeam forest. Both habitats supported species associated with forest, open and forested areas, and open areas. The species represented all zoogeographical elements found in Poland. However, their proportions differed between the two habitats. There was also a difference in the pattern of activity, with peak activity of the mixed forest assemblage seen in August and in the oak-hornbeam forest, in July. Species diversity, measured with the Shannon-Wiener index (H'), and evenness, measured with the Pielou index (J'), were H' = 1.8981, J' = 0.5897 for the mixed mesic forest assemblage and H' = 2.1328, J' = 0.6546 for the oak-hornbeam forest site. Catch results were analysed with regard to such factors as air temperature, ground temperature, precipitation, relative air humidity, and air levels of SO_2 and NO_2. There was a statistically significant positive correlation between the Carabidae catch yield and air temperature at 2 metres in both habitats: for the mixed forest, r = 0.4, df = 38, p = 0.01; for the oak-hornbeam forest, r = 0.35, df = 38, p = 0.02; and between catch yield and ground temperature at a depth of 0.05 m: for the mixed forest, r = 0.49, df = 38, p = 0.001; for the

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oak-hornbeam forest, r = 0.42, df = 38, p = 0.007. Correlations between catch yield and the remaining factors were less marked and reached statistical significance only in three cases.

Keywords: Carabidae, nature monitoring, atmospheric factors, environmental pollution

Introduction

For several dozen years, base stations of the Integrated Natural Environment Monitoring System have been responsible for the measurement of environmental indices in the area where they are situated. The underlying methodological assumption behind Integrated Natural Environment Monitoring is that the field stations carry out focused stationary research on selected elements of the natural environment [1], including physical, chemical and biological factors. The monitoring of physical and chemical factors in conjunction with biological monitoring may be an important tool for analysing changes in the environment provided it is carried out over the long term and follows a unified methodology to allow for comparison of results obtained within one object as well as between objects [2]. The biological monitoring includes observations of epigeic fauna, including ground beetles (Carabidae, Coleoptera), as this taxon is eligible for monitoring [3–8].

In view of the above, the aim of the present study was to analyse Carabidae assemblage structures and correlations between structural indices (*ie* the number of species and individuals captured) and the following selected abiotic factors: air temperature at 0.05 m and 2 m above ground, ground temperature at a depth of 0.05 m; 0.1 m; 0.2 m; 0.5 m; and 1 m, mean monthly precipitation, mean monthly relative air humidity, and mean monthly air pollutant (SO_2, NO_2) levels. Correlations were verified with the t test and significance was assessed with Pearson's linear correlation coefficient. Assemblage diversity was also assessed with Shannon-Wiener's index of species diversity (H') [9], and assemblage evenness was measured with Pielou's index of evenness (J') [10].

Material and methods

The research was carried out between 2004 and 2011 within the Integrated Natural Environment Monitoring Base Station in Puszcza Borecka (catchment area of Lake Lekuk, located in the mesoregion of Pojezierze Elckie, macroregion of Pojezierze Mazurskie, subprovince of Pojezierze Wschodniobaltyckie – Fig. 1), in Borki Forest Inspectorate, Diabla Gora forest district, Przerwanki surveying section. Samples were collected in two habitat variants: a mixed forest site (variant I) and an oak-hornbeam forest site (variant II). (Fig. 1). The mixed forest grew on rusty pseudogley and rusty brown pseudogley soils. It was composed of hornbeam (30 %), spruce (20 %) and oak (20 %) trees, all aged 117 years, with admixtures of linden (10 %) and birch (10 %) aged 117 years and 24-year-old hornbeam (10 %). The oak-hornbeam forest was composed of spruce (30 %), oak (30 %) and hornbeam (30 %) aged 119 years growing on pseudogley soils, with admixtures of 39-year-old hornbeam trees (10 %). Three permanent study sites were established in both types of forest. Five Barber pitfall traps

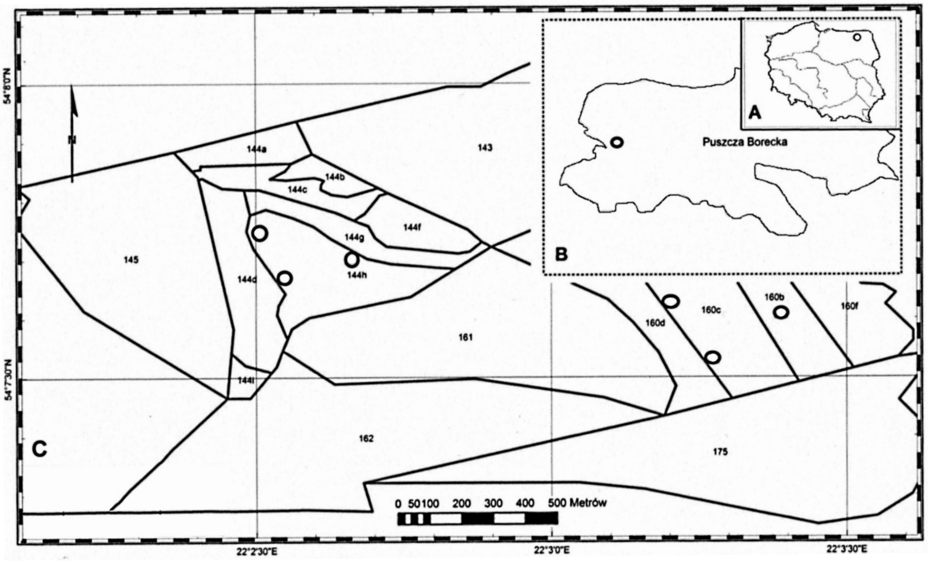

Fig. 1. Location of the study area (A, B) and permanent study sites (C) [□ – divisions, ○ – study sites]

were buried in the ground at 3-metre intervals at each site and filled with ethylene glycol to a third of their height. Carabidae were collected in five monthly catch cycles from May to October.

Abundance was determined on the basis of the catch yield of species and individuals, dominance patterns were represented by a dominance index [11], dominance distribution by Simpson's dominance index [12], constancy was characterised with a constancy index [13], and a fidelity index was used to study fidelity [13]. Carabidae were classified into particular ecological categories on the basis of papers by such authors as Koch [14], Larsson [15], Lindroth [16], Sarova [17, 18], Szyszko [19], and Aleksandrowicz [20].

Correlations were investigated between the following combinations of catch yield (abundance) data and selected abiotic factors: a) catch yield (abundance) of Carabidae individuals in particular months of the growing season in a given year vs. mean monthly values of a given factor (separately for each year in the period 2004–2011); b) overall catch yield in particular months of the growing season in consecutive years of study vs. the value of a given factor in a given month (Table 1); c) catch yield in a given year vs. mean value of a given factor in the growing season; d) catch yield in a given year vs. mean value of a given factor in that year. The analyses were then replicated with regard to species classified as dominants (eudominants, dominants, subdominants) collectively and separately for each species of that group. The t test was used to determine the significance of Pearson's linear correlation coefficient. Correlations were also assessed with canonical correspondence analysis (CCA) [21]. Assemblage diversity was also investigated, using Shannon-Wiener's index of species diversity (H') [9], while assemblage evenness was evaluated with Pielou's index of evenness (J') [10].

Table 1

Correlation between Carabidae abundance and selected abiotic environmental factors in the years 2004–2011

Factor		TP [°C]		TG [°C]					RH	PR	S-SO$_2$	N-NO$_2$
		2 m	0.05 m	0.05 m	0.1 m	0.2 m	0.5 m	1 m	[%]	[mm]	[µg/m^3]	[µg/m^3]
Mixed forest	r	0.403	0.494	0.486	0.486	0.468	0.400	0.265	0.112	0.279	0.004	0.061
	p	0.0099	0.0012	0.0015	0.0015	0.0023	0.0105	0.0980	0.5082	0.0817	0.9783	0.7099
Oak-hornbeam forest	r	0.355	0.356	0.417	0.417	0.391	0.294	0.126	0.018	0.187	–0.012	0.010
	p	0.0247	0.0240	0.0074	0.0074	0.0125	0.0651	0.4403	0.9164	0.2489	0.9395	0.9518

Key to symbols: r – Pearson's linear correlation coefficient, p – level of significance, RH – air humidity, TG – ground temperature, TP – air temperature, PR – precipitation.

Results

The total catch yield during the study was 13.227 individuals of Carabidae representing 31 species (Table 2). In the mixed forest the total abundance was 8.023 individuals representing 25 species (Table 3). The figures varied over time, but displayed a general growing trend (Fig. 2). As regards dominance patterns, *P. niger* accounted for a constantly high proportion of the assemblage.

It was clearly the dominant species and maintained a eudominant rank throughout the study period (Table 7), with annual contributions from 18.01 % in 2004 to 59.51 % in 2009. Another species that was also a eudominant in individual years of the study period was *P. melanarius*, whose contribution to the assemblage ranged from 11.90 % in 2007 to 39.88 % in 2004, falling into the dominant range only once (5.37 % in 2006). The abundance of the remaining 22 species varied considerably, placing them into the recendent, subdominant, dominant or eudominant bracket depending on the particular species and year. The distribution of individuals between species was assessed with Simpson's index. These figures are presented in Table 7 and reveal the greatest evenness for the assemblage of the year 2005. The only species in the highest constancy class of euconstant species was *P. niger* (92.5 %) (Table 9), while three species: *P. melanarius* (72.5 %), *C. hortensis* (61 %) and *P. oblongopunctatus* (53 %), were classified as constant species. The accessory species group comprised *C. granulatus* (48 %), *N. brevicolis* (44.2 %), *A. assimile* (40.8 %), *C. caraboides* (37.5 %), *C. violaceus* (36 %), *C. coriaceus* (32 %) and *C. cancellatus* (25 %). The remaining 14 species (56 % of all species recorded in the mixed forest) were classified as accidental. An analysis of fidelity revealed 16 characteristic species, of which 6 were selective and 10 were preferential. Unique exclusive species (100 %) comprised *Carabus convexus, C. glabratus, A. plebeja, A. ater,* and *A. viduum*, with exclusive species including also *P. nigrita* (80.28 %). Selective characteristic species comprised *C. caraboides* (74.01 %), *P. aethiops* (70 %), *P. melanarius* (69.45 %), *N. brevicolis* (65.41 %), *C. violaceus* (64.11 %), *P. niger* (64.01 %), *C. granulatus* (63.61 %), *C. hortensis* (52.04 %), *C. coriaceus* (50.99 %), *H. latus* (50 %).

Overall peak activity of the carabid assemblage from the mixed forest site during the entire period of study was noted in August (Fig. 3). As regards ecological indices, the dominant habitat preference of the constituent species was for forest habitats (Table 5), while in terms of humidity preferences the dominant type was mesohygrophilous species, with large zoophages dominating with regard to feeding habits and spring breeders being the dominant breeding type.

A variety of zoogeographic elements were noted (Table 6), with the Palaearctic element dominant among the 8 types recorded. Species diversity, measured as Shannon-Wiener's index (H'), and evenness, measured as Pielou's index (J'), were H' = 1.8981 (H' max = 3.2189) and J' = 0.5897, respectively, in the mixed forest assemblage.

The total abundance of ground beetles in the oak-hornbeam site was 5,204 individuals representing 26 species (Table 4). Abundance varied between years of study, but as with the mixed forest habitat, a clear tendency towards increasing catch yield was noted (Fig. 2).

Table 2

Zoogeographic and ecological characteristics of Carabidae and overall species abundance in the entire study period in both habitat variants

No.	Species	Ecological category				Zoogeo-graphic element	Abundance
		Habitat	Feeding habits	Humidity preferences	Breeding type		
1	*Carabus coriaceus* L.	F	Lz	Mh	A	Efp	151
2	*C. violaceus* L.	F	Lz	Mh	S	Pal	170
3	*C. convexus* Fabr.	OFa	Lz	Mh	S	Esb	1
4	*C. granulatus*L.	OFa	Lz	Hg	S	Esb	742
5	*C. cancellatus* Ill.	OFa	Lz	Mh	S	Esb	210
6	*C. arcensis* Herbst	F	Lz	Mh	S	Pal	250
7	*C. nemoralis* O. F. Müller	F	Lz	Mh	S	Efp	155
8	*C. hortensis* L.	F	Lz	Mh	A	Efp	513
9	*C. glabratus* Payk.	F	Lz	Mh	S	Ear	2
10	*Cychrus caraboides* (L.)	F	Lz	Mh	S	Ear	153
11	*Leistus piceus* Frol.	F	Sz	Hg	S	Efpm	11
12	*Nebria brevicollis* (Fabr.)	OFa	Sz	Hg	A	Emd	1064
13	*Patrobus atrorufus* (Stroem)	F	Sz	Hg	S	Esb	58
14	*Amara plebeja* (Gyll.)	Oa	Hz	Hg	S	Pal	2
15	*Pterostichus oblongopunctatus* (Fabr.)	F	Sz	Mh	S	Pal	674
16	*P. niger* (Schall.)	F	Lz	Mh	S	Esb	5460
17	*P. melanarius* (L.)	OFa	Lz	Mh	A	Esb	2239
18	*P. nigrita* (Fabr.)	OFa	Sz	Hg	S	Pal	142
19	*P. strenuus* (Panz.)	F	Sz	Mh	S	Pal	4
20	*P. aethiops* (Panz.)	F	Sz	Mh	S	Eca	10
21	*Abax carinatus* (Duft.)	F	Sz	Mh	S	Efpm	8
22	*A. ater* (Pill. et Mitt.)	F	Sz	Mh	A	Efp	1
23	*Calathus micropterus* (Duft.)	F	Sz	Mh	A	Pal	3
24	*Agonum muelleri* (Herbst)	OFa	Sz	Mh	S	Hol	57
25	*A. viduum (*Panz.)	Oa	Sz	Hg	S	Esb	1
26	*A. assimile* (Payk.)	F	Sz	Hg	S	Pal	1084
27	*A. obscurum* (Herbst)	F	Sz	Hg	S	Hol	1
28	*A. gracile* Sturm	Oa	Sz	Hg	S	Pal	3
29	*Harpalus rufipes* (De Geer)	Oa	Hz	Mh	A	Pal	1
30	*H. latus* (L.)	F	Hz	Mh	S	Pal	56
31	*H. guadripunctatus* Dej.	F	Hz	Mh	S	Pal	1
	Total						13227

Key: F – Forest (species), Oa – open-area, OFa – open and forested area; Lz – Large zoophage, Sz – Small zoophage, Hz – Hemizoophage; Hg – Hygrophilous, Mh – Mesohygrophilous; S – Spring breeder, A – Autumn breeder; Hol – Holarctic; Pal – Palaearctic, Esb – Euro-Siberian, Eca – Euro-Central Asian, Ear – Euro-Arctic, Emd – Euro-Mediterranean, Efp – European Forest Province, Efpm – European Forest Province (montane).

Table 3

Abundance of Carabidae species collected in mixed forest by year of study

No.	Species	Number of individuals								
		Mixed forest (Variant I)								
		2004	2005	2006	2007	2008	2009	2010	2011	Total
1	*C. coriaceus* L.	3	5		24	15	11	7	12	77
2	*C. violaceus* L.		4	9	32	19	18	24	3	109
3	*C. convexus* Fabr.					1				1
4	*C. granulatus* L.	139	115	57	85	54	22			472
5	*C. cancellatus* Ill.	16	22	2	1		5	38	2	86
6	*C. arcensis* Herbst							25	87	112
7	*C. nemoralis* O. F. Müller	15	11	18	14	6	1	3	7	75
8	*C. hortensis* L.	14	25	66	54	30	30	29	19	267
9	*C. glabratus* Payk.								2	2
10	*Cychrus caraboides* (L.)	11	6	7	35	18	12	11	14	114
11	*Leistus piceus* Frol.					3		1		4
12	*Nebria brevicollis* (Fabr.)	20	55		68	238	101	90	124	696
13	*Patrobus atrorufus* (Stroem)				5	5	6	2	4	22
14	*Amara plebeja* (Gyll.)						2			2
15	*Pt. oblongopunctatus* (Fabr.)	96	67	68	31	14	18	22	20	336
16	*P. niger* (Schall.)	154	220	208	640	439	741	712	381	3495
17	*P. melanarius* (L.)	341	87	272	141	75	161	226	252	1555
18	*P. nigrita* (Fabr.)	3				9	102			114
19	*P. aethiops* (Panz.)	7								7
20	*A. ater* (Pill. et Mitt.)			1						1
21	*Calathus micropterus* (Duft.)	1								1
22	*Agonum muelleri* (Herbst)						2	1		3
23	*A. viduum* (Panz.)						1			1
24	*A. assimile* (Payk.)	35	49	41	54	4	11	167	82	443
25	*H. latus* (L.)		1	25		1	1			28
	Total	855	667	774	1184	931	1245	1358	1009	8023
	Number of species collected	14	13	12	13	16	18	15	14	25

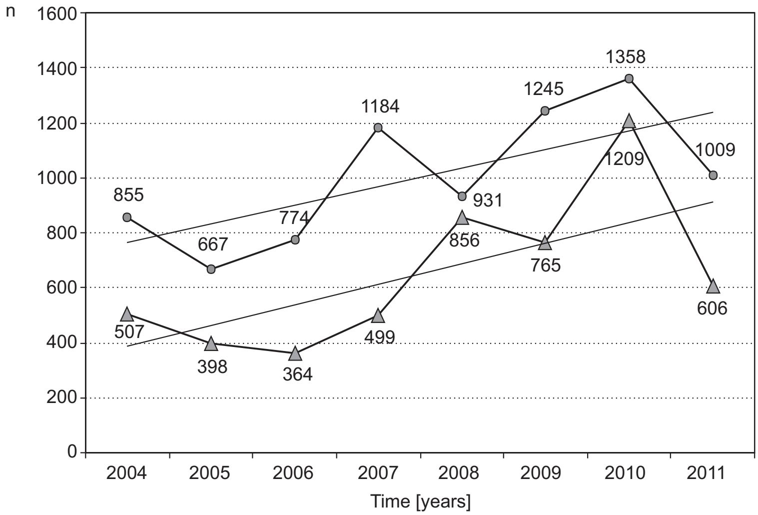

Fig. 2. Carabid abundance in oak-hornbeam forest and mixed forest by year of study (● – mixed forest, ▲ – oak-hornbeam forest)

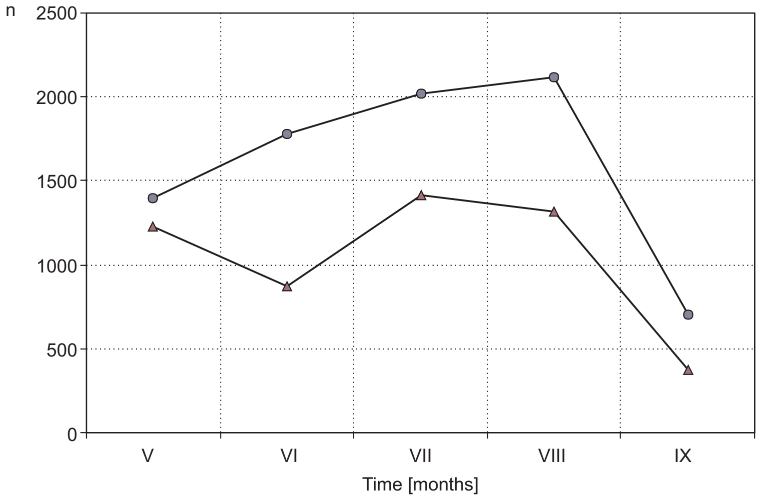

Fig. 3. Activity of Carabid assemblages in oak-hornbeam forest and mixed forest in years 2004–2011 (from May (V) to September (IX), ● – mixed forest, ▲ – oak-hornbeam forest)

Also in this variant, *P. niger* was a eudominant throughout the study period, with contributions to assemblage abundance from 11.39 % in 2005 to 55.42 % in 2009. *P. melanarius* was a eudominant in almost every year of the study period (except 2008 and 2010), as was *A. assimile* (except 2005 and 2009, when it was a subdominant). *N. brevicollis* and *C. granulatus* also had constantly high contributions to overall abundance. The dominance patterns of the remaining 21 species demonstrated con-

Table 4

Abundance of Carabidae species collected in the oak-hornbeam forest by year of study

No.	Species	Number of individuals								
		Oak-hornbeam forest (Variant II)								
		2004	2005	2006	2007	2008	2009	2010	2011	Total
1	*Carabus coriaceus* L.	7	11	10	12	15	13	1	5	74
2	*C. violaceus* L.	2		8	20	14	8	6	3	61
3	*C. granulatus*L.	32	88	40	40	43	27			270
4	*C. cancellatus* Ill.	12	13	3			4	81	11	124
5	*C. arcensis* Herbst							37	101	138
6	*C. nemoralis* O. F. Müller	26	8	16	11	5	4	4	6	80
7	*C. hortensis* L.	17	4	17	27	53	47	53	28	246
8	*Cychrus caraboides* (L.)	5	1	7	9	10	4		3	39
9	*Leistus piceus* Frol.	1				1		5		7
10	*Nebria brevicollis* (Fabr.)	6	11		8	201	64	46	32	368
11	*Patrobus atrorufus* (Stroem)	15			4	1	8	2	6	36
12	*Pt. oblongopunctatus* (Fabr.)	76	56	68	31	16	13	41	37	338
13	*P. niger* (Schall.)	140	45	88	203	388	424	474	203	1965
14	*P. melanarius* (L.)	75	130	53	67	46	108	104	101	684
15	*P. nigrita* (Fabr.)	27				1				28
16	*P. strenuus* (Panz.)		1				3			4
17	*P. aethiops* (Panz.)	2	1							3
18	*Abax carinatus* (Duft.)		8							8
19	*Calathus micropterus* (Duft.)	2								2
20	*Agonum muelleri* (Herbst)	20			13	16		5		54
21	*A. assimile* (Payk.)	37	19	40	49	44	37	346	69	641
22	*A. obscurum* (Herbst)	1								1
23	*A. gracile* Sturm	3								3
24	*Harpalus rufipes* (De Geer)					1				1
25	*H. latus* (L.)		2	14	5	1	1	4	1	28
26	*H. guadripunctatus* Dej.	1								1
	Total	507	398	364	499	856	765	1209	606	5204
	Number of species collected	21	15	12	14	17	15	15	14	26

siderable variability between species and between years. The distribution of individuals between species was evaluated with Simpson's index. These data are presented in Table 8 and show that the most uniform distribution of species was noted in 2010 and also in 2004 and 2006.

P. niger (79.2 %) was the sole member of the group of the most constant species (euconstant), while *P. melanarius* (61.2 %) was classified as a constant species. Accessory species were represented by *N. brevicolis* (42.5 %), *P. oblongopunctatus* (42.5 %), *C. hortensis* (40.8 %), *C. granulatus* (38.3 %), *A. assimile* (36.7 %), *C. nemoralis* (33.3 %), *C. cancellatus* (29.1 %), and *C. coriaceus* (28.3 %) (Table 9). The remaining 16 species (61 % of all species captured in the oak-hornbeam habitat) were classified as accidental. A fidelity analysis revealed 16 characteristic species, including 7 exclusive and 9 preferential species. Unique exclusive (100 %) species comprised *P. strenuus, A. carinatus, A. obscurum, A. gracile, H. rufipes*, and *H. guadripunctatus*. *A. müelleri* (94.73 %) was classified as exclusive, while the group of preferential characteristic species comprised *C. micropterus* (66.66 %), *L. piceus* (63.63 %), *P. atrorufus* (62.06 %), *A. assimile* (59.13 %), *C. cancellatus* (59.04 %), *C. arcensis* (55.2 %), *C. nemoralis* (51.61 %), *P. oblongopunctatus* (50.14 %), and *H. latus* (50 %).

The highest activity of this assemblage was noted in July (Fig. 3). The dominant ecological elements were the same as in the mixed forest assemblage, with the exception of ranking by feeding habits, as it was small zoophages which were the dominant element in this dimension in qualitative analysis (Table 5), accounting for 50 % of the assemblage, while the proportion of large zoophages was 38.5 %.

Table 5

Ecological characteristics of Carabid assemblages

Ecological category	Ecological element	Variant I (Mixed forest)				Variant II (Oak-hornbeam forest)			
		M	[%]	N	[%]	M	[%]	N	[%]
Habitat	F	16	64.0	5093	63.480	18	69.231	3672	70.561
	OFa	7	28.0	2927	36.483	6	23.077	1528	29.362
	Oa	2	8.0	3	0.037	2	7.692	4	0.077
Feeding habits	Lz	12	48.0	6365	79.334	10	38.462	3681	70.734
	Sz	11	44.0	1628	20.292	13	50.000	1523	29.266
	Hz	2	8.0	30	0.374	3	11.538	30	0.576
Humidity requirements	Hg	8	32.0	1754	21.862	8	30.769	1354	26.018
	Mh	17	68.0	6269	78.138	18	69.231	3850	73.982
Breeding type	S	19	76.0	5426	67.631	20	76.923	3829	73.578
	A	6	24.0	2597	32.369	6	23.077	1375	26.422

Key: F – Forest (species), Oa – open-area, OFa – open and forested area; Lz – Large zoophage, Sz – Small zoophage, Hz – Hemizoophage; Hg – Hygrophilous, Mh – Mesohygrophilous; S – Spring breeder, A – Autumn breeder; M – number of species, N – number of individuals, % – percentage of assemblage.

Zoogeographic variety in the oak-hornbeam site assemblage was at the same level (8 elements) and the dominant element was also Palaearctic (Table 6). The indices of species diversity and evenness amounted to H' = 2.1328 (H' max = 3.2581) and J' = 0.6546, respectively.

Table 6

Contribution of zoogeographic elements to Carabidae abundance

Zoogeographic element	Variant I (Mixed forest)				Variant II (Oak-hornbeam forest)			
	M	[%]	N	[%]	M	[%]	N	[%]
Holarctic	1	4.0	3	0.037	2	7.692	55	1.057
Palaearctic	8	32.0	1145	14.271	11	42.308	1245	23.924
Euro-Siberian	7	28.0	5632	70.198	5	19.231	3079	59.166
Euro-Arctic	2	8.0	116	1.446	1	3.846	39	0.749
Euro-Central Asian	1	4.0	7	0.087	1	3.846	3	0.058
Euro-Mediterranean	1	4.0	696	8.675	1	3.846	368	7.071
European Forest Province	4	16.0	420	5.235	3	11.538	400	7.686
Euro. Forest Province (montane)	1	4.0	4	0.050	2	7.692	15	0.288

Key: M – number of species, N – number of individuals, % – percentage of assemblage.

There was a statistically significant positive correlation between the number of carabids captured in particular months during the growing season and mean monthly air temperature at 2 m in both habitat variants: for the mixed forest (r = 0.4, df = 38, p = 0.01) and for the oak-hornbeam forest (r = 0.35, df = 38, p = 0.02); and also between the number of carabids captured in particular months during the growing season and mean monthly ground temperature at a depth of 0.05 m: for the mixed forest (r = 0.49, df = 38, p = 0.001) and for the oak-hornbeam forest (r = 0.42, df = 38, p = 0.007). Data from different years of study show that in 2010 there was a strong positive correlation between carabid abundance in the mixed forest and mean monthly values of the following indices: air temperature at 2 m (r = 0.95, df = 3, p = 0.012) and at 0.5 m (r = 0.95, df = 3, p = 0.013), and ground temperature at a depth of 0.05 m (r = 0.96, df = 3, p = 0.008), 0.1 m (r = 0.96, df = 3, p = 0.011) and 0.2 m (r = 0.95, df = 3, p = 0.015).

With regard to SO_2 and NO_2 levels in the air, a significant negative correlation was noted between carabid abundance and mean monthly SO_2 levels in the air in the mixed forest in 2004 (r = –0.89, df = 3, p = 0.043), while a positive correlation with regard to this index was noted in 2008 (r = 0.95, df = 3, p = 0.013). Of note is also a positive and significant correlation between carabid abundance and mean monthly NO_2 levels in the air in the mixed forest in 2006 (r = 0.95, df = 3, p = 0.015).

An analysis of correlations between the capture rates of dominant species and the abiotic factors revealed that changes in the abundance of *Pterostichus niger* in both

Table 7

Dominance patterns in Carabidae assemblages in mixed forest by year of study

No.	Species	Dominance [%]							
		Mixed forest							
		2004	2005	2006	2007	2008	2009	2010	2011
1	*Carabus coriaceus*	0.35	0.75		2.03	1.61	0.88	0.52	1.19
2	*C. violaceus*		0.60	1.16	2.70	2.04	1.45	1.77	0.30
3	*C. convexus*					0.11			
4	*C. granulatus*	16.26	17.24	7.36	7.18	5.80	1.77		
5	*C. cancellatus*	1.87	3.30	0.26	0.08		0.40	2.80	0.20
6	*C. arcensis*							1.84	8.62
7	*C. nemoralis*	1.75	1.65	2.33	1.18	0.64	0.08	0.22	0.69
8	*C. hortensis*	1.64	3.75	8.53	4.56	3.22	2.41	2.14	1.88
9	*C. glabratus*								0.20
10	*Cychrus caraboides*	1.29	0.90	0.90	2.96	1.93	0.96	0.81	1.39
11	*Leistus piceus*					0.32		0.07	
12	*Nebria brevicollis*	2.34	8.25		5.74	25.56	8.11	6.63	12.29
13	*Patrobus atrorufus*				0.42	0.54	0.48	0.15	0.40
14	*Amara plebeja*						0.16		
15	*Pt. oblongopunctatus*	11.23	10.04	8.79	2.62	1.50	1.45	1.62	1.98
16	*P. niger*	18.01	32.98	26.87	54.05	47.15	59.52	52.43	37.76
17	*P. melanarius*	39.88	13.04	35.14	11.91	8.06	12.93	16.64	24.98
18	*P. nigrita*	0.35				0.97	8.19		
20	*P. aethiops*	0.82							
22	*A. ater*			0.13					
23	*Calathus micropterus*	0.12							
24	*Agonum muelleri*						0.16	0.07	
25	*A. viduum*						0.08		
26	*A. assimile*	4.09	7.35	5.30	4.56	0.43	0.88	12.30	8.13
30	*H. latus*		0.15	3.23		0.11	0.08		
	λ*	0.23	0.18	0.23	0.32	0.30	0.39	0.32	0.23

* Simpson's dominance index.

Table 8

Dominance patterns in Carabidae assemblages in oak-hornbeam forest by year of study

No.	Species	Dominance [%]							
		Oak-hornbeam forest							
		2004	2005	2006	2007	2008	2009	2010	2011
1	*Carabus coriaceus*	1.38	2.76	2.75	2.40	1.75	1.70	0.08	0.83
2	*C. violaceus*	0.39		2.20	4.01	1.64	1.05	0.50	0.50
3	*C. granulatus*	6.31	22.11	10.99	8.02	5.02	3.53		
4	*C. cancellatus*	2.37	3.27	0.82			0.52	6.70	1.82
5	*C. arcensis*							3.06	16.67
6	*C. nemoralis*	5.13	2.01	4.40	2.20	0.58	0.52	0.33	0.99
7	*C. hortensis*	3.35	1.01	4.67	5.41	6.19	6.14	4.38	4.62
8	*Cychrus caraboides*	0.99	0.25	1.92	1.80	1.17	0.52		0.50
9	*Leistus piceus*	0.20				0.12		0.41	
10	*Nebria brevicollis*	1.18	2.76		1.60	23.48	8.37	3.80	5.28
11	*Patrobus atrorufus*	2.96			0.80	0.12	1.05	0.17	0.99
12	*Pt. oblongopunctatus*	14.99	14.07	18.68	6.21	1.87	1.70	3.39	6.11
13	*P. niger*	27.61	11.31	24.18	40.68	45.33	55.42	39.21	33.50
14	*P. melanarius*	14.79	32.66	14.56	13.43	5.37	14.12	8.60	16.67
15	*P. nigrita*	5.33				0.12			
16	*P. strenuus*		0.25				0.39		
17	*P. aethiops*	0.39	0.25						
18	*Abax carinatus*		2.01						
19	*Calathus micropterus*	0.39							
20	*Agonum muelleri*	3.94			2.61	1.87		0.41	
21	*A. assimile*	7.30	4.77	10.99	9.82	5.14	4.84	28.62	11.39
22	*A. obscurum*	0.20							
23	*A. gracile*	0.59							
24	*Harpalus rufipes*					0.12			
25	*H. latus*		0.50	3.85	1.00	0.12	0.13	0.33	0.17
26	*H. guadripunctatus*	0.20							
	λ*	0.14	0.19	0.14	0.21	0.27	0.34	< 0.01	0.19

* Simpson's index of dominance.

Table 9

Fidelity and constancy of occurrence of Carabidae species in the two habitat variants

No.	Species	Fidelity [%]		Constancy [%]	
		Mixed forest	Oak-hornbeam forest	Mixed forest	Oak-hornbeam forest
1	*Carabus coriaceus*	50.99	49.01	32.21	28.32
2	*C. violaceus*	64.12	35.88	36.72	21.63
3	*C. convexus*	100.00		0.81	
4	*C. granulatus*	63.61	36.39	48.11	38.31
5	*C. cancellatus*	40.95	59.05	25.32	29.13
6	*C. arcensis*	44.80	55.20	11.06	15.82
7	*C. nemoralis*	48.39	51.61	27.03	33.33
8	*C. hortensis*	52.05	47.95	61.26	40.83
9	*C. glabratus*	100.00		1.73	
10	*Cychrus caraboides*	74.51	25.49	37.52	19.26
11	*Leistus piceus*	36.36	63.64	2.51	2.54
12	*Nebria brevicollis*	65.41	34.59	44.24	42.54
13	*Patrobus atrorufus*	37.93	62.07	11.72	7.53
14	*Amara plebeja*	100.00		0.81	
15	*P. oblongopunctatus*	49.85	50.15	53.23	42.52
16	*P. niger*	64.01	35.99	92.58	79.28
17	*P. melanarius*	69.45	30.55	72.51	61.26
18	*P. nigrita*	80.28	19.72	4.23	5.84
19	*P. strenuus*		100.00		1.62
20	*P. aethiops*	70.00	30.00	4.23	2.53
21	*Abax carinatus*		100.00		1.62
22	*A. ater*	100.00		0.81	
23	*Calathus micropterus*	33.33	66.67	0.81	1.62
24	*Agonum muelleri*	5.26	94.74	2.51	7.53
25	*A. viduum*	100.00		0.81	
26	*A. assimile*	40.87	59.13	40.83	36.72
27	*A. obscurum*		100.00		0.81
28	*A. gracile*		100.00		1.62
29	*Harpalus rufipes*		100.00		0.81
30	*H. latus*	50.00	50.00	6.66	11.73
31	*H. guadripunctatus*		100.00		0.81

habitat variants correlated significantly with changes in mean monthly air temperatures at the height of 2 m in the mixed forest (r = 0.585, df = 38, p = 0.0001) and in the oak-hornbeam forest (r = 0.622, df = 38, p = 0.0001), and at the height of 0.05 m in the mixed forest (r = 0.669, df = 38, p = 0.0001) and in the oak-hornbeam forest (r = 0.634, df = 38, p = 0.0001). There was also a correlation with changes in mean monthly

Table 10

Trophic structure of Carabidae assemblages in the years 2004–2007

T	2004				2005				2006				2007			
	M	[%]	N	[%]	M	[%]	N	[%]	M	[%]	N	[%]	M	[%]	N	[%]
Mixed forest																
Lz	8	0.57	693	0.81	9	0.69	495	0.73	8	0.67	639	0.83	9	0.69	1026	0.87
Sz	6	0.43	162	0.19	3	0.23	171	0.26	3	0.25	110	0.14	4	0.31	158	0.13
Hg					1	0.08	1	0.01	1	0.08	25	0.03				
Oak-hornbeam forest																
Lz	9	0.43	316	0.62	8	0.53	300	0.75	9	0.75	242	0.66	8	0.57	389	0.78
Sz	11	0.52	190	0.37	6	0.40	96	0.24	2	0.17	108	0.30	5	0.36	105	0.21
Hg	1	0.05	1	0.01	1	0.07	2	0.01	1	0.08	14	0.04	1	0.07	5	0.01

Key: T – feeding habits, Lz – large zoophage, Sz – small zoophage, Hg – hemizoophage, M – number of species, N – number of individuals, % – percentage of assemblage.

Table 11

Trophic structure of Carabidae assemblages in the years 2008–2011

T	2008				2009				2010				2011			
	M	[%]	N	[%]	M	[%]	N	[%]	M	[%]	N	[%]	M	[%]	N	[%]
Mixed forest																
Lz	9	0.56	657	0.71	9	0.50	1001	0.80	9	0.60	1075	0.79	10	0.71	779	0.77
Sz	6	0.38	273	0.28	7	0.39	241	0.19	6	0.40	283	0.21	4	0.29	230	0.23
Hg	1	0.06	1	0.01	2	0.11	3	0.01								
Oak-hornbeam forest																
Lz	8	0.47	574	0.67	9	0.60	639	0.84	8	0.53	760	0.63	10	0.71	493	0.81
Sz	7	0.41	280	0.32	5	0.33	125	0.15	6	0.40	445	0.36	3	0.21	112	0.18
Hg	2	0.12	2	0.01	1	0.07	1	0.01	1	0.07	4	0.01	1	0.07	1	0.01

Key: T – feeding habits, Zd – large zoophage, Zm – small zoophage, Hg – hemizoophage, M – number of species, N – number of individuals, % – percentage of assemblage.

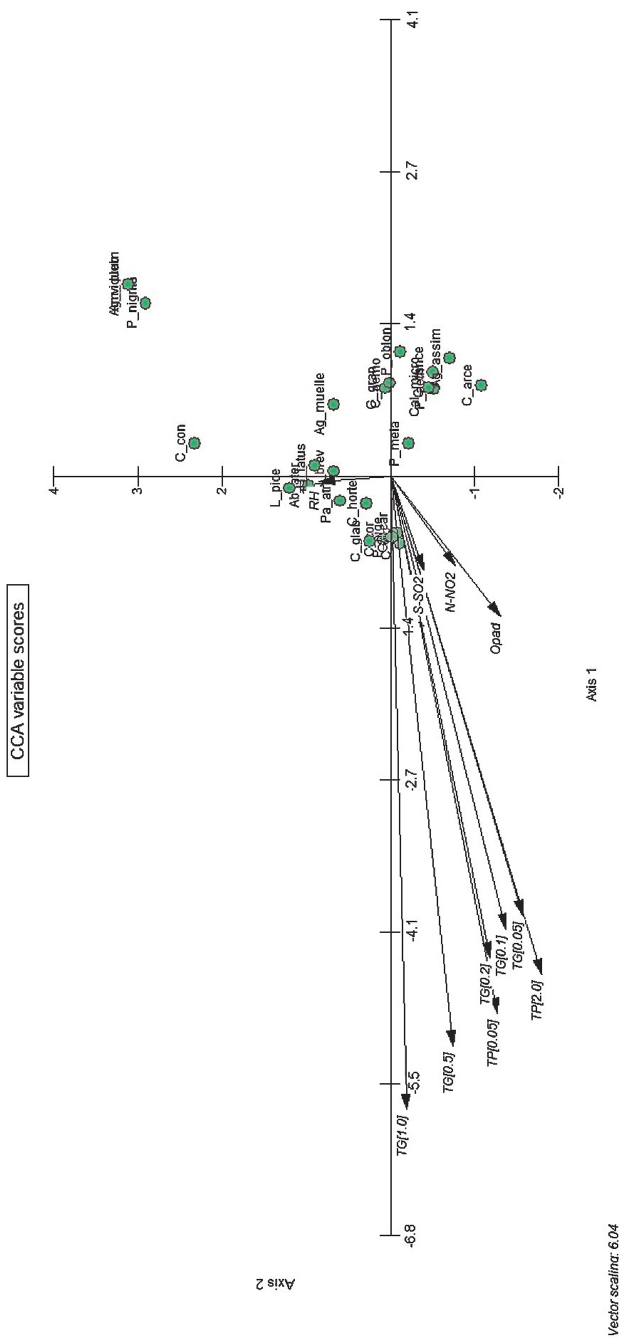

Fig. 4. Canonical correspondence analysis (CCA) diagram for Carabidae assemblages from the mixed forest

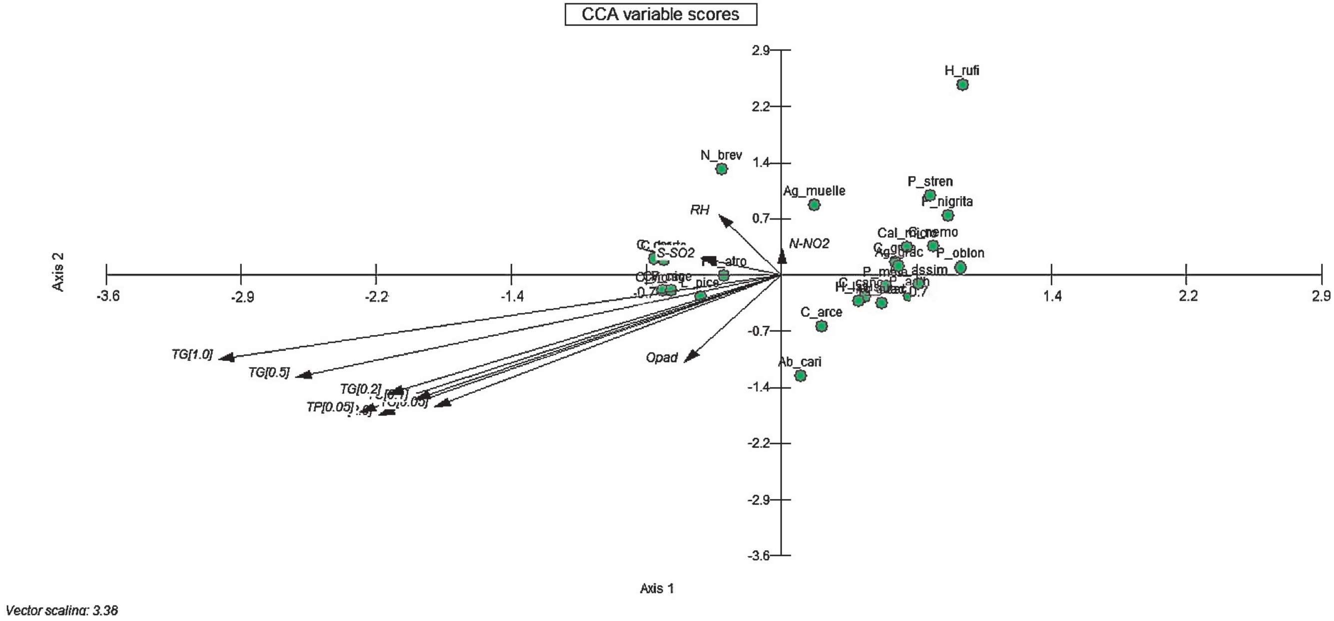

Fig. 5. Canonical correspondence analysis (CCA) diagram for Carabidae assemblages from the oak-hornbeam forest

temperatures of the ground at various depths: at 0.05 m (mixed forest: r = 0.579, df = 38, p = 0.0001; oak-hornbeam forest: r = 0.611, df = 38, p = 0.0001), 0.1 m (mixed forest: r = 0.584, df = 38, p = 0.0001; oak-hornbeam forest: r = 0.621, df = 38, p = 0.0001), 0.2 m (mixed forest: r = 0.601, df = 38, p = 0.0001; oak-hornbeam forest: r = 0.636, df = 38, p = 0.0001), 0.5 m (mixed forest: r = 0.631, df = 38, p = 0.0001; oak-hornbeam forest: r = 0.661, df = 38, p = 0.0001), 1 m (mixed forest: r = 0.593, df = 38, p = 0.0001; oak-hornbeam forest: r = 0.609, df = 38, p = 0.0001). *Carabus arcensis* also merits a note as the only dominant species to demonstrate a positive and significant correlation between its abundance and mean monthly precipitation in the oak-hornbeam forest in the study period 2004–2011 (r = 0.329, df = 38, p = 0.038).

Canonical correspondence analysis (CCA), whose results reflect correlations between the presence of a particular species and environmental factors [21], revealed, for both forest habitat variants studied, that the greatest effect on the Carabidae assemblages was exerted by ground temperature (Fig. 4, Fig. 5).

Discussion

The 8 years of research yielded quite abundant Carabidae material representing a variety of species, ecological categories and zoogeographic elements. The trapability index (number of Carabidae individuals captured daily into one trap) reached medium values similar to those reported from other studies [22, 23].

The Carabidae assemblage inhabiting the oak-hornbeam forest site demonstrated greater variety than its counterpart from the mixed forest site. The index of similarity for the two assemblages was 0.645. The assemblages differed distinctly with regard to peak activity, with the mixed forest assemblage displaying one peak in August and the oak-hornbeam forest assemblage showing two peaks of activity: a minor one in May and a major one in July. Ecological analysis showed a predominance of the same ecological elements in both assemblages with regard to the various categories, except for feeding habits. Representatives of 8 zoogeographic elements were found, *ie* all elements listed by Leśniak [24, 25].

Correlations were also studied between abundance and selected abiotic factors. With regard to temperature, which ranks among the most important ecological factors, it was revealed that higher catch yields correlated positively and significantly with temperature in both habitat variants throughout the study period. Szujecki [26] points out that every insect species has an optimal temperature range where its basic life functions are at their best and most efficient. A The existence of a strong correlation between the activity of epigeic Carabidae and changes in soil temperature has been indicated by [27], Saska and Honek [28] and Tuf et al [29]. Williams and Gormally [30], in a study involving continuous monitoring of ground temperature, found a positive correlation between the abundance of *Carabus clatratus* and ground temperature (with higher temperatures being associated with increased abundance of that species). In a 4-year study, Olszewski [31] found a highly positive correlation between Carabidae abundance and air temperature. At the same time, Gutowski [32] failed to observe significant correlations between Carabidae catch counts and meteorological indices including mean air temperature during the growing season in Puszcza Białowieska in the years 1988–1999. The same authors indicate (Olszewski [31], Gutowski [32]) the absence of a correlation between the amount and distribution of precipitation in a given year and the year before vs. the number of Carabidae captured into Barber's traps. Still another study failed to show an association between Carabidae catch yield and precipitation [23, 33]. Besides temperature, humidity is also a very important abiotic factor that plays a major role in the development of soil-dwelling insects and influences these animals in a complex and multifaceted manner [34]. A positive correlation between soil humidity and the activity of selected species of Carabidae was shown by Nčve [35], with increased soil humidity causing an increase in the abundance of species preferring such habitat conditions. The present study did not analyse the correlation between soil humidity and abundance of Carabidae since soil humidity data were not available. However, we studied the link between relative air humidity and Carabidae catch yield, finding no significant correlations between these factors. This finding is partially attributable to the fact that, while air humidity is an important factor in insect life [26], the association between this factor and abundance is more difficult to grasp.

As regards SO_2 and NO_2 content in the air, only three instances of significant correlations between these factors and Carabidae catch yield were identified. These findings appear quite explicable in view of the fact that daily air concentrations of SO_2 and NO_2 did not exceed levels considered harmless to human health [36]. It might be speculated that air levels of sulphur dioxide and nitrogen dioxide that pose no harm to humans are also not harmful to plants or animals, which may explain the lack of significant correlations between these factors and catch yield for most analytical pairs. Other studies have revealed a strong negative correlation between Carabidae abundance and SO_2 and $NO_{2,}$ concentrations, which were, however, determined in the soil (ground beetle abundance fell with increasing levels of these compounds in the soil) [31, 37–40].

Faunal monitoring within the system of INEM Base Stations aims also to assess the condition of the environment by evaluating a particular taxon or a group of taxa. In the case of Carabidae, indices serving to evaluate environmental health include abundance, dominance patterns, contributions of particular zoogeographic elements to an assemblage, trophic structure [3–8], geographical ranges of particular species [41] or mean individual biomass [42]. The monitoring of Carabidae by the INEM Base Stations involves such indices as the number of individuals and species, trapability, dominance, trophic structure, diversity and evenness. Of these, dominance patterns (Tables 7 and 8) and trophic structure (Tables 10 and 11) were found to be most useful for environmental monitoring. It is believed that good environmental conditions are paralleled by balanced dominance relations, with no species showing excessive quantitative advantage over the others. As regards the feeding habits of species in a community, it is believed that good environmental health is accompanied by a predominance of zoophagous species, especially large zoophages, while progressive destruction of a forest ecosystem shifts this index in favour of hemizoophages [7]. Our data (Tables 7 and 8 and Tables 10 and 11) allow the conclusion that the pressure of negative factors in the study habitats may not be high, as indicated by low values of Simpson's index and a predominance of zoophages in the two assemblages.

Conclusions

Studies of Carabidae assemblages conducted by INEM Base Stations are of considerable value owing to the continuity of data and the determination of abiotic factors at the site of collection of Carabidae, which affords unique possibilities for studying the correlation between a given factor and the catch yield. There are not many opportunities for carrying out such studies outside the base station network.

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CARABIDAE (COLEOPTERA) WYBRANYCH ŚRODOWISK PRZYRODNICZYCH W PUSZCZY BORECKIEJ

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Abstrakt: W pracy przedstawiono wyniki badań nad Carabidae lasu mieszanego oraz grądu prowadzonych w latach 2004–2011 w obrębie Stacji Bazowej ZMŚP w Puszczy Boreckiej. Celem pracy była charakterystyka struktur zgrupowań Carabidae funkcjonujących w wymienionych środowiskach na tle wybranych abiotycznych czynników środowiska. Podczas badań odłowiono łącznie 13227 osobników Carabidae należących do 31 gatunków. W lesie mieszanym odłowiono łącznie 8023 osobniki należące do 25 gatunków, w grądzie 5204 osobniki należące do 26 gatunków. Różnorodność gatunkowa mierzona wskaźnikiem Shannona-Wienera (H') oraz równomierność mierzona wskaźnikiem Pielou (J') wyniosły w przypadku lasu mieszanego H' = 1,8981; J' = 0,5897 a dla grądu odpowiednio H' = 2,1328; J' = 0,6546. Wyniki odłowów analizowano na tle takich czynników, jak temperatura powietrza, gruntu, opady atmosferyczne, wilgotność względna powietrza, zawartość w powietrzu SO_2, NO_2. Stwierdzono istotną statystycznie, dodatnią korelację między liczebnością odłowów Carabidae a temperaturą powietrza na wysokości 2 m w obydwu badanych środowiskach: las mieszany – r = 0,4, df = 38, p = 0,01, grąd – r = 0,35, df = 38, p = 0,02; jak również między liczebnością odłowów biegaczowatych a temperaturą gruntu na głębokości 0,05 m, odpowiednio: dla lasu mieszanego – r = 0,49, df = 38, p = 0,001 oraz grądu – r = 0,42, df = 38, p = 0,007. Zależności między liczebnością odłowów, a wartością pozostałych czynników były słabo wyrażone, tylko w trzech przypadkach istotne statystycznie.

Słowa kluczowe: Carabidae, monitoring przyrodniczy, temperatury, opady, SO_2, NO_2 w powietrzu

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HEAVY METAL CONCENTRATIONS IN WATER AND BOTTOM SEDIMENTS OF SMALL WATER RESERVOIR IN KANIOW

KONCENTRACJA METALI CIĘŻKICH W WODZIE I OSADACH DENNYCH MAŁEGO ZBIORNIKA WODNEGO W KANIOWIE

Abstract: The paper presents the results of investigations on concentrations of ions of selected heavy metals (Fe, Cr, Zn, Ni, Mn, Cu, Pb, Cd, and Hg) in sediments and water in a small dammed reservoir located in Kaniow (the Swietokrzyskie Province, Poland) on the stream flowing from Borowa Gora (Borowa Mount). The sediments and water were collected from the upper, middle, and lower part of the reservoir. The samples were collected twice, first in July 2012, and then in March 2013. The content of trace elements in water did not exceed: 801 $\mu g \cdot dm^{-3}$ (Fe), 0.064 $\mu g \cdot dm^{-3}$ (Cr), 13.5 $\mu g \cdot dm^{-3}$ (Zn), 0.106 $\mu g \cdot dm^{-3}$ (Ni), 285 $\mu g \cdot dm^{-3}$ (Mn), 2.35 $\mu g \cdot dm^{-3}$ (Cu), 6.98 $\mu g \cdot dm^{-3}$ (Pb), and 3.35 $\mu g \cdot dm^{-3}$ (Cd). As regards bottom sediments, the maximum recorded concentrations of heavy metals were: 872 $mg \cdot kg^{-1}$ (Fe), 15.19 $mg \cdot kg^{-1}$ (Cr), 26.26 $mg \cdot kg^{-1}$ (Zn), 16.26 $mg \cdot kg^{-1}$ (Ni), 750 $mg \cdot kg^{-1}$ (Mn), 3.58 $mg \cdot kg^{-1}$ (Cu), 82.56 $mg \cdot kg^{-1}$ (Pb), 0.73 $mg \cdot kg^{-1}$ (Cd), and 0.0031 $mg \cdot kg^{-1}$ (Hg). An attempt to determine the mathematical relationship between the content of organic matter in sediments and heavy metals was made and the possibility of agricultural utilization of sediments after removal from the bottom was determined.

Keywords: heavy metals, bottom sediments, reservoir

Water reservoirs are the sites where intense deposition of suspensions transported by watercourses is observed. Sediments accumulated within the bowls of reservoirs contribute to the reduction of their capacity, and thus they limit the function those objects perform. Small water reservoirs are particularly affected by intensive silting [1, 2], as a result after a few years, or dozens of years of operation, they have to be

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subjected to dredging. Every time the reservoir is desilted, the bottom sediments need to be properly managed, which depends on their physical and chemical properties [3].

The chemical composition of bottom sediments results from many factors, both natural and anthropogenic ones. It primarily depends on the basin geological structure, the basin management and use, and also on climatic and hydrological conditions. Those are decisive for the pattern and intensity of the erosion processes in the basin, for the migration and accumulation of suspensions, and also of trace elements [4–7]. In non-industrialized basins, shallow deposits of minerals may provide a source of heavy metals, while in areas subjected to strong anthropogenic pressures, heavy metals originate in man's industrial and agricultural activities [8–10]. Uncontrolled discharges of domestic sewage and stormwater directly into rivers and reservoirs may become a major source of heavy metals. The investigations carried out by Bak et al., and also by Gorska and Sikorski [11, 12] indicate that stormwater from urban areas is strongly polluted with lead, zinc, nickel, chromium, cadmium, and copper compounds.

As a result of chemical and biochemical processes, noxiuos substances contained in bottom sediments can undergo desorption to water, which poses a great hazard to aquatic biota. At high flows, warp containing harmful substances can be deposited within the flood routes, and thus contaminate the areas adjacent to water [13, 14].

The study presents the characteristics of physical and chemical properties of bottom sediments and water from a small dammed reservoir located in Kaniow. The content of heavy metals was compared with the threshold values of permissible concentrations specified in the regulation of the Minister of the Environment, the guidelines developed by the Institute of Soil Science and Plant Cultivation (IUNG) and LAWA classification. The assessment of the content of heavy metals in the sediments will provide a basis to evaluate the possibility of agricultural use of sediments, and also the hazard they cause to the aquatic environment.

Material and methods

Facility description

Kaniow reservoir was put into operation in the 1980s. It was constructed by damming the watercourse flowing from the hill called Borowa Gora at km 0 + 920 with an earth dam, 80 m in length. It is a valley, flow-through reservoir, which serves recreational purposes. At the normal water level, the reservoir volume is 40.0 thousand m^3 and the water table area is 2.0 ha. The average depth of this water body is approx. 2.0 m. The basin area is equal to 7.37 km^2. The land use structure involves forests which dominate in the reservoir basin covering over 80 % of its area and forming spruce and fir, pine, and hornbeam and spruce dense complexes. Meadows constitute 8.1 %, arable land – 10.9 % and built-up area – 0.7 % of the area. Grain is the dominant crop, whereas root crops (mainly potatoes) have a lower share. In the reservoir basin, low-fertility soils of V and VI valuation class are mostly found. In the years 2001–2002, Kaniow reservoir was modernised. As a result of the construction works, the front dam of the reservoir was elevated and strengthened. The reservoir was also desilted.

Methodology of investigations

Samples of bottom sediments from Kaniow reservoir were collected twice, first in July 2012, and then in March 2013. Sampling was performed from the boat. Samples were collected into transparent cylinders, 57 mm in internal diameter and 1200 mm in length, using the Beeker-type sampler. It is equipped with a cutting head which allows closing the cylinder bottom, thus collecting the samples in undisturbed state. Water samples for analysis were collected, using the bathometer, at the middle of the water depth at the given site. Sites of bottom sediment and water sampling are shown in Fig. 1. The cylinders were transferred to the laboratory, then the warp was dried at the temperature of 60 ^{o}C. After that, it was ground in a mill to a fraction below 0.063 mm, and finally subjected to complete mineralisation. The total content of ions of selected heavy metals (Pb, Cr, Cd, Cu, Ni, Zn, Fe, and Mn) in sediment and water samples was determined. Depending on the level of concentrations and the matrix received, the determination was performed using the atomic absorption spectrometry method with SavantAA Sigma or SavantAA Zeeman spectrometers.

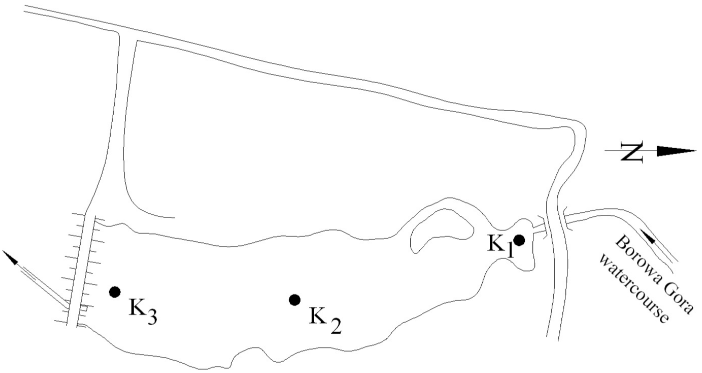

Fig. 1. Outline of Kaniow reservoir with water and bottom sediment sampling sites

The granulometric composition of sediments was determined with the sieve method and the areometric Casagrande's method modified by Pruszyński. 1.5 g of anhydrous sodium carbonate (Na_2CO_3), acting as deflocculant, were added to the solution in the form of sediment suspension. The analysis provided information on the percentage content of argillaceous ($d \leq 0.002$ mm), dust ($0.002 < d \geq 0.05$ mm), and sand ($0.05 < d \geq 2$ mm) fractions. The percentage content of organic matter was determined by sample annealing.

The quality assessment of bottom sediments in Kaniow reservoir was performed by comparing the content of heavy metals accumulated in the sediments with the values given in the binding Regulation of the Minister of Environment of 9 Sept. 2002 [J. of Laws No. 165, item 1359] [15] and the guidelines drawn up by the Institute of Soil Science and Plant Cultivation (IUNG) in Puławy [16]. Employing the guidelines developed by IUNG makes it possible to indicate the possibility of sediment use in agriculture, depending on the chemical composition and granulometric variation of sediments.

In order to assess the degree of contamination of the aquatic environment components, German LAWA classification was employed [17]. In this classification, seven purity classes were specified with reference to bottom sediment contamination with heavy metals. Permissible content of heavy metals for individual classes is shown in Table 1.

Table 1

Permissible content of heavy metals in sediments in accordance with LAWA classification

Metal	Purity class							Sediment classification
	I	I–II	II*	II–III	III	III–IV	IV	
Zinc	≤ 100	≤ 200	≤ 400	≤ 800	≤ 1600	≤ 3200	3200	I – non-contaminated
Lead	≤ 25	≤ 50	≤ 100	≤ 200	≤ 400	≤ 800	800	I–II – non-contaminated/ moderately contaminated
Copper	≤ 20	≤ 40	≤ 80	≤ 160	≤ 320	≤ 640	640	II – moderately contaminated
Nickel	≤ 30	≤ 60	≤ 120	≤ 240	≤ 480	≤ 960	960	II–III – moderately contaminated/ heavily contaminated
Chromium	≤ 80	≤ 160	≤ 320	≤ 640	≤ 1280	≤ 2560	2560	III – heavily contaminated
Cadmium	≤ 0.3	≤ 0.6	≤ 1.2	≤ 2.4	≤ 4.8	≤ 9.6	9.6	III–IV – heavily/very heavily contaminated IV – very heavily contaminated

* Recommended permissible value.

Results of investigations

On the basis of analyses of granulometric composition, it was found out that bottom sediments of Kaniow reservoir comprise finely grained deposits with a low content of organic matter that does not exceed 2 % (Table 2). In samples K_2 and K_3, grains 0.25–0.5 mm in diameter (medium sand) constituted 62 % and 53 %, respectively, of the whole sample mass. Dust and argillaceous fractions in both samples did not exceed 0.2 %. In sample K_1, collected at the upper part of the reservoir, grains of 0.05–0.25 mm range (fine sand, 58 % of the mass sample) and 0.002–0.05 mm range (dust fraction, 14 % of the mass sample) prevailed.

Analysis of heavy metal content in sediments showed that the highest variation in results, in all samples, was found for nickel and lead (Table 2). The content of those metals in the sediments did not exceed $16.26\ \mathrm{mgNi \cdot kg^{-1}}$ and $82.56\ \mathrm{mgPb \cdot kg^{-1}}$, additionally, the highest values both for nickel and lead, were observed at station K_2, and the lowest values – at station K_1 for nickel, and K_3 – for lead. A wide variation in results is also observed for chromium, zinc, copper and cadmium. The highest content of chromium was noted at K_2, and the lowest – in the samples collected in the upper part of the reservoir (station K_1). Zinc content in samples did not exceed 26.26 mgZn · kg^{-1}. The maximum value of this element compounds was found at station K_3 in 2013. Copper content in the samples did not exceed 3.56 mgCu · kg^{-1}, and cadmium 0.79 mgCd · kg^{-1}. Additionally, in 2012, the maximum cadmium content was found at station K_1, and a year later – at K_3. The highest content of iron compounds was discovered in bottom sediments collected in the central part of the reservoir (866 mgFe · kg^{-1} – 2012, 881 mgFe · kg^{-1} – 2013), the lowest iron content was found in the samples from the lower part of the reservoir (762 mgFe · kg^{-1} – 2012, 734 mgFe · kg^{-1} – 2013). A similar situation was observed for manganese compounds. Mercury content ranged from 0.002 to 0.003 mgHg · kg^{-1}.

Table 2

Content of heavy metal ions in bottom sediments

Station	Fe	Cr	Zn	Ni	Mn	Cu	Pb	Cd	Hg	Content of organic substances
	[mg · kg^{-1}]									[%]
2012										
K_1	828	8.45	22.00	6.94	701	3.56	51.25	0.73	0.003	1.79
K_2	866	15.19	18.43	14.98	750	2.25	82.56	0.55	0.003	0.39
K_3	762	10.23	24.54	9.03	690	3.30	35.86	0.62	0.002	0.35
2013										
K_1	852	8.98	20.47	7.36	717	2.98	53.15	0.79	0.003	2.00
K_2	881	14.62	17.50	16.26	758	2.19	77.36	0.58	0.003	0.28
K_3	734	11.16	26.26	9.85	673	3.54	39.16	0.69	0.002	0.45

As regards water samples, such a great variation in the content of individual compounds of heavy metals was not observed, as it was the case for sediments. Chromium and lead ions were characterised by the highest variation. The highest content of these metals was found in water samples collected from sites denoted as K_1 for chromium (0.06 μgCr · m^{-3} – 2012 and 2013), and K_3 for lead (6.98 μgPb · m^{-3} – 2013). Iron, zinc, manganese and cadmium ions showed a similar range of variation. The highest values for iron and also zinc compounds were noted at station K_1 both in 2012 and 2013. The highest concentrations of manganese and cadmium were observed in the samples collected at the dam-adjacent part of the reservoir, whereas the lowest – in water samples collected next to the reservoir inlet, both in 2012 and in 2013. The content of these elements ranged 229–285 μgMn · m^{-3} and 2.66–3.35 μgCd · m^{-3} (Table 3). The content of nickel and copper demonstrated the lowest variation throughout the whole measurement cycle. The content of these elements in water ranged 0.09–0.11 μg · m^{-3} for nickel, and 2.11–2.35 μg · m^{-3} for chromium. In all samples of concern, mercury content was below the detection threshold.

Table 3

Content of heavy metal ions in water

Station	Fe	Cr	Zn	Ni	Mn	Cu	Pb	Cd	Hg	pH	Suspension
	[μg · kg^{-1}]										[g · m^{-3}]
2012											
K_1	801	0.06	13.51	0.10	229	2.11	5.21	2.66	—	7.40	17.68
K_2	649	0.06	12.21	0.10	229	2.11	6.11	3.06	—	7,20	12.54
K_3	702	0.05	12.21	0.09	244	2.35	6.52	3.35	—	7.25	15.25
2013											
K_1	743	0.06	13.26	0.10	264	2.25	4.37	2.66	—	7.35	18.11
K_2	693	0.04	12.97	0.11	273	2.15	6.23	2.97	—	7.40	11.11
K_3	721	0.05	12.35	0.09	285	2.32	6.98	3.25	—	7.20	19.78

The pH of the water sampled for testing fluctuated around the near neutral values, and the content of suspensions did not exceed 19.78 g·m^{-3} (Table 3).

Discussion of results

Table 4 shows the content of heavy metals in the bottom sediments of Kaniow reservoir. It can be stated that in accordance with the LAWA classification, bottom sediments did not exceed the permissible level in any sample. With respect to chromium, zinc, nickel and copper, the threshold values for class I of deposits cleanliness were not exceeded. Only for lead (stations K_1 and K_2) and cadmium (stations K_1 and K_3), bottom sediments of the reservoir were classified as moderately contaminated.

Table 4

Classification of bottom sediments from Kaniow reservoir

Station	Cr	Zn	Ni	Cu	Pb	Cd	Hg	Criterion
K_1	I	I	I	I	II*	II*	—	LAWA classification
K_2	I	I	I	I	II*	I–II	—	
K_3	I	I	I	I	I–II	II*	—	
K_1	—	0	0	0	I	I	—	IUNG guidelines
K_2	—	0	I	0	II	I	—	
K_3	—	0	0	0	I	I	—	
K_1	A	A	A	A	B	A	A	Ministerial regulation
K_2	A	A	A	A	B	A	A	
K_3	A	A	A	A	A	A	A	

* Recommended permissible value.

In accordance with the guidelines developed by the Institute of Soil Science and Plant Cultivation (IUNG), sediments deposited in the lake basin of Kaniow reservoir can be classified as uncontaminated, with the natural content of trace metals, for zinc, nickel and copper. With respect to lead (except for station K_2) and cadmium, the reservoir bottom sediments can be classified as soils with elevated metal content. The sediments collected from the central part of the reservoir (station K_2) should be classified as weakly contaminated as regards the lead content. Therefore, while planning the agricultural use of the sediments, it is necessary to exclude certain horticultural crops, due to the hazard of the chemical contamination of plants. Nonetheless, the cultivation of cereals, root crops and fodder is allowed on so fertilised land.

In accordance with the soil quality standards set in the regulation [15], permissible limits were exceeded only for lead (stations K_1 and K_2). Thus, sediments deposited on the reservoir bottom correspond to soils classified as farmland (group B) as regards chemical composition.

Table 5 shows the content of heavy metals in bottom sediments from two small dammed reservoirs located in the Swietokrzyskie Province (Suchedniow reservoir and Kielce artificial lake), also from Krempna water reservoir, which is characterised by a percentage content of forests in the total basin area similar to that of Kaniow reservoir, and finally Zeslawice reservoir, the basin of which is agricultural in character as arable land constitutes approx. 80 % of its area.

Table 5

Content of heavy metal ions in selected small water reservoirs

Fe	Cr	Zn	Ni	Mn	Cu	Pb	Cd
$[mg \cdot kg^{-1}]$							
Suchedniow reservoir							
10911–21948	37.36–63.92	177.49–216.94	29.01–31.41	499.76–698.45	26.39–42.97	59.39–110.02	5.02–10.75
Artificial lake in Kielce							
—	3.7–64	5.2–68	—	—	1.2–28	34.2–291	2.32–22.6
Krempna reservoir							
—	38.2–70	39.7–79.5	28.3–52.3	—	14.2–33.2	9.3–17.2	0.14–0.32
Zeslawice reservoir							
—	29.0–29.9	55.0–72.6	5.9–9.0	—	5.1–7.7	9.7–13.9	0.27–0.41

Comparing the sets of data in Table 5 and Table 2, it can be concluded that bottom sediments of Kaniow reservoir were contaminated with heavy metals to a far lesser extent than bottom sediments of Suchedniow reservoir and Kielce artificial lake. The latter are located in urban areas and subjected to strong anthropogenic pressure. In addition, their function is to receive stormwater, which undoubtedly affects the quality of sediments in those reservoirs. Bottom sediments of Kaniow reservoir are characterised by a lower content of Cr, Zn, Ni and Cu when compared with sediments from Krempna reservoir. The content of Pb and Cd compounds was higher than in the sediments of Kaniow reservoir. A similar situation was observed when bottom sediments from Kaniow and Zeslawice reservoirs were compared.

The literature data [18] show that heavy metals can be absorbed by sediment organic matter. The results of statistical analyses indicate the occurrence of a strong correlation dependence, which is statistically relevant for the level $p < 0.05$, for the pairs: Fe – organic matter and Ni – organic matter. Correlation coefficients for these pairs were 0.94 for iron and –0.87 for nickel (Fig. 2a and b). In all remaining cases, this dependence was not statistically significant.

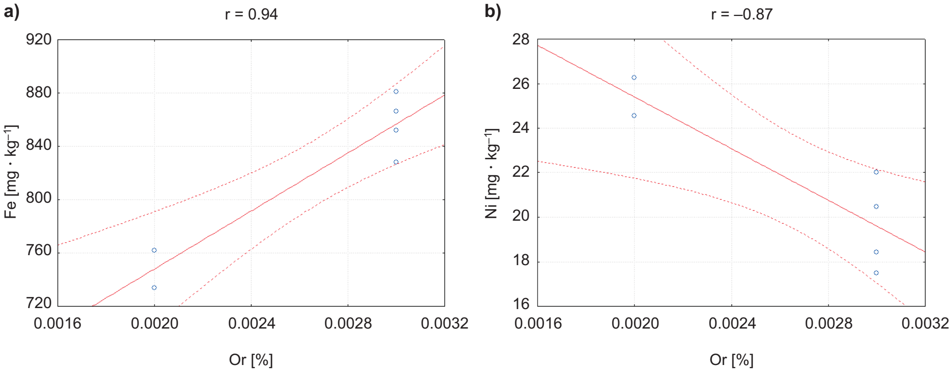

Fig. 2. Correlation dependence: a) Fe – organic matter; b) Ni – organic matter

The waters of Kaniow reservoir satisfy the basic chemical requirements (content of heavy metals) that should be met by potable water. In the investigations, the highest permissible values of concentrations of heavy metals, stipulated in the Regulation of the Minister of Health of 27 March 2007 [19], were not exceeded.

Conclusions

1. The bottom sediments of Kaniow reservoir are only weakly contaminated with heavy metals, additionally spatial differentiation in their concentrations can be observed. It should be noted that the highest concentrations of Fe, Cr, Ni, Mn and Pb were found in the sample collected in the central part of the reservoir. In other samples, the content of those metals was much lower. At present, it is not possible to adequately explain the reason for that difference, therefore it is necessary to continue monitoring the state of the reservoir bottom sediments.

2. In accordance with the LAWA classification, the content of heavy metal ions in the reservoir bottom sediments did not exceed the permissible limits.

3. Values of lead and cadmium concentrations in all analysed samples corresponded to level I–II contamination in accordance with the IUNG classification. For the

remaining elements, it was the 0 level (except for station K_2, where level I contamination was observed). II level indicates weak soil contamination, therefore it is possible to grow only cereals, root crops and fodder on such land, due to the hazard of the chemical contamination of plants. Significant correlation was found between iron and organic matter, and also nickel and organic matter.

4. The concentrations of ions of heavy metals in Kaniow reservoir water do not exceed the maximum limits for potable water, in accordance with the Regulation of the Minister of Health of 27 March 2007.

5. The examined sediments contain a substantial amount of sandy material and small amounts of particulate and argillaceous materials. The sandy fraction in the samples constituted from 86 % to 99.8 % of the total mass.

Acknowledgements

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KONCENTRACJA METALI CIĘŻKICH W WODZIE I OSADACH DENNYCH MAŁEGO ZBIORNIKA WODNEGO W KANIOWIE

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Abstrakt: W artykule przedstawiono wyniki badań stężeń jonów wybranych metali ciężkich (Fe, Cr, Zn, Ni, Mn, Cu, Pb, Cd, Hg) w osadach i wodzie małego zbiornika zaporowego zlokalizowanego w miejscowości Kaniów na cieku od Borowej Góry. Do badań pobrano osady i wodę z części górnej, środkowej i dolnej zbiornika. Próby pobrano dwukrotnie, po raz pierwszy w lipcu 2012 r., a następnie w marcu 2013 r. Zawartość pierwiastków śladowych w wodzie nie przekraczała: 801 $\mu g \cdot dm^{-3}$ (Fe), 0,064 $\mu g \cdot dm^{-3}$ (Cr), 13,5 $\mu g \cdot dm^{-3}$ (Zn), 0,106 $\mu g \cdot dm^{-3}$ (Ni), 285 $\mu g \cdot dm^{-3}$ (Mn), 2,35 $\mu g \cdot dm^{-3}$ (Cu), 6,98 $\mu g \cdot dm^{-3}$ (Pb), 3,35 $\mu g \cdot dm^{-3}$ (Cd). W przypadku osadów dennych maksymalne odnotowane stężenia metali ciężkich wynosiły: 872 $mg \cdot kg^{-1}$ (Fe), 15,19 $mg \cdot kg^{-1}$ (Cr), 26,26 $mg \cdot kg^{-1}$ (Zn), 16,26 $mg \cdot kg^{-1}$ (Ni), 750 $mg \cdot kg^{-1}$ (Mn), 3,58 $mg \cdot kg^{-1}$ (Cu), 82,56 $mg \cdot kg^{-1}$ (Pb), 0,73 $mg \cdot kg^{-1}$ (Cd), 0,0031 $mg \cdot kg^{-1}$ (Hg). Podjęto również próbę określenia matematycznej zależności między zawartością części organicznych w osadach dennych zbiornika a metalami ciężkimi oraz określono możliwość rolniczego wykorzystania osadów po ich wydobyciu z dna zbiornika.

Słowa kluczowe: metale ciężkie, osady denne, zbiornik wodny

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DETERMINATION OF SELECTED NONSTEROIDAL ANTI-INFLAMMATORY DRUGS IN THE AQUATIC ENVIRONMENT

OZNACZANIE WYBRANYCH NIESTEROIDOWYCH LEKÓW PRZECIWBÓLOWYCH I PRZECIWZAPALNYCH W ŚRODOWISKU WODNYM

Abstract: The presence of pharmaceutical compounds in the aquatic environment requires the development of an effective monitoring method. The paper presents the developed procedure of determination of pharmaceutical compounds selected from the group of nonsteroidal anti-inflammatory drugs – ibuprofen and diclofenac. To isolate selected compounds from the tested water the solid phase extraction (SPE) was employed. The quantitative and qualitative analysis was carried out by high performance liquid chromatography HPLC (UV) as well as gas chromatography coupled with mass spectrometry with electron ionization GC-MS (EI). Described analytical procedures allows to separate and quantificat pharmaceutical mixtures with a satisfactory reproducibility and accuracy. The values of the recovery degree confer the possibility of the complete quantitative control of tested pharmaceuticals in water samples. The described method can be used to control the analytical processes of natural water purification and wastewater treatment from pharmaceutical compounds.

Keywords: diclofenac, ibuprofen, SPE, HPLC (UV), GC-MS (EI)

Introduction

Pharmaceutical substances and endocrine disruptors (EDCs) are the most frequently identified organic pollutants in the environment [1]. The greatest interest of researchers focuses on acidic non-steroidal analgesics, *ie* ibuprofen, diclofenac, ketoprofen, naproxen and phenolic EDCs including *eg* triclosan, bisphenol, octylphenol and nonylphenol [2]. In numerous review papers these compounds were assessed as priority compounds which deteriorate the quality of many ecosystems, including the aquatic

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ecosystem, due to their widespread use and toxicity to aquatic organisms [3–5]. Over 3000 different drugs are registered on the European Union pharmaceutical market [6]. In contrast, the pharmaceutical industry itself is one of the fastest growing industries in the European Union. The market analysis conducted by PharmaExpert [7] shows that the total sales on the Polish pharmaceutical market in June 2014 amounted to 2271 million PLN, which is a 4.5 % increase in sales compared to this period in 2013. Total sales of all over-the-counter drugs including non-steroidal analgesics and anti-inflammatory drugs in pharmacies amounted to 893 million PLN and increased by 5.9 % compared to June 2013. The forecast for 2014 assumes that the value of the whole market at the end of the year will amount to 28.5 billion PLN, and will be about 3 % higher than in the previous year [7]. The increase in the production of pharmaceuticals is dictated by the lengthening of life expectancy related to advances in the diagnosis and treatment of diseases and the wide availability of drugs, particularly those available over the counter.

Numerous studies [8–10] have shown that the majority of pharmaceutical compounds is not completely removed from wastewater during conventional treatment processes based on activated sludge or from surface waters which are a potential source of drinking water. This is due to their polar nature and poor susceptibility to biochemical decomposition [11, 12]. This results in the continuous release of these chemicals and their metabolites to the environment. As it was already mentioned, the presence of pharmaceutical compounds in the aquatic environment is a potential threat to these ecosystems and thus to human health [13, 14]. It is difficult to predict the specific environmental consequences which may arise from the presence of pharmaceutical compounds in aquatic ecosystems. Drugs apart from acute and chronic toxicity can be genotoxic [15], contribute to the growth of pathogenic bacteria resistant to the commonly known and used antibiotics [16] and can cause endocrine disorders [17]. In addition, some pharmaceuticals such as antidepressants and antibiotics have the ability to bioconcentrate/bioaccumulate in aquatic organisms, especially fish [18, 19]. Therefore, the presence of drugs in the aquatic environment, especially in surface waters used as a source for the production of drinking water, must be continually monitored and considered as an important problem in terms of safety to human health. Satisfactory removal efficiencies for pharmaceuticals are achieved in the advanced treatment systems, which include, among others, membrane techniques – nanofiltration and reverse osmosis [20] and advanced oxidation processes – ozonation [21] and the photocatalytic oxidation [22, 23].

The quantification of pharmaceutical compounds in the environment is a difficult procedure because of their low concentrations within the range of tens of $\mu g/dm^3$ to several ng/dm^3, and the coexistence of pharmaceutical active compounds from different treatment groups. The identification of individual compounds may also interfere with other organic micropollutants. The challenge is therefore to develop an analytical procedure which is characterized by high sensitivity and accuracy, allowing the determination of the largest possible number of pharmaceutical micropollutants and by-the products of their decomposition during the treatment of water streams. One of the chromatographic techniques available for determining the concentration of trace polar mixtures of pharmaceutical compounds in environmental samples is liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) [24, 25]. Due to the lower cost of analysis and a much wider availability of equipment the most commonly used analytical techniques are, however, high performance liquid chromatography (HPLC) with a spectrophotometric (UV) or fluorescence (F) detector [26], and gas chromatography with mass spectrometry (GC-MS) with different detection systems [27, 28]. GC-MS is an analytical method which is considered to be more sensitive while maintaining the minimum value of the measurement uncertainty as compared with liquid chromatography (HPLC) [29].

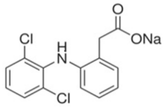
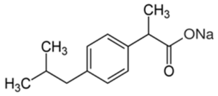
This paper presents a comparison of two procedures used for determination of the concentration of selected non-steroidal analgesic and anti-inflammatory pharmaceutical compounds occurring in the mixture: diclofenac (aminophenylacetic acid derivative), and ibuprofen classified as a propionic acid derivative. The presented analytical methods are based on the isolation of analytes from water samples by means of solid phase extraction (SPE) and their determination using HPLC (UV) as well as gas chromatography coupled with mass spectroscopy with electron ionization GC-MS (EI). The developed analytical procedures can be used to determine the concentration of pharmaceuticals in the aquatic environment, and evaluate the effectiveness of water treatment and wastewater polishing processes for the elimination of pharmaceutical micropollutants.

Materials and methods

Analytical standards of non-steroidal anti-inflammatory drugs and analgesics in the form of diclofenac (DCL)sodium salt and ibuprofen (IBU)sodium salt with a purity of > 98 % was purchased from Sigma-Aldrich (Poznan, Poland) (Table 1).

Table 1

Physicochemical characteristics of the selected pharmaceuticals

Pharmaceutical compound	Diclofenac sodium salt	Ibuprofen sodium salt
Structural formula		
Molecular formula	$C_{14}H_{10}Cl_2NNaO_2$	$C_{13}H_{17}O_2Na$
Molecular weight [g/mol]	318.13	228.26
Solubility in water [mg/dm^3]	50	100
pK$_a$	4.15	4.91
log K$_{ow}$	4.51	3.97

N-methyl-N-(trimethylsilyl)-trifluoroacetamid (MSTFA) was selected as a derivatising agent (silylation reaction) and was purchased from Sigma-Aldrich (Poznan, Poland). Additionally, methanol was used in the study and was purchased from Avantor Performance Materials Poland S.A. (Gliwice, Poland). During solid phase extraction (SPE) disposable Supelclean™ ENVI-8 cartridges with a volume of 6 cm^3 (1.0 g) and an SPE pressure chamber purchased from Supelco (Poznan, Poland) were used.

In the study of the extraction process two aqueous solutions prepared using deionised water and the IBU and DCL standards at a concentration of 0.5 mg/dm^3 and 1 mg/dm^3. The pH of the water was adjusted to pH 7 with 0.1 mol/dm^3 HCl or 0.1 mol/dm^3 NaOH. However, DCL and IBU at concentrations of 0.2 to 2 mg/cm^3 were analysed chromatographically in order to assess the linearity of the mass detector response and the instrumental detection limit. Analytical standards were subjected only to silylation reaction.

In order to determine the analytes by the HPLC (UV) and GC-MS (EI) chromatography in 20 cm^3 (pH = 7) water samples the tested pharmaceuticals were extracted by solid phase extraction (SPE) in cartridges with octylsilane (C_8) packing. The bed prior to extraction was washed with 5 cm^3 of methanol and conditioned with deionised water at pH = 7. Next water sample was added to the extraction cartridge. After the extraction the packing was dried for 5 min under vacuum. The eluent was eluted with 3 cm^3 of methanol and subjected to drying in a stream of nitrogen.

For the determinations by high performance liquid chromatography using a Varian apparatus (UV detector, wavelength λ = 220 nm), the eluent was dissolved in 100 μm^3 of methanol. Sample injections into Hypersil GOLD column from Thermo Scientific with a length of 25 cm, a diameter of 4.6 mm and a pore size of 5 µm were performed manually using a 50 μm^3 Hamilton microsyringe. The mobile phase was a mixture of acetonitrile/water in the proportions of 85:15 (v/v).

The determinations using a gas chromatograph coupled with a mass detector (GC-MS with EI) model Saturn Varian 2100 T (Warszawa, Poland) were performed after derivatisation of the eluents obtained by SPE. 10 μm^3 of MSTFA was used as a silylating reagent. After 35-min reaction time at 55 ^oC trimethylsilyl (TMS) derivatives of the analysed pharmaceuticals were obtained and were next analysed chromatographically. The determinations were carried out using the chromatograph. The eluent was separated in the SLB^{TM} – 5 ms column from the Supelco Company (Poznan, Poland) with dimensions of 30 m × 0.25 mm × 0.25 µm at the following temperature settings of the column oven: 80 ^oC (8 min), 10 ^oC/min up to 300 ^oC (5 min). The other temperature parameters were as follows: injector 230 ^oC, ion trap 180 ^oC, ion source 290 ^oC. Helium was the carrier phase and the flow rate was 1.1 cm^3/min. Injections of the sample with a volume of 1 μm^3 ware performed manually using a 10 μm^3 Hamilton microsyringe. The mass detector operated in the ion recording mode in the range of 70 to 400 m/z.

Results and discussion

Calibration curves, based on the standard solution of diclofenac sodium salt and ibuprofen sodium salt dissolved in methanol in a concentration range of 0.2 to 2 mg/cm^3, were constructed in order to calibrate the UV detector of the HPLC chromatograph and the mass detector. The linearity of the detector response was examined by linear regression (Fig. 1 – HPLC (UV), Fig. 2 – GC-MS (EI)).

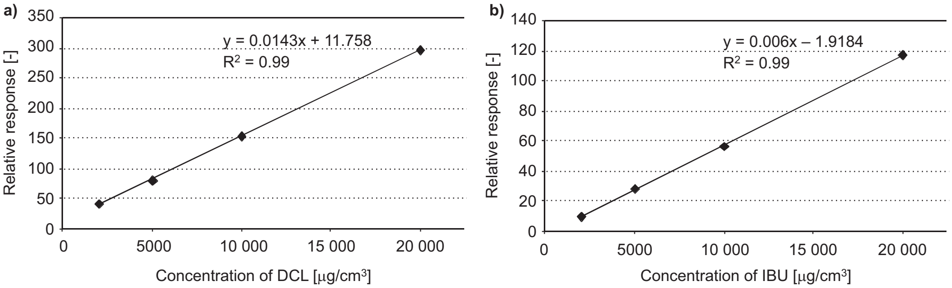


Fig. 1. Calibration curve of DCL a) and IBU b) determination by the HPLC method

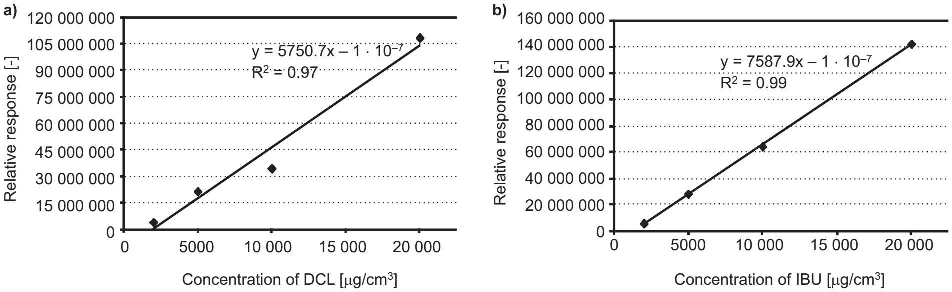


Fig. 2. Calibration curve of DCL a) and IBU b) determination by the GC-MS method

Retention times and characteristic ions, which are listed in Table 2, were determined based on chromatograms and mass spectra obtained during the HPLC (UV) (Fig. 3) and GC-MS (EI) (Fig. 4) analyses of silyl derivatives of the pharmaceuticals (Fig. 5 and Fig. 6). Instrumental Detection Limit (IDL) for the test compounds was determined by assuming the ratio of signal (S) to noise (N) to be ≥ 3 (Table. 2).

Table 2

Parameters of the qualitative analysis

Method	Compound	Characteristic ions [m/z]	Retention time, $t_R \pm$ SD (n = 5)*	CV [%] (n = 5)	IDL [ng/μm^3]
HPLC (UV)	IBU	—	3.190 ± 0.010	0.08	5
	DCL	—	2.150 ± 0.010	0.02	2
GC-MS (EI)	IBU-$(TMS)_2$	350, 263, 234, 161, 117	18.429 ± 0.059	2.82	2
	DCL-TMS	352, 277, 242, 214, 179	24.319 ± 0.063	1.50	11

* n – number of replicates.

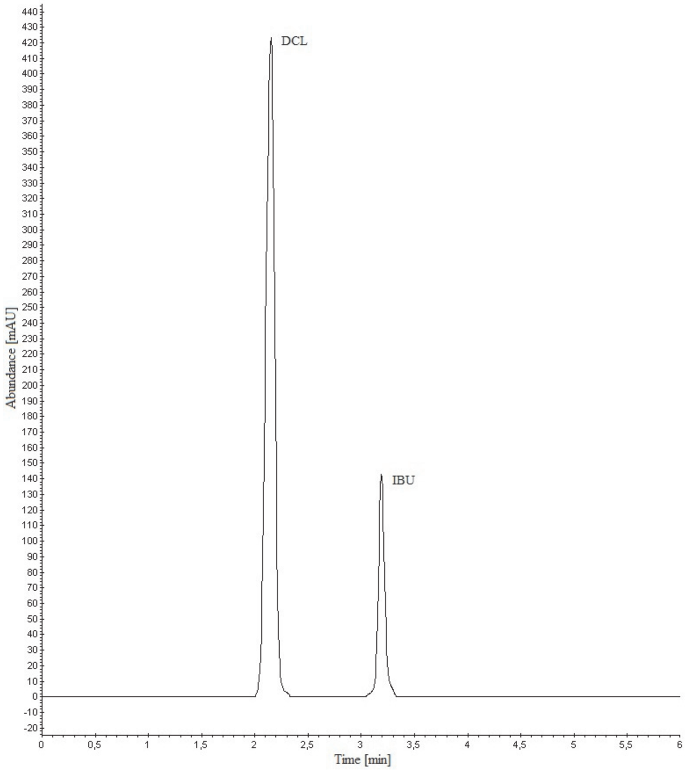


Fig. 3. HPLC (UV) chromatogram of ibuprofen and diclofenac sodium salts

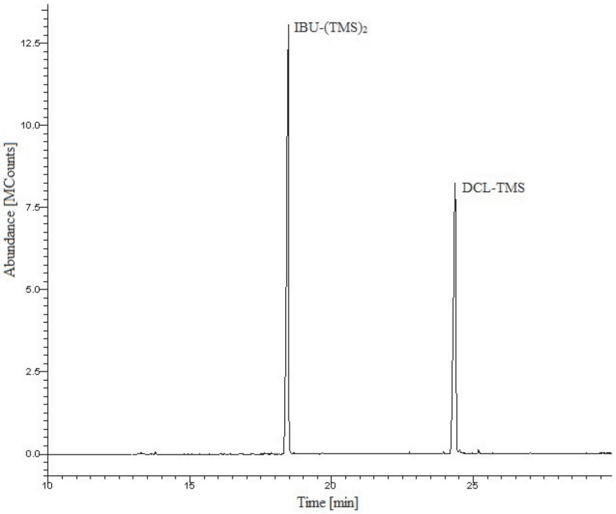


Fig. 4. GC-MS chromatogram of the silyl derivatives of ibuprofen (IBU-$(TMS)_2$) and diclofenac (DCL-TMS)

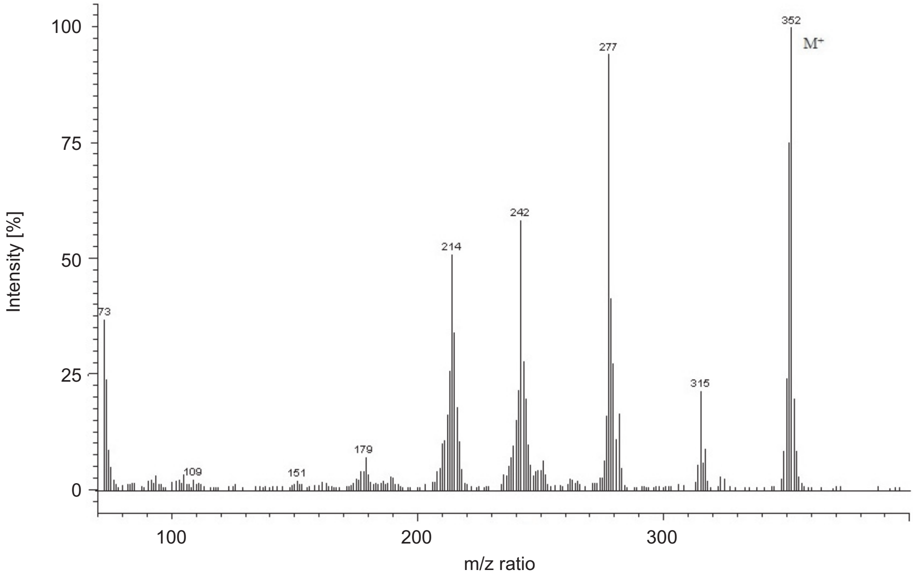


Fig. 5. Mass spectrum of the silyl derivative of diclofenac (M^+ – molecular ion)

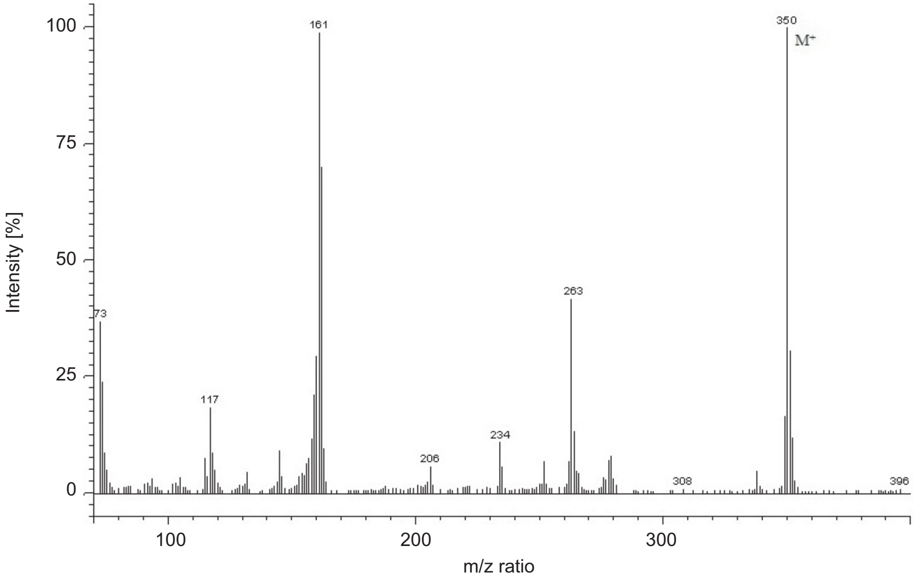
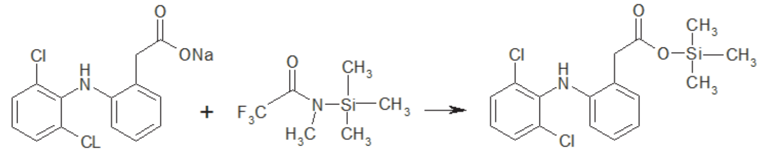
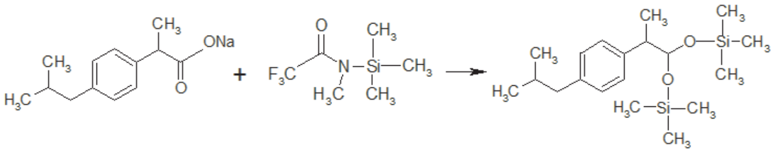


Fig. 6. Mass spectrum of the silyl derivative of ibuprofen (M^+ – molecular ion)

Based on the analysis of the obtained mass spectra it can be assumed that the reactions of formation of the trimethylsilyl derivatives of diclofenac (1), and ibuprofen (2) occur in accordance with the following equations:



(1)



(2)

The applied analytical procedure allowed separating the silyl derivatives of the pharmaceutical compounds. The peaks of the derivatives of ibuprofen and diclofenac have different retention times, and are characterized by the ions of different m/z masses. The low value of the standard deviations for the retention time of both compounds implies very small difference between the values of the parameter obtained for the number of replicates of chromatographic separation. This applies both to the HPLC (UV) and GC-MS (EI) analyses. This allows for a very precise identification of the pharmaceutical compounds present in water samples on the basis of a comparison of their retention time determined during the analysis of the standard and a sample. The values of coefficient of variation (CV, %), which is a measure of the reproducibility of the HPLC (UV) and GC-MS (EI) measurements do not exceed 1 and 3 %, respectively, which is very satisfactory.

Reproducibility of the quantification plays a crucial role during identification and assessment of concentrations of pharmaceutical compounds in surface water and wastewater being treated. The quantitative analysis of DCL and IBU was carried out based on the determination of the area of the peak for the derivatives of the pharmaceuticals. Quantitative analysis of DCL IBU was carried out based on the determination of the area of the peaks of the pharmaceutical compounds (HPLC (UV)) and the areas of the peaks for derivatives of pharmaceuticals (GC-MS (EI)). Table 3 shows the results of the calculation of the precision of the UV spectrophotometric and MS detector response for four levels of concentration of the pharmaceutical derivatives injected to the chromatographic column.

Table 3

Precision of the detector response

Method	Compound	Concentration [μg/μm^3]			
		0.2	0.5	1	2
		CV [%] (n = 5)			
HPLC (UV)	IBU	3	2	0.1	0.1
	DCL	1	1	0.1	0.1
GC-MS (EI)	IBU	1	1	5	3
	DCL	1	2	1	2

n – number of replicates.

The coefficient of variation for the HPLC (UV) and GC-MS (EI) measurements in most cases does not exceed 3 %. The exception is the determination of IBU by the GC-MS (EI) analysis for a concentration of 1 μg/μm^3 (CV = 5 %). These results confirm high reproducibility of the measurements.

Another parameter characterizing the discussed analytical methods is the degree of recovery of the compounds and the method reproducibility for the analyses of real water samples. Aqueous solutions containing analytical standards of the pharmaceuticals at concentration of 0.5 mg/dm^3 and 1 mg/dm^3 were analyzed (Table 4). Method limit of quantification (LOQ) was also determined (Table 5).

Table 4

Repeatability of the analytical methods in the determination process
of analytical standards of IBU and DCL

Compound	Concentration in model water			
	0.5 mg/dm^3		1 mg/dm^3	
	Recovery [%] (n = 5)	SD [%]	Recovery [%] (n = 5)	SD [%]
IBU	55	4	60	9
DCL	78	3	85	4

* for S/N = 10.

Table 5

Limit of quantification LOQ in the determination process
of analytical standards of IBU and DCL

Compound	Method	
	HPLC-UV	GC-MS (EI)
	[μg/dm^3]	[ng/dm^3]
IBU	2.8	16
DCL	0.8	44

The accuracy of the determinations according to the developed analytical method, which was determined based on the calculated degree of recovery, was very good in the case of aminophenylacetic acid derivative (DCL) – the degree of recovery for the solutions with higher concentrations of pharmaceutical standards was 85 %. Lower degree of recovery (60 %) was obtained for propionic acid derivative (IBU). Reproducibility of results determined on the basis of the standard deviation was satisfactory, and its value was within the range of 3 to 9 %.

Limit of quantification LOQ for the HPLC (UV) method for IBU was 2.8 μg/dm^3, and for DCL 0.8 μg/dm^3. The LOQ value for the GC-MS (EI) method was respectively 16 and 44 ng/dm^3, which indicates much higher sensitivity of the method in comparison with high performance liquid chromatography with UV detector.

Conclusions

– The presented analytical procedures allow for separation and quantification of mixtures of two non-steroidal anti-inflammatory drugs present in water at a concentration of 1 mg/dm^3 and 0.5 mg/dm^3 with satisfactory accuracy and repeatability. The GC-MS (EI) technique has a higher sensitivity than HPLC (UV). The LOQ values for the studied pharmaceuticals in the case of this technique did not exceed 44 ng/dm^3. However, for both techniques, the determinations were characterized by high precision.

– The values of the degree of recovery enable full control over the quantification of the studied pharmaceuticals in water samples.

– Analytical procedure based on the HPLC (UV) technique can be used for screening of test compounds. Furthermore, determination of the studied pharmaceuticals in the environment at low concentration levels is possible by the procedure employing the GC-MS (EI) chromatography.

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OZNACZANIE WYBRANYCH NIESTEROIDOWYCH LEKÓW PRZECIWBÓLOWYCH I PRZECIWZAPALNYCH W ŚRODOWISKU WODNYM

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Abstrakt: Obecność związków farmaceutycznych w środowisku wodnym wymusza opracowanie skutecznej metody ich monitoringu. W pracy przedstawiono opracowany sposób oznaczenia wybranych związków farmaceutycznych z grupy niesteroidowych leków przeciwbólowych i przeciwzapalnych, tj. ibuprofenu i diklofenaku. Do wydzielenia analitów zastosowano ekstrakcję do fazy stałej (SPE), a do analizy jakościowo-ilościową wysokosprawną chromatografię cieczową HPLC (UV) oraz chromatografię gazową sprzężoną z detektorem masowym (GC-MS). Opracowana procedura analityczna umożliwia rozdział i oznaczenie ilościowe mieszaniny związków farmaceutycznych z zadowalającą powtarzalnością i dokładnością. Wyznaczone wartości stopni odzysku dają możliwość pełnej kontroli ilościowego oznaczenia badanych farmaceutyków w próbkach wodnych. Powyższa metoda może znaleźć zastosowanie do kontroli analitycznej przebiegu procesów uzdatniania wód naturalnych i doczyszczania ścieków pod kątem eliminacji związków farmaceutycznych.

Słowa kluczowe: diklofenak, ibuprofen, ekstrakcja SPE, HPLC (UV), GC-MS (EI)

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COMPARATIVE STUDIES ON ELIMINATION OF ESTROGENS AND XENOESTROGENS BY THE OXIDATION PROCESSES

BADANIA PORÓWNAWCZE ROZKŁADU ESTROGENÓW I KSENOESTROGENÓW W PROCESACH UTLENIANIA

Abstract: The presence of organic micropollutants, especially the substances interfering with hormonal processes termed as endocrine disrupting chemicals EDCs, in surface waters is a potential threat to life and living organisms, including humans. Hazardous biological activity of these compounds (including toxicity) and high resistance to biodegradation necessitate research on their removal in conventional and non-conventional water treatment and wastewater treatment processes. The presented research was motivated by the increasingly frequent reports in the literature regarding the effectiveness of different advanced oxidation processes used for the elimination of micropollutants. In the present study the degradation efficiency for 17β-estradiol (E2), 17α-ethinylestradiol (EE2) and bisphenol A (BPA) in the photolysis process (UV) and in the coupled photolysis and ozonation process (UV/O_3) was evaluated in real and artificial municipal wastewater treatment plant effluent. The obtained results were compared for the degradation efficiency of the test compounds in deionised water. It was determined that both the use of the UV and UV/O_3 processes enabled to achieve high degradation efficiencies for 17β-estradiol and 17α-ethinylestradiol regardless of the treated solution. Degradation of the compounds in the processes occurred in a very short time. On the other hand, the efficiency of elimination of bisphenol A depended on the applied process and the physico-chemical composition of the solution. It was shown that the combination of photolysis with ozonation allowed achieving higher degree of degradation of the test compound than in the case of a single UV process. The study allowed also the identification of substances supporting the degradation of bisphenol A in the $UV\text{-}O_3$ process.

Keywords: elimination of organic micropollutants, EDCs, 17β-estradiol, 17α-ethinylestradiol, bisphenol A, wastewater treatment; photolysis, photolysis and ozonation

Introduction

Endocrine disrupting chemicals (EDCs) are chemicals that cause adverse effects on the endocrine systems of animals and humans. This group comprises natural estrogens

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(*eg* 17β-estradiol), synthetic estrogens (*eg* 17α-ethinylestradiol), natural androgens, plant hormones – the so-called phytoestrogens, and other industrial compounds termed as xenoestrogens (*eg* bisphenol A) [1]. Previous studies have shown that EDCs in living organisms can cause fertility problems and increase the risk of cancer [2]. The source of EDCs for living organisms is mainly food and water. Due to the fact that the conventional activated sludge wastewater treatment process does not allow for complete elimination of EDC [3–5], the use of advanced oxidation processes (AOPs) in this aspect has been studied in recent years. Recently, in the studies on AOPs it has been attempted to use the phenomenon of synergism of various oxidants such as ozone (O_3) and UV radiation, which is designed to increase the effects and the rate of decomposition of organic substances [6].

The aim of this study was to compare the decomposition efficiency for three compounds belonging to the group of EDCs, *ie* 17β-estradiol (E2), 17α-ethinylestradiol (EE2) and bisphenol A (BPA) in the photolysis processes (UV) and the coupled photolysis and ozonation process (UV/O_3) in real and artificial municipal wastewater treatment plant effluent. The obtained results were compared for the degradation efficiency of the test compounds in deionised water. The selected test compounds differed mainly in the origin and stability in aqueous solution. 17β-estradiol is an estrogenic natural sex hormone, and 17α-ethinylestradiol is a synthetic sex hormone used as a component of most modern contraceptives and drugs used in hormone replacement therapy. In contrast, bisphenol A is an organic chemical compound from the group of phenols. It is produced by human and exhibits estrogenic activity. In this study it was attempted also to identify the substances supporting or inhibiting the decomposition of organic micropollutants in the UV/O_3 process. The degradation efficiency of the studied EDCs was determined using the GC-MS (EI) technique after prior separation of the compounds from water using solid-phase extraction (SPE) and conversion of the analytes into their silyl derivatives (preparatory phase prior to chromatographic determination).

Materials and methods

Analytical standards of the test compounds (17β-estradiol, 17α-ethinylestradiol and bisphenol A) were purchased from Sigma-Aldrich (Poznan, Poland). N-methyl-N--(trimethylsilyl)-trifluoroacetamid (MSTFA) was used as a derivatising agent (silylation reaction) and was purchased from Sigma-Aldrich (Poznan, Poland). Methanol of > 99.8 % purity and acetonitrile of > 99.5 % purity were used in the study and was purchased from Avantor Performance Materials Poland S.A. (Gliwice, Poland). For solid phase extraction (SPE) disposable Supelclean™ ENVI-18 cartridges with a volume of 6 cm^3 (1.0 g) and an SPE pressure chamber purchased from Supelco (Poznan, Poland) were used.

The solutions that were treated in the UV and UV/O_3 were prepared in deionised water and were also artificial and real effluent after the biological treatment. All of the solutions were spiked with analytical standards of the studied compounds at a constant concentration of 500 $\mu g/dm^3$. The real effluent was collected from domestic wastewater treatment plant located in western Poland operated in a mechanical-biological mode. The treated real effluent originally contained no test compounds. Physico-chemical characterisation of the test solutions is presented in Table 1.

Table 1

The physical and chemical characteristics of the investigated solutions including EDCs

Treated solution	pH*	Conductivity [μS/cm]	Absorbance(UV$_{254}$) [1/cm]	Total organic carbon (TOC) [mg/dm^3]
Deionised water	7.0	0.518	0.000	0.00
Artificial effluent		0.793	0.055	24.41
Real effluent		0.985	0.218	33.01

* adjusted with of 0.1 mol/dm^3 hydrochloric acid HCl solution or 0.1 mol/dm^3 sodium hydroxide NaOH solution.

Multiparameter inoLab® 740 meter manufactured by WTW (Wroclaw, Poland) was used for the pH and conductivity measurements. The absorbance was measured at a wavelength of 254 nm using UV-VIS Cecil 1000 spectrophotometer from Analytik Jena AG (Poznan, Poland), and the concentration of total organic carbon was determined by HiPerTOC analyser from Thermo Electron (Gliwice, Poland).

In order to determine the studied EDCs by GC-MS chromatography in 100 cm^3 (pH = 7) water samples of the tested pharmaceuticals were extracted by solid phase extraction (SPE). The cartridge packing prior to extraction was conditioned with 5 cm^3 of methanol and 5cm^3 of acetonitrile and next it was washed with 5 cm^3 of deionised water. The extracted compounds were eluted with 1 cm^3 of acetonitrile/methanol mixture (40/60, v/v). Subsequently, the eluent was dried in a gentle stream of nitrogen, and then subjected to silylation with MSTFA in an amount of 30 mm^3. After 35-min reaction time at 55 ^{o}C the obtained silyl derivatives of the analysed pharmaceuticals were analysed chromatographically.

The chromatographic analyses were performed by a gas chromatograph coupled with a mass detector with electron ionisation GC-MS (EI) (ion trap type), Saturn 2100 T model from Varian (Warszawa, Poland). The analytes was separated in the SLB^{TM} – 5 ms column from the Supelco Company (Poznan, Poland) with dimensions of 30 m × 0.25 mm × 0.25 μm at the following temperature settings: 80 ^{o}C (1 min), 20 ^{o}C/min up to 300 ^{o}C (3.5 min). The other temperature parameters were as follows: injector 300 ^{o}C, ion trap 180 ^{o}C, ion source 290 ^{o}C. Helium was the carrier phase and the flow rate was 1.4 cm^3/min. Injections of the sample with a volume of 1 mm^3 were performed manually using a 10 mm^3 Hamilton microsyringe. The mass detector operated in the ion recording mode in the range of 70 to 400 m/z.

The applied analytical procedure is characterised by the EDCs recovery in the range of 66 (bisphenol A) and 100 % (17β-estradiol and 17α-ethinylestradiol), which was determined for deionised water with the addition of analytical standards of the studied compounds at 500 μg/dm^3. The limit of quantification (in ng/dm^3) was as follows: 20 for bisphenol A, 40 for 17α-ethinylestradiol and 200 for 17β-estradiol.

The photolysis process was carried out at 20 ^{o}C in a batch reactor from the Heraeus Company (Warszawa, Poland) with a medium-pressure immersion lamp with the power of 150 W. The irradiation was carried out continuously for 30 minutes. Ozone was introduced into the reactor through a ceramic diffusor with a time shift in relation to the irradiation onset, which was designed to allow comparison of the efficiency of a single process of irradiation and of the coupled process of UV and O_3. Ozone was produced from the air in the Ozoner FM 500 (WRC Multiozon; Sopot, Poland) generator with a capacity of 0.14 mg/s. The ozone dose was constant and was 3 mgO_3/dm^3.

In order to identify the substances supporting or inhibiting the degradation of low-molecular weight organic micropollutants a series of experiments using an artificial effluent was conducted. The artificial effluent was prepared using dry nutrient broth (C_0 concentration 10.5 mg/dm^3), casein peptone (C_0 = 15.6 mg/dm^3), NH_4Cl (C_0 = 2 mg/dm^3), NaCl (C_0 = 0.7 mg/dm^3), $CaCl_2 \cdot 6H_2O$ (C_0 = 0.7 mg/dm^3), $MgSO_4 \cdot 7H_2O$ (C_0 = 0.2 g/dm^3), K_2HPO_4 (C_0 = 2 g/dm^3) and KH_2PO_4 (C_0 = 5 mg/dm^3). During the tests the concentration of two organic components (casein peptone and dry nutrient broth) and two inorganic compounds (NH_4Cl and K_2HPO_4) was modified in order to determine the effect of each of these substances on the decomposition of bisphenol A in the UV/O_3 process. The highest concentration of a component in the artificial effluent (C_0) was assumed to be the selection criterion. In each experiment, which was carried out separately, the concentration of only one of the selected components was modified (assuming $0.5 \cdot C_0$ and 0.25 C_0). In this manner it was determined which component of the artificial effluent may support or inhibit the decomposition of bisphenol A. In addition, analysis of variance (ANOVA) was performed for the results from the data sets containing different concentrations of the same component of the artificial effluent. The analysis was carried out to test the hypothesis that each analysed sample (test series) originates from the same basic probability distribution with respect to the alternative hypothesis, which, in turn, states that the basic probability distributions are not the same for all tests. Consequently, the selected method allowed to state explicitly whether the difference in the degree of decomposition of bisphenol A in the samples with different concentrations of a selected component of the artificial effluent was statistically significantly. Prior to performing the ANOVA test, the detection of outliers was performed, the data were checked for the normality of distribution (assumed probability α = 0.05) and the hypothesis of homogeneity of variance was verified (assumed α = 0.05) by the Barlett test.

Results and discussion

In the first stage of the study the efficiency of decomposition of the studied EDCs was compared for a single photolysis process carried out for the real effluent (Fig. 1). It was determined that with the increasing irradiation time of the solution the concentration of 17β-estradiol, 17α-ethinylestradiol and bisphenol A decreased. The greatest reduction in the concentration of the studied estrogens was observed during the initial irradiation. Already after 5 minutes of the process the decomposition degree for 17α-ethinylestradiol was approx. 93 %, and for 17β-estradiol was 94 %. In contrast,

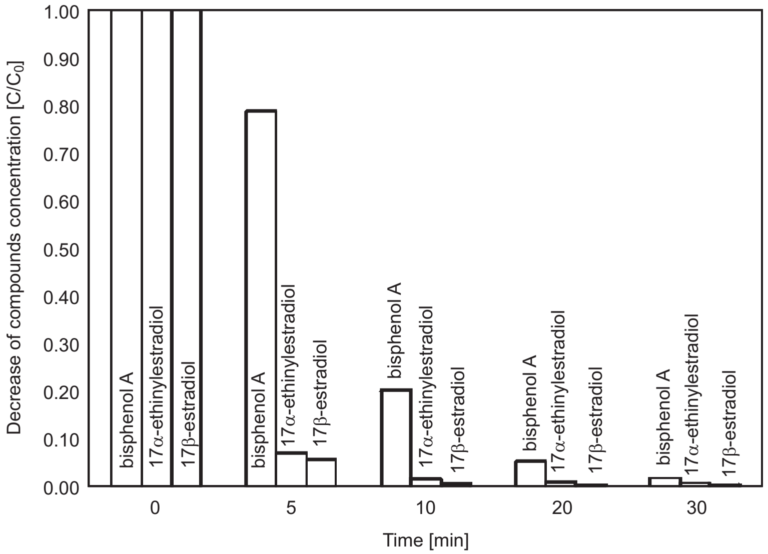

Fig. 1. The influence of radiation time on the decrease of EDCs concentration in the photolysis process of the real effluent

comparable reduction in the concentration of bisphenol A was observed only after 20 minutes of irradiation. This phenomenon may stem from different structural composition and physico-chemical properties of the test compounds, which directly affects their stability or reactivity in aqueous solutions [7]. It is noteworthy that bisphenol A is a compound characterised by high stability in aqueous media [8–11].

The decomposition efficiency for the estrogens was comparable in the real effluent and in deionised water (the results are not presented in this work). In the case of bisphenol A the decomposition of the compound in deionised water was much lower than in the real effluent regardless of the irradiation time. The explanation of this phenomenon is provided later in this work.

In the further stage of the experiment, deionised water containing the test compounds was treated by the coupled processes of photolysis and ozonation. The introduction of ozone into the reactor with a time delay in relation to the onset of irradiation was intended to allow for the comparison of the efficiency of a single irradiation process and the coupled process of irradiation and ozonation. The obtained results are shown in Fig. 2. It was observed that the degree of degradation of both 17β-estradiol and 17α-ethinylestradiol did not increase when ozone was added to the reactor. In contrast, in the case of bisphenol A, the decomposition degree of the compound was more than 75 % after the introduction of ozone (an increase of approx. 20 % compared to the single UV process). Then, in spite of continued irradiation of the solution the concentration of the compound remained at a similar level. The results obtained for 17β-estradiol and 17α-ethinylestradiol demonstrated that the synergistic effect of various processes is not always observed. It should be noted, however, that the

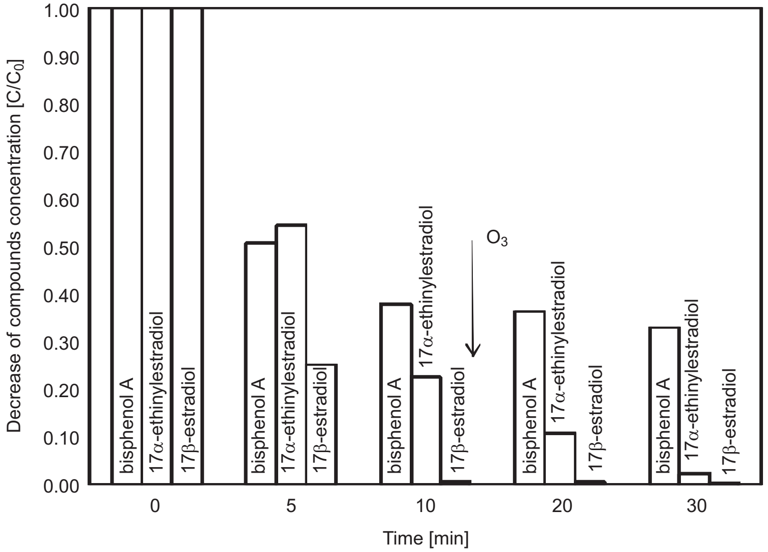

Fig. 2. The influence of the radiation time on EDCs decomposition in a combined process of photolysis and ozonation of the deionized water

efficiency of the degradation of estrogens was already high in the single photolysis process. However, it could be assumed that after the addition of ozone, these compounds would be completely degraded. Also Esplugas et al [12] did not observe the synergistic effect of various combinations of oxidation processes (O_3/H_2O_2, O_3/UV and O_3/UV/H_2O_2) used for the removal of phenol from aqueous solutions. This may stem from the fact that the efficiency of the oxidation process depends on many factors, such as pH and physico-chemical composition of the solution, the concentration of the substance to be removed or the properties of the reagent itself [13].

In the next part of the study, the process of UV and O_3 was used for the treatment of the real effluent (Fig. 3). The obtained results indicate that in the case of 17β-estradiol and 17α-ethinylestradiol, there was no synergistic effect of the processes also for the real effluent. In contrast, after the introduction of ozone into the reactor, the concentration of bisphenol A was reduced significantly to approx. 98 % (an increase of approx. 40 % compared to the single UV process).

The observed higher degree of decomposition of bisphenol A for the real effluent (Fig. 3) compared to deionised water (Fig. 2) resulted probably from the fact that the effluent after biological treatment contained chemicals that were showing a similar effect to photosensitizers supporting the process of oxidation. This phenomenon was described by Neamtu and Frimmel [14]. Photosensitizers are chemical compounds having the ability to absorb the energy of electromagnetic radiation and transfer it to other substances taking part in photochemical reactions. For this reason, in the final stage of the study an attempt was made to identify the substances supporting or inhibiting the bisphenol A oxidation process.

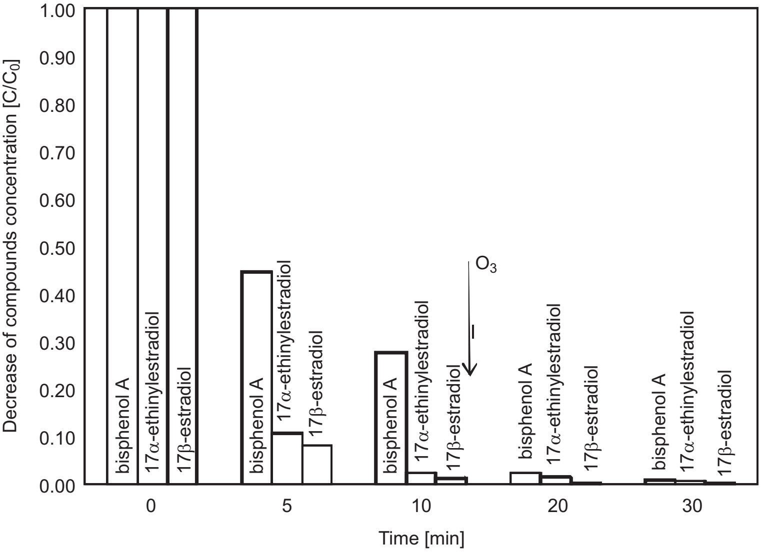

Fig. 3. The influence of the radiation time on EDCs decomposition in a combined process of photolysis and ozonation of the real effluent

Based on the obtained results (Fig. 4) it can be seen that the concentration of peptone exerted the greatest effect on the reduction of the degradation efficiency for bisphenol A in the coupled photolysis and ozonation process. When the peptone concentration was

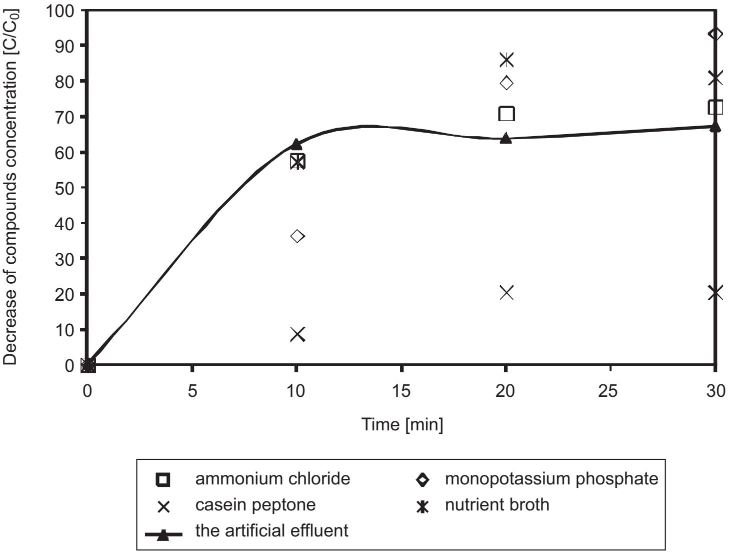

Fig. 4. The influence of change in concentration (0.5·C$_0$) of individual components of the simulated effluent on EDCs decomposition in a combined process of photolysis and ozonation of the simulated effluent

reduced twice ($0.5 \cdot C_0$), the bisphenol A degradation efficiency was also reduced by approx. 50 % at each time point. No change in the degree of decomposition of bisphenol A was observed in the process of UV/O_3 at the initial irradiation time (0 to 20 minutes), when the concentration of broth and ammonium chloride was reduced two-fold. The sample containing two times lower concentration of monopotassium phosphate (V) the decomposition efficiency for bisphenol A was approx. 35 % after 10 minutes of the process. In contrast, after 20 minutes of the process, the degree of degradation of BPA in the case of all three substances increased by respectively approx. 20 % for broth, approx. 10 % for ammonium chloride for and for disodium phosphate (V) by more than 20 %.

Based on the results presented above, it can be concluded that peptone can exert the effect similar to the effect of photosensitizers. In order to strengthen this hypothesis, an analysis of variance was performed at each sampling time point for the process of UV/O_3, which included the following concentrations of peptone: C_0, $0.5 \cdot C_0$ and $0.25 \cdot C_0$. Table 2 shows the results of the ANOVA test.

Table 2

Results of the ANOVA

Parametr	Sum of squared deviations from the mean (SS)	Number of degrees of freedom (df)	Mean square (MS)	Value of the F-Snedecor statistic (F)	Probability (p)
Among	4961.32	2	2480.66	13.78	0.0057
Within	1080.39	6	180.06	—	—
Total	6041.71	8	—	—	—

The test statistic for the collected data takes the value of F-Snedecor statistics at the level of 13.78. The calculated value of the test statistic exceeds the critical value equal to 10.92 (value for the F-Snedecor distribution) for the probability of $\alpha = 0.05$. Hence, the final conclusion for the statistical analysis is that the degree of decomposition of bisphenol A in the UV/O_3 is statistically different at different concentrations of peptone.

Conclusions

It was determined that both the use of the UV and UV/O_3 processes allowed achieving similar degrees of decomposition of female estrogens 17β-estradiol and 17α-ethinylestradiol. Degradation of the compounds in the processes occurred in a very short time. For the elimination of estrogens additional dosing of ozone is unnecessary. The estrogens were not completely removed from the solution despite the use of a complex treatment process. No distinct differences were observed among the treated solutions (deionised water, real effluent). In the case of bisphenol A, it was shown that the combined use of irradiation and ozonation is more advantageous than the use of the single photolysis process. The degradation of bisphenol A during the treatment of the real effluent was higher than in the case of deionised water. This is related probably to the presence of photosensitizers in the real effluent, which supports the process of decomposition of bisphenol A. In this study it was proved that peptone is an example of such a substance.

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Abstrakt: Obecność mikrozanieczyszczeń organicznych, a szczególnie substancji zakłócających procesy hormonalne z ang. *Endocrine Disrupters Compounds* EDCs w wodach powierzchniowych stanowi potencjalne zagrożenie dla zdrowia i życia organizmów żywych, w tym również dla człowieka. Niebezpieczna aktywność biologiczna tych związków (w tym również toksyczność) oraz duża odporność na biodegradację stwarza konieczność prowadzenia badań nad ich usuwaniem w konwencjonalnych i niekonwencjonalnych procesach uzdatniania wody i oczyszczania ścieków. W oparciu o coraz częstsze doniesienia w literaturze przedmiotowej dotyczące skuteczności zastosowania różnych technik zaawansowanego utleniania do eliminacji mikrozanieczyszczeń podjęto badania nad oceną efektywności eliminacji 17β-estradiolu (E2), 17α-etynyloestradiolu (EE2) oraz bisfenolu A (BPA) z rzeczywistego i modelowego odpływu z oczyszczalni ścieków komunalnych w procesie fotolizy (UV) oraz w procesie łącznym fotoliza i ozonowanie (UV/O_3). Uzyskane wyniki badań porównano pod kątem skuteczności rozkładu badanych związków dla wody zdejonizowanej. Określono, że zarówno zastosowanie procesu UV, jak i UV/O_3 umożliwiło osiągnięcie wysokich stopni rozkładu dla 17β-estradiolu oraz 17α-etynyloestradiolu bez względu na rodzaj oczyszczanego roztworu.

Rozkład związków następował w bardzo krótkim czasie trwania procesów. Natomiast, efektywność eliminacji bisfenolu A zależała od zastosowanego procesu oraz składu fizykochemicznego roztworu. Wykazano, że połączenie fotolizy z ozonowaniem umożliwiło osiągnięcie wyższego stopnia rozkładu badanego związku, niż w przypadku pojedynczego procesu UV. Przeprowadzone wyniki badań umożliwiły również identyfikację substancji wspomagających rozkład bisfenolu A w procesie UV/O_3.

Słowa kluczowe: eliminacja mikrozanieczyszczeń, EDCs, bisfenol A, 17β-estradiol, 17α-etynyloestradiol, oczyszczenie ścieków, fotoliza, fotoliza i ozonowanie

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ANALYSIS AND EVALUATION OF SEWAGE TREATMENT PLANT OPERATION

ANALIZA I OCENA FUNKCJONOWANIA OCZYSZCZALNI ŚCIEKÓW

Abstract: The paper presents the analysis and evaluation of six sewage treatment plants. The effectiveness of wastewater treatment was assessed for the following treatment facilities: Barcza (O1), Opatow (O2), Ostrowiec (O3), Starachowice (O4), Sandomierz (O5) and Piaseczno (O6). The evaluation of plant operation was based on the amount of reduction of five selected pollution indicators: BOD_5, COD, total suspension, total nitrogen and total phosphorus. In addition, COD/BOD_5 ratios were determined, the reliability coefficient (RC) was calculated and the degree of use of sewage treatment plant capacity, in relation to pollutant loads, was assessed. The paper also analyzes the non uniformity coefficient that characterizes variation in sewage flow.

Keywords: waste water treatment plant, BOD_5, biological treatment, removal efficiency

Introduction

Wastewater introduced into a water body should not cause any physical, chemical and biological changes [1]. The task of wastewater treatment plants is, among others, to ensure proper functioning of the environment and to protect clean water resources. This can be done only by a correctly operating sewage treatment plant [2]. The reliability of a sewage treatment plant is defined as its ability to dispose of the estimated amount of sewage. The latter need to be purified to the extent required by the wastewater receiver, under specified conditions of the plant operation, during the intended period of the plant service, and at random changes in the characteristics of the plant functional components [3]. When sewage composition and properties are altered (removal of pollutants such as nutrients, refractory compounds, pathogenic microorganisms, easily falling and colloidal suspensions, organic compounds), it is possible to discharge sewage into the receiver with a minimal risk to human and animal health [4]. According to the data [5], approx. 24.6 thousand dam^3 liquid waste was collected in 2010, which constitutes an

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increase of 5 % on the previous year. Besides, the Treaty of Accession provides that the European Union legislation on the disposal and treatment of urban wastewater will fully come into effect in Poland from 31 December 2015 [6]. Consequently, sewage treatment plants will not only have to comply with more restrictive EU regulations, but also to cope with an increasing amount of sewage. It is likely that not all facilities will be able to meet new requirements, and some irregularities in the operation sewage plants may occur. Incorrectly operating treatment plants can pose a threat to the safety of the people living in the vicinity of the plant, and also to recreational use of wastewater receivers, lakes and rivers, by the public [7]. The paper presents the evaluation of five sewage treatment plants in the Swietokrzyskie Province and one in the Mazowieckie Province. Sewage treatment plants under consideration are located in: Barcza (O1), Opatow (O2), Ostrowiec (O3), Starachowice (O4), Sandomierz (O5) and Piaseczno (O6).

General characteristics of the objects O1–O6

The sewage treatment plant O1 is located in the south-eastern part of the Zagnańsk Commune, in the grounds of Gruszka village. It is intended for treatment of domestic sewage from the villages: Barcza, Gruszka, Jaworze, Kajetanow, Lekomin, Zablocie. The plant O1 was put into service in 2003, and its target capacity is expected to reach 520 m^3/d. Because of the degree of sewerage in the catchment, the plant has been designed to operate in two stages. Currently, the sewage treatment plant operation is described as stage I ($Q_{davrg.}$ = 250 m^3/d). The object O1 is a mechanical-biological treatment plant intended for integrated removal of carbon, nitrogen and phosphorus compounds. It is based on EvU-Pearl technology, which combines a three-phased activated sludge method with separate anaerobic, anoxic, aerobic zones and whirled biological sludge – moving-bed biofilter methods. Biofilter which fills the biological reactor are EVU-Pearl cylindrical hollow forms of bulk volume specific surface 800 m^2/m^3. Biological phosphorus removal process may be assisted by chemical precipitation with ferric sulfate (PIX) coagulant. Treated effluent is discharged into the receiver, which is the river Lubrzanka. The plant is equipped with a sludge treatment line, where aerobically stabilized sludge is subjected to gravitational compaction and mechanical dewatering.

The wastewater treatment plant (WWTP) O2 is located in the Sempolowskiej street in Opatow. Sewage from the town of Opatow flow through gravity sewers into the raw sewage pumping station, from where it is pumped into the expansion chamber, situated within the facility. Then the wastewater flows by gravity through the mechanical and manual screens, and also through a vertical grit chamber, where wastewater is cleaned mechanically. In the next stage, wastewater is directed to the activated sludge chamber, where under varying substrate and aerobic conditions, it receives full biological treatment. In addition, phosphorus removal can be fostered by dosing PIX 113 formulation. A mixture of treated sewage and the activated sludge is further transferred to the vertical secondary settlement tanks, in which sedimentation takes place. After sedimentation, the sludge is pumped through the recirculated sludge chamber back to the activated sludge chamber. Cleaned effluent flows by gravity through measurement flume into the receiver, which in this case is the river Opatowka.

The municipal wastewater treatment plants O3 is located at Mostowa Street 72, within the administrative boundaries of the town of Ostrowiec, in the south-east of the town. Municipal sewage from the town is delivered by the municipal collecting sewer to the wastewater treatment plant. The facility O3 is a mechanical-biological treatment plant. The mechanical component of the facility starts with a coarse screen mounted in a collection well, from where the sewage flows down by gravity to the raw sewage pumping station I°. The latter is equipped with submersible pumps, which force the sewage through two channels into the stilling chamber, and further to the step grates. A sand separator receiving sand pulp from the vortex grit chamber is also located in the screening building. From the grit chamber, sewage flows by gravity to the pumping station II, and then it is forced into the flow splitting chamber situated before primary circular sedimentation basin. Clarified effluent from preliminary settling tanks is piped by the gravity through overflows and overflow troughs to the multifunctional reactors in two parallel treatment lanes. Each of the reactors consists of four chambers, namely those of: dephosphatation, predenitrification, denitrification and nitrification. From the biological reactor, effluent flows through an overflow to the secondary settling tanks. Treated effluent from the wastewater plant is discharged through the measurement flume to the river Kamienna.

The WWTP O4 has been in service since 1962. For the first time, the expansion and modernization of the wastewater treatment was carried out in the 80s. The next upgrading of the facility took place in 1999–2002 and was aimed at increasing the efficiency of wastewater treatment and removal of nutrients. The separation of floating and dragged pollutants is carried out in the screening building on the mechanical screen. The material retained (screenings) is transported to the press-rinse; pressed screenings are emptied to the container. The removal of sand takes place in two vertical sand traps. The sand collected in the hopper is removed periodically by pumping through a sand separator and scrubber into an above-ground tank located in the pumping station. The separation of suspended solids is performed in the primary circular sedimentation basin. Clarified effluent is discharged into the sewage pumping station II, and the drained sludge is passed to the sludge pumping station, and further to the existing sludge management facilities. Floating parts are removed to the pumping station and to the existing sludge facilities. The process of biologically activated sludge treatment in the O4 is performed on two process lines, each consisting of a biological reactor, secondary settling tank, recirculation pumping station, and the blowers station. The biological reactor is a three-chamber tank with activated sludge, in which the process of biological removal of carbon compounds, nitrogen and phosphorus takes place. The separation of activated sludge from treated wastewater is performed in the circular secondary clarifier. Clarified effluent is discharged by gravity through a measuring device into the receiver. The sludge separated in the secondary clarifier is recirculated through the sludge recirculation pumping to the biological reactor and the excess of the sludge is transferred, after mechanical compaction, to the existing sludge facilities. It is possible to promote the removal of total phosphorus by dosing the solution of iron (III) sulphate (VI).

The sewage treatment plant O5 provides treatment services to a larger part of the town, the wastewater is carried there, by gravity, from the left and right bank of the river. In addition, approx. 900 tanks without drainage are located in the city. The O5 is a mechanical-biological treatment plant, located on the left bank of the Vistula River and its nominal capacity is 7500 m^3/d. The plant operation has continued without interruption since 1998. The technological system of the wastewater treatment plant comprises mechanical and biological parts. The mechanical part employs the processes of separation of floating and dragged pollutants trailing on the step grate, as also the mechanical plane grating and sedimentation of sand in the vortex girt chamber. The grit chamber installed in a separate room in the screening building is used for sand separation. The biological method is based on activated sludge with the enhanced removal of nutrients. The basic elements of this part are two integrated multi-function circular bioreactors, which comprise a centrally located secondary clarifier and activated sludge chambers located along the circumference.

The wastewater treatment plant O6 is located in the eastern part of the town of Piaseczno, between Żeromskiego Street and Mazurska Street. It purifies the sewage flowing through the separate sewer system and the sewage transported to the discharge point at the sewage treatment plant with road tanker vehicles. The facility uses mechanical-biological treatment of wastewater with the biological dephosphatation, denitrification and nitrification, and with simultaneous chemical precipitation of phosphorus. It has a technological line, where, in the first stage, wastewater is subjected to mechanical methods of purification. The second stage involves biological treatment in the dephosphatation chamber and four chambers of activated sludge with an option of simultaneous phosphorus removal. Two of those chambers, together with two secondary clarifiers constitute the emergency system of the treatment plant. From denitrification and nitrification chambers, wastewater is carried to the circular secondary clarifier, from where, when the sludge is settled and clarified, it is discharged into the receiver.

Quantitative analysis of wastewater

The basic flow describing discussed treatment plants O1–O6 are summarized in Table 1.

Table 1

Characteristic wastewater flows in objects O1–O6

Treatment plant	Q_{davrg} [m^3/d]	Q_{dmax} [m^3/d]	Q_{hmax} [m^3/h]
O1	250	300	25
O2	959	1 950	210
O3	13 137	20 200	630
O4	15 200	18 200 (dry weather) 24 000 (wet weather)	1 100
O5	7 500	10 000	625
O6	17 152	24 870	—

The facility O1 is the smallest of the plants discussed. Presently, the sewage load expressed as the population equivalent amounts to PE = 2250, but from the design data PE is expected to increase to 3833. In accordance with the design data, the O2 sewage load population equivalent is 21125, but the actual PE-based value of the maximum average weekly load for the calendar year 2010 was 15243. For the wastewater treatment plant O3, the design predicts the sewage load population equivalent to amount to 88060. The actual PE value for the O3 facility, based on the maximum average weekly load, is 50142. The wastewater treatment plant O4 [8], as well as the facilities O2–O4, belong to the fourth group with PE ranging from 15000 to 99999. As regards the treatment plant O5, the PE real data was 31933. The treatment plant O6 sewage load expressed with the population equivalent is currently 121779, and its target size is estimated at PE = 163500. Variability of wastewater flow is defined by the non-uniformity coefficient. It represents the ratio of the maximum daily flow of sewage to the average daily flow:

$$N_{dmax} = Q_{dmax} \; / \; Q_{davrg} \tag{1}$$

The value of the coefficient of daily nonuniformity N_{dmax} depends on the number of inhabitants and according to [9] are:

- in large cities (R > 25000) 1.2,
- in medium-sized cities (10000 < R < 25000) 1.5,
- in small towns (5000 < R <10000) 1.75,
- in small settlements (R <5000) 2.0.

Summary of ratio values for each object N_{dmax} O1–O6 are presented in Fig. 1. N_{dmax} values according to Fig. 1 are in the range 1.20–1.58. High value refers to the object O4 and the lowest to the treatment O1. The calculations for the analyzed objects O1–O6 is clear that the values N_{dmax} according to the literature data are somewhat higher than those calculated for the actual values. This means that the treatment plants O1–O6 are not so vulnerable to changing flow conditions – so their work is more stable.

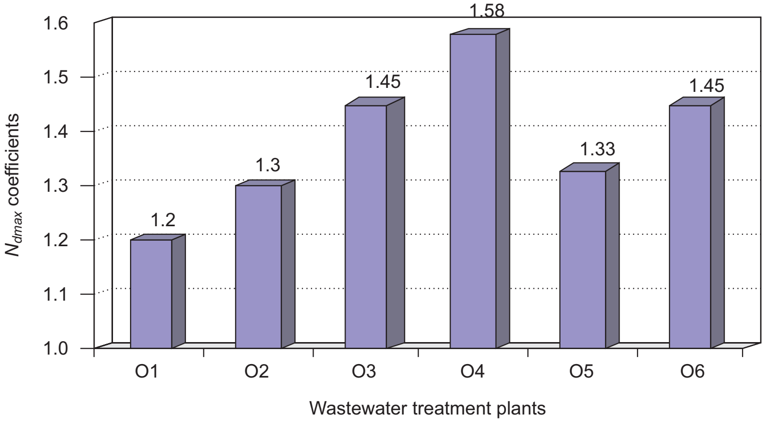

Fig. 1. Coefficient of nonuniformity N_{dmax} for objects O1–O6

Qualitative analysis of wastewater

Malfunctioning of wastewater treatment plants usually leads to environmental pollution. The resultant deterioration of water quality in the receiver depends on the type of irregularities or failures and their duration [10]. The mere fact that a sewage treatment plant has been constructed does not relieve the operators from the obligation to maintain appropriate quality of the effluent. It is necessary to collect raw sewage and effluent samples and then to determine the values of pollution indicators on current basis. With these data, it is possible to assess the effectiveness of removing pollutants and to determine the reliability coefficients for the various indicators of sewage pollution [11]. On the basis of [8], it should be noted that, in terms of the PE size, sewage treatment plants under consideration can be categorized into three different groups. Therefore, the requirements for effluent quality are different, which is shown in Table 2.

Table 2

The highest permissible values of indicators and minimum percentages of pollutant reduction in accordance with [8]

Indicator	The highest permissible values of indicators and minimum percentages of pollutant reduction for PE		
	from 2000 to 9999 (O1)	from 15000 to 99999 (O2–O5)	100000 and above (O6)
BOD_5	25 mgO_2/dm^3 or 70–90 %	15 mgO_2/dm^3 or 90 %	15 mgO_2/dm^3 or 90 %
COD	125 mgO_2/dm^3 or 75 %	125 mgO_2/dm^3 or 75 %	125 mgO_2/dm^3 or 75 %
Total suspension	35 mg/dm^3 or 90 %	35 mg/dm^3 or 90 %	35 mg/dm^3 or 90 %
Total nitrogen	15 mgN/dm^3	15 mgN/dm^3 or 80 %	10 mgN/dm^3 or 85 %
Total phosphorus	2 mgP/dm^3	2 mgP/dm^3 or 85 %	1 mgP/dm^3 or 90 %

Each of the sewage treatment plants has a valid sewage effluent disposal consent for effluent discharge into the receiver. In accordance with the document mentioned above, effluent discharged into a water body or soil can not exceed certain values. Actual values are at the same level (O4–O6), and in two cases they are lower (O2 and O3) than those stated by the regulation [8].

The criterion adopted to assess the plant efficiency is a percent to which the values of individual pollution indicators are lowered

$$\eta = (S_i - S_e) / S_\mathrm{i} \cdot 100\ \% \tag{2}$$

where: S_i – concentration of a particular pollutant type in wastewater flowing into the wastewater treatment plant [mg/dm^3],

S_e – concentration of a particular pollutant type in effluent treated and discharged to the receiver [mg/dm^3].

As shown in Fig. 2, the percent of pollution reduction in the wastewater plants under consideration is within the following ranges:

– for BOD$_5$, from 93.2 % (O4) to 99.6 % (O1),
– for the COD, from 80.5 % (O3) to 97.9 % (O2),
– for suspended solids, from 81.3 % (O3) to 99.6 % (O6),
– for total nitrogen, from 57.2 % (O4) to 95.3 % (O6),
– and for total phosphorus, from 76.2 % (O1) to 98.3 % (O6).

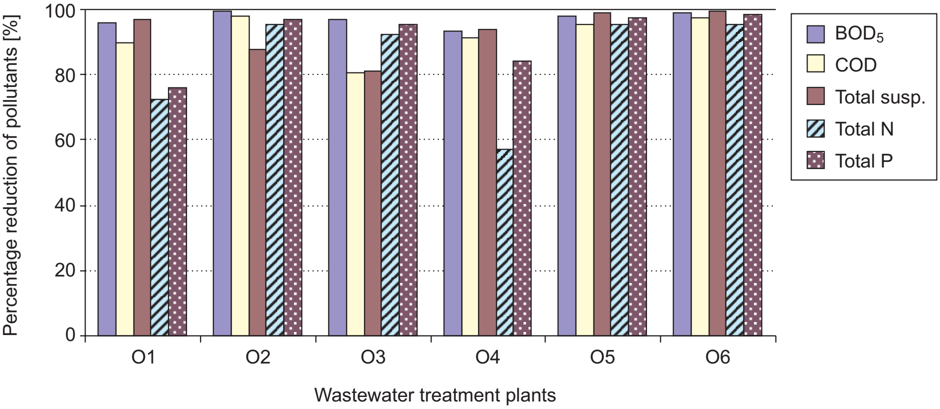

Fig. 2. The percentage reduction in the value of individual indicators of pollution

In the case of the treatment plant O1, although high percents of reduction in COD and total nitrogen have been achieved, the other indicator values in the effluent are higher than those recommended by the regulation [8]. The sewage plant O3 has been highly effective in nutrient removal (Fig. 2), which makes it possible to abandon using coagulants to assist the dephosphatation process, resulting in lower sludge production. Alarmingly low levels of total nitrogen reduction present in the case of O4 may result in penalties for failure to comply with the sewage effluent disposal consent. That can also lead to the accumulation of nitrogen compounds in the receiver, which in turn, can cause the intensification of eutrophic processes.

To determine the reliability of the wastewater treatment plants, a reliability ratio (WN) [11] was used, presented in Table 3. It was calculated for pollution indicators in all objects using the formula [11]:

$$WN = \mu_x / X_{lim} \tag{3}$$

where: μ_x – mean value of pollution indicator in treated effluent [mg/dm^3],
X_{lim} – permissible value of pollution indicator in treated effluent [mg/dm^3].

Low values of the reliability coefficient for the analyzed pollutants indicate effective operation of sewage treatment plants. Excessively high values of the coefficient occur in

Table 3

Reliability coefficient for sewage treatment plant O1–O6

Indicator, for which reliability coefficient is calculated	O1	O2	O3	O4	O5	O6
BOD_5	0.9	0.3	0.4	0.8	0.8	0.3
COD	1.0	0.3	0.2	0.4	0.3	0.2
Total suspension	0.7	0.2	0.2	0.5	0.1	0.1
Total nitrogen	1.9	0.4	0.6	1.7	0.3	0.4
Total phosphorus	2.5	1.1	0.4	0.6	0.1	0.4

the following treatment plants: O1 (for COD, total N, and total P), O2 (for total P), and also O4 (for total N). Despite high values of the reliability coefficient, relatively high values of pollution reduction are obtained in all the treatment plants, which is shown in Fig. 2.

To test the quality of the analysis of raw and treated wastewater, the correlations between indicators of pollution, *ie* COD and BOD_5, are established. For raw sewage, two cases are considered: $COD/BOD_5 < 2$ (readily biodegradable wastewater) and $COD/BOD_5 > 2$ (hard biodegradable wastewater) [12]. For the effluent $COD/BOD_5 = 5–6$.

In sewage plants O2 and O5 (Fig. 3), raw sewage is easily biodegradable ($COD/BOD_5 < 2$). In the remaining four cases, the ratio of COD to BOD_5 is greater than 2, which means the wastewater is hard biodegradable. In objects O2 and O6, the COD/BOD_5 ratio in treated sewage is higher (7.3 and 6.7, respectively) than those described in the literature, also in the O5, the value is lower than in the literature and amounts to only 3.5.

Typically, while designing objects such as sewage treatment plants, prognostic data on wastewater quantity and quality are relied on. Consequently, when the facility has

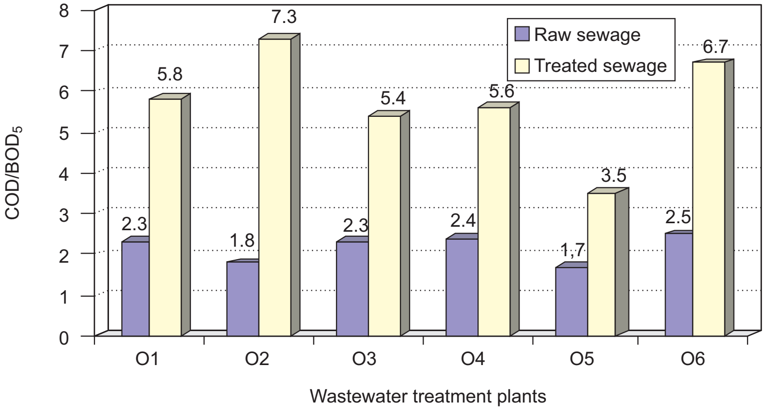

Fig. 3. Correlations between COD and BOD_5 pollution indicators

started operating, it may turn out that the actual data differ significantly from the design data. Discrepancies between the design and real data may result in irregularities in the operation of wastewater treatment plants [13].

The utilization percent of sewage treatment capacity with respect to pollution loads was calculated from the dependence [13]:

$$\omega_i = L_R / L_P \cdot 100 \ \% \tag{4}$$

where: L_R – the actual average daily load of pollutants contained in wastewater influent to the treatment plant expressed in kg/d;
L_P – the average daily load of pollutants contained in wastewater influent to the treatment plant according to the design data.

Table 4 clearly indicates that all these facilities operate under extremely changeable conditions varying from underload to overload with various pollution types. The lack of stability in influent loads may significantly affect the quality of treated wastewater and sewage treatment plant operation. Therefore, it is necessary to control the inflowing loads, so that they were at a fairly constant level, and as close as possible to the design assumptions. It should also be emphasized that in spite of differences in ω_i for individual pollutant, the objects achieved very high levels of pollution reduction. Also, in most cases, they did not exceed the pollutant limit for treated wastewater discharged into the receiver.

Table 4

The utilization percent of sewage treatment capacity in O1–O6

Indicator, for which ω_i is calculated	O1	O2	O3	O4	O5	O6
BOD_5	74.9	101.7	58.9	66.3	59.6	—
COD	65.6	109.6	84.2	87.4	65.6	—
Total suspension	39.4	45.9	64.8	138.8	63.8	—
Total nitrogen	—	163.7	80.3	88.5	65.3	—
Total phosphorus	—	66.7	63.6	93.5	49.4	—

Summary and conclusions

The sewage treatment plants O1–O6 can be categorized into three different groups with respect to load expressed by the population equivalent (from 2250 to 121779). Despite differences in load size, all facilities operate satisfactorily. They produce very good results in wastewater treatment and comply with the limit values and sewage effluent disposal consent. Only in the treatment plant O1, in spite of achieving high degrees of pollution reduction for COD and total nitrogen, effluent values are higher than those recommended by the regulation. In most cases, low reliability coefficients for the analyzed pollutants were obtained, which indicates correct operation of sewage treatment plants. It should also be noted that although working under highly variable load conditions as regards individual pollutants, all treatment plants reached very high levels of reduction.

Lack of sewage treatment plants or sewerage would be a major barrier to the development of any region. That would lower the attractiveness of an area to potential residents and investors. Due to the operation of sewage treatment plants, uncontrolled wastewater discharge into water and soil has been substantially reduced. To conclude, positive effects produced by wastewater treatment plants fully balance their possible adverse impact on the environment.

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ANALIZA I OCENA FUNKCJONOWANIA OCZYSZCZALNI ŚCIEKÓW

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Abstrakt: W pracy przedstawiono analizę oraz ocenę funkcjonowania sześciu oczyszczalni ścieków. Ocenie efektywności systemu oczyszczania poddano oczyszczalnie ścieków Barcza (O1), a także w Opatowie (O2), Ostrowcu Świętokrzyskim (O3), Starachowicach (O4), oraz w Sandomierzu (O5) i Piasecznie (O6). Oceny poprawności działania oczyszczalni oparto na wielkości redukcji 5 wybranych wskaźników zanieczyszczeń: BZT$_5$, ChZT, zawiesina ogólna, azot ogólny i fosfor ogólny. Poza tym dokonano obliczeń stosunku

ChZT/BZT$_5$, jak i również obliczono współczynnik niezawodności WN oraz stopień wykorzystania przepustowości oczyszczalni ścieków ze względu na ładunki zanieczyszczeń. W pracy przeanalizowano również wartość współczynnika nierównomierności, który charakteryzuje zmienność dopływu ścieków.

Słowa kluczowe: oczyszczalnia ścieków, BZT$_5$, biologiczne oczyszczanie ścieków, skuteczność usuwania zanieczyszczeń

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IMPROVING OF ANAEROBIC DIGESTION BY DRY ICE DISINTEGRATION OF ACTIVATED SLUDGE

DEZINTEGRACJA OSADU CZYNNEGO SUCHYM LODEM W CELU INTENSYFIKACJI FERMENTACJI METANOWEJ

Abstract: Disintegration by dry ice has a positive effect on the degree and rate of surplus activated sludge anaerobic digestion. By applying thermal disintegration the lysis of cells occurs in minutes instead of days. The intracellular and extracellular components are set free and are immediately available for biological degradation which leads to an improvement of the subsequent anaerobic process. Thermal disintegration by dry ice of the surplus activated sludge results in organic matter and a polymer transfer from the solid phase to the liquid. During the disintegration process, soluble chemical oxygen demand (SCOD) value and proteins concentration increase about 583 mg/dm^3 and 265 mg/dm^3, respectively. At the same time the concentration of carbohydrates increase about 53 mg/dm^3. In addition the degree of thermal disintegration changed from 13 % for the volume ratio of dry ice to surplus activated sludge 0.25:1 to 49 % for the volume ratio of dry ice to sludge 1:1.

The addition of thermal disintegrated sludge (30 %SASDI of volume) to the digestion process leads to increased biogas production about 49 %.

Keywords: dry ice, soluble chemical oxygen demand, proteins, carbohydrates, anaerobic digestion, biogas

Introduction

The aim of wastewater treatment is to mineralise organic matter and enhance nutrient removal. Anaerobic digestion is a common method for surplus activated sludge stabilization resulting in the reduction of sludge volatile matter and the production of biogas. Anaerobic degradation of biomass is considered to follow a sequence of four phases: hydrolysis, acidogenesis, acetogenesis and methanogenesis. The slow degradation rate of surplus activated sludge in the anaerobic digestion process is due to the

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rate-limiting step of sludge hydrolysis. Therefore, the disintegration pretreatment of a surplus activated sludge process using physics (thermal) [1, 2], chemical (using *eg* acids) [3], mechanical (ball mill, ultrasonic) [4–6], oxidation (ozone and hydrogen peroxide) [7, 8], or biological (using enzymes) [9–11] and thermal (heat treatment, freezing/thawing) [12–14] treatment processes, can improve the subsequent anaerobic digestion. Although the methods are different, the aim of all of them is partial or complete bacterial cell rupture, *ie* destruction of the cell wall and release of organic substances present inside the cells to the liquid phase. Thermal disintegration by dry ice has a positive effect on the degree and rate of sludge anaerobic digestion.

The freeze/thaw frequently found in nature, which leads to changes in soil. This process is used in the municipal wastewater treatment for dewatering of sewage sludge. Sludge dewatering by freezing is done by separating the solid and liquid fractions during the formation of ice crystals. It was also found that the refrigeration mechanism is conducive to converting the form of flocs in a more compact, dense [15].

Freezing/thawing using by dry ice is an effective technique for dewatering, sewage sludge and its attention was already 1990 by Vesilind and Martel [16]. Dry ice transforms the structure of flocs into larger agglomerates and reducing water-related because the process of freeze/thaw by dry ice is becoming more and more popular [17–21].

Basic mechanisms of freeze/thaw sludge used as a method of conditioning of sludge prior to anaerobic stabilization are increasing interest due to the reduction of pathogenic bacteria, reduce sediment and increase the biomass production of biogas [22, 23].

Dry ice is a completely natural product. It is produced in the form of granules for compressing gaseous carbon dioxide to liquid form, removing the heat generated by compression, and then rapid expansion. This expansion and rapid evaporation of carbon dioxide gas remaining fluid which cooled to the melting point and freezing in the CO_2 "snow", which takes the form of beads or prills. The dry ice sublimes at $-78.5\ ^{o}C$ and a pressure of 1013.25 hPa. Its heat of sublimation is 573 kJ, which means that it is approximately 3.3 times more effective coolant than water ice (with the same volume). Its specific gravity comprises in the range from 1.2 kg/dm^3 to 1.6 kg/dm^3, and its hardness on the Mohs scale is 2, which corresponds to the hardness of gypsum. It is anhydrous, non-flammable, non-toxic and has no smell or taste. It is intended as a catering, refrigeration, to clean all types of machines and laboratories to slow exothermic reactions [24, 25].

The new concept described in this paper is based on the combined process of surplus activated sludge disintegration by dry ice prior to anaerobic digestion. Thermal disintegration by dry ice can activate the biological hydrolysis process and therefore significantly increase the biogas production in anaerobic stabilization.

Materials and methods

Experimental material was surplus activated sludge taken from the municipal wastewater treatment plant in the south of Poland, working according to the Enhanced Biological Nutrient Removal (EBNR). The plant was designed for a flow of 120 000

m^3/d. At present the amount of treated wastewater is about 90 000 m^3/d. Solid retention time (SRT) is about 14 days and concentration of mixed liquid suspended solids (MLSS) 4.3–4.7 g/dm^3.

Thermal disintegration

The disintegration of surplus activated sludge, the following ratios by volume of dry ice to sludge, *ie*: 0.25:1; 0.5:1; 0.75:1; 1:1.

The chemical analysis

Soluble chemical oxygen demand (SCOD) and proteins value was determined for samples before and after each time of disintegration according to *Standard Methods* [26]. Carbohydrate concentration was determined according to Anthron methods [27].

The degree of disintegration

In order to obtain a quantitative measure of the effects of disintegration, Kunz and Wagner [28] have proposed a coefficient which they called the degree of disintegration (DD). Later this coefficient was modified by Müller [29]. In this paper the degree of sludge disintegration was determined according to that given by Müller [30] as follows:

$$DD = [(SCOD_1 - SCOD_2) / (SCOD_3 - SCOD_2)] \cdot 100 \ [\%],$$

where: DD – the degree of disintegration;
$SCOD_1$ – the SCOD of the liquid phase of the disintegrated sample;
$SCOD_2$ – the SCOD of the original sample;
$SCOD_3$ – the value after the chemical disintegration.

Chemical disintegration was done in this case by treating the sludge samples for 10 min at 90 °C after adding NaOH, 1 M, in a ratio of 1:2. Centrifugation in all cases was done for 10 min at 30 000 g.

The fermentation experiments

The anaerobic digestion experiments were performed in six glass fermenters (3 dm^3) operated in parallel at a temperature of 35 ± 1°C. Residence time was 23 days. The volume of producing biogas was determined by the liquid displacement method ever each day. Different rates of raw and disintegrated surplus activated sludge were applied:

– **Fermenter 1** was feed with surplus activated sludge (70 % volume of fermenter; 70 %SAS) and digested sludge (30 % volume of fermenter; 30 %DS),

– **Fermenter 2** was feed with 50 %SAS and 30 %DS and with surplus activated sludge after dry ice disintegration (20 % volume of fermenter; 20 %SASDI),

– **Fermenter 3** was feed with 40 %SAS and 30 %DS and 30 %SASDI,

– **Fermenter 4** was feed with 30 %SAS and 30 %DS and 40 %SASDI,
– **Fermenter 5** was feed with 20 %SAS and 30 %DS and 50 %SASDI,
– **Fermenter 6** was feed with 30 %DS and 70 %SASDI.

The aim of carrying out the experiment of sludge digestion was to show the possibilities to improve and accelerate the anaerobic process. The investigations presented here were performed in 5 stages, and arithmetic average and standard deviation were established. The standard deviation was determined according to the estimator of the highest credibility in STATISTICA 6.0.

Results and discussions

Increase of volume of freezing water in the cytoplasm of microorganisms causes them disruption by mechanical damage of the walls and cell membranes, osmotic shock and destruction of cellular organelles. These mechanical damage is caused by the formation of ice crystals in the environment surrounding of cells as well as in their interior, which leads to changes in their properties (denaturation) [31, 32]. These crystals cause damage and changes in properties of microbial cells, which leads to release of intracellular substances into the environment.

The disintegration of surplus activated sludge by dry ice resulted in the release of organic matter (expressed as SCOD). Disrupter of flocs and microbial cells of activated sludge leads to the release of intracellular organic compounds to liquid phase. The value of SCOD in supernatant liquid of surplus sludge 63 mg/dm^3 (Fig. 1), while during the dry ice disintegration process increase:

– for the volume ratio of dry ice to sludge 0.25:1, the value of SCOD was 210 mg/dm^3 (Fig. 1),

– for the volume ratio of dry ice to sludge 0.5:1, the value of SCOD was 363 mg/dm^3 (Fig. 1),

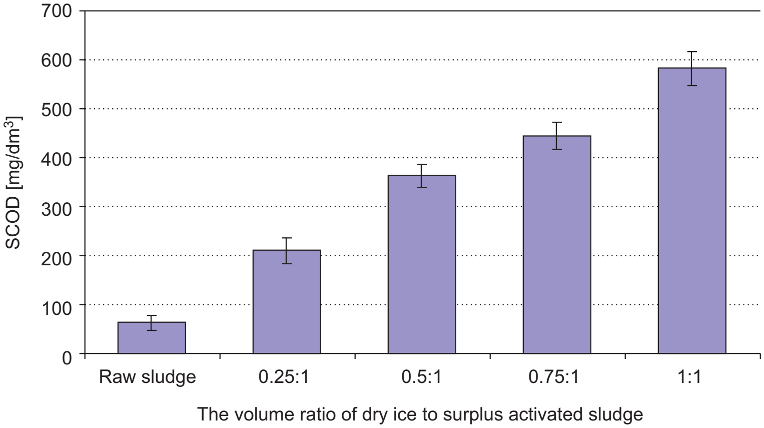

Fig. 1. Changes of SCOD value in the sludge supernatant before and after the disintegration

– for the volume ratio of dry ice to sludge 0.75:1, the value of SCOD was 445 mg/dm^3 (Fig. 1),

– for the volume ratio of dry ice to sludge 1:1, the value of SCOD was 583 mg/dm^3 (Fig. 1).

Freezing/thawing by dry ice of surplus activated sludge caused disruption of flocs structure and resulted in a different degree of disintegration (DD). The effect of dry ice (various volumes) on the sludge degree of disintegration was studied. The results of the experiments are presented in Fig. 2.

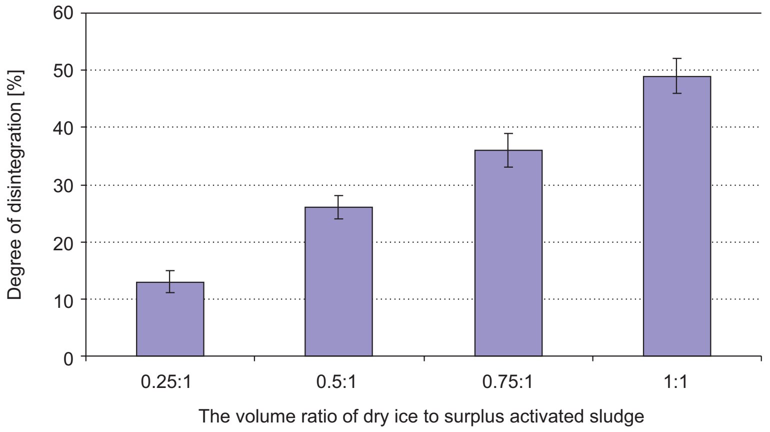

Fig. 2. Degree of surplus activated sludge disintegration by dry ice

Within the range of dry ice volume, between 0.25:1 to 1:1, the degree of disintegration increased most rapidly. The achieved degree of sludge disintegration was about 49 % (Fig. 2).

The effectiveness of the dry ice disintegration effect of these relations by volume of sludge to dry ice were as follows:

– for the volume ratio of dry ice to sludge 0.25:1, DD was 13 % (Fig. 2),

– for the volume ratio of dry ice to sludge 0.5:1, DD was 26 % (Fig. 2),

– for the volume ratio of dry ice to sludge 0.75:1, DD was 36 % (Fig. 2),

– for the volume ratio of dry ice to sludge 1:1, DD was 49 % (Fig. 2).

The disintegration of raw sludge resulted in the release of proteins. The increase of proteins concentration were associated with the destructive effects of dry ice in the cells, which resulted in the release of organic matter into the liquid phase. The proteins and carbohydrates concentration in untreated surplus activated sludge supernatant was 39 mg/dm^3 and 15 mg/dm^3, respectively. These concentrations increase with volume of dry ice (Fig. 3).

Freezing/thawing disintegration by dry ice accelerates the biological degradation of sludge. The cell liquid contains components, which upon being released, can be easily assimilated. The released organic substances (expressed here, as SCOD) as a result of

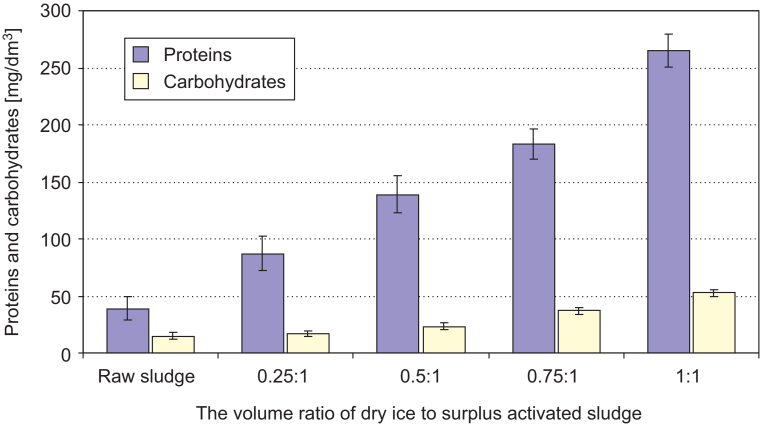

Fig. 3. Changes proteins and carbohydrates concentration before and after disintegration of dry ice

surplus activated sludge flocs disintegration, lead to a substantial increase of biogas production in the subsequent anaerobic sludge digestion process (Fig. 4).

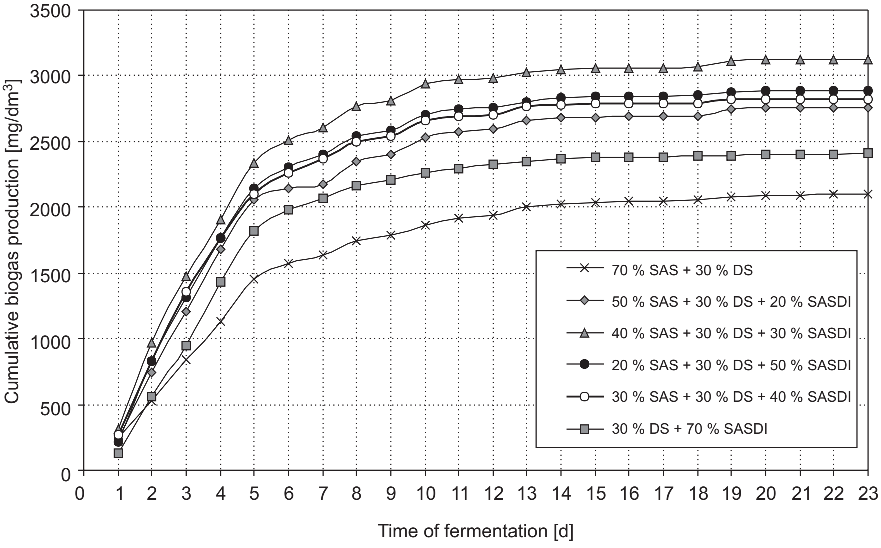

Fig. 4. Changes of cumulative biogas production during anaerobic digestion

Significantly higher amounts of biogas were produced in the fermenters feed with disintegrated activated sludge (20 %, 30 %, 40 %, 50 % or 70 % volume of fermenter). The gas production during sludge digestion depends on volatile solids, degree of disintegration (expressed as COD) and fermentation time.

Figure 4 shows the development of biogas production after 23 days of fermentation. Comparing the results it can be concluded that in the case of sample with addition disintegrated sludge in volume of 70 %, there was a smaller production of biogas (2 408 cm^3/dm^3) than in sample involving 40 % (2826 cm^3/dm^3) and 50 % (2890 cm^3/dm^3) of volume of surplus activated sludge after thermal destruction. Low biogas production is probably due to too high load of organic matter introduced into the digester. The samples with 30 % of volume of disintegrated surplus activated sludge produced 3 124 cm^3/dm^3 of biogas, which gives a 49 % more of biogas in comparison to the blank sample (70 % SAS + 30 % DS) (Fig. 4).

The organic matter transferred by dry ice treatment from the sludge solids into the liquid phase was readily biodegradable. Surplus activated sludge consists mainly of heterotrophic bacteria. The gradual break–up of the bacterial cell walls limits the degradation process. By applying freezing/thawing disintegration the lysis of cells occurs in minutes instead of days. The intracellular and extracellular components are set free and are immediately available for biological degradation which leads to an improvement of the subsequent anaerobic process. In Fig. 4, this is shown by comparing the increase of biogas production in the anaerobic digestion, post thermal disintegration. A calculation of energy consumption and cost shows that the disintegration by dry ice is an economically viable process. The surplus gas can be used for power and heat production. This energy yield can be used for the thermal disintegration of surplus activated sludge. The reduced cost of the sludge disposal, enhanced fermentation rates and acceleration of biogas production should lead to the practical use of disintegration by dry ice as a new technology.

Conclusions

The experiments have clearly demonstrated that thermal disintegration is a suitable method to destroy surplus activated sludge micro-organisms. In this study, the addition of disintegrated by dry ice raw sludge was examined in order to improve the anaerobic digestion process. The most important conclusions are:

– The disintegration by dry ice surplus activated sludge destroys the flocs structure of sludge and ruptures the cells of the micro-organisms. As a result of sludge disintegration, organic matter is transferred from the sludge, solid phase into the liquid phase (expressed as SCOD). SCOD increased from 63 mg/dm^3 to 583 mg/dm^3 in direct proportion with the time needed for disintegration.

– With increasing doses of dry ice, the degree of disintegration and increased volume ratio of the excess sludge to a dry ice 1:1 was 49 %.

– As a result of disintegration of surplus activated sludge dry ice, the destruction of cell structures of microorganisms and thereby increasing the concentration of proteins. In the settlement is not subjected to excessive disintegration of the dry ice, the concentration of protein amounted to 39 mg/dm^3, and the volume ratio of dry ice to precipitate 1:1, protein concentration was 265 mg/dm^3.

– An additional effect of the destructive action of dry ice on microorganisms excessive sludge was released into the supernatant fluid concentrations of organic matter expressed carbohydrates. With the increasing volume of dry ice to precipitate obtained significantly increasing the concentration of carbohydrates. For the ratio of the volume of dry ice to precipitate 1:1 carbohydrate concentration increased by 38 mg/dm^3.

– The thermal disintegration of surplus activated sludge leads to a higher degree of degradation and higher biogas production. Addition of disintegrated sludge (30 % volume of fermenter) to the anaerobic digestion stage resulted in increased biogas production, about 49 %.

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DEZINTEGRACJA OSADU CZYNNEGO SUCHYM LODEM W CELU INTENSYFIKACJI FERMENTACJI METANOWEJ

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Abstrakt: Jednym z podstawowych problemów występujących w układach przeróbki osadów ściekowych jest zwiększenie dostępności i podatności substancji organicznych na biodegradację, co można osiągnąć poprzez dezintegrację osadu.

W pracy wykorzystano dezintegrację osadu nadmiernego suchym lodem oraz określono jej wpływ na uwalnianie materii organicznej i na efektywność fermentacji metanowej wyrażonej produkcją biogazu.

Zamrażanie/rozmrażanie osadu suchym lodem powodowało wzrost wartości uwolnionego ($UChZT_{Cr}$) o 520 mg O_2/dm^3, a stopień dezintegracji (SD) wyniósł 49 %. W wyniku destrukcji osadu przy pomocy suchego lodu do cieczy nadosadowej zostały również uwolnione proteiny oraz węglowodany, co świadczyło o skuteczności procesu. Stężenia tych parametrów wyniosły odpowiednio 265 mg/dm^3 i 53 mg/dm^3.

Poddanie fermentacji mieszanki: osadu zdezintegrowanego w objętości 30 %, osadu niezdezintegrowanego w objętości 40 % i 30 % osadu przefermentowanego, spowodowało wzrost wydajności produkcji biogazu o 49 %.

Słowa kluczowe: suchy lód, $ChZT_{Cr}$, proteiny, węglowodany, fermentacja metanowa, biogaz

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ZINC UPTAKE FROM SOILS AT VARIOUS TIMES POLLUTED WITH HEAVY METALS

POBIERANIE CYNKU W RÓŻNYM CZASIE Z GLEB ZANIECZYSZCZONYCH METALAMI CIĘŻKIMI

Abstract: The interest in the subject of trace element concentrations and their behaviour in the environment is increasing, therefore investigations focused at determining abilities for harmful metals accumulation in plants growing on soil permanently or freshly polluted with heavy metals, contribute to development of this area of research. The paper aimed at an assessment of yielding and accumulation of zinc in buckwheat (*Fagopyrum esculentum* Moench) and sunflower (*Helianthus annuus* L.) cultivated on soils at various times polluted with this metal.

Much lower yields of buckwheat and sunflower were obtained on light soils, irrespective of supplementary mineral fertilization or the level and date of soil pollution with heavy metals. A toxic effect of heavy metals “freshly” applied to the soil was visible only on light soil, additionally fertilized with NPK. Plants cultivated on soils with higher content of these metals usually accumulated at least several times higher amounts of zinc, both in their shoots and roots. Additional NPK fertilization often increased Zn content. The largest amounts of zinc were absorbed by plants from light soils freshly and permanently polluted with heavy metals, on which additional mineral (NPK) fertilization was applied, and next from medium soil, freshly polluted with heavy metals and fertilized with NPK.

Keywords: zinc, pot experiment, heavy metals, sunflower, buckwheat

Introduction

Zinc is the element widely common in the environment, which constitutes about 0.007 % of the earth’s crust. It is a component of almost 100 enzymes and fulfills numerous important functions in plant metabolism [1]. Among others it influences plant resistance to drought and diseases, regulates cell membrane permeability and pro-

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portions of the key component content in cell [2]. Considering the microelements crucial for living organisms, zinc deficiencies are the cause of the greatest losses in crop yields worldwide [3].

Zinc presence in plants is connected not only with its content in soil, but also with soil ability to bind this element, which may result in either deficiency or sometimes excess, *ie* its toxic effect upon plants [4].

Plants show deficiency of this element at its contents from 15 to 20 mg · kg^{-1} d.m. through reduction of their biomass, leaf chlorosis, poor flower setting and early ageing of older leaves [5, 6]. Zinc deficiency in plan tissues causes production of reactive oxygen species, mainly immediately in result of disturbed ratio of this element concentration to Cu. The outcome of this process is inhibition of protein synthesis and increase in Fe accumulation leading to damage of cell membranes, chlorophyll and enzymes participating in the photosynthesis process, which is visible as chlorotic lesions on plant leaves [7].

Human activity, such as ore extracting and processing, generation of municipal and industrial sewage or agriculture, contribute to pollution with zinc of considerable areas of agricultural lands [8–12]. Beside anthropogenic pollution, in some regions so called fault soils, which normally contain considerable amounts of this element [13]. However, bioaccumulation of Zn from the soil in these regions is not the major hazard to plant or animal organisms. On the other hand, their correct development is threatened by cadmium and lead simultaneously present in the rock, which have a toxic effect both on plants and animals [14, 15]. Therefore, in order to reduce, among others, Zn accumulation in the food chain in these areas, a recultivation by means of phytoremediation has been suggested [12]. The process is effective at properly selected plant species and the best are so called hyperaccumulators which have the ability of taking up about 1 % of the available amount of metal available from the soil during one vegetation period [16, 17].

The paper aimed at an assessment of yielding and zinc accumulation in buckwheat (*Fagopyrum* Mill.) and sunflower (*Helianthus annus* L.) cultivated on soil at different times polluted with this metal.

Materials and methods

The pot experiment was conducted in the vegetation hall of the Faculty of Agriculture and Economics, University of Agriculture in Krakow, at Mydlniki near Krakow. Plastic pots with 7 kg of soil d.m. were used for the experiment. Light and medium soil with heavy metal content close to natural (collected in Mydlniki area) and light and medium soils polluted with heavy metals (collected in Bukowno region) were used for the experiment. Detailed data concerning physicochemical properties of the soil materials used for the experiment were presented in Table 1.

Zn concentrations in the soils originating from Bukowno area were generally several times higher in comparison with the soils from Mydlniki. The soils from Mydlniki were characterized by a neutral reaction, cation exchange capacity (assessed using Kappen's method) on the level of 105.5 mmol(+) · kg^{-1} (light soil – ML) and 504.2

mmol(+) · kg^{-1} (medium soil – MM) and organic carbon content (assessed by Tiurin's method) 12.1 (ML) and 25.2 g · kg^{-1} soil d.m. (MM), respectively.

Table 1

Physicochemical properties of soils used for the experiment

Parameter		Unit	Bukowno		Krakow-Mydlniki	
			Light soil	Medium soil	Light soil	Medium soil
Use		—	Coppice	Meadow	Wasteland	Meadow
Granulometric group		—	Loose sand	Sandy loam	Light loamy sand	Medium loam
pH_{H2O}		—	5.73	7.40	6.96	6.81
Hydrolytic acidity (Hh)		[mmol(+) · kg^{-1}]	29.8	8.40	10.50	5.70
Cation exchange capacity (CEC)			168.0	483.6	105.5	504.2
Organic C		[g · kg^{-1} d.m.]	16.4	29.3	12.1	25.2
Cd	Total form	[mg · kg^{-1} d.m.]	1.22	5.53	0.78	2.50
Pb			29.1	311.6	21.9	66.1
Zn			188.6	440.6	66.4	101.9

The soil materials from Bukowno area somewhat differed by the determined physicochemical properties. Light soil from Bukowno (BL) revealed pH = 5.73, cation exchange capacity 16.0 mmol(+) · kg^{-1} and organic C content 16.4 g · kg^{-1} soil d.m., whereas in case of medium soil (BM) pH was 7.40, cation exchange capacity 483.6 mmol(+) · kg^{-1} soil d.m. and organic C content 29.3 g · kg^{-1} d.m.

Water solutions of $CdSO_4 \cdot 7H_2O$, $(CH_3COO)_2Pb \cdot 3H_2O$ and $ZnSO_3 \cdot 7H_2O$ were added to light soil from Mydlniki (objects 9–12) to compare the possibilities of heavy metal uptake by plants from the soils "freshly" polluted and for a long time polluted with zinc. In this way cadmium, lead and zinc contents in these soils were equaled to the values assessed in the soils from Bukowno. In case of zinc, 122.2 mg Zn was added to the light soil and 338.7 mg Zn · kg^{-1} to medium soil (Table 2). The second factor was additional mineral fertilization applied in the objects 5–8 and 11–12.

Fertilizer components were applied under the forecrop (buckwheat) as NH_4NO_3, KH_2PO_4 and KCl in the following amounts:1.4 gN, 0.4 gP_2O_5 and 1.3 gK_2O per pot. Solutions of fertilizer NPK components and heavy metals were mixed with the whole soil mass in a pot one week prior to the first plant sowing.

The test plant, buckwheat (*Fagopyrum esculentum* Moench), "Panda" cv., was sown on 26 May 2011 and after its harvesting, an aftercrop – sunflower (*Helianthus annuus* L.) was sown on 25.07.2011 in the amount of 12 seeds per pot. During the plant vegetation the soil moisture was maintained on the level of 50 % (at the initial period) and then 60 % of the maximum water capacity. Water losses were supplemented with distilled water.

Table 2

Pot experiment design

No.	Object description	Object symbol
1	Bukowno light soil	BL
2	Bukowno medium soil	BM
3	Mydlniki light soil	ML
4	Mydlniki medium soil	MM
5	Bukowno light soil + NPK	BL + NPK
6	Bukowno medium soil + NPK	BM + NPK
7	Mydlniki light soil + NPK	ML + NPK
8	Mydlniki medium soil + NPK	MM + NPK
9	Mydlniki light soil + heavy metals	ML + h.m.
10	Mydlniki medium soil + heavy metals	MM + h.m.
11	Mydlniki light soil + heavy metals + NPK	ML + h.m. + NPK
12	Mydlniki medium soil + heavy metals + NPK	MM + h.m. + NPK

Zinc content was assessed in the harvested plant material (tops and roots) after previous mineralization in a muffle furnace and digestion in nitric(V) and hydrochloric acids, using ICP-OES Optima 7300 DV, atomic emission spectrometer (PerkinElmer).

Results and discussion

The yield of buckwheat and sunflower and zinc content in the yields were statistically verified using analysis of variance, while a multiple comparison procedure was conducted basing on Tukey’s test, at $\alpha \leq 0.05$. Columns marked with the same letter do not differ significantly at significance level $\alpha \leq 0.05$. Yielding and zinc concentrations were presented separately for the tops and roots, whereas Zn uptake as a sum of this element amount absorbed from a pot with tops and roots yield of buckwheat and sunflower.

On the control objects, *ie* on the soils from both Bukowno and Mydlniki, without an additional NPK fertilization and without heavy metal supplement, a similar yielding of the test plant shoots and roots was registered, however the yields of tops gathered particularly from light soils were visibly smaller (Fig. 1).

Applied mineral fertilization of the analyzed soils, both those strongly polluted with heavy metals and with natural content of these elements, markedly increased the cultivated crops yielding. It was more noticeable for buckwheat, under which NPK fertilization was applied and light soil from which about 4-fold bigger total dry mass yields were harvested jointly from both cultivated crops on the objects receiving NPK treatment. Application of heavy metal salts solutions to the soils from Mydlniki (ML and MM), equalizing their level to the content registered in the soils from Bukowno (BL and BM), did not decrease the yield of buckwheat tops and roots or sunflower cultivated as an aftercrop.

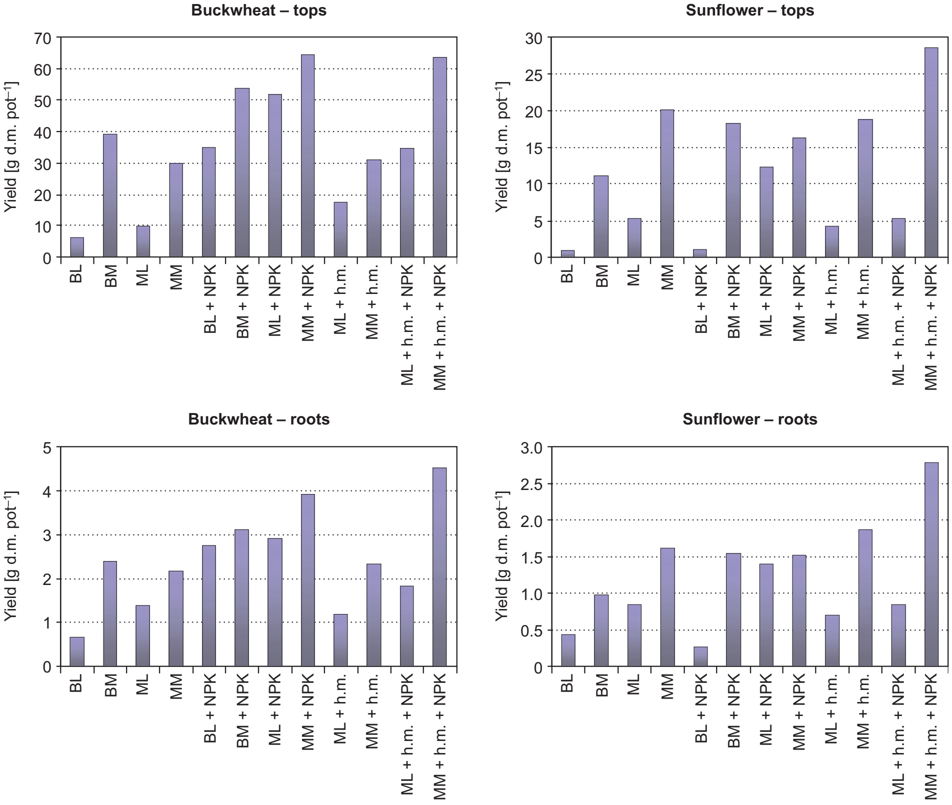

Fig. 1. Yields of buckwheat and sunflower tops and roots

A comparison of crop yielding on the artificially polluted objects (ML and MM) with plant biomass harvested from "soils naturally polluted with heavy metals"(BL and BM), did not reveal a decline in yield in result of introducing soluble forms of the analyzed metals to the soil from Mydlniki. On the other hand, a toxic effect of heavy metals "freshly" applied to the soil became evident in the object with light soil additionally fertilized with NPK (ML+h.m.+NPK) where significantly smaller yield of both buckwheat and sunflower was gathered. In case of medium soil, not such effect was noted and even a larger yield of sunflower aboveground parts was harvested from the soil fertilized with NPK with heavy metal supplement.

As might have been expected, zinc accumulation in plants cultivated on the soil from the lead-zinc ore extraction region (BL and BM) was much higher than in the plants cultivated on soil from the vicinity of Krakow (ML and MM) (Table 3).

The shoots of buckwheat and sunflower gathered from light soil from Bukowno (BL) accumulated over 12-fold more of zinc that assessed in the analogous soil materials from Mydlniki (ML). In case of medium soil, the differences were about 2.5-fold. Additionally NPK fertilization of the analyzed soils, despite markedly increased yielding, did not lead to so called "dilution effect" of the element in a larger yield [5, 18].

Table 3

Zinc (Zn) content in tops and roots of buckwheat and sunflower [mg · kg^{-1} d.m.]

Objects	Buckwheat		Sunflower	
	tops	roots	shoots	roots
BL	574.1^{c}	239.0^{ef}	911.5^{bc}	1573.6^{c}
BM	121.9^{a}	52.4^{abc}	89.1^{a}	81.2^{a}
ML	47.4^{a}	88.9^{abc}	72.1^{a}	116.6^{a}
MM	50.9^{a}	22.4^{a}	36.4^{a}	40.5^{a}
BL + NPK	538.4^{c}	247.4^{g}	1424.9^{c}	1990.8^{d}
BM + NPK	99.9^{a}	65.7^{abc}	95.2^{a}	70.7^{a}
ML + NPK	95.5^{a}	55.8^{abc}	150.6^{a}	97.2^{a}
MM + NPK	67.3^{a}	47.5^{ab}	48.6^{a}	48.3^{a}
ML + h.m.	225.3^{b}	94.8^{bc}	551.4^{bc}	747.9^{b}
MM + h.m.	229.6^{b}	121.8^{cd}	217.2^{a}	222.5^{a}
ML + h.m. + NPK	588.0^{c}	172.3^{de}	542.5^{b}	635.6^{b}
MM + h.m. + NPK	220.9^{b}	112.3^{bc}	197.9^{a}	166.8^{a}

Means marked with the same letter in columns do not differ significantly according to Tukey's test at $\alpha \leq 0.05$.

In case of sunflower cultivated on light soil polluted with heavy metals (BL + NPK), some synergic effect was observed, *ie* increased uptake and accumulation of zinc, both in the tops and roots.

A similar phenomenon occurred for buckwheat cultivated on soils freshly polluted with heavy metals. A supplement of these metals to both soils with initial natural heavy metal contents (ML + h.m and MM + h.m.) generally increased several times zinc accumulation in the test plants. It was also observed that applied supplementary mineral (NPK) fertilization increased zinc content in the tops and roots of buckwheat growing on light soil (ML + h.m. + NPK) and in these plants' tops when grown on medium soil (MM + h.m. + NPK).

Element uptake by plants and its removal from soil is a ratio of the yield of cultivated plants and the elements content in the yield [4]. In conditions of the presented experiment, the biggest amounts of zinc were absorbed by buckwheat yield from light soils: permanently and freshly polluted with heavy metals and additionally fertilized (BL + NPK and ML + h.m. + NPK), but also from medium soil freshly polluted and additionally fertilized (MM + h.m. + NPK) (Table 4). These amounts were several times bigger than absorbed even from the soils permanently polluted with heavy metals but without any supplementary fertilization. In case of sunflower cultivated as an aftercrop, the highest quantities of zinc were taken up by the plants cultivated on the soil in MM + h.m. + NPK object and from the MM + h.m. object, *ie* with the same freshly polluted soil, but without supplementary fertilization.

Table 4

Total uptake of zinc with yield of buckwheat and sunflower tops and roots [$\mu g \cdot pot^{-1}$]

Object	Buckwheat	Sunflower	Total
BL	3 725	1 806	5 531
BM	4 889	1 079	5 968
ML	585	479	1 064
MM	1 573	794	2 367
BL + NPK	19 664	2 138	21 802
BM + NPK	5 580	1 863	7 443
ML + NPK	5 121	2 057	7 178
MM + NPK	4 479	859	5 338
ML + h.m.	4 082	2 759	6 841
MM + h.m.	7 245	4 478	11 723
ML + h.m. + NPK	20 717	3 394	24 111
MM + h.m. + NPK	14 549	6 114	20 663

Analyzed plant material: shoots of buckwheat and sunflower were assessed for their forage usefulness. Considering assumed [18] standards of zinc content, it should be stated that the harvested material of sunflower plants growing both on medium and light soils from Mydlniki without supplements and additionally fertilized with NPK, fulfilled the requirements for forage use which for Zn range from 50 to 100 mg · kg^{-1} d.m. of plant material [18]. In case of buckwheat growing on soils polluted for a long and short time, the forage criterion of the analyzed material was fulfilled only in case of plants growing on medium soil from Bukowno additionally fertilized with NPK. Shoots of both buckwheat and sunflower gathered from the other objects did not fulfill the standards of forage usefulness and therefore should be destined for industrial processing.

In conclusion it should be stated that the ability to absorb the analyzed element by the test plants of buckwheat and sunflower depended to the greatest extent on the form in which zinc occurred in the substratum. Plants growing on soils from Bukowno region, *ie* polluted for a long time, usually absorbed smaller amounts of Zn in comparison with the quantities taken up from the soils freshly polluted with this element. The relationship is probably due to much bigger content of exchangeable zinc, soluble in the soil from Mydlniki freshly polluted with this metal in comparison with Zn amount in soil from Bukowno. Jiang et al [19] in their research on solubility of various Zn salts, stated that *Sedum alfredii* and maize (*Zea mays* L.), which they cultivated in hydroponics were taking up the greatest quantities of Zn salts which after application to the substratum became a part of exchangeable and soluble fraction of this element. Also the kind of soil caused an increase of Zn uptake, *ie* light soil has a poor ability for metal sorption, in comparison with medium soil. Cation exchange capacity of soil sorption complex depends on many factors, according to Miretzky et al [20] and Galletti et al [21], mainly on: soil pH, organic matter content, content of soil clay fraction and soil moisture. Author's own studies confirmed these relationships: plants harvested from objects growing on light soils, *ie* with lesser abilities for immobilizing this element contained much higher amounts of Zn in comparison with plants gathered from the objects with medium soil, which probably absorbed a considerable part of Zn introduced to the soil.

Conclusions

1. In conditions of conducted experiment much smaller yields of buckwheat and sunflower were obtained on light soil in comparison with medium one, irrespective of supplementary mineral fertilization, as well as the level and date of soil pollution with heavy metals. Toxic effect of heavy metals "freshly" applied to the soil became apparent only on light soil additionally fertilized with NPK.
2. Plants cultivated on soils with bigger content of these metals accumulated usually at least several fold bigger quantities of zinc both in their tops and roots, irrespective of the date of pollution with heavy metals. Additional NPK treatment often increased Zn content.
3. The largest amounts of zinc were taken up by plants from light soil freshly and permanently polluted with heavy metals on which supplementary mineral NPK fertilization was applied and next from medium soil, freshly polluted with heavy metals and fertilized with NPK.

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POBIERANIE CYNKU W RÓŻNYM CZASIE Z GLEB ZANIECZYSZCZONYCH METALAMI CIĘŻKIMI

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Abstrakt: Stale wzrasta zainteresowanie tematyką zawartości pierwiastków śladowych i ich zachowaniem w środowisku, a badania, których celem jest określenie zdolności do akumulowania pierwiastków szkodliwych w roślinach rosnących na glebach trwale oraz świeżo zanieczyszczonych tymi metalami, przyczyniają się do rozwoju tego obszaru badawczego. Celem pracy była ocena plonowania oraz kumulacji cynku w gryce (*Fagopyrum esculentum* Moench) oraz w słoneczniku (*Helianthus annuus* L.) uprawianych w różnym czasie na glebach zanieczyszczonych tym metalem.

Znacznie mniejsze plony gryki i słonecznika uzyskano na glebie lżejszej, niezależnie od dodatkowego nawożenia mineralnego oraz poziomu i terminu zanieczyszczenia gleby metalami ciężkimi. Toksyczny efekt „świeżo" zastosowanych do gleby metali ciężkich ujawnił się jedynie na glebie lekkiej dodatkowo nawożonej NPK. Rośliny uprawiane na glebach z większą zawartość tych metali nagromadzały zwykle co najmniej kilkakrotnie większe ilości cynku zarówno w częściach nadziemnych, jak i korzeniach. Dodatkowe nawożenie NPK często zwiększało tę koncentrację. Największe ilości cynku zostały pobrane przez rośliny z gleby lekkiej świeżo oraz trwale zanieczyszczonej metalami ciężkimi, do której zastosowano dodatkowe nawożenie mineralne (NPK) i z kolei z gleby średniej, świeżo zanieczyszczonej metalami i nawożonej NPK.

Słowa kluczowe: cynk, doświadczenie wazonowe, metale ciężkie, słonecznik, gryka

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INFLUENCE OF COMPOST FROM FISH BY-PRODUCTS ON NUTRIENT SUPPLY IN RADISH

WPŁYW KOMPOSTÓW Z PRODUKTÓW UBOCZNYCH GOSPODARKI RYBACKIEJ NA ZAWARTOŚĆ MAKROSKŁADNIKÓW W RZODKIEWCE

Abstract: Processing undesirable species of lake fish into compost can accompany the catching and removal of such fish, which aims to restrict the eutrophication of lakes. A pot experiment with radishes was conducted in order to assess composts containing caught fish from the Cyprinidae family (minnows), sawdust, straw, bark and brown coal. The experiment encompassed two series: I – composts at a dose of 1gN of compost per pot, II – composts and 0.5 g of urea. On average, the application of composts resulted in a 52.68 % increase in radish crop yield in the series without additional mineral fertilization and a 35.42 % increase in the series with additional mineral fertilization when compared to the control group. The average content of P, K, Ca, Na, and Mg in leaves was found to be higher in the series without mineral fertilization than in the control series. Radish roots of plants subjected to mineral fertilization contained, on average, more P, K, Ca, Na, and Mg as compared to the control. In the series with additional mineral fertilization, the average content of P, K, Ca, and Mg increased whilst Na decreased.

Keywords: fish by-products, fertilization, macroelements, radish

Processes aimed at restricting the eutrophication of lakes can be accompanied by processing the low-grade lake fish into composts on location where they had been caught. There is an imbalance between the individual elements of the trophic ladder in eutrophized water basins [1–3]. A decrease in the consumption of seaweed has been observed due to the disappearance of large forms of zooplankton, which plankton-eating fish feed on. There is a necessity to regulate the population of small minnows (the bleak *Alburnus alburnus*, the roach *Rutilus rutilus*, the bream *Abramis brama* and silver bream *Blicca bjoerkna*) by catching these undesirable species of fish, which have

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dominated eutrophized waters and this results in an increasing amount of fish waste [4]. The continuous search for ecological, environmentally-friendly methods of managing such waste material have given rise to developing a solution as to its management by composting low-grade minnows on location where they had been fished and compost produced in such a way has been subjected to assessment in terms of its suitability for application in agriculture [5–6].

The aim of the presented research is to determine the influence that the addition of composts produced from caught minnows and containing different variants of additives in accordance to the study method has on the content of macroelements in the above-ground plant parts and roots of radish (*Raphanus sativus* L.).

Materials and methods

The composts used in the experiment came from an experiment set up outdoors in 2010 which was conducted in specially constructed wooden crates measuring 50 × 60 × 60 cm. The basic substrate for obtaining compost were small cyprinid fish form Lake Kortowskie (Olsztyn, Poland): the bleak (*Alburnus alburnus*), common roach (*Rutilus rutilus*), common bream (*Abramis brama*) and white bream (*Blicca bjoerkna*). In order to realize the assumed study aims, and experiment was carried out on the following types of compost: C_1: 80 % fish + 20 % sawdust; C_2: 80 % fish + 20 % straw; C_3: 80 % fish + 20 % bark; C_4: 79.3 % fish + 19.7 % sawdust + 1 % brown coal; C_5: 79.3 % fish + 19.7 % straw + 1 % brown coal; C_6: 79.3 % fish + 19.7 % bark + 1 % brown coal. Selected chemical properties of the experimental materials are given in Table 1.

Table 1

Chemical properties of substances used for composting [$g \cdot kg^{-1}$ d.m.]

Parameter	Sawdust	Straw	Bark	Brown coal
Total organic matter	566.2	422.6	410.0	705.0
Total N	1.92	5.53	7.04	8.40
Phosphorous	0.40	1.12	0.62	—
Potassium	0.94	12.44	1.05	—
Magnesium	1.12	1.16	1.32	—
Calcium	1.22	2.62	1.51	—

During the course of the composting process, the humidity of materials was maintained at a constant level of 60–70 %, and the compost mass was mixed once a week to aerate it. Chemical properties of composts before application are given in Table 2. The vegetation pot experiment was conducted at a steady temperature of 22 ^{o}C ± 2 ^{o}C in the greenhouse facility of the University of Warmia and Mazury in Olsztyn (Poland). PCV pots filled with 5 kg of soil mixed that had been previously mixed with composts in accordance with the study procedure were used for this purpose. The soil used in the experiment was characterized by a pH of 5.7 and

Table 2

Mean chemical properties of final composts before application [$g \cdot kg^{-1}$ d.m.]

Compost[a]	N_{tot}	P	K	Na	Ca	Mg	C_{org}	C:N
Compost C_1	9.21	2.61	3.07	0.30	3.75	0.60	425.74	46.2
Compost C_2	12.13	2.56	3.74	0.31	3.19	0.62	418.59	34.5
Compost C_3	11.46	2.43	3.77	0.33	3.17	0.65	382.80	33.4
Compost C_4	9.93	2.53	3.15	0.31	4.04	0.61	413.38	41.6
Compost C_5	12.59	2.49	3.95	0.34	3.70	0.67	428.71	34.1
Compost C_6	12.22	2.50	3.99	0.35	3.68	0.63	369.96	30.3
Mean C_1–C_6	11.26	2.52	3.61	0.32	3.59	0.63	406.53	36.7

[a] C_1: 80 % fish + 20 % sawdust; C_2: 80 % fish + 20 % straw; C_3: 80 % fish + 20 % bark; C_4: 79.3 % fish + 19.7 % sawdust + 1 % brown coal; C_5: 79.3 % fish + 19.7 % straw + 1 % brown coal; C_6: 79.3 % fish + 19.7 % bark + 1 % brown coal.

granulometric composition of 86 % sand (2.0–0.05 mm), 11.2 % dust (0.05–0.002 mm) and 2.2 % suspended fraction (< 0.002 mm) obtained from the arable layer of a farm field. The content of organic carbon and nitrogen in this soil was: C_{org} – 6.30 $g \cdot kg^{-1}$, N_{tot} – 0.54 $g \cdot kg^{-1}$, absorbable elements: phosphorus – 84.76 $mg \cdot kg^{-1}$, magnesium – 76.37 $mg \cdot kg^{-1}$ and potassium – 57.89 $mg \cdot kg^{-1}$ soil. The soil samples were air-dried and passed through a 5-mm sieve prior to the greenhouse pot experiment. The experiment consisted of two series, each conducted in four replicates. In the first, composts were applied in the amount of 1 gN compost per pot. In the second, 0.5gN-min. in the form of urea was applied in addition to the compost. Control objects were accounted for – not fertilized nor subjected to mineral fertilization. The effect of fertilizations was tested on radish (*Raphanus sativus* L.) of the Rowa variety. The density was 5 plants per pot. During the vegetation soil humidity was maintained at the level of 60 % capillary water capacity. The radishes were picked upon reaching full maturity.

The collected plant samples was analyzed in order to determine yield of aerial parts, mass of above-ground parts as well as roots from each pot. Plant samples were fragmented, dried at 60 °C, ground, and mineralized in concentrated sulphuric (VI) acid with hydrogen peroxide added as a catalyst. After mineralization of the plant samples, the content of phosphorus and potassium was analysed with the vanadium-molybdenum method [7], calcium and sodium with atomic emission spectroscopy – AES on FLAPHO 4 CZJ model [8]. Magnesium with atomic absorption spectroscopy – AAS [8], dry mass by means of the dryer method at a temperature of 105 °C. All reagents were of analytical reagent grade unless otherwise stated. Double deionized water (Milli-Q Millipore 0.055 µS/cm resistivity) was used for all dilutions.

The results were subjected to statistical analyses, calculating the average values and significance of differences between average macroelement contents in the above-ground plant parts and roots of radish by means of one factor analysis of variance based on the smallest significant differences using Statistica version 9.0.

Results and discussion

The growth and crop yield of radish (*Raphanus sativus* L.) indicated a clear differentiation, which was influenced by the type of compost (C_1–C_6) as well as mineral fertilization (Table 3). The average crop yield of above-ground plant parts and roots in the series without additional mineral fertilization was 52.68 % higher, and in the N-min. series – 35.42 % higher, when compared to the control objects. The influence of varied organic and mineral fertilization on the crop yield of radish has been confirmed by studies conducted by Islam et al [9], Ebid et al [10], Liao et al [11] and Akoumianakis et al [12]. Asghar et al [13] reported a positive effect of composts from organic wastes along with mineral fertilizers on the crop yield of above-ground parts and roots of radish plants. The composts applied in the experiment resulted in different crop yields of above-ground radish parts and roots. The highest crop yield of plants subjected to additional mineral fertilization was noted in the case of applying compost containing bark with the addition of brown coal. The addition of brown coal (composts C_4–C_6) significantly influenced the crop yield of above-ground plant parts and roots in both of the study series as compared to composts which did not contain it (C_1–C_3). Mineral fertilization increased mainly the crop yield of above-ground parts in the control objects.

Table 3

Effect of fish compost application on the yield above part and root of radish (*Raphanus sativus* L.) fresh matter

Fertilization	Series without mineral N				Series with mineral N			
	Yield [g]			Relative to control [%]	Yield [g]			Relative to control [%]
	Leaves	Root	Total		Leaves	Root	Total	
Without fertilization	42.40	30.12	72.52	100.00	54.13	32.19	86.32	100.00
Compost C_1	60.39	37.20	97.59	134.56	68.35	41.94	110.29	127.77
Compost C_2	65.24	43.51	108.75	149.96	66.46	40.40	106.86	123.80
Compost C_3	68.57	38.99	107.56	148.32	66.50	40.81	107.31	124.32
Compost C_4	64.74	45.14	109.88	151.52	75.59	47.49	123.08	142.59
Compost C_5	70.40	47.33	117.73	162.34	71.13	51.15	122.28	141.66
Compost C_6	74.36	48.45	122.81	169.35	78.50	53.03	131.53	152.37
Mean C_1–C_6	67.28^a	43.44^b	110.72^c	152.68	71.01^a	45.80^b	116.81^c	135.42
$LSD_{0.05}$	14.71	10.04	20.45		15.62	11.01	19.66	

$^{a–a,\ b–b,\ c–c}$ – significant differences at $p < 0.05$.

Dry mass content took on varied levels, which were influenced by the variant of fertilization (Table 4). Nitrogen fertilization influences the fresh mass yield more than it does the dry mass [11, 12, 14]. The application of composts resulted in decreasing the dry mass content in radish roots in both of the series compared to the control objects, while significant differences were not found between the dry matter content of the

Table 4

Dry matter content [%] in above-ground parts of radish (*Raphanussativus* L.) accounting for the fertilization variant

Fertilization	Series without mineral N		Series with mineral N	
	Leaves	Root	Leaves	Root
Without fertilization	4.25	7.15	4.26	7.19
Compost C_1	4.15	6.78	4.19	6.79
Compost C_2	4.00	6.68	4.16	6.81
Compost C_3	4.07	6.74	4.15	6.77
Compost C_4	3.98	6.65	4.10	6.54
Compost C_5	3.90	6.48	4.07	6.44
Compost C_6	3.89	6.23	4.05	6.21
Mean C_1–C_6	4.00^{a}	6.59^{b}	4.12^{a}	6.59^{b}
$\text{LSD}_{0.05}$	n.s.	0.77	n.s.	0.81

$^{a–a}$ – insignificant differences at $p < 0.05$, $^{b–b}$ – significant differences at $p < 0.05$.

above-ground plant parts in both experimental series. Similar dependencies were revealed by Lu et al [15] when subjecting radish, tomato and Chinese cabbage to intense organic and mineral fertilization. The applied fertilization resulted in various contents of macroelements in the dry mass of above-ground plant parts of radish (Table 5).

Table 5

Macroelement content in radish leaves [$g \cdot kg^{-1}$ d.m.]

Fertilization	Series without mineral N					Series with mineral N				
	P	K	Ca	Na	Mg	P	K	Ca	Na	Mg
Without fertilization	3.23	17.01	22.08	3.64	2.88	3.41	11.25	25.98	4.32	3.59
Compost C_1	4.57	19.78	20.60	4.70	3.85	4.14	18.27	27.82	4.62	3.86
Compost C_2	3.44	23.79	21.94	3.19	3.09	3.90	26.30	24.62	2.89	3.28
Compost C_3	3.84	13.76	28.28	4.47	3.75	4.21	16.77	26.90	4.02	3.67
Compost C_4	3.88	18.27	25.54	3.72	3.67	3.66	14.76	30.62	3.27	3.61
Compost C_5	3.42	16.27	24.62	3.19	3.48	4.47	21.78	24.64	3.42	3.37
Compost C_6	3.22	14.26	22.38	3.42	3.07	3.74	18.77	24.18	3.64	3.32
Mean C_1–C_6	3.73^{a}	17.69^{b}	23.0^{c}	3.78^{d}	3.49^{e}	4.02^{a}	19.44^{b}	26.46^{c}	3.64^{d}	3.52^{e}
$\text{LSD}_{0.05}$	0.81	3.92	4.10	0.84	0.59	0.72	3.98	3.44	0.83	0.69

$^{a–a,\ b–b,\ c–c,\ d–d,\ e–e}$ – significant differences at $p < 0.05$.

These factors influenced the quantitative changes in the individual elements as well as their totals, although to different degrees. The average content of the sum of macroelements: phosphorus (P), potassium (K), sodium (Na), calcium (Ca), and Magnesium (Mg) in the series with additional mineral fertilization was 10.43 % higher in the series with additional mineral fertilization than in the series to which only composts had been applied. In terms of the individual elements, a positive influence of N-min was observed in the increasing values of average phosphorus, potassium, calcium and magnesium contents in both analyzed plant organs. The average content of phosphorus, potassium, calcium, sodium and magnesium in the above-ground plant parts of radish was higher in the series containing only composts, whereas the series with additional mineral fertilization led to an increase in the average content of phosphorus, potassium, and calcium but decrease in sodium and magnesium. Phosphorus content (4.57 g · kg^{-1} d.m.) in the above-ground parts of the analyzed plant in the series without mineral fertilization was most affected by the addition of compost containing fish waste and sawdust. The lowest content of phosphorus totaling 3.22 g · kg^{-1} d.m. in the same series was observed in plants grow in soil containing compost, bark and brown coal. Asghar et al [13] report that fertilization with vegetable and fruit compost alone has little influence on the phosphorus content in the above-ground plant parts of radish. Urea fertilization increased the accumulation of phosphorus and potassium in the above-ground parts of the tested plant. In the series with mineral fertilization, phosphorus content in the above-ground plant parts ranged from 11.25 g · kg^{-1} d.m. in the series without fertilization to 26.30 g · kg^{-1} d.m. upon the application of compost containing fish waste and straw (C_2). The above-ground plant parts of radish grown on compost composed of a mixture of fish waste and sawdust without additional mineral fertilization were characterized by the lowest content of calcium, determined to be 20.60 g · kg^{-1} d.m. The highest value (30.62 g · kg^{-1} d.m.) occurred in the second series of the experiment, when compost produced from fish waste, sawdust and the addition of brown coal was used. The biggest differences in terms of sodium content were observed in the above-ground parts of the analyzed plant, where its value ranged from 3.19 g · kg^{-1} d.m. in plants fertilized with compost containing fish waste and straw to 4.70 g · kg^{-1} d.m. in the case of compost with fish waste and sawdust. Magnesium content was lowest in the series without N-min. (2.88 g · kg^{-1} d.m.) and highest in plants fertilized with compost containing fish waste and sawdust (C_1) (3.86 g · kg^{-1} d.m.). The obtained results can be compared to studies carried out by Krzebietke [16] in which the author showed that the content of macroelements in the leaves of butter lettuce subjected to nitrogen fertilization to be as follows: 4.9–6.0 g · kg^{-1} d.m. phosphorus, 40.2–45.2 g · kg^{-1} d.m. potassium, 17.1–23.0 g · kg^{-1} d.m. calcium, 0.8–7.3 g · kg^{-1} d.m. sodium and 2.8–4.0 g · kg^{-1} d.m. magnesium. The type of compost as well as the application a mineral fertilizer significantly modified the contents of selected macroelements in the roots of radish (*Raphanus sativus* L.) (Table 6). A positive influence of mineral fertilization was observed only in the case of the average potassium content in the above mentioned plant organ. The roots of radish fertilized with composts without additional mineral fertilization contained more phosphorus, potassium, calcium, sodium, and magnesium than those in the control group. In the case of plants that underwent additional mineral fertilization, the average content of phosphorus, potassium, calcium and magnesium were shown to increase, accompanied by a decrease in sodium. The lowest (3.22 g · kg^{-1} d.m. in the control group) as well as highest (4.49 g · kg^{-1} d.m. in the group with C3 compost) phosphorus content in radish roots were noted in the series without additional mineral fertilization. The content of potassium in

Table 6

Macroelements content in radish roots [g · kg^{-1} d.m.]

Fertilization	Series without mineral N					Series with mineral N				
	P	K	Ca	Na	Mg	P	K	Ca	Na	Mg
Without fertilization	3.94	22.77	3.95	2.13	1.89	3.96	22.28	4.02	2.43	2.07
Compost C_1	4.19	26.30	3.98	2.88	2.16	4.46	27.30	4.33	2.28	2.04
Compost C_2	4.08	28.31	4.23	1.93	2.15	4.38	29.81	4.65	1.63	1.89
Compost C_3	4.49	25.80	4.82	2.63	2.16	4.26	26.30	4.37	2.18	2.34
Compost C_4	4.44	30.32	4.67	2.53	2.32	4.42	30.81	4.65	2.03	2.17
Compost C_5	4.44	28.31	4.70	1.93	2.07	4.29	29.31	4.33	1.94	1.93
Compost C_6	4.30	26.80	4.93	2.03	1.91	4.28	27.30	4.37	2.13	2.28
Mean C_1–C_6	4.32^a	27.64^b	4.56^c	2.32^d	2.13^e	4.35^a	28.47^b	4.45^c	2.03^d	2.11^e
LSD$_{0.05}$	n.s.	4.92	0.72	0.56	0.34	0.42	3.94	0.55	0.43	0.42

$^{a–a,\ c–c,\ d–d,\ e–e}$ – significant differences at $p < 0.05$, $^{b–b}$ – insignificant differences at $p < 0.05$.

the roots of plants grown with a mineral fertilizer ranged from 22.28 g · kg^{-1} d.m. (control) to 30.81 g · kg^{-1} d.m. (compost C_4). The group of plants grown in compost made of fish waste, sawdust and the addition of brown coal was characterized by the highest concentration of calcium (4.82 g · kg^{-1} d.m.) in the roots of radish planted in pots with additional mineral fertilization was noted in of the group in where compost was made of fish waste, sawdust, and the addition of brown coal. There was some variety in the sodium content of radish roots depending on the type of fertilization applied, and the lowest value (1.63 g · kg^{-1} d.m.) of this macroelement occurred in those plants which had been fertilized with urea (N-min) and compost containing fish waste and straw. Magnesium content was lowest in the control object (without fertilization) and determined to be 1.89 g · kg^{-1}. Similar values of macrolements in radish roots were reported by Asghar et al [13] and Djurovka et al [17]. In their studies on the influence of fertilization with compost and mineral fertilizers on the content of macroelements in radish roots, Asghar et al [13] determined that the concentration of phosphorus increased in objects fertilized with compost along with doses of urea. Potassium content in the roots however, was not influenced by neither organic nor mineral fertilization. In studies conducted by Lu et al. [14] on the concentration of potassium and phosphorus in radish shoots and the fleshy root of radish, potassium content did not depend on the type of mineral fertilization or different doses of manure, but did appear to increase along with increased organic fertilization. Islam et al [9] revealed that organic mineral fertilization has a beneficial effect on the absorption of potassium and phosphorus by radish when compared to organic fertilization by itself.

The quality of the edible plant parts depends not only on the concentration of macro- and microelements but also on their ratios, which can depend not only on the species of plant but also the proportions of cations in the fertilizers [18]. The optimal proportions between the individual elements should be higher than Ca: P – 2; Ca: Mg – 3; K: (Ca + Mg) – 1.6–2.2; K: Mg – 6; K: Ca – 2 [19, 20]. The studies confirmed that the

Table 7

Mass ratios between macroelements in radish roots accounting for fish compost fertilization

Fertilization	Series without mineral N					Series with mineral N				
	K : Ca	K : Mg	Ca : P	Ca : Mg	K : (Ca + Mg)	K : Ca	K : Mg	Ca : P	Ca : Mg	K : (Ca + Mg)
Without fertilization	5.76	12.05	1.00	2.09	3.90	5.54	10.76	1.02	1.94	3.66
Compost C_1	6.61	12.18	0.95	1.84	4.28	6.30	13.38	0.95	2.12	4.29
Compost C_2	6.69	13.17	1.04	1.97	4.44	6.41	15.77	1.06	2.46	4.56
Compost C_3	5.35	11.94	1.07	2.23	3.70	6.02	11.24	1.03	1.87	3.92
Compost C_4	6.49	13.07	1.05	2.01	4.34	6.63	14.20	1.05	2.14	4.52
Compost C_5	6.02	13.68	1.06	2.27	4.18	6.77	15.19	1.01	2.24	4.68
Compost C_6	5.44	14.03	1.15	2.58	3.92	6.25	11.97	1.02	1.92	4.11
Mean C_1–C_6	6.10	13.01	1.05	2.15	4.14	6.39	13.63	1.02	2.13	4.35

radish roots were characterized by an wider ranges of K: Ca, K: (Mg + Ca) and K: Mg (Table 7).

The ratios between Ca:P and Ca:Mg were favorable. Tariq and Mott [21] stated that the addition of boron (B) and calcium (Ca) has an influence on the ratios of K:Mg and K:Na cations in the leaves and roots of radish. Composts, in both series of the experiment, extended all cation ratios, however, the effect of individual composts varied. An exception to this was the ratio between Ca:P in the series with N-min., where the average effect of composts did not result in its change. The most significant reduction of K:Ca, K:Mg and K: (Mg + Ca) ratios in the first series was observed in plants in the group fertilized with compost containing fish waste and bark (C_3), and in the case of Ca:Mg – in the group containing C_2 (fish waste and straw). In the second series of the experiment, narrower proportions were noted only in the group of plants with compost made of a mixture of fish waste, straw and brown coal (C_5) – Ca:P and that with compost which contained bark (C_3) – Ca:Mg as compared to the control group.

Conclusions

The conducted experiment showed that the application of composts produced from by-products of the fishing industry had a positive effect on the crop yield of above-ground plant parts and roots of radish (*Raphanus sativus* L.). Additional mineral fertilization effected crop yield only to a small degree. This indicates that plants make good use of nitrogen. The highest crop yield was obtained upon the application of compost the contents of which included bark and the addition of brown coal. The addition of this component was also shown to have a positive effect in the other composts variants. In the series not subjected to mineral fertilization, the above-ground parts of radish contained, on average, more phosphorus, potassium, calcium, sodium and magnesium than the control group. The roots too were characterized by the highest phosphorus, potassium, calcium, and magnesium contents in the series without mineral fertilization as compared to the control group, but the sodium content was shown to decrease.

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WPŁYW KOMPOSTÓW Z PRODUKTÓW UBOCZNYCH GOSPODARKI RYBACKIEJ NA ZAWARTOŚĆ MAKROSKŁADNIKÓW W RZODKIEWCE

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Abstrakt: Przetwarzanie małocennych gatunków ryb jeziorowych na komposty może towarzyszyć zabiegom ich odłowów stosowanych przy ograniczaniu eutrofizowacji jezior. W sposób mało kosztowny można uzyskać nawóz organiczny. W celu oceny kompostów, w których składzie znajdowały się odłowione ryby karpiowate, trociny, słoma, kora i węgiel brunatny założono doświadczenie wazonowe z rzodkiewką (*Raphanus sativus* L.). Doświadczenie obejmowało dwie serie: I – komposty w dawce 1 gN kompostu na wazon, II – komposty i 0,5 g mocznika. Stosowanie kompostów spowodowało średni wzrost plonu rzodkiewki o 52,68 % w obiektach bez dodatkowego nawożenia mineralnego, a w serii z dodatkowym nawożenie mineralnym o 35,42 % w stosunku do obiektów kontrolnych. Stwierdzono korzystne działanie węgla brunatnego w kompostach na plon zarówno części nadziemnych, jak i korzeni rzodkiewki. Liście zawierały średnio w stosunku do serii kontrolnej więcej P, K, Ca, Na i Mg w serii bez nawożenia mineralnego. W serii z mocznikiem wzrosła średnia zawartość P, K i Ca, a zmalała Na i Mg. Korzenie rzodkiewki zawierały średnio w stosunku do kontroli więcej P, K, Ca, Na i Mg w serii bez nawożenia mineralnego. W serii z dodatkowym nawożeniem mineralnym wzrosła średnia zawartość P, K, Ca i Mg, a zmalała Na.

Słowa kluczowe: odpady rybne, nawożenie, makroskładniki, rzodkiewka

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EFFECT OF FORM AND DOSE OF SELENIUM ON YIELDING AND CONTENTS OF MACRONUTRIENTS IN MAIZE

WPŁYW FORMY I DAWKI SELENU NA PLONOWANIE ORAZ ZAWARTOŚĆ MAKROSKŁADNIKÓW W KUKURYDZY

Abstract: The purpose of the conducted experiment was to estimate the influence of the form and dose of selenium on yielding and N, P, K, Ca, Mg and S content in the above ground parts of maize. The experiment was established in Wagner type pots with a capacity of 5 kg of soil in three replications. The substrate was a slightly acidic light loam with an average content of P, K, Mg and S and low Se content. Selenium was applied in the form of Na_2SeO_4 and Na_2SeO_3, and doses of: 0; 0.10; 0.25; 0.50; 0.75; 1.0; 2.0; 3.0 and 4.0 mg · kg^{-1} of soil. The 'Lober' strain of maize was used as a test plant and was cut down during the BBCH 73 phase.

The conducted research demonstrated that the after entering into the soil 0.1 mgSe · kg^{-1}, form in which the element was used, did not affect the weight of the harvested crop corn. At all the rest selenium dosage levels the biomass cultivated in soil enriched with sodium selenate was higher than the biomass obtained after the application of sodium selenite. The application of selenium in the form of selenate (Na_2SeO_4) and selenite (Na_2SeO_3) caused a substantial increase in the content of this trace element in maize. The effect of the selenate was such that average Se content in plants was 13 times higher than the predetermined average for maize cultivated in soil with the selenite supplement. The introduction of selenium into the soil, especially in higher doses, triggered changes in the chemical composition of the maize. With the greater doses of Se(VI) the content of nitrogen, potassium and calcium content in plants decreased. The impact of that form of selenium upon phosphorus and sulphur content depended on the size of the applied doses. Selenium introduced in doses of 0.1–0.5 mg · kg^{-1} increased the content of P and limited the amount of S. At greater doses the reverse relationship was observed. Maize cultivated in soil enriched with Na_2SeO_3 contained less nitrogen and sulphur but more phosphorus, potassium, and calcium than that cultivated in soil with the selenate supplement.

Keywords: Na_2SeO_4, Na_2SeO_3, maize, yielding, macronutrients content

Introduction

Selenium belongs to the group of elements essential to the normal development and functioning of human and animal organisms. However, it is yet to be shown that it is

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indispensable to the majority of plants [1]. The average selenium content in plants that are not its natural depository is 0.05 mg · kg^{-1}. However, when those plants are cultivated in soils or nutrient media with high Se content they can accumulate significant amounts of this microelement [1–6]. Selenium content in soil varies enormously, but in most soils it averages 0.01–2 mg · kg^{-1} [7]. Research conducted in recent years has shown that most soils in Poland contain low amounts of selenium [8, 9]. According to Piotrowska [10], Se content varies from 0.04 to 0.65 mgSe · kg^{-1}, for arable soils in the Wroclaw region Patorczyk-Pytlik and Kulczycki [9] provide a range of 0.081–0.494 mgSe · kg^{-1}, and in the soils of the Pomerania and Kujawy regions Borowska et al [8] estimated from 0.035 to 0.332 mgSe · kg^{-1} soil.

An additional reason for a low plant intake of selenium is the soils' significant acidity [11–14]. The form in which selenium appears in soils, the changes it undergoes, and its availability for plants are determined by many physicochemical factors such as: soil pH, oxidoreduction potential, contents of humus, clay minerals, Fe oxides and other elements, microbiological activity and also the nature and character of the absorbing surfaces [3, 13–15]. The best selenium uptake is from alkaline, well-aired soils, and the lowest from acidic, gleyic soils, which contain lerge amounts of organic matter [3, 11, 13, 14].

The average plant selenium content is 0.05 mg · kg^{-1}, with the exception of plants originating from regions commonly referred to as the selenium regions, seldom exceeds 1.0 mg · kg^{-1} of dry mass [3, 7, 15]. The plant selenium content is influenced mainly by three factors: Se content in soil, agents that create a Se form of appearance and also specific properties of plants [4, 13–16]. Selenium uptake occurs mainly through plant roots in the form of selenate, to a lesser extent in the form of selenite, and also in an organic form as selenocysteine and selenometionine [17]. The reason for the lower uptake of selenite than selenate may be its higher rate of absorption in soil [18]. Hawkesford and Zhao [19] report that selenates are characterized by higher solubility and mobility than selenites, and are also more often subject to washout into deeper soil layers [20]. Additionally, the uptake mechanism of these two forms is not the same. Selenate is absorbed from soil solution during an active process with use of sulphur carriers through the plasma membrane of plant roots. Selenite uptake in turn occurs during a process of passive diffusion [3, 19, 21]. Rosen and Liu [22], Li et al [23], as well as Hopper and Parker [24] report that, selenate intake proceeds like that of sulfates, and selenite intake like that of phosphates. In addition, selenite is accumulated mainly in plant roots, whereas selenate is rapidly translocated to the above ground parts [6, 25–28].

It has been shown in many studies that selenium applied in small doses (0.1 to 1.0 mg · kg^{-1}) has a beneficial effect upon plant growth and development. This may be a result of selenium impact on the glutathione peroxidase (GSH-Px) activity [2, 28], and the increase of plant stress resistance to salinity [29], UV radiation [2, 30, 31] and drought [32]. Hartikainen et al [2] in their research confirmed the stimulating effect of small doses of selenium on a common ryegrass yield, and Turakainen [33] observed the same effect on potato yield. Broadley et al [34], Ducsay et al [35], Curtin et al [36] and Grant et al [37] showed no effect of selenium on a yield of wheat.

Plants cultivated in soils/water culture with high selenium content can accumulate significant amounts of this element in their tissues. That in turn may inhibit the plants' growth and development [2, 4]. This is caused by factors such as a decrease in the amount of chlorophyll and carotenoids [6], a suppression of protein synthesis [3], a decrease in the starch content [38], disorders in the intake of the macro and microelements [5, 6, 16, 39–41].

Most of the studies regarding the impact of selenium on the accumulation of other elements is conducted using the water culture method [6, 24, 39–42] and most often encompasses the impact of one particular chemical form of selenium. It allows the precise determination of the effect of the tested agent, but does not take into account the changes, which will be underwent selenium compounds applied to the soil. Properties of the soil can substantially modify the form of the Se presence, and thereby the availability of this component for the plants.

The purpose of the research conducted was the comparison of the impact of different selenium doses introduced into the soil on the maize yield and content of Se in the above ground parts of maize, as well as the macroelements content in maize biomass. Selenium doses were introduced into the soil in the form of Na_2SeO_4 or $Na_2SeO_{4.}$

Material and methods

The experiment was conducted in Wagner pots with a capacity of 5 kg of soil. It was carried out using a completely random system with three repetitions. The substrate used was a light loam with an average content of the available phosphorus, potassium, sulphur and a low content of the available magnesium (Table 1).

Table 1

Selected properties of soil substrate

pH_{KCl}	Organic C	Available form			Total S	SO_4-S	Total Se	DTPA Se
		P	K	Mg				
	$[g \cdot kg^{-1}]$	$[mg \cdot kg^{-1}]$					$[\mu g \cdot kg^{-1}]$	
6.1	7.6	57	118	22	310	11.4	143	13.8

The total selenium content (Table 1) oscillated below the average value determined by Piotrowska [10] (280 $\mu g \cdot kg^{-1}$) and Patorczyk-Pytlik and Kulczycki [9] (206 $\mu g \cdot kg^{-1}$) for light loam. The identical mineral fertilization was applied in all the pots. The pre-sowing fertilization was applied in doses: 0.5 gN (NH_4NO_3), 0.4 gP and 0.5 gK (KH_2PO_4) and 0.3 gMg ($MgSO_4 \cdot 7H_2O$) per pot, and the top dressing with doses: 1.5 gN (NH_4NO_3), 0.5 gK (KCl) and 0.3 gMg ($MgSO_4 \cdot 7H_2O$) per pot. During the first phase of the experiment selenium was introduced into soil in the form of a water solution of Na_2SeO_4 and Na_2SeO_3, in the following doses: 0.10; 0.25; 0.50; 0.75; 1.0; 2.0; 3.0 and 4.0 mgSe $\cdot$ kg^{-1} of soil. The control object was soil with natural selenium content. When planning the amount of input of selenium in the soil mostly included the recommended dose (0.1 mg · kg^{-1}) and an amount that lets to enhance the content to the level approved as medium to high (0.25–3.0 mg · kg^{-1}) and high (4 mg · kg^{-1}) [19].

The 'Lober' strain of maize was used as a test plant. Plants were sown in a distribution of 12 seeds per pot and after 10 days that number was reduced to 6 per pot. Maize was cut down during the BBCH 73 phase. The vegetation period lasted 90 days. In order to avoid selenium losses through methylation at a temperature higher than 30 ^{o}C, the collected biomass was dried at room temperature. In the dry mass were determined: the total nitrogen content after the mineralization of the samples with the salicylic-sulphuric acid using the Kjeldahl method, and the sulphur content – using the Butters-Chenery method. After the mineralization of the samples at a temperature of 450 ^{o}C the ash was dissolved in a 1 mol · dm^{-3} of HNO_3 solution. In obtained solutions the following elements were determined: the phosphorus by the calorimetric vanadium-molybdenum method, the potassium and calcium content by the emissive spectrophotometric method using a AAS 3 apparatus, and the magnesium by the atomic-absorption spectrophotometric method using a Spectra 220 FS Varian apparatus. The selenium content was determined according to procedures specified in the annex to an ordinance from the Ministry of Agriculture dating on 23rd January, 2003 [43]. The measurement of the selenium concentration was done by the AAS method in conjunction with a generation of hydrides using the SpectrAA FS Varian apparatus with a VGA-76 add-on.

All the measurements were performed twice for each pot and the results shown in the tables are their averages. The certified plant material INCT-TL-1 was used to appraise the mineralization and measurement conditions. The statistical analysis was performed using the STATISTICA 8.0 and Microsoft Office Excel 2010 programs. The simple correlation coefficients were calculated at a significance level of $p \leq 0.05$ and the two-factor variation analysis was performed using the Duncan test.

Results and discussion

The research conducted showed that the amount of maize yield was dependent on the form and dose of the applied selenium (Table 2).

After entering into the soil 0.1 mgSe · kg^{-1}, form in which the element was used, did not affect the harvested crop of maize. At all the rest selenium dosage yield of maize cultivated in soil enriched with sodium selenate was higher than amount of biomass obtained after the application of sodium selenite. Ramos et al [28] and Xue et al [31] confirmed a similar dependency in their research. However, in studies conducted by Sharma et al [44] rape in the blooming phase produced more mass when it was cultivated in soil with added Na_2SeO_3, than with Na_2SeO_4. Also Cartes et al [26] showed that selenite applied in doses up to 10 mg · kg^{-1} of soil did not have any effect upon ryegrass yield. However, a dose of 2 mg · kg^{-1} of selenium introduced in the form of selenate inhibited plant growth. This shows that different plant species react differently to selenium present in soil.

Comparing the yield-forming activity of both selenium chemical forms shows that the application of this element in the form of Na_2SeO_4 did not limit plant growth. The

Table 2

Effects of form and dose of selenium on the amount of yield and on the Se content in above ground parts of maize

Dose of Se [mg · kg^{-1} of soil]	Yield [g d.m. per pot]			Content of selenium [mg · kg^{-1} d.m.]		
	Na_2SeO_4	Na_2SeO_3	Means for doses	Na_2SeO_4	Na_2SeO_3	Means for doses
0	217.3		217.3	0.095		0.095
0.1	219.2	220.2	219.7	0.758	0.184	0.47
0.25	232.2	218.5	225.4	1.524	0.215	0.87
0.50	215.0	212.0	213.5	2.573	0.246	1.41
0.75	211.5	201.8	206.7	3.842	0.303	2.07
1.0	213.7	195.6	204.7	5.061	0.448	2.75
2.0	212.4	185.7	199.1	10.780	0.645	5.71
3.0	210.6	186.0	198.3	18.796	1.050	9.92
4.0	209.6	185.0	197.3	20.469	1.774	11.12
Means for forms	215.7	202.5	—	7.100	0.551	—
$LSD_{0.05}$ for:	Se form – 2.32; Se dose – 8.04; interaction – 7.80			Se form – 0.060; Se dose – 0.21; interaction – 0.28		

maize yield grown in soil with a dose up to 0.25 mgSe · kg^{-1} applied was significantly greater than that from the control object. A positive impact of small selenium doses on plant yield was also confirmed by other authors [2, 7, 28, 42]. In the experiment the test plants reacted differently to selenite. The addition of low selenium doses (0.1 and 0.25 mgSe · kg^{-1}) as Na_2SeO_3 to the soil did not affected the biomass yield of maize harvested in comparison with the control object. However, the increase of the applied Se in that form limited the maize yield. The yield of plants cultivated in soils with the addition of doses 2.0; 3.0 and 4.0 mgSe · kg^{-1} was the same and about 15 % less than that obtained from the control object.

The research conducted confirmed, as cited in the literature [1–4, 6, 14–16, 18, 27, 35, 38, 42], the high plant ability to accumulate selenium when cultivated in soils with high Se content. It was shown that the addition of selenium to soil in both forms (Na_2SeO_4 and Na_2SeO_3) contributed to the significant increase of that element content in maize above ground parts (Table 2). In the conditions of executed test, the average Se content in the above ground parts of plants taken from objects with Se(VI) addition was almost 13 times higher than the predetermined average for maize collected from soil where selenite had been added. Similar dependencies were also shown by other authors [26, 28, 44]. After addition of 0.1 mgSe · kg^{-1} in the form of selenate, this element content in plants was 8 times higher, and after the highest dose (4 mgSe · kg^{-1}) was over 200 times higher than the predetermined content in maize from the control treatment (Table 2). Under the same conditions selenite application caused an increase of Se content of 2 and 18 times, respectively. The reason for the smaller plant uptake of selenium from selenite than from selenate probably is the significant sorption of that form in soil [18, 45], and also different mechanisms of their uptake [3, 22–24, 28], as was previously mentioned.

The introduction of selenium into soil, especially at higher doses caused changes in the chemical composition of the cultivated maize. The maize nitrogen content decreased together with an increase of the selenium doses (Table 3).

Table 3

Effect of form and dose of selenium on the N, K and Ca content in maize

Se dose [mg · kg^{-1}]	N			K			Ca		
	[g · kg^{-1} d.m.]								
	Na_2SeO_4	Na_2SeO_3	Mean	Na_2SeO_4	Na_2SeO_3	Mean	Na_2SeO_4	Na_2SeO_3	Mean
0	11.7		11.7	12.4		12.4	0.504		0.504
0.1	11.2	9.8	10.5	10.6	11.6	11.1	0.550	0.519	0.534
0.25	10.7	9.6	10.2	10.1	11.2	10.7	0.488	0.670	0.579
0.5	9.8	9.3	9.6	9.0	10.6	9.8	0.460	0.632	0.546
0.75	10.0	9.1	9.6	8.6	8.8	8.7	0.435	0.550	0.493
1.0	9.8	9.1	9.5	8.4	8.4	8.4	0.426	0.552	0.489
2.0	9.4	9.0	9.2	8.2	8.0	8.1	0.407	0.552	0.479
3.0	9.3	8.6	8.9	8.0	8.0	8.0	0.405	0.524	0.465
4.0	9.2	7.5	8.4	7.9	8.0	7.9	0.394	0.526	0.460
Mean	10.1	9.3	—	9.3	9.7	—	0.452	0.559	—
$LSD_{0.05}$ for:	form – 0.12; dose – 0.23; interaction – 0.32			form – 0.14; dose – 0.20; interaction – 0.29			form – 0.005; dose – 0.012; interaction – 0.017		

It was found that selenite limited the accumulation of N more than selenate, since after the application of its highest dose in the form of Na_2SeO_4 the nitrogen content decreased by 21 % in comparison with the plants from the control object. After it application in the form of Na_2SeO_3 the nitrogen content was smaller by 36 %. The reduction of the nitrogen content in mustard with increasing higher doses of selenium was confirmed by Singh [46], and in barley by IIbas et al [16].

The introduction of increasingly higher selenium doses caused a decrease of the potassium content in maize (Table 3). A similar dependency was demonstrated by other authors [5, 39, 42]. However, Zembala et al [40] did not confirm an impact of selenium on the accumulation of potassium. In the studies conducted by Kopsell et al [41] an increase of potassium content was observed after the addition of Na_2SeO_4 to the nutrient medium. The differences between the impact of selenate and selenite on the accumulation of potassium occurred only after the application of small doses. The potassium content in plants collected from soils to which doses of 0.1; 0.25 and 0.5 mgSe · kg^{-1} were applied in the form of Na_2SeO_3 was lower than the predetermined content in the plants from the control treatment. However, it was significantly higher than in maize grown in soil to which the Se doses were introduced in the form of Na_2SeO_4.

The greater average calcium content was observed in plants cultivated in soils with the addition of selenite than after selenate application (Table 3). In comparison with the content determined in the control plants, an increase in the content of calcium occurred only in maize collected from soil with the addition of selenium in the form of Na_2SeO_4 and a dose of 0.1 $mg \cdot kg^{-1}$. The continued increase of the amount of selenium caused a decrease in the Ca accumulation. Plants collected from soil with 4.0 $mgSe \cdot kg^{-1}$ addition contained 22 % less Ca than those from the control object. Filek et al [39] also demonstrated a diminish in the calcium content in rape after the addition of Na_2SeO_4 to the nutrient medium. However, in the studies of Zembala et al [40] selenate did not cause any difference in the amounts of Ca accumulated in the above ground parts of rape and wheat.

In comparison with the control object, only when the dose of 0.25 $mgSe \cdot kg^{-1}$ was applied in the form of Na_2SeO_3, increased calcium contents in the plants were found. Bigger doses of selenium introduced into soil caused a slow decrease in the amount of accumulated calcium. However, these amounts were significantly greater than those determined in the plant biomass from the control treatment.

The average magnesium content in the above ground parts of plants collected from soils where selenates and selenites were added, were about the same but higher than those found in maize from the control object (Table 4).

Table 4

Effect of form and dose of selenium on the Mg, P and S content in maize

Se dose [$mg \cdot kg^{-1}$]	Mg			P			S		
	[$g \cdot kg^{-1}$ d.m.]								
	Na_2SeO_4	Na_2SeO_3	Mean	Na_2SeO_4	Na_2SeO_3	Mean	Na_2SeO_4	Na_2SeO_3	Mean
0	0.158		0.158	2.62		2.62	1.275		1.275
0.1	0.167	0.151	0.159	2.85	3.15	3.00	1.091	1.362	1.227
0.25	0.161	0.173	0.167	2.91	3.62	3.27	0.997	1.025	1.011
0.5	0.166	0.178	0.172	3.05	3.94	3.50	0.819	0.997	0.908
0.75	0.169	0.180	0.174	2.94	4.20	3.57	1.113	0.822	0.968
1.0	0.163	0.182	0.172	2.96	4.28	3.62	1.171	0.579	0.875
2.0	0.170	0.168	0.169	2.85	4.25	3.55	1.544	0.506	1.025
3.0	0.176	0.161	0.168	2.74	4.40	3.57	1.828	0.647	1.238
4.0	0.179	0.160	0.170	2.62	4.35	3.49	1.950	0.806	1.378
Mean	0.168	0.168	—	2.84	3.87	—	1.310	0.891	—
$LSD_{0.05}$ for:	form – i.d.; dose – 0.005; interaction – 0.008			form – 0.13; dose – 0.18; interaction – 0.26			form – 0.026; dose – 0.042; interaction – 0.061		

Explanation: i.d. – insignificant difference.

The magnesium content in plants increased with the selenate dose. In maize cultivated in soil enriched with Na_2SeO_3, such dependency occurred only up to a dose of 1.0 mgSe · kg^{-1}. With higher selenium doses the magnesium content oscillated around the same level for all objects (Table 4). The research of Feng et al [5] with *Pteris vittata* L. as the test plant, shows that addition of selenium in the form of Na_2SeO_3 and a dose of up to 20 mg · kg^{-1} of soil limited magnesium accumulation. Similar dependency for rape cultivated in a nutritional medium enriched with Na_2SeO_4 was demonstrated by Filek et al [39] and for wheat by Zembala et al [40].

Se addition also caused a difference in the phosphorus content of maize (Table 4). In comparison with plants grown in soil fertilized with selenate, the average P content was greater in plants from objects with selenite application. The lower doses of Na_2SeO_4 (0.1–2.0 mgSe · kg^{-1}) caused an increase, while the higher ones limited the phosphorus content in the above ground parts of maize in comparison with plants collected from the control object. After the application of 4 mgSe · kg^{-1} of soil the P content became the same as that determined in maize collected from the control object. The diminish in phosphorus content as a result of increased selenium(VI) amount in medium was also discovered by Kopsell et al [2000] in *Brassica oleracea* and Filek et al [39] in rape.

As opposed to the results obtained by Hopper and Parker [24], who discovered the phenomenon of antagonism between SeO_3^{2-} and PO_4^{2-} ions in their research using the water culture method, the phosphorus content in plants would increase with higher doses of Na_2SeO_3.

Similar geochemical properties of sulphur and selenium are the reason that their uptake by plants is determined by the quantitative ratio of their contents in soil or in the nutrient medium [3, 24, 47–49]. This dependency was confirmed by the research conducted (Table 5).

Table 5

Simple correlation coefficients between amount of yield, selenium content, and macroelement content

Specification	Content of Se after application of	
	Na_2SeO_4	Na_2SeO_3
Yield	–0.52	–0.76
N	–0.76	–0.78
P	–0.37	0.58
K	–0.70	–0.71
S	0.91	–0.49
Ca	–0.78	i.d.
Mg	0.76	i.d.

Explanation: Significant difference at $p < 0.05$; i.d. – insignificant difference.

On the basis of sulfur and selenium contents in the soil used as substrate in the experiment (Table 1) and the quantity of delivered Se dose was calculated that as a result of Se addition was narrowing S:Se ratio from 2168:1 in control soil to 75:1 after application of 4 mgSe · kg^{-1}.

The calculated average of object sulphur content in maize (Table 4) showed that Se introduced into soil in the form of selenite limits in larger degree S accumulation by maize than the selenate. Hawrylak-Nowak [6] and Rios et al [49] observed similar dependencies for lettuce.

In comparison with the plants collected from the control object, the sulphur content decreased with an increase of selenate doses up to 0.5 mgSe · kg^{-1}. An introduction of 0.75 and 1.0 mgSe · kg^{-1} into the soil caused the greater Se accumulation in maize biomass than that specified in plants collected from the soil with a supplement of 0.5 mgSe · kg^{-1}, but substantially smaller than that determined in plants grown in the control object.

Only when the selenium addition to the substrate was increased to 2.0; 3.0 and 4.0 mgSe · kg^{-1}, a significant increase in the sulphur content in maize was observed. A distinct reaction was observed in maize grown in soil enriched with Na_2SeO_3. The introduction of 0.1 mgSe · kg^{-1} of soil generated a significant increase in the sulphur content in those plants in comparison with the plants grown in the control object. A substantial decrease of the sulphur content in the above ground parts of maize followed an increase of Se doses from 0.25 to 2 mgSe · kg^{-1}. The biggest dose of selenate (4 mgSe · kg^{-1}) resulted in an increase of the S content in above ground parts of maize in comparison to a smaller dose of this element, but was still smaller than that specified in the control plants.

The presence of the ionic antagonistic/synergistic reaction according to the ratio of these elements in soil or nutrient medium and the applied form of selenium was also discovered by the other authors [6, 24, 47, 48].

The statistical analysis conducted demonstrated that the plant yield was significantly and negatively correlated with the selenium content in maize (Table 5). The greater value of correlation coefficient values stated for selenite than for selenate demonstrate that first Se form limited more plant yield. The effect of selenium introduced into soil on the content of the studied macroelements in maize biomass depended on the form in which it was applied. The phosphorus content in the above ground parts of maize was significantly and negatively correlated with the selenium quantity introduced in the form of selenite, however positively correlated when selenate was applied. The opposite dependency was determined for the sulphur content in plants. The Na_2SeO_4 introduced into the soil limited the content of N, P, K and Ca in maize biomass but stimulated the accumulation of S and Mg. After the application of Na_2SeO_3 the N, K and S content in maize turned out to be negatively correlated with the selenium content and the opposite affected phosphorus content which was positively correlated with dose of S applied as selenite.

Conclusions

The research conducted confirmed that maize should be classified as a group of plant which in the conditions of their cultivation in soils with a high content of selenium will contain substantial amounts of this element. This property should be taken into account when planning fertilization with this element. Even the highest Se dose (4 mg · kg^{-1})

used in a form of Na_2SeO_4 was not critical one for the yield of maize, while 1 mgSe · kg^{-1} in the form of Na_2SeO_3 resulted in a 10 % decrease in the amount of harvested mass. The content of selenium in maize grown on soil with 0.1 to 0.75 mgSe · kg^{-1} addition in form of Na_2SeO_3 was optimal for the quality of feed, but already 0.1 mgSe · kg^{-1} in the form of Na_2SeO_4 resulted in exceeding this value. The application of both selenium compounds resulted in changes of the chemical composition of a plant, from that selenite to a much greater extent than selenate limited the content of nitrogen and sulfur, however resulted in increase of calcium and phosphorus content in maize biomass.

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WPŁYW FORMY I DAWKI SELENU NA PLONOWANIE ORAZ ZAWARTOŚĆ MAKROSKŁADNIKÓW W KUKURYDZY

Katedra Żywienia Roślin
Uniwersytet Przyrodniczy we Wrocławiu

Abstrakt: Celem przeprowadzonych badań była ocena wpływu formy i dawki wprowadzonego do gleby selenu na plonowanie oraz zawartość N, P, K, Ca, Mg i S w częściach nadziemnych kukurydzy. Doświadczenie założono w wazonach typu Wagnera o pojemności 5 kg gleby, w trzech powtórzeniach. Podłożem była lekko kwaśna glina lekka, wykazująca średnią zasobność w P, K, Mg i S oraz niską zasobność w Se. Selen zastosowano w formie Na_2SeO_4 i $Na_2 SeO_3$ w dawkach: 0; 0,1; 0,25; 0,50; 0,75; 1,0; 2,0; 3,0 i 4,0 mg · kg^{-1} gleby. Rośliną testową była kukurydza odmiany 'Lober', zebrana w fazie BBCH 73.

Przeprowadzone badania wykazały, że po wprowadzeniu do gleby 0,1 mgSe · kg^{-1}, forma w jakiej ten pierwiastek był zastosowany, nie miała wpływu na wielkość zebranego plonu biomasy kukurydzy. Pozostałe dodatki tego mikroelementu zwiększały istotnie plony biomasy roślin uprawianych na glebie z dodatkiem selenianu(VI) sodu w większym stopniu niż po zastosowaniu selenianu(IV) sodu. Zastosowanie selenu zarówno w postaci Na_2SeO_4, jak i Na_2SeO_3 spowodował istotne zwiększenie zawartości tego mikroelementu w kukurydzy. Pod wpływem selenianu(VI) średnia zawartość Se w roślinach była 13 razy większa od średniej zawartości w biomasie kukurydzy uprawianej na glebie z dodatkiem selenianu(IV). Wprowadzenie do gleby selenu, zwłaszcza jego większych dawek, spowodowało zmiany składu chemicznego uprawianej kukurydzy.

Wraz ze zwiększaniem dawki selenianu(VI) zmniejszała się zawartość azotu, potasu i wapnia w częściach nadziemnych roślin. Wpływ tej formy Se na zawartość fosforu i siarki zależał od wielkości zastosowanej dawki. Selen wprowadzony w dawkach 0,1–0,5 mg · kg^{-1} zwiększał zawartość P oraz ograniczał zawartość S. Po zastosowaniu większych dawek selenu zanotowano odwrotną zależność. Kukurydza uprawiana na glebie wzbogaconej w Na_2SeO_3 zawierała mniej azotu i siarki, a więcej P, K i Ca niż uprawiana na glebie z dodatkiem selenianu(VI).

Słowa kluczowe: Na_2SeO_4, Na_2SeO_3, kukurydza, plonowanie, zawartość makroskładników

Varia

INVITATION FOR ECOpole ’14 CONFERENCE

CHEMICAL SUBSTANCES IN ENVIRONMENT

We have the honour to invite you to take part in the 23rd annual Central European Conference ECOpole ’14, which will be held in 15–18.10.2014 (Wednesday–Saturday) in Hotel Ziemowit in Jarnoltowek, PL.

The Conference Programme includes oral presentations and posters and will be divided into four sections:

- SI Chemical Pollution of Natural Environment and Its Monitoring
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- SIII Forum of Young Scientists and Environmental Education in Chemistry
- SIV Impact of Environment Pollution on Food and Human Health

The Conference Opening Lecture:

Elementary steps in heterogeneous catalysis: the basis for environmental chemistry

will be delivered by the Nobel Prize Winner in Chemistry 2007
Professor Dr. Gerhard ERTL

The Conference language is English.

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- full papers will be published in successive issues of the *Ecological Chemistry and Engineering/Chemia i Inżynieria Ekologiczna* (Ecol. Chem. Eng.) ser. A or S.

The deadline for sending the Abstracts is **15th July 2014** and for the Extended Abstracts: **1st October 2014**. The actualized list (and the Abstracts) of the Conference contributions accepted for presentation by the Scientific Board, one can find (starting from **31st July 2014**) on the Conference website:

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The Conference fee is 400 € (covering hotel, meals and transportation during the Conference). It could be reduced (to 250 €) for young people actively participating in the

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Further information is available from:
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Będzie to **dwudziesta trzecia z rzędu** konferencja poświęcona badaniom podstawowym oraz działaniom praktycznym dotyczącym różnych aspektów ochrony środowiska przyrodniczego. Odbędzie się ona w Hotelu Ziemowit w Jarnołtówku.

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