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SEVERITY OF POTATO TUBERS DISEASES IN TREATMENTS WITH FOLIAR FERTILIZATION

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K e y w o r d s: potato tubers, diseases, foliar fertilization, mineral fertilization.

A b s t r a c t

The study was conducted over the years 2004–2006 in experimental plots located in Bałcyny. A multi-purpose, late potato cultivar, Jasia, was grown. The experimental factors were as follows: I – mineral fertilization levels: *A* (N – 80 kg ha^{-1}, P – 80 kg ha^{-1}, K – 120 kg ha^{-1}), *B* (N – 120 kg ha^{-1}, P – 144 kg ha^{-1}, K – 156 kg ha^{-1}), II – foliar fertilization: *1* (Basfoliar 12-4-6 – 8 dm^3 ha^{-1}), *2* (ADOB Mn – 4 dm^3 ha^{-1}), *3* (Solubor DF – 2 dm^3 ha^{-1}), *4* (ADOB Mn – 2 dm^3 ha^{-1} + Basfoliar 12-4-6 – 4 dm^3 ha^{-1}), *5* (ADOB Mn – 2 dm^3 ha^{-1} + Solubor DF – 1 dm^3 ha^{-1}), *6* (Basfoliar 12-4-6 – 4 dm^3 ha^{-1} + Solubor DF – 1 dm^3 ha^{-1}), *7* (Basfoliar 12-4-6 – 2.7 dm^3 ha^{-1} + ADOB Mn – 1.3 dm^3 ha^{-1} + Solubor DF – 0.7 dm^3 ha^{-1}), *8* (control treatment without foliar fertilization).

The health status of potato tubers was studied after five-month storage. The rates of tuber infection by *Streptomyces scabies* and *Rhizoctonia solani* were estimated on 100 tubers selected randomly of particular treatments, according to a nine-point scale, and were presented as a percentage infection index. The symptoms of soft rot (*Pectobacterium carotovorum* subsp. *carotovorum*), late blight (*Phytophthora infestans*) and dry rot (*Fusarium* spp.) were evaluated in 5 kg samples for each treatment. The results were expressed as a percentage of the mass of infected tubers.

Foliar fertilization and the levels of mineral fertilization NPK did not affect the severity of common scab symptoms. Significantly higher rates of infection by *R. solani* were observed in tubers from the control treatment without foliar fertilization and from the treatment with a lower level of mineral fertilization (*A*). The symptoms of soft rot (4.6% of the mass of infected tubers) and late blight (1.3%) were strongest in 2006, while the symptoms of dry rot (2.7%) – in 2005. The severity of diseases caused by the above pathogens was greater in tubers grown in plots with a higher level of mineral fertilization (*B*) – N 120 kg ha^{-1}, P 144 kg ha^{-1}, K 156 kg ha^{-1} (1.3 to 4.1% of the mass of infected tubers) than in tubers grown in plots with a lower level of mineral fertilization (*A*) – N 80 kg ha^{-1}, P 80 kg ha^{-1}, K 120 kg ha^{-1}, (0.8 to 3%). The combined application of foliar fertilizers reduced the percentage mass of tubers infected by *P. carotovorum* subsp. *carotovorum* and *P. infestans* to the highest degree.

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NASILENIE WYBRANYCH CHORÓB BULW ZIEMNIAKA NAWOŻONEGO DOLISTNIE

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S ł o w a k l u c z o w e: bulwy ziemniaka, choroby, nawożenie dolistne, nawożenie mineralne.

A b s t r a k t

Badania przeprowadzono w latach 2004–2006 na poletkach doświadczalnych w Bałcynach. Uprawiano wszechstronnie użytkowaną, późną odmianę ziemniaka Jasia. W doświadczeniu uwzględniono dwa czynniki: I – poziomy nawożenia mineralnego: *A* (N – 80 kg ha^{-1}, P – 80 kg ha^{-1}, K – 120 kg ha^{-1}), *B* (N – 120 kg ha^{-1}, P – 144 kg ha^{-1}, K – 156 kg ha^{-1}) i II – nawożenie dolistne: *1* (Basfoliar 12-4-6 – 8 dm^3 ha^{-1}), *2* (ADOB Mn – 4 dm^3 ha^{-1}), *3* (Solubor DF – 2 dm^3 ha^{-1}), *4* (ADOB Mn – 2 dm^3 ha^{-1} + Basfoliar 12-4-6 – 4 dm^3 ha^{-1}), *5* (ADOB Mn – 2 dm^3 ha^{-1} + Solubor DF – 1 dm^3 ha^{-1}), *6* (Basfoliar 12-4-6 – 4 dm^3 ha^{-1} + Solubor DF – 1 dm^3 ha^{-1}), *7* (Basfoliar 12-4-6 – 2,7 dm^3 ha^{-1} + ADOB Mn – 1,3 dm^3 ha^{-1} + Solubor DF – 0,7 dm^3 ha^{-1}), *8* (kontrola bez nawożenia dolistnego). Po 5-miesięcznym przechowywaniu bulw przeprowadzano ocenę ich zdrowotności. Nasilenie parcha zwykłego (*Streptomyces scabies*) i ospowatości bulw (*Rhizoctonia solani*) określano na 100 bulwach z kombinacji, według 9^o skali, a wyniki podano w % jako indeks porażenia. Objawy mokrej zgnilizny (*Pectobacterium carotovorum* subsp. *carotovorum*), zarazy ziemniaka (*Phytophthora infestans*) i suchej zgnilizny bulw (*Fusarium* spp.) oceniano w 5-kilogramowej próbie bulw z każdej kombinacji. Wyniki przedstawiono w procentach masy porażonych bulw.

Nawożenie dolistne oraz poziomy nawożenia mineralnego NPK nie różnicowały nasilenia objawów parcha zwykłego. Istotnie wyższe porażenie *R. solani* zanotowano na bulwach w kombinacji kontrolnej bez nawożenia dolistnego i z nawożeniem mineralnym w niższej dawce (poziom *A*). Największe objawy mokrej zgnilizny bulw (4,6% masy porażonych bulw) i zarazy ziemniaka (1,3%) stwierdzono w 2006 r., a suchej zgnilizny (2,7%) – w 2005 r. Zanotowano silniejsze objawy chorób powodowanych przez wymienione patogeny na bulwach pochodzących z roślin uprawianych w kombinacji z zastosowanym wyższym nawożeniem mineralnym doglebowym *B* – N 120 kg ha^{-1}, P 144 kg ha^{-1}, K 156 kg ha^{-1}, (1,3 do 4,1% masy porażonych bulw) niż u roślin w kombinacji z niższym nawożeniem *A* – N 80 kg ha^{-1}, P 80 kg ha^{-1}, K 120 kg ha^{-1} (od 0,8 do 3%). Łączne stosowanie nawozów dolistnych najbardziej ograniczało procent masy bulw porażonych *P. carotovorum* subsp. *carotovorum* i *P. infestans*.

Introduction

Next to chemical control, the soil and foliar application of mineral fertilizers is the main determinant of the health status of potato plants and the yield and quality of tubers (GRZEŚKIEWICZ, TRAWCZYŃSKI 1999, LAMBERT et al. 2005, MILLER, ROSEN 2005, STACHOWICZ 2007, MALAKOUTI 2008). In Poland, the following foliar fertilizers are most commonly applied to potato plants: Plonvit K, Wuxal Top N, Ekosol, Insol ZBR, Basfoliar, Solubor, Tytanit, and ADOB (WIERCZEWSKA, SZTUDER 2004). The foliar application of mixed microelement fertilizers is advisable under stress conditions. According to KAPSA (2002), MILLER and ROSEN (2005), the combined application of fertilizers and

crop protection chemicals allows to reduce total production costs and contributes to potato protection against pathogenic factors, including *P. infestans*. OSOWSKI (2005) reported that Basfoliar 12-4-6 used together with the fungicides Antracol 70 WG and Unikat 75 WG reduced the incidence of Alternaria blight on potatoes during the growing season. The effects of foliar application of mixed fertilizers on the health status of potato tubers have been discussed by, among others, BORÓWCZAK and GŁADYSIAK (1999), JABŁOŃSKI (2003), RĘBACZ and BORÓWCZAK (2007). The objective of this study was to determine the effect of two levels of mineral fertilization and foliar fertilizer types on the severity of selected diseases on the tubers of potato cv. Jasia, stored for five months.

Material and Methods

The experimental materials comprised potato tubers stored for five months at a temperature of 5°C, harvested during a three-year exact plot experiment (randomized split-plot design, four replications) established in 2004 in Bałcyny, on grey-brown podsolic soil developed from light silty loam of complex 4, class III, by the Department of Agrotechnology and Crop Production Management, University of Warmia and Mazury in Olsztyn. A multi-purpose, late potato cultivar with a high starch content, Jasia, was grown. The experimental factors were as follows:

I – mineral fertilization levels:
- *A* (N – 80 kg ha^{-1}, P – 80 kg ha^{-1}, K – 120 kg ha^{-1}),
- *B* (N – 120 kg ha^{-1}, P – 144 kg ha^{-1}, K – 156 kg ha^{-1}),

II – foliar fertilization:
- *1* (Basfoliar 12-4-6 – 8 dm^3 ha^{-1}),
- *2* (ADOB Mn – 4 dm^3 ha^{-1}),
- *3* (Solubor DF – 2 dm^3 ha^{-1}),
- *4* (ADOB Mn – 2 dm^3 ha^{-1} + Basfoliar 12-4-6 – 4 dm^3 ha^{-1}),
- *5* (ADOB Mn – 2 dm^3 ha^{-1} + Solubor DF – 1 dm^3 ha^{-1}),
- *6* (Basfoliar 12-4-6 – 4 dm^3 ha^{-1} + Solubor DF – 1 dm^3 ha^{-1}),
- *7* (Basfoliar 12-4-6 – 2.7 dm^3 ha^{-1} + ADOB Mn – 1.3 dm^3 ha^{-1} + Solubor DF – 0.7 dm^3 ha^{-1}),
- *8* (control treatment without foliar fertilization).

Healthy, certified seed tubers purchased from a seed production company were planted. Cereal crops were grown as a forecrop. The same agricultural practices (recommended by the Institute of Soil Science and Plant Cultivation in Puławy) and protection measures against agrophages (recommended by the Institute of Plant Protection in Poznań) were carried out in all plots.

The rates of tuber infection by *Streptomyces scabies* and *Rhizoctonia solani* were estimated on 100 tubers selected randomly of particular treatments, according to a nine-point scale (1 – no symptoms, 9 – most severe symptoms, *Metodyka obserwacji*... 1999), and were presented as a percentage infection index. The symptoms of soft rot (*Pectobacterium carotovorum* subsp. *carotovorum*), late blight (*Phytophthora infestans*) and dry rot (*Fusarium* spp.) were evaluated in 5 kg samples collected from particular plots in each treatment. The results were expressed as a percentage of the mass of infected tubers.

Meteorological data for the experimental period are shown in Table 1. The growing seasons of 2004 and 2006 were characterized by higher precipitation totals than the growing season of 2005, in which mean monthly precipitation remained within normal limits over the summer, reaching the upper limit in July. Precipitation was accompanied by moderate temperatures, only in July in the last two years temperatures ranged from 19 to 21°C.

The results were verified statistically by an analysis of variance for a randomized block design (STATISTICA® 8.0 2007–2008 software). Means were compared by Duncan's test (significance level 0.05).

Table 1

Meteorological data according to Meteorological Station Bałcyny

Month	Mean monthly temperature °C			Mean for 1960–90	Mean monthly rainfall in mm			Σ rainfall 1960–90
	2004	2005	2006		2004	2005	2006	
May	11.0	12.5	12.5	12.4	87.1	68.2	93.2	56.7
June	14.5	14.9	16.0	15.7	90.6	35.4	83.5	68.3
July	16.2	18.9	21.0	15.3	78.8	83.9	27.1	81.3
August	18.2	16.8	17.3	17.9	89.3	39.6	141.7	78.1

Results and Discussion

Significantly higher intensity of soft rot, common scab, late blight and rhizoctoniosis was observed on potato tubers harvested in 2004 and 2006, compared with 2005, while weather conditions during the growing season of 2005 contributed to higher rates of infection by *Fusarium* spp. Disease occurrence on stored tubers is affected by growing season weather conditions (REPSIENE, MINEIKIENE 2006).

The severity of tuber diseases was determined by the experimental factors, i.e. NPK fertilization levels and foliar fertilizer types. The highest infection rates were noted for common scab and rhizoctoniosis. During the wet growing seasons of 2004 and 2006, the degree of tuber infection by *S. scabies* was

similar at both NPK fertilization levels. The values of infection indices increased along with an increase in fertilizer rates only in 2005, when precipitation totals were below normal limits (Table 2). REPSIENE and

Table 2

Intensity of common scab *S. scabies* and black scurf *R. solani* (index of infestation in %)

Level NPK	Foliar fertilization	Common scab				Black scurf			
		2004	2005	2006	X	2004	2005	2006	X
*A**	*1***	13.8^{b-h***}	13.3^{b-h}	14.2^{a-g}	13.8^{a-d}	13.7^{jkl}	6.3^{mn}	17.8^{d-h}	12.6^{c-f}
	2	13.2^{b-h}	12.2^{f-i}	14.4^{a-f}	13.3^{bcd}	15.7^{g-k}	6.7^{mn}	21.8^{ab}	14.7^{b}
	3	15.2^{a-e}	10.3^{i}	15.4^{a-d}	13.6^{a-d}	15.5^{g-k}	7.2^{mn}	18.4^{c-g}	13.7^{bc}
	4	12.8^{c-i}	11.4^{ghi}	13.3^{b-h}	12.5^{cd}	15.6^{g-k}	7.5^{mn}	19.9^{b-e}	14.3^{b}
	5	13.4^{b-h}	11.0^{hi}	13.8^{b-h}	12.7^{cd}	14.3^{i-l}	6.1^{mn}	19.1^{b-f}	13.2^{b-e}
	6	14.5^{a-f}	12.8^{c-i}	14.6^{a-f}	14.0^{abc}	14.7^{h-l}	5.7^{mn}	20.5^{a-d}	13.7^{bc}
	7	13.7^{b-h}	10.3^{i}	13.8^{b-h}	12.6^{cd}	13.2^{jkl}	5.8^{mn}	17.5^{d-h}	12.2^{c-g}
	8	15.1^{a-e}	13.3^{b-h}	16.7^{a}	15.0^{a}	18.5^{c-g}	8.9^{m}	23.3^{a}	16.9^{a}
X for treatments		14.0^{ab}	11.8^{d}	14.5^{a}	13.5^{a}	15.2^{c}	6.8^{e}	19.8^{a}	13.9^{a}
B	*1*	13.3^{b-h}	12.5^{e-i}	13.9^{b-g}	13.2^{bcd}	11.8^{l}	6.4^{mn}	16.3^{f-j}	11.5^{efg}
	2	12.8^{c-i}	13.2^{b-h}	14.2^{a-g}	13.4^{bcd}	13.7^{jkl}	6.8^{mn}	19.3^{b-f}	13.3^{bcd}
	3	14.5^{a-f}	13.0^{c-i}	15.9^{ab}	14.5^{ab}	13.0^{kl}	7.1^{mn}	17.3^{e-i}	12.5^{c-g}
	4	12.7^{c-i}	12.4^{e-i}	13.4^{b-h}	12.8^{cd}	13.9^{jkl}	6.0^{mn}	16.3^{f-j}	12.1^{c-g}
	5	12.2^{f-i}	12.7^{c-i}	12.7^{c-i}	12.5^{cd}	12.7^{kl}	5.8^{mn}	14.8^{h-l}	11.1^{fg}
	6	14.2^{a-g}	13.4^{b-h}	13.8^{b-h}	13.8^{a-d}	12.0^{l}	5.2^{n}	15.2^{h-k}	10.8^{g}
	7	12.6^{d-i}	12.6^{d-i}	11.8^{f-i}	12.3^{d}	11.9^{l}	6.0^{mn}	17.7^{d-h}	11.9^{d-g}
	8	14.0^{a-g}	14.2^{a-g}	15.5^{abc}	14.6^{ab}	15.6^{g-k}	7.4^{mn}	21.2^{abc}	14.7^{b}
X for treatments		13.3^{bc}	13.0^{c}	13.9^{b}	13.3^{a}	13.1^{d}	6.3^{e}	17.3^{b}	12.2^{b}
X for foliar fertilization									
1		13.6^{c-f}	12.9^{c-g}	14.1^{bcd}	–	12.8^{cd}	6.4^{ef}	17.1^{b}	
2		13.0^{c-g}	12.7^{d-g}	14.3^{a-d}	–	17.7^{c}	6.8^{ef}	20.6^{a}	
3		14.9^{abc}	11.7^{fg}	15.7^{ab}	–	14.3^{cd}	7.2^{ef}	17.9^{b}	
4		12.8^{d-g}	11.9^{efg}	13.4^{c-g}	–	14.8^{c}	6.8^{ef}	18.1^{b}	
5		12.8^{d-g}	11.9^{efg}	13.3^{c-g}	–	13.5^{cd}	6.0^{f}	17.0^{b}	
6		14.4^{a-d}	13.1^{c-g}	14.2^{bcd}	–	13.4^{cd}	5.5^{f}	17.9^{b}	
7		13.1^{c-g}	11.5^{g}	12.8^{d-g}	–	12.6^{d}	5.9^{f}	17.6^{b}	
8		14.6^{a-d}	13.8^{cde}	16.1^{a}	–	17.1^{b}	8.2^{e}	22.3^{a}	
X for years		13.6^{b}	12.4^{c}	14.2^{a}	–	14.1^{b}	6.6^{c}	18.5^{a}	

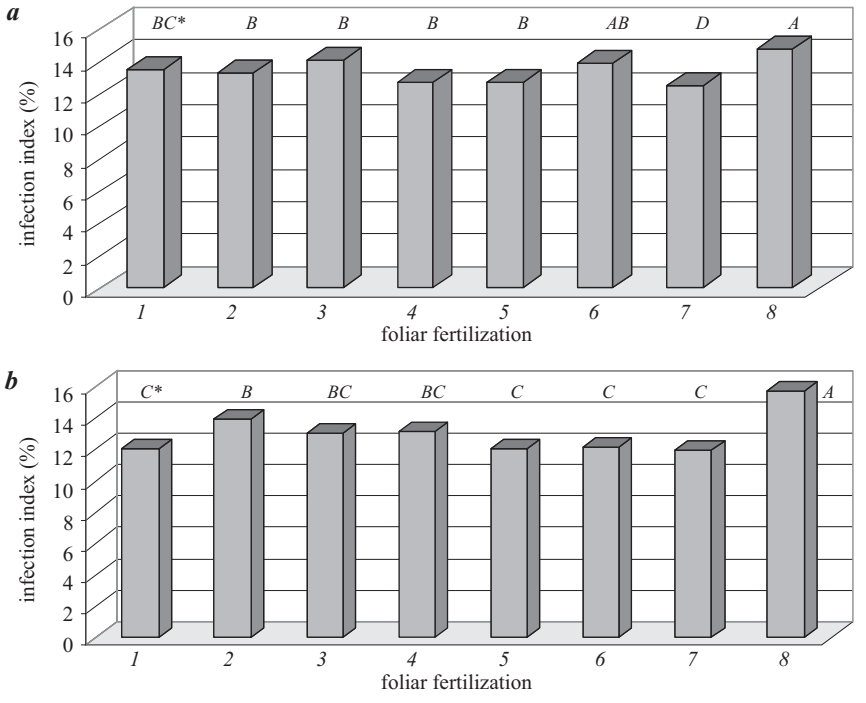
* level of NPK: *A* – N 80 kg ha^{-1}, P 80 kg ha^{-1}, K 120 kg ha^{-1}; *B* – N 120 kg ha^{-1}, P 144 kg ha^{-1}, K 156 kg ha^{-1});

** foliar fertilization: *1* – Basfoliar 12-4-6, *2* – ADOB Mn, *3* – Solubor DF, *4* – ADOB Mn + Basfoliar 12-4-6, *5* – ADOB Mn + Solubor DF, *6* – Basfoliar 12-4-6 + Solubor DF, *7* – Basfoliar 12-4-6 + ADOB Mn + Solubor DF, *8* – control without fertilization;

*** homogeneous groups according to Duncan test for comparison of means within factors and their interactions

MINEIKIENE (2006) observed no differences in the severity of common scab on fertilized and non-fertilized potato tubers, but found that mineral fertilization reduced the incidence of rhizoctoniosis. In the present study significantly lower rates of potato infection by *R. solani* (except for the year 2005) were noted in the treatment with a higher level of NPK fertilization, compared with that with a lower fertilization level. PUA and ABZA (2005) demonstrated that mineral and organic fertilizers alleviated the symptoms of rhizoctoniosis. However, in a study conducted by HONEYCUTT et al. (1996) nitrogen rates of 0 to 250 kg ha^{-1} had no effect on the intensity of infection caused by *R. solani*. KLIKOCKA et al. (2005) reported that sulfur fertilization at a rate of 50 kg ha^{-1} significantly reduced tuber infection by *S. scabies* and *R. solani*.

The foliar fertilizers applied in this experiment had no significant effect on the severity of common scab on potato tubers. However, an analysis of the mean values obtained for both levels of mineral fertilization (*A* and *B*) and for foliar fertilization shows that the infection rates were lower in the treatments in which ADOB Mn was applied together with Basfoliar 12-4-6 (treatment *4*) or with Solubor DF (*5*), and when all three foliar fertilizers were applied (*7*), compared with the other treatments (Figure 1a). The results regarding the effect of mixed foliar fertilizers on the health status of potato tubers, reported in literature, are inconclusive. According to KEINATH and LORIA (1989), the application of manganese can alleviate the symptoms of common scab. JABŁOŃSKI (2003) demonstrated that ADOB Mn and Basfoliar 36 E had no significant effect on the severity of common scab, internal rust spot, brown center and hollow heart. On the other hand, BOLIGŁOWA (2003) reported that the combined application of Insol 7 and urea increased the rates of tuber infection by *S. scabies*. The results of other studies suggest that common scab severity was affected neither by the foliar application of lime salpeter /$Ca(NO_3)_2$/ (SZUTKOWSKA and LUTOMIRSKA 2002) nor by potato growing with or without foliar fertilization (BORÓWCZAK and GŁADYSIAK 1999). BORÓWCZAK and GŁADYSIAK (1999) noted stronger symptoms of rhizoctoniosis on tubers from non-fertilized plants. RĘBACZ and BORÓWCZAK (2007) observed the weakest symptoms of rhizoctoniosis in a high-input production system with the foliar application of Mikrosol U, compared with medium- and low-input systems without foliar fertilization. In the present experiment the strongest symptoms of rhizoctoniosis were noted in the treatment without foliar fertilization, and the mean infection indices for both levels of mineral fertilization were significantly higher, compared to the treatment with the application of foliar fertilizers. The mean values obtained in treatments with foliar fertilization are indicative of similar results (Figure 1b).



* homogeneous groups according to Duncan test for comparison of means

Fig. 1. Infection of tubers: *a* – *S. scabies, b* – *R. solani; 1* – Basfoliar 12- 4-6, *2* – ADOB Mn, *3* – Solubor DF, *4* – ADOB Mn + Basfoliar 12-4-6, *5* – ADOB Mn + Solubor DF, *6* – Basfoliar 12-4-6 + Solubor DF, *7* – Basfoliar 12-4-6 + ADOB Mn + Solubor DF, *8* – control without fertilization

In this study potato tubers showed symptoms of rot caused by *P. carotovorum* subsp. *carotovorum* and species of the genus *Fusarium*, as well as symptoms of infection caused by *P. infestans*. These symptoms were more severe in treatments with higher rates of mineral fertilizers (*B* – N 120 kg ha^{-1}, P 144 kg ha^{-1}, K 156 kg ha^{-1}), in comparison with treatments with lower fertilization rates (*A* – N 80 kg ha^{-1}, P 80 kg ha^{-1}, K 120 kg ha^{-1}). The percentage mass of tubers infected by particular pathogens was as follows: *P. carotovorum* subsp. *carotovorum* – 4.1 and 3.0%, *P. infestans* – 1.3 and 0.8%, *Fusarium* spp. – 2.6 and 1.4% (Table 3 and Table 4), and the respective differences were statistically significant. The effect of foliar fertilizers on the severity of the above diseases varied (Figure 2). Higher rates of mineral fertilizers affected the symptoms of soft rot in treatments with foliar fertilization. The highest percentage mass of infected tubers – 4.7 (mean of the experimental period) was noted in the control treatment, and the difference between this treatment and the remaining fertilization treatments was

Table 3

Intensity of soft rot *P. carotovorum* subsp. *carotovorum* and of late blight of potato tubers *P. infestans* (percentage of mass of infected tubers)

Level NPK	Foliar fertilization	Soft rot				Late blight			
		2004	2005	2006	X	2004	2005	2006	X
A	*1*	3.0^{opq}	1.5^{wz}	3.8^{i-n}	2.8^{fg}	1.3^{f-i}	0.6^{l-o}	1.2^{f-j}	1.0^{bcd}
	2	2.8^{pqr}	2.1^{s-w}	4.2^{f-k}	3.0^{ef}	1.5^{d-g}	0.3^{op}	0.9^{i-m}	0.9^{cde}
	3	3.5^{mno}	1.4^{z}	5.1^{bcd}	3.3^{e}	0.6^{l-o}	0.6^{l-o}	1.0^{h-l}	0.7^{e}
	4	3.3^{nop}	1.7^{uwz}	3.3^{nop}	2.8^{fg}	0.7^{k-o}	0.5^{mno}	1.2^{f-j}	0.8^{de}
	5	4.0^{h-k}	2.2^{r-u}	3.5^{mno}	3.2^{e}	1.1^{g-k}	0.4^{nop}	0.7^{k-o}	0.7^{e}
	6	3.8^{i-n}	1.8^{t-z}	4.1^{g-l}	3.2^{e}	0.7^{k-o}	0.3^{op}	1.0^{h-l}	0.7^{e}
	7	2.4^{qrs}	1.7^{uwz}	3.2^{nop}	2.4^{g}	0.8^{j-n}	0^{p}	0.5^{mno}	0.4^{f}
	8	2.8^{pqr}	2.0^{t-z}	4.8^{b-f}	3.2^{e}	1.3^{f-i}	0.5^{mno}	0.7^{k-o}	0.8^{de}
X for treatments		3.2^{d}	1.8^{f}	4.0^{c}	3.0^{b}	1.0^{c}	0.4^{d}	0.9^{c}	0.8^{b}
B	*1*	4.5^{d-h}	2.2^{r-u}	4.7^{c-g}	3.8^{d}	1.0^{h-l}	0.8^{j-n}	0.9^{i-m}	0.9^{cde}
	2	3.8^{i-n}	2.7^{p-s}	6.3^{a}	4.3^{bc}	1.8^{b-e}	1.4^{e-h}	1.9^{bcd}	1.7^{a}
	3	4.6^{d-h}	2.3^{r-u}	4.7^{c-g}	3.9^{d}	1.5^{d-g}	1.3^{f-i}	2.4^{a}	1.7^{a}
	4	4.8^{b-f}	3.3^{nop}	5.4^{b}	4.5^{ab}	1.1^{g-k}	1.0^{h-l}	1.4^{e-h}	1.2^{b}
	5	4.4^{e-i}	2.7^{p-s}	5.0^{b-e}	4.0^{cd}	1.2^{f-j}	1.1^{g-k}	1.0^{h-l}	1.1^{bc}
	6	3.7^{j-n}	3.5^{mno}	6.0^{a}	4.4^{ab}	0.8^{j-n}	0.9^{i-m}	1.6^{c-f}	1.1^{bc}
	7	3.5^{l-o}	2.1^{s-w}	4.2^{f-k}	3.3^{e}	1.0^{h-l}	1.1^{g-k}	1.4^{e-h}	1.2^{b}
	8	5.1^{bcd}	3.6^{j-o}	5.3^{bc}	4.7^{a}	2.0^{abc}	0.4^{nop}	2.2^{abc}	1.5^{a}
X for treatments		4.3^{b}	2.8^{e}	5.2^{a}	4.1^{a}	1.3^{b}	1.0^{c}	1.6^{a}	1.3^{a}
X for foliar fertilization									
1		3.8^{cd}	1.9^{i}	4.3^{b}	–	$1.2^{bc}d$	0.7^{f-i}	1.1^{cde}	
2		3.3^{ef}	2.4^{h}	5.3^{a}	–	1.7^{a}	0.9^{d-g}	1.4^{ab}	
3		4.1^{bcd}	1.9^{i}	4.9^{a}	–	1.1^{cde}	1.0^{def}	1.7^{a}	
4		4.1^{bcd}	2.5^{h}	4.3^{b}	–	0.9^{d-g}	0.8^{e-i}	1.3^{bc}	
5		4.2^{bc}	2.5^{h}	4.3^{b}	–	1.2^{bcd}	0.8^{e-i}	0.9^{d-g}	
6		3.8^{cd}	2.7^{gh}	5.1^{a}	–	0.8^{e-i}	0.6^{ghi}	1.3^{bc}	
7		3.0^{fg}	1.9^{i}	3.7^{de}	–	0.9^{d-g}	0.6^{hi}	1.0^{def}	
8		4.0^{bcd}	2.8^{gh}	5.1^{a}	–	1.7^{a}	0.5^{i}	1.5^{ab}	
X for years		3.8^{b}	2.3^{c}	4.6^{a}	–	1.2^{b}	0.7^{c}	1.3^{a}	

Explanations as in Table 2

statistically significant. At both levels of mineral fertilization, the weakest symptoms of soft rot were observed in the treatment with combined application of foliar fertilizers. According to BAIN et al. (1996), the optimum rates of mineral fertilizers (e.g. calcium and magnesium) may reduce plant infection by *P. carotovorum* subsp. *carotovorum*, thus decreasing the incidence of soft rot on stored tubers. CZAJKA et al. (2006) demonstrated that nitrogen applied at excessive rates stimulated the development of selected potato diseases,

Table 4

Intensity of dry rot *Fusarium* spp. (percentage of mass of infected tubers)

Level NPK	Foliar fertilization	2004	2005	2006	X
A	*1*	1.6^{lm-p}	1.9^{j-m}	1.8^{j-n}	1.8^{cd}
	2	1.2^{o-s}	1.7^{k-o}	0.6^{tu}	1.2^{g}
	3	1.4^{m-q}	2.2^{h-k}	1.6^{l-p}	1.7^{cde}
	4	1.4^{m-q}	2.0^{i-l}	1.0^{q-u}	1.5^{ef}
	5	1.1^{p-t}	2.3^{g-j}	1.4^{m-q}	1.6^{def}
	6	0^{w}	1.0^{q-u}	0.5^{u}	0.5^{h}
	7	0.8^{r-u}	0.7^{stu}	0.7^{stu}	0.7^{h}
	8	2.1^{i-l}	1.8^{j-n}	2.0^{i-l}	2.0^{c}
X for treatments		1.2^{e}	1.7^{d}	1.2^{e}	1.4^{b}
B	*1*	0.8^{r-u}	1.7^{k-o}	1.0^{q-u}	1.2^{g}
	2	3.1^{def}	4.8^{ab}	3.4^{d}	3.8^{a}
	3	3.3^{de}	4.2^{c}	3.6^{d}	3.7^{a}
	4	0.8^{r-u}	2.0^{i-l}	1.2^{o-s}	1.3^{fg}
	5	2.7^{fgh}	4.5^{bc}	2.5^{ghi}	3.2^{b}
	6	2.8^{efg}	5.2^{a}	3.1^{def}	3.7^{a}
	7	1.2^{o-s}	3.3^{de}	1.4^{m-q}	2.0^{c}
	8	1.3^{n-r}	3.1^{def}	1.4^{m-q}	1.9^{c}
X for treatments		2.0^{c}	3.6^{a}	2.2^{b}	2.6^{a}
X for foliar fertilization					
1		1.2^{hi}	1.8^{ef}	1.4^{gh}	–
2		2.2^{cde}	3.3^{a}	2.0^{def}	–
3		2.4^{bcd}	3.2^{a}	2.6^{b}	–
4		1.1^{hi}	2.0^{def}	1.1^{hi}	–
5		1.9^{ef}	3.4^{a}	2.0^{ef}	–
6		1.4^{gh}	3.1^{a}	1.8^{ef}	–
7		1.0^{i}	2.0^{def}	1.1^{hi}	–
8		1.7^{fg}	2.5^{bc}	1.7^{fg}	–
X for years		1.6^{b}	2.7^{a}	1.7^{b}	–

Explanations as in Table 2

including soft rot. MILLS et al. (2006) reported that potassium sorbate, potassium alum and copper sulfate exerted a fungistatic effect on the above bacteria under *in vitro* conditions. Potato tubers treated with the above compounds before storage showed weaker infection symptoms than control tubers.

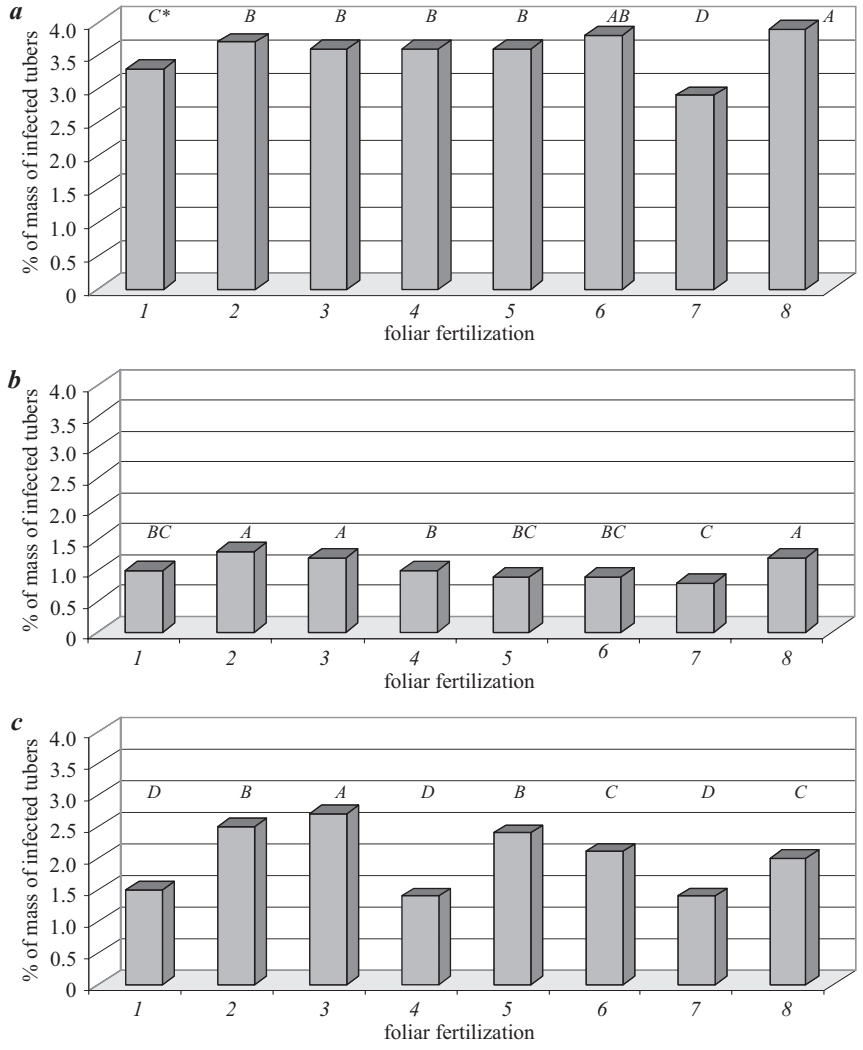


Fig. 2. Infection of tubers: *a* – *P. carotovorum* subsp. *carotovorum*, *b* – *P. infestans*, *c* – *Fusarium* spp. Explanations as in Figure 1

In the present experiment, late blight symptoms were noted on 0.4 to 1% of potato tubers, in the treatment with the application of all three foliar fertilizers and in that with the application of Basfoliar 12-4-6 and ADOB Mn respectively (Table 3). Slightly higher infection rates (significant differences) were reported in treatments with a higher level of mineral fertilization and the application of ADOB Mn or Solubor DF and in the control treatment (1.5–1.7%), compared with the other treatments.

Mineral fertilization, including the application of mixed foliar fertilizers, affects late blight occurrence both during the growing season and storage. The foliar application of phosphorus reduces the incidence of infection caused by *P. infestans* (COOKE, LITTLE 2002) and *P. erytroseptica* (JOHNSON et al. 2004). RĘBACZ and BORÓWCZAK (2007) demonstrated that nitrogen applied at rates comparable to those used in this experiment had no effect on the percentage of tubers infected by *P. infestans,* while a significant decrease in their percentage share was noted at higher nitrogen rates (180 kg N ha^{-1}). ANN (2001) found that the infection of potato plants by the above pathogen was reduced following the combined application of fungicides and the foliar fertilizers Nur-Phite P and Guard PK. Also KAPSA (2002) reported that foliar fertilizers applied together with fungicides protected potato plants against *P. infestans*. The authors of earlier studies (BÓROWCZAK and GŁADYSIAK 1999) observed comparable severity of late blight symptoms in treatments with and without foliar fertilization.

The highest rates of infection by fungi of the genus *Fusarium* (5.2% of the total mass of tubers) were noted on potato tubers harvested in 2005 in the treatment with a higher level of mineral fertilization and foliar application of Basfoliar 12-4-6 and Solubor DF (treatment *6*, Table 4). The strongest symptoms of dry rot were observed in treatments with a higher level of mineral fertilization and the application of ADOB Mn or Solubor DF. The weakest disease symptoms were reported in treatments with the application of Basfoliar 12-4-6, ADOB Mn together with Basfoliar 12-4-6, and the combined application of all three foliar fertilizers (Figure 2c).

Some species of the genus *Fusarium* (*F. avenaceum, F. culmorum, F. equiseti, F. sambucinum, F. solani* var. *coeruleum*) are dangerous pathogens causing substantial tuber yield loss and tuber quality deterioration during storage (KURZAWIŃSKA 1997, PETERS et al. 2008, STEVENSON et al. 2001). The findings of MECTAU et al. (2002) indicate that certain salts (sodium carbonate, aluminum chloride) inhibit the development of dry rot on potato tubers.

Conclusions

1. Higher rates of tuber infection by *P. carotovorum* subsp. *carotovorum, P. infestans* and fungi of the genus *Fusarium*, and lower rates of infection by *R. solani* and *S. scabies* were noted in treatments with a higher level of mineral NPK fertilization, compared with treatments with a lower fertilization level.

2. Foliar fertilizers exerted a varied effect on the severity of tuber diseases. Lower rates of infection by *R. solani* were observed in fertilized treatments than in the control treatment.

3. The combined application of three foliar fertilizers had the most beneficial effect on the health status of potato tubers.

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CONTENT OF CADMIUM IN MAIZE (*ZEA MAYS* L.) AND SOILS FERTILIZED WITH SEWAGE SLUDGES AND MIXTURES OF SEWAGE SLUDGE AND PEAT

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K e y w o r d s: cadmium, sewage sludge, maize.

A b s t r a c t

The investigations aimed at an assessment of treatment with sewage sludges and mixtures of sewage sludge and peat effect on cadmium content in maize and soils with diversified texture. The research was conducted in conditions of pot experiment. Fertilization with sewage sludge and sludge mixtures with peat had a more beneficial effect on maize yields than treatment with mineral salts. As compared to fertilization with mineral salts, organic fertilizers applied to the soil did not increase cadmium concentrations in maize biomass. Soil pH affected cadmium mobility more than applied sewage sludge. Mixtures of sewage sludge and peat (in comparison to sewage sludge as such) slightly better influenced maize biomass yield and had a comparably cadmium content in plant biomass.

ZAWARTOŚĆ KADMU W KUKURYDZY (*ZEA MAYS* L.) I W GLEBACH NAWOŻONYCH OSADAMI ŚCIEKOWYMI I MIESZANINAMI OSADÓW ŚCIEKOWYCH I TORFU

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Słowa kluczowe: kadm, osady ściekowe, kukurydza.

Abstrakt

Celem badań była ocena wpływu nawożenia osadami ściekowymi oraz mieszaninami osadów ściekowych i torfu na zawartość kadmu w kukurydzy i w glebach o zróżnicowanym składzie granulometrycznym. Badania przeprowadzono w warunkach doświadczenia wazonowego. Nawożenie

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osadami ściekowymi i mieszaninami osadów z torfem działało korzystniej na plony kukurydzy niż nawożenie solami mineralnymi. Zastosowane doglebowo materiały organiczne w porównaniu z nawożeniem solami mineralnymi nie zwiększyły zawartość kadmu w biomasie kukurydzy. Większy wpływ na mobilność kadmu wywierał odczyn gleb niż zastosowane osady ściekowe. Mieszaniny osadów ściekowych z torfem (w porównaniu z samymi osadami ściekowymi) działały nieznacznie korzystniej na plon biomasy kukurydzy oraz porównywalnie na zawartość kadmu w biomasie roślin.

Introduction

Progressing economic and civilization development leads to generation of larger amount of sewage and in consequence sewage sludges. Still insufficient utilization of the materials necessitates their storage, which not only incurs considerable costs for the sludge generator but is also only temporary solution to this problem.

Environmental application of sewage sludge is often reduced by microbiological factor but also by its high heavy metal concentrations posing hazards to the cleanliness of the soil environment and crop quality (SOMMERS 1977, SMITH 1994).

Transformations of bioavailability of heavy metals supplied to the soil with sewage sludge are conditioned by the soil properties (HAGHIRI 1974, DIJKSHORN et al. 1981, MCGRATH, LANE 1989, BHOGAL et al. 2003, HOLM et al. 2003, QURESHI et al. 2004) and by the properties of each element (BERTI, JACOBS 1996), which apparently makes difficult prediction of heavy metal migration from soil to plants (BASTA et al. 2005). Therefore, the investigations were undertaken to assess the effect of fertilization with sewage sludge on cadmium concentrations in maize cultivated in soils with diversified texture.

Material and Methods

The assessment of fertilization effect on cadmium concentrations in maize was made in a pot experiment conducted in 2003–2005. The experimental design comprised the following treatments in four replications on three soils: control (0); mineral treatment – (NPK); farmyard manure – (FYM); sewage sludge *A* – (SSA); sewage sludge *B* – (SSB) and mixtures of the sewage sludges with peat (MSSA, MSSB). The soil material used for the experiment comprised: weakly loamy sand (GI), sandy silt loam (GII) and medium silt loam (GIII), collected from the arable layer (0–20 cm) of ploughlands from the Krakow neighbourhood. The sewage sludge used for the experiment originated from two different mechanical-biological municipal sewage treatment plants. Sewage sludges were mixed with peat in the 1 : 1 weight ratio in conversion to

material dry mass. The peat containing 408 g kg^{-1} dry matter was characterized by the contents of 88 g kg^{-1} of ash, 34.4 g N, 0.91 g P, 1.14 g K and 0.56 mg Cd kg^{-1} d.m. The characteristics of the chemical composition of the soil material and organic materials were given in Table 1 and Table 2 (values converted into dry matter determined at 105°C).

Table 1

Chemical composition of materials used in experiment

Determination		FYM	Sewage sludge (SSA)	Sewage sludge + peat (MSSA)	Sewage sludge (SSA)	Sewage sludge + peat (MSSB)
Dry matter g kg^{-1}		189	310	343	418	372
pH (H_2O)		6.22	6.12	5.57	5.73	5.20
Organic matter g kg^{-1} d.m.		679	353	652	552	771
Total forms						
N	g kg^{-1} d.m.	21.6	17.0	24.7	37.4	35.1
P		22.60	5.48	3.00	19.32	7.64
K		26.69	2.71	1.88	2.81	1.64
Cd	mg kg^{-1} d.m.	1.28	2.71	1.45	2.25	1.03

Table 2

Some properties of soils before the establishment of the experiment

Determination			Soil		
			(GI)	(GII)	(GIII)
Granulometric composition Ø	1.0–0.1 mm	%	78	42	28
	0.1–0.02 mm		13	33	29
	< 0.02 mm		9	25	43
pH KCl			6.21	5.69	5.30
Hydrolitic acidity		mmol(+) kg^{-1} d.m.	11.2	23.4	33.2
Sum of alkaline cation			39.9	86.8	128.4
Total N		g kg^{-1} d.m.	0.96	1.25	1.72
Organic C			9.37	13.36	17.68
Total S			0.16	0.28	0.32
Total Cd		mg kg^{-1} d.m	0.68	0.65	0.78
Available forms					
P		mg kg^{-1} d.m.	79	217	29
K			166	359	138
Mg			134	154	126
S-SO_4			13.4	11.9	11.4

PVC pots used for the experiment contained 5.50 kg of air-dried soil material. The soils were gradually moistened prior to the experiment outset to obtain 30% of maximum water capacity. After this period sandy silt loam and medium silt loam were limed to obtain pH stated by the decree. The measure was applied separately in each pot. Chemically pure CaO was used in a dose calculated on the basis of soil hydrolytic acidity. Subsequently all soils were left for 4 weeks and water loses were supplemented occasionally. After this period fertilization in organic form was introduced in the amount corresponding to 1.20 g N pot^{-1} . Phosphorus and potassium quantities were supplemented with solutions of chemically pure salts [P – $Ca(H_2PO_4)_2$ H_2O and K – KCl] to equalize the amounts of these components supplied with the organic materials. The identical nitrogen, phosphorus and potassium doses were used on mineral (NPK) treatment as on the treatments receiving organic materials. Doses of N, P and K were, respectively: 1.20 g N pot^{-1} as NH_4NO_3, 1.26 g P pot^{-1} as $Ca(H_2PO_4)_2$ H_2O and 1.48 g K pot^{-1} as KCl. Taking into consideration the residual fertilizer effect and the soils abundance in bioavailable phosphorus and potassium, in the second and third year of the experiment, the following doses of fertilizer components were applied corresponding to: 0.80 g N; 0.2 g P and 1.40 g K $pot^{-1} \cdot year^{-1}$ as chemically pure salts.

Maize, San c.v. (FAO 240) was cultivated each year and 5 plants were left per pot. Maize was always harvested at the stage of 7–9 leaves. Plant growing period in the subsequent years was as follows: 47 days in the first year, 66 days in the second and 54 days in the third. The plants were watered with distilled water throughout the experiment to 50% of the maximum soil water capacity. After the harvest the plants were dried (at 70°C) to constant weight and the yield of dry mass of shoots and roots was determined. Subsequently, the dried biomass was crushed in a laboratory mill and mineralized in a muffle furnace (at 450°C for 5 hours). The remains were dissolved in diluted nitric acid 1:2 (v/v) (OSTROWSKA et al. 1991). The soil material collected each year after completed vegetation period was analyzed with reference to the changes of physicochemical properties occurring in result of the applied fertilization. Cadmium concentrations were determined in dried material sifted through a sieve with 1 mm mesh after extraction with 1 mol dm^{-3} NH_4NO_3 solution (DEL CASTHILO, RIX 1992). Cadmium was assayed in the obtained solutions of plant material and soil extracts using ICP-AES method on JY 238 Ultrace apparatus (France). Plant reference material – NCS DC73348 (China National Analysis Center for Iron & Steel) and soil reference – AG-2 (*Agro*MAT) was added to each analyzed series. The results were elaborated according to a fixed model where fertilization or soil was the factor. Statistical computations were made using single factor ANOVA and the significance of differences were estimated by NIR Fisher test at the significance level $p < 0.05$ (STANISZ 1998).

Results and Discussion

Average yields of maize biomass (the aboveground parts and roots) from treatments obtained over the three-year period on the soil, which belonged to light soil class GI were markedly smaller (by over 20% – irrespectively of the plant part) than the yields from the other two, heavier soils GII and GIII (Figure 1). The difference in yield obtained on sandy loam and medium loam was not significant for the aboveground parts, whereas an average root biomass yield did not differ at all between these two soils.

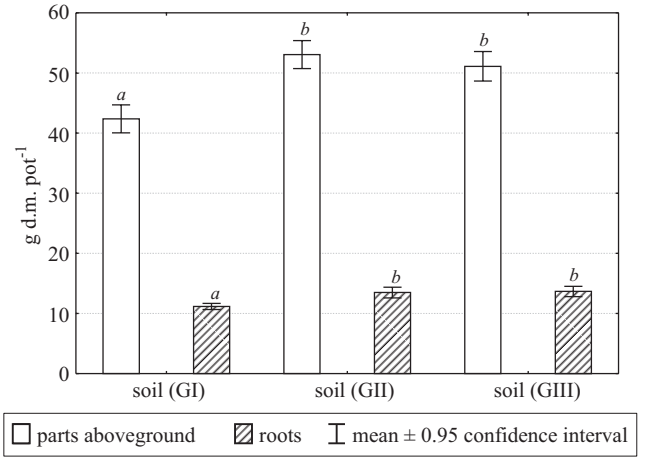

Fig. 1. Mean yields of parts aboveground and roots of maize from fertilization objects from period 3 of years
Means followed by the same letters did not differ significantly at $p < 0.05$ according to the Fisher test

Analysis of variance confirmed an advantageous effect of organic treatment on maize biomass yield (Table 3). Fertilization with sewage sludge or its mixtures with peat and farmyard manure allowed to obtain significantly larger yield than harvested on the object fertilized exclusively with mineral compounds. In both cases when mixtures of sewage sludge and peat were used, larger yield was produced than on the sludge used separately. Fertilizer effectiveness of organic materials is determined mainly by nitrogen content, particularly its mineral forms (SZULC et al. 2004). Nitrogen in the first place determines the amount of obtained biomass yield. However, disturbed relationships between other nutrients may directly affect plant mineral economy. Fertilization with organic materials applied over the three-year period of investigations produced better results visible as the amount of biomass yields,

than mineral salt treatment. This effect cannot be fully ascribed to the activity of applied sewage sludge or its mixtures with peat. It resulted from the consequent effect of organic materials and supplementary treatment with mineral salts used in the second and third year of investigations. The factor determining plant yielding might have been also introducing to the soil of other components of organic materials such as sulphur, magnesium or micro-elements, whose amounts were not balanced. According to WOŁOSZYK (2003), application of natural or organic fertilizers not always causes increase in crop yield in result of so called consequent effect. Research conducted by DRAB and DERENGOWSKA (2003) demonstrated a positive effect of sewage sludge on plat yielding, at the same time revealing that the amount of yield, irrespective of soil is conditioned by the sewage sludge dose. WIATER et al (2004) reported worse direct effect of sewage sludge granulate on maize yield than mineral treatment but the consequent effect of granulate activity was better.

Irrespective of applied fertilization or soil, higher cadmium concentrations were assessed in maize roots (Table 3, Figure 2). The greatest amounts of cadmium were determined in the biomass of aboveground parts and roots of plants from mineral salt treatment. Mean cadmium concentration in biomass for the three years on this treatment was 0.84 mg Cd kg^{-1} d.m. in the aboveground parts and was by over 100% higher than the content assessed in the aboveground parts of maize fertilized with organic materials. Assuming a permissible cadmium content in biomass of plants for fodder on the level of 0.5 mg Cd kg^{-1} d.m., it may be seen that except the biomass from mineral salt treatment, this element concentrations in the aboveground parts did not limit its usability for fodder (KABATA-PENDIAS et al. 1993). Smaller diversification between the treatments was registered for cadmium concentrations in maize root biomass. Significant diversification was registered in maize biomass cadmium concentrations depending on soil (Figure 2). In medium silt loam GIII cadmium content was the largest in the aboveground parts, while in weakly loamy sand GI it was the highest in roots. Results of analyses conducted by PIOTROWSKA and GAŁCZYŃSKA (1990) and PATORCZYK-PYTLIK (2001) reveal that excessive heavy metal accumulation in plants, including cadmium, occurs when large doses of sewage sludge heavily loaded with these elements are supplied with fertilizers. According to WOŁOSZYK (2003) application of moderate doses of sewage sludge does not cause excessive cumulation of heavy metals in plants. Author's own research revealed that significantly greatest quantities of cadmium, irrespective of maize parts, were found in plants fertilized with mineral salts. Presented results corroborate with these published by LOGAN and CHANEY (1983). According to CHANEY (1982) cadmium is the element which is not affected by so called soil-plant barriers, which means that plants tolerate in their organisms (without any toxicity symptoms) the amounts of cadmium which are normally toxic for animals consuming

these plants. The above-mentioned statement explains why no decline in plant yield was noted on mineral salt treatment where this element content was the highest. On the other hand KABATA-PENDIAS and PENDIAS (1999) claim that at its increased uptake cadmium mostly accumulates in roots. It most probably results from plant, particularly the unicotyledonous, ability to complex among others cadmium in their roots (INOUHE et al. 1994).

Table 3

3-year period mean yields of dry mass of parts aboveground and roots plants of total cadmium content in maize and mobile forms cadmium in soils

Fertilization	Yield of biomass		Cadmium in the plant		Cadmium in the soil
	g d.m. pot^{-1}		g Cd kg^{-1} d.m.		mg Cd kg^{-1} d.m.
	(PAG)	(K)	(PAG)	(K)	
Control (0)	22.1^{a}	7.4^{a}	0.19^{a}	1.47^{ab}	0.024^{b}
NPK	42.5^{b}	10.1^{b}	0.84^{d}	2.43^{c}	0.031^{c}
FYM	48.0^{c}	13.1^{cd}	0.30^{ab}	1.22^{a}	0.018^{a}
Sewage sludge A (SSA)	47.3^{bc}	11.8^{c}	0.42^{bc}	1.67^{ab}	0.020^{ab}
Sewage sludge A + peat (MSSA)	49.3^{c}	11.8^{c}	0.46^{c}	1.72^{b}	0.021^{ab}
Sewage sludge B (SSB)	50.4^{c}	14.1^{d}	0.37^{bc}	1.49^{ab}	0.022^{ab}
Sewage sludge B + peat (MSSB)	55.6^{d}	15.8^{e}	0.36^{bc}	1.57^{ab}	0.023^{b}

(PAG) parts aboveground; (K) roots

Means followed by the same letters in columns did not differ significantly at $p < 0.05$ according to the Fisher test

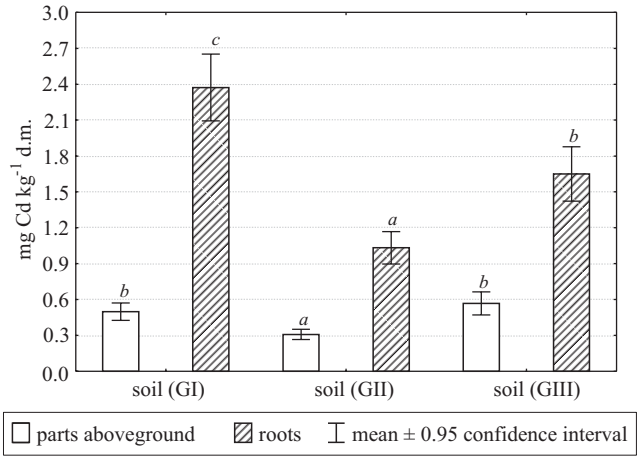

Fig. 2. Mean content of cadmium in parts aboveground and roots maize from fertilization objects from period 3 of years

Means followed by the same letters did not differ significantly at $p < 0.05$ according to the Fisher test

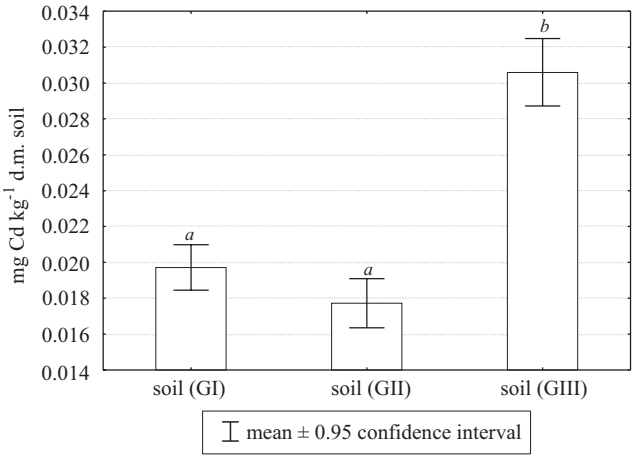

Fig. 3. Mean content of mobile forms of cadmium in soils from fertilization objects from period 3 of years
Means followed by the same letters did not differ significantly at $p < 0.05$ according to the Fisher test

Mean content of mobile cadmium forms in soil after the experiment completion was the greatest in the treatment of the heaviest texture GIII (Figure 3). The conducted research showed that on average the content of this element mobile forms in soils after the application of organic materials was significantly smaller than the concentrations assessed in soils of mineral salt treatments (NPK) – Table 3. It should be emphasized that mean content of cadmium mobile forms in soils from the treatments where organic materials were used was almost the same, whereas soil pH significantly affected this element mobility (SUKREEYAPONGSE et al. 2002) – Figure 4. Heavy metal bioavailability in soils is different for various elements. Heavy metal passing into the soil solution depends on the soil properties and the element itself (BASTA et al. 2005). Presented research revealed progressing soil acidification, especially in result of mineral salt application, irrespective on soil agronomic category, which undoubtedly had a strong influence on improving cadmium bioavailability (WILLIAMS et al. 1987, NARWAL et al. 1983, GONDEK 2008). The results obtained by the Author do not correspond with the results of PATORCZYK-PYTLIK (2001) who does not associate the changes of solubility of among others cadmium compounds in soil with the soil pH, which, according to the quoted author, did not change much after the application of either crude or composted sewage sludge.

In Figures 5–7 placed changes of dry weight yield, its cadmium concentrations and this element mobile forms in soil for the individual years of the experiment. Presented results point to a comparable effect of sewage sludge treatment on cadmium content in maize biomass and the content of mobile

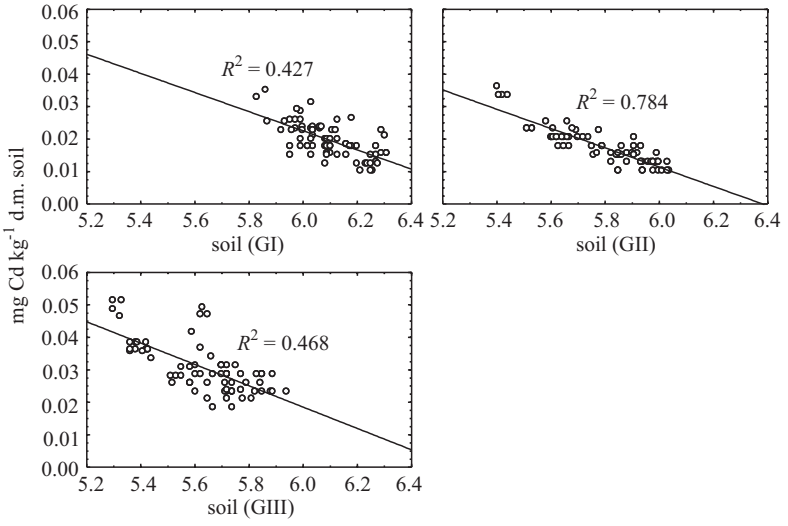

Fig. 4. Relationship between soil pH and mobile forms cadmium content in soil

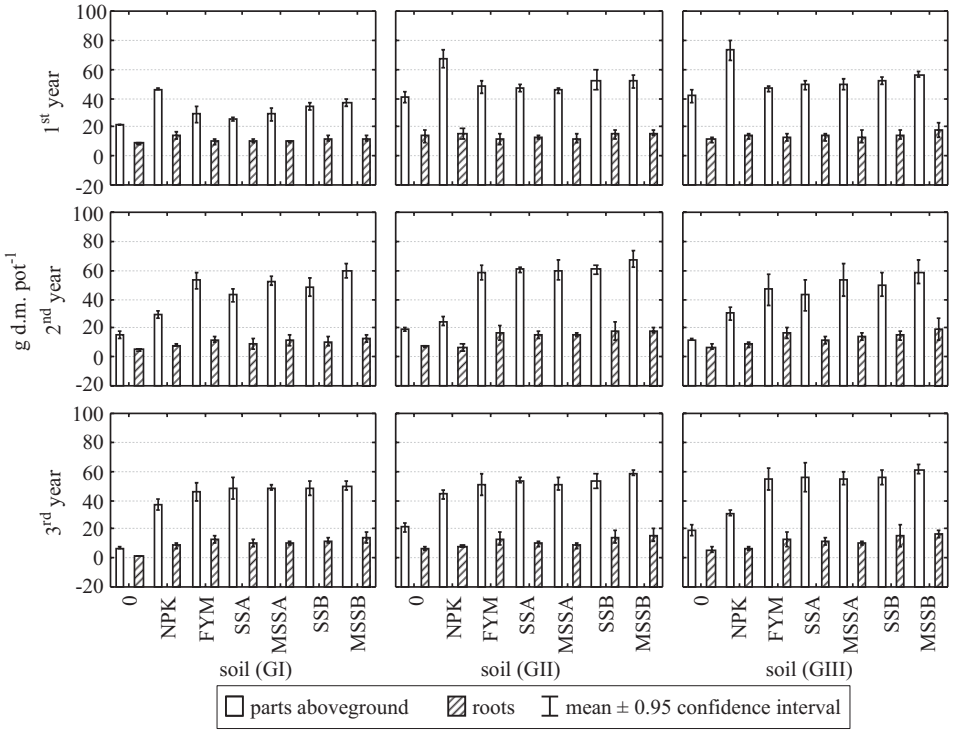

Fig. 5. Yield of parts aboveground and roots of maize from three years of the experiment on three soils

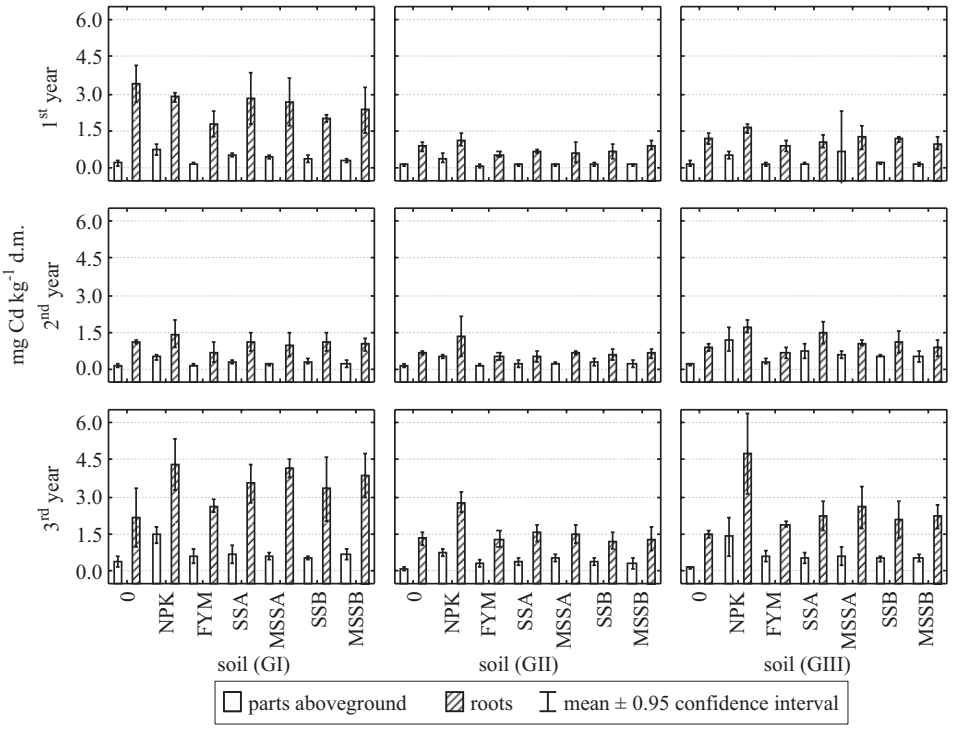

Fig. 6. Content of cadmium in parts aboveground and roots of maize from three years of the experiment on three soils

cadmium forms in soil in relation to fertilization with mixtures of sewage sludge and peat. Plant response expressed by the quantity of yield from mineral salt (NPK) treatments deserves attention. Greater yields from this treatment were registered in the first year of the investigations. In the subsequent years yield response to fertilization with organic materials was positive despite blurring differences on lighter soils. It resulted from so called consequent effect of organic fertilizers which additionally reinforced by supplementary doses of mineral fertilizers positively affected maize yield.

Conclusion

1. In comparison with organic materials supplied to the soil, fertilization with mineral salts significantly increased cadmium concentrations in maize biomass.

2. Soil pH affected cadmium mobility more than fertilization with sewage sludge and its mixtures with peat.

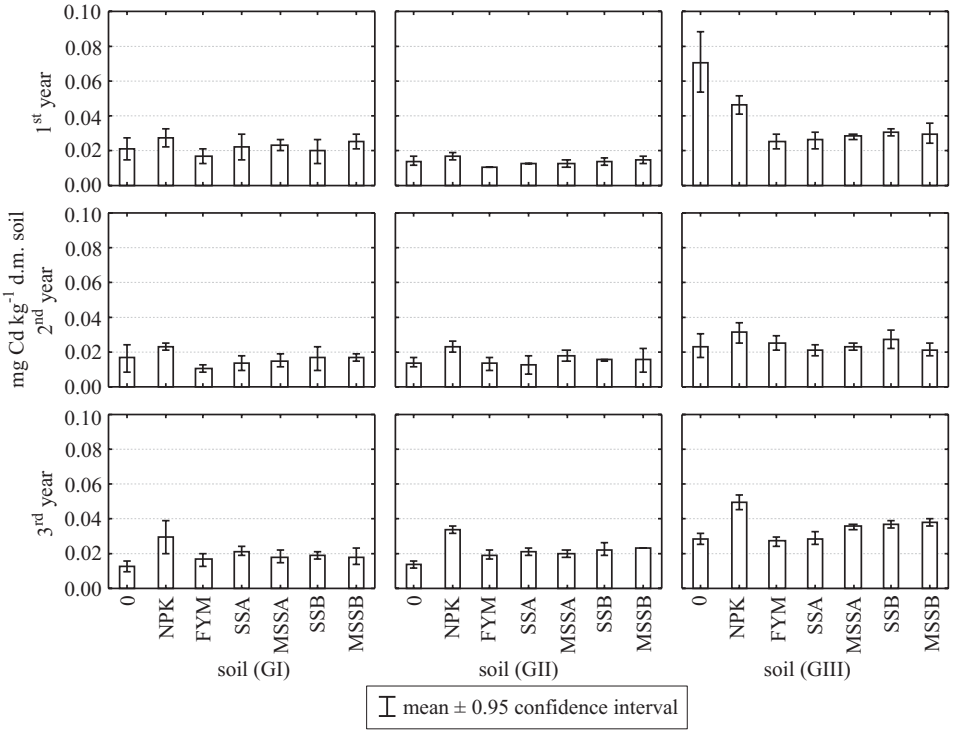

Fig. 7. Content of mobile forms of cadmium in soils from three years of experiment on three soils

3. Mixtures of sludge and peat (in comparison with sewage sludge applied separately) slightly more positively affected maize biomass yield and comparably cadmium content in the plant biomass.

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EFFECT OF APPLICATION OF POLYALUMINIUM CHLORIDE ON REDUCING EXPLOITATION PROBLEMS AT THE WASTEWATER TREATMENT PLANT IN OLSZTYN

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K e y w o r d s: *Microthrix parvicella*, sludge bulking, biological origin foam, filamentous organisms, polyaluminium chloride.

A b s t r a c t

The paper contains an assessment of the effectiveness of polyaluminium chloride (PAX-18) application in improving sedimentation properties of activated sludge at the wastewater treatment plant in Olsztyn. The causes of bulking and foaming of activated sludge have been identified. The effect of adding doses of polyaluminium chloride on the sludge volume index (SVI) and counts of *Microthrix parvicella* has been analysed. Application of doses of PAX-18 within the range of 0.63 and 2.13 g Al^{+3} kg^{-1} sdmd (sludge dry mass daily) to the system, for the duration of 10 to 89 days, proved to be an ineffective way of reducing SVI. However, in most cases, the applied treatments reduced the foaming of activated sludge in multi-functional reactors.

WPŁYW DAWKOWANIA CHLORKU POLIGLINU NA OGRANICZENIE PROBLEMÓW EKSPLOATACYJNYCH W OCZYSZCZALNI ŚCIEKÓW W OLSZTYNIE

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Słowa kluczowe: *Microthrix parvicella*, puchnięcie osadu, piana pochodzenia biologicznego, organizmy nitkowate, chlorek poliglinu.

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Abstrakt

W pracy oceniono skuteczność stosowanych zabiegów dawkowania chlorku poliglinu (PAX-18) na poprawę właściwości sedymentacyjnych osadu czynnego w oczyszczalni ścieków w Olsztynie. Określono przyczynę puchnięcia i pienienia osadu czynnego. Przeanalizowano wpływ dozowania chlorku poliglinu na indeks objętościowy osadu czynnego (SVI) i liczebność *Microthrix parvicella.* Dawkowanie PAX-18 w zakresie od 0,63 do 2,13 g Al^{+3} kg^{-1} smod w układzie, w przedziałach czasowych od 10 do 89 dni, okazało się nieskutecznym sposobem na obniżenie SVI. Stosowane zabiegi w większości przypadków ograniczały natomiast wpienianie osadu czynnego w reaktorach wielofunkcyjnych.

Introduction

The technological changes broadly introduced in the 1990s to Polish wastewater treatment plants operating on activated sludge that would enable improved removal of nitrogen and phosphorus from sewage and wastewater have compounded the problem of foaming and bulking of activated sludge. This is a result of the fact that the conditions which facilitate the removal of biogens promote the development of specific forms of filamentous bacteria in activated sludge, mostly responsible for its foaming and bulking. One of the most common representative of such filamentous microorganisms is *Microthrix parvicella* (KALISZ et al. 2005, DRZEWICKI et. al. 2008). Excessive growth of this bacterium creates many exploitation difficulties, such as a disturbed sludge sedimentation process, difficulties in maintaining the adequate age of sludge and recirculant concentrations, impeded dewatering of sludge, inferior production of biogas, problems related to safety in closed fermentation chambers, and many other drawbacks up to inferior visual qualities of the wastewater treatment facilities. Excessive growth of *Microthrix parvicella* in activated sludge used for removal of biogenic substances is very difficult to halt. The bacterium proliferates well owing to the long age of sludge and associated low load. The microorganism demonstrates high affinity to oxygen (LEMMER 1992). Under both aerobic and anaerobic conditions, it is capable of binding and utilizing long-chain fatty acids, present in raw sewage, as a source of carbon atoms and energy (ANDREASEN, NIELSEN 2000, SLIJKHUIS, DEINEMA 1988, SLIJKHUIS et al. 1984). All these factors make it difficult to control the causes of the growth and development of *Microthrix parvicella* in wastewater plants using technologies for improved removal of nitrogen and phosphorus from sewage. It seems that application of polyaluminium chloride could be an effective way of controlling the bacterium. However, this is an expensive method, which also involves generation of excess sludge and can be harmful to the whole activated sludge biomass as well as to the natural environment in that that is acts selectively and creates better conditions for

growth of subordinate filamentous organisms. Therefore, each wastewater plant needs to work out its own strategy for the frequency of application and concentration of used doses of this reagent.

The purpose and scope of the study

The purpose of this study has been to assess the effectiveness of adding doses of polyaluminium chloride PAX-18 in improving sedimentation properties of activated sludge in the wastewater treatment plant in Olsztyn.

The study involved:

– determination of the causes of foaming and bulking of activated sludge;

– analysis of the effects produced by the applied doses of polyaluminium chloride on sludge volume index (SVI) and population of *Microthrix parvicella*.

Materials and Methods

The wastewater treatment plant

The Łyna Wastewater Treatment Plant in Olsztyn receives from 32 to 37,000 m^3 d^{-1} of municipal sewage from the towns of Olsztyn and Barczewo as well as from four rural communes: Barczewo, Dywity, Gietrzwałd and Stawiguda. Of the total volume of received sewage and wastewater, only 0.35% is delivered in septic vehicles. Some of the characteristics of the received sewage can be found in Table 1. The plant facilities were refurbished in 2004, and now the technological line consists of two automated dense stepped screens, a horizontal grit chamber with two parallel channels, two circular Dorr type primary sedimentation tanks, a primary sludge digester, a recirculated sludge denitrification chamber, a dephosphatation chamber, five

Table 1

Physicochemical characteristics of sewage according to the data supplied by the WTP laboratory

Parameters	Units	Raw sewage			Treated sewage		
		2006	2007	2008	2006	2007	2008
BOD_5	mg O_2 dm^{-3}	401	583	583	5.5	7.5	4.3
COD_{Chr}	mg O_2 dm^{-3}	664	954	973	40	50.8	58
Suspended soilds	Mg dm^{-3}	224	476	516	3.1	18	4.5
Nitrogen	mg N_{og} dm^{-3}	72	81	90	11.3	9.2	14
Phosphophrus	mg P_{og} dm^{-3}	11	17	16.8	1.5	1.3	0.9

All data are presented as means from 12 analyses performed at monthly intervals

multi-functional chambers of bioreactors each equipped with four Passavant rotors, and three secondary sedimentation tanks. Sewage sludge is inactivated in two closed fermentation chambers and three open fermentation tanks to be finally dewatered on a mechanical press. The post-fermentation waters are discharged to the Łyna River. The values of pollutants in the treated wastewater are presented in Table 1.

Exploitation problems

The analysis of the collected information and results of own observations enable us to conclude that the exploitation problems encountered at the wastewater treatment plant in Olsztyn are related to the poor properties of the activated sludge. This becomes particularly evident in winter and spring, when the SVI exceeds 190 $cm^3\ g^{-1}$ (Figure 1). Bulked sludge has an adverse effect on

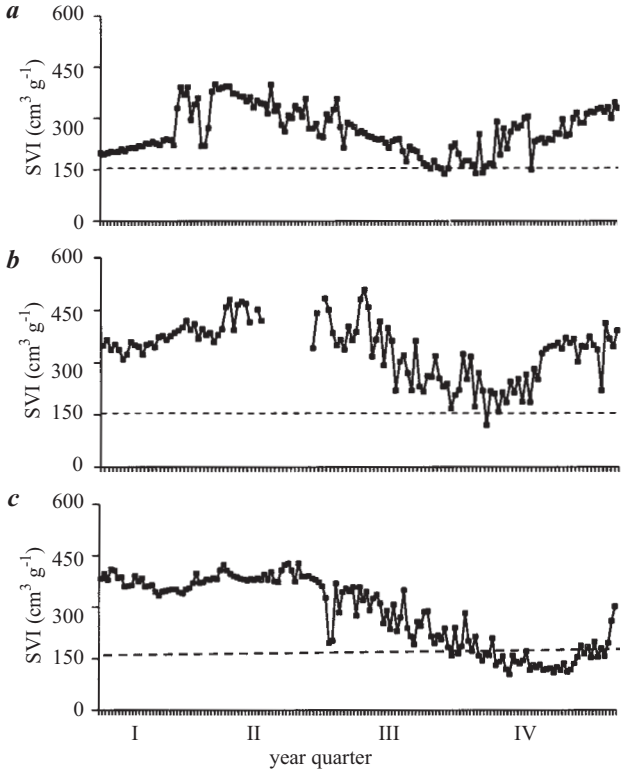

Fig. 1. The curve of the sludge volume index in: *a* – 2006, *b* – 2007, *c* – 2008

the operation of the sludge processing facilities. It is more difficult to dewater sludge on filter presses, which causes the hydraulic overload of these devices. During the fermentation process in closed fermentation chambers thick foam is created, which disturbs inner recirculation and makes it difficult to transport fermenting sludge to open fermentation tanks. Foam causes periodical blockage of gas pipes and airlocking of the pumps.

Biological assay methods

The microscopic analysis of sludge and foam samples collected from the multi-functional chambers was performed on live material, immediately on receiving the samples at the laboratory and in preparations stained with Gram and Neisser methods. For identification of the microorganisms, we referred to publications by EIKELBOOM (2000) and JENKINS et al. (2004). Counts of filamentous microorganisms were assessed according to the estimation scale elaborated by Jenkins, which comprises the range FI = 0–6.

Analytical methods

The results of the physicochemical analysis of activated sludge, such as dry matter of activated sludge, temperature of sewage in multi-functional chambers, sludge volume index, have been obtained from the laboratory at the wastewater treatment plant in Olsztyn.

Results and Discussion

Problem of activated sludge and foam

Analyses of activated sludge and foam have demonstrated that the cause of the high SVI at the wastewater treatment plant in Olsztyn and resultant exploitation problems is the excessive growth of filamentous bacteria *Microthrix parvicella*. The microscopic images revealed very numerous and long filaments of *M. parvicella* inside flocs (Table 2, Figure 2). They impede sedimentation and compaction of flocs, and periodically make sludge particle adhere to gas bubbles, which causes permanent foaming of sludge (Figure 3).

Table 2

Estimated counts of filamentous microorganisms

Filamentous microorganisms	Date				
	2006	2007	2008		
	5. 04	7. 05	18. 03	31. 03	22. 04
Microthrix parvicella	5	5	5	5	5
Type 0092	–	–	2	2	2
Typ 0041	2	–	2	1	1
Nostocoida limicola	–	–	–	–	1
Haliscomenobacter hydrossis	–	1	–	–	–
Typ 021 N	–	1	–	–	–
Thiothrix sp.	–	1	–	–	–
Typ 0411	–	1	–	–	–

1 – few, 2 – some, 3 – common, 4 – very common, 5 – abundant

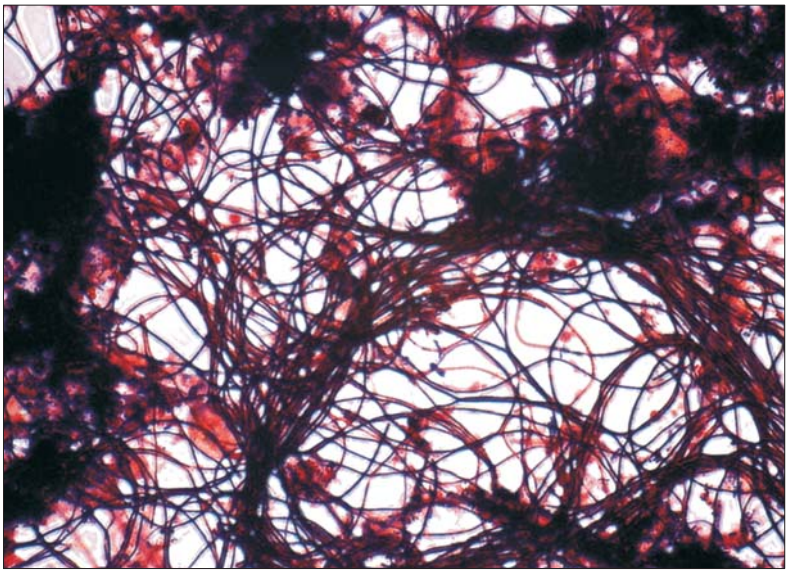

Fig. 2. The Gram stained filamentous microorganism *Microthrix parvicella* dominant in the activated sludge and foam, 1000x

Fig. 3. Foam produced by *Microthrix parvicella*

Experiments with doses of PAX

In order to depress the index of activated sludge and eliminate foam, polyaluminium chloride PAX-18 was introduced to the discharge canal, situated between the multi-functional reactors and secondary treatment tanks. The frequency, duration and rates of applied doses (expressed as aluminium ions) of the compound are presented in Table 3. The effect of the treatments on the sludge volume index (SVI) is illustrated in the Figure 4 a–c).

Table 3

Frequency and duration of the application of PAX-18 as well as its rates converted to aluminium ions in 2006–2008

Time period		g AL^{3+}/kg dsm/d
2006	13.01–22.01	1.06–1.70
	31.03–1.05	1.06–1.70
	27.10–6.11	0.64–1.06
2007	28.02–15.03	1.06–1.70
	27.03–29.04	0.85–1.70
	12.05–01.06	1.70–2.13
	17.10–29.10	0.85–1.70
2008	8.01–22.01	1.06–1.70
	01.02–0 6.05	0.85–1.70
	13.10–22.12	0.85–1.70

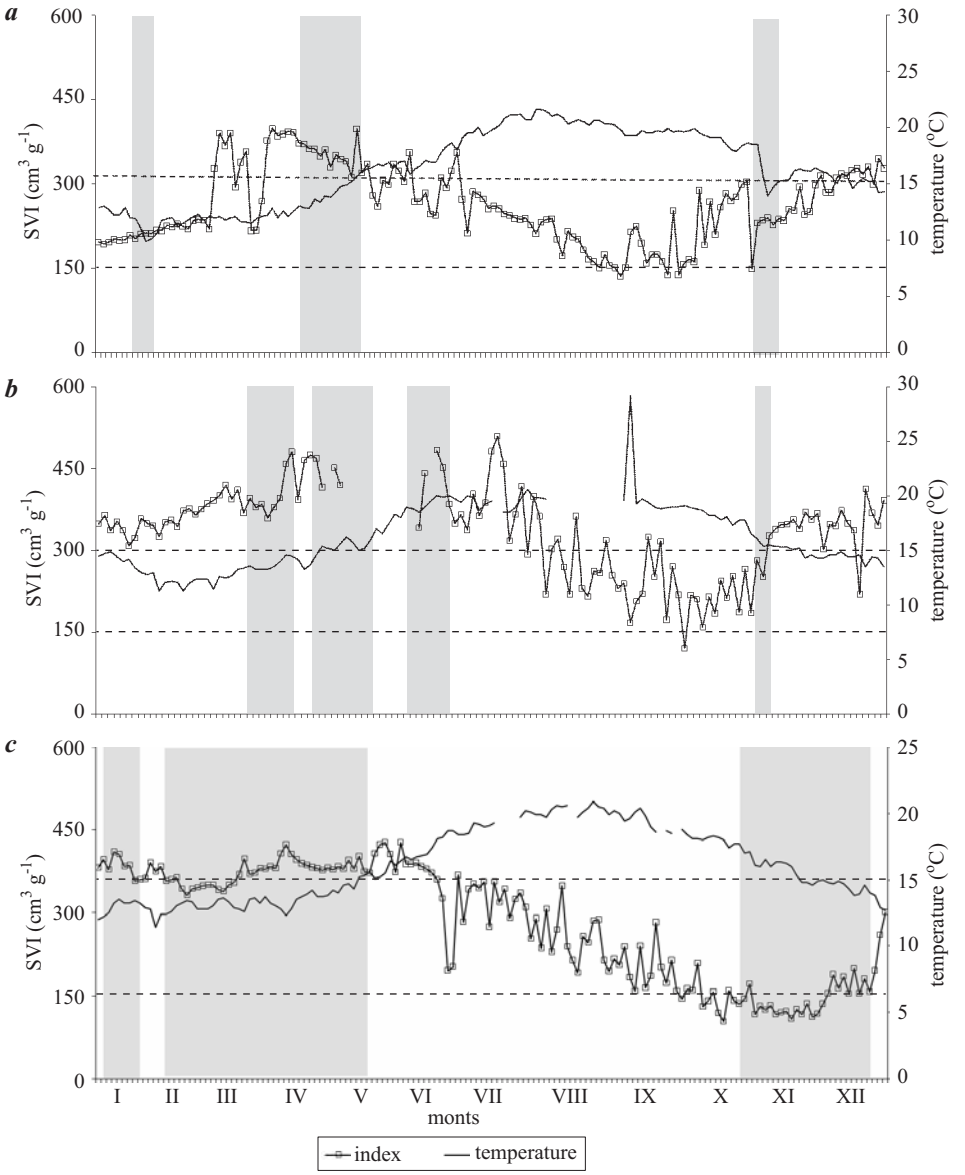

Fig. 4. The curve of the sludge volume index and temperature in: *a* – 2006, *b* – 2007, *c* – 2008; the grey colour indicates when PAX-18 was applied

In 2006, PAX was dosed three times, using between 0.64 and 1.70 g Al^{+3} kg^{-1} of sdmd (sludge dry mass daily). Despite the application of the reagent, in all the cases the value of SVI considerably exceeded 150 cm^3 g^{-1}. The index did

not tend to fall. On the contrary, during the longest, 32-day, application of the compound, it rose from 392 to 398 $cm^3\ g^{-1}$.

In 2007, the dose of PAX applied during four treatments ranged from 0.85 to 2.13 g $Al^{+3}\ kg^{-1}$ of sdmd. As in 2006, the value of SVI was much above 150 $cm^3\ g^{-1}$. One day before the termination of the longest, 34-day, application of PAX, the SVI reached 420 $cm^3\ g^{-1}$.

In 2008, the applied dose of PAX reached 0.85–1.70 g $Al^{+3}\ kg^{-1}$ sdmd. The compound was applied three times. The treatments did not bring about any desirable effects. Although the reagent was added for nearly the whole first half-year, the values of SVI would typically exceed 350 $cm^3\ g^{-1}$. In all the cases, however, while dosing PAX the foaming was observed to be less intense.

Discussion

The references (ROELS et al. 2002, CZERWIONKA et al. 2003) suggest that dosing polyaluminium chloride is an effective method for limiting the consequences of excessive growth of *M. parvicella*, such as foaming and bulking of activated sludge. The analysis of our results, however, indicate that application of polyaluminium chloride PAX-18 at the wastewater treatment plant in Olsztyn failed to lower values of sludge volume index (SVI) below 150 $cm^3\ g^{-1}$. The improvement of sedimentation properties of sludge observed in the second half-year is attributable to higher temperature of sludge in multi-functional chambers. *Microthrix parvicella* prefers the temperature < 15°C (KNOP, KUNST 1998). A possible reason for the failure of the applied treatments is the insufficiently low doses of PAX-18 introduced to the facilities, which did not guarantee an adequate concentration of the metal relative to the quantities of activated sludge. Another possible reason is that the duration of the treatments was too short. Most of the observed filaments of *M. parvicella* in activated sludge did not reveal any morphological changes under the influence of the reagent (Figure 5). According to the reference data, the minimum application period for PAX should be three weeks (ROELS et al. 2002). The initial rate should be no less than 3 g $Al^{+3}\ kg^{-1}$ sdmd (GENEJA, CZERWIONKA 2003). The treatments carried out at the Olsztyn Wastewater Treatment Plant did result, however, in the visible reduction of foaming in the multi-functional chambers. Nonetheless, they were unsuccessful in eliminating the cause of the negative event such as the excessive growth of *M. parvcella* in activated sludge.

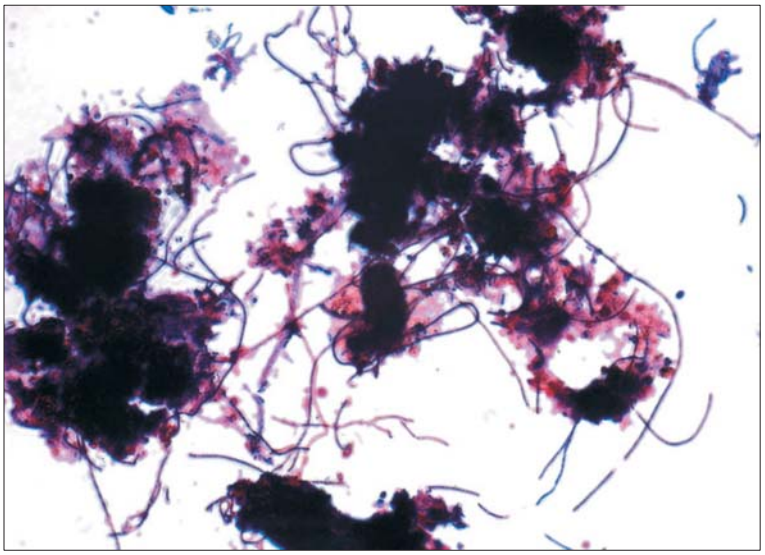

Fig. 5. Image of a Gram stained population of *Microthrix parvicella* during the application of polyaluminium chloride, 1000x

Conclusions

1. The cause of the main exploitation problems encountered at the Olsztyn WTP such as bulking and foaming of activated sludge was the excessive growth of a population of the filamentous bacteria *Microthrix parvicella*.

2. Application of polyaluminium chloride (PAX-18) in the rates of 0.64 to 2.13 g Al^{+3} g^{-1} sdmd (expressed as aluminium ions) did not lower the SVI. The sludge volume index would typically remain at a level above 150 cm^3 g^{-1} sdm.

3. Most of the observed filaments of *M. parvicella* sampled from activated sludge revealed no changes in their morphology caused by the prolonged application of PAX.

4. In most of the cases, the application of PAX reduced the foaming of activated sludge n the multi-functional chambers.

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APPLICATION DNA FINGERPRINT ANALYSIS FOR IDENTIFICATION OF MIXED GROUPS OF SIBERIAN STURGEON (*ACIPENSER BAERI* BRANDT)*

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K e y w o r d s: fingerprinting, gynogenetic offspring, microsatellite DNA, Siberian sturgeon.

A b s t r a c t

DNA finferprinting analysis based on microsatellites was applied for separation of mixed gynogenetic offspring of Siberian sturgeon (*Acipenser baeri*) and individuals from commercial production. Variation at 11 microsatellite DNA loci was surveyed for parent of gynogenetic offspring. Thus microsatellite DNA profiles in studied loci were known and this key-point was aplied in segregation analysis of mixed fish. In results 108 individuals of 281 studied were verified as gynogenetic offspring. The present survey of microsatellite variation demonstrated a reliable tool for separation of mixed group of fish.

ZASTOSOWANIE ANALIZY GENETYCZNEGO ODCISKU PALCA DO SEPARACJI MIESZANYCH GRUP JESIOTRA SYBERYJSKIEGO (*ACIPENSER BAERI* BRANDT)

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S ł o w a k l u c z o w e: genetyczny odcisk palca, jesiotr syberyjski, mikrosatelitarny DNA, potomstwo gynogenetyczne.

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Abstrakt

W opracowaniu zastosowano analizę genetycznego odcisku palca (DNA fingerprinting) do odseparowania gynogenetycznego potomstwa jesiotra syberyjskiego (*Acipenser baeri* Brandt) oraz ryb tego samego gatunku pochodzących z komercyjnej produkcji. W badaniach zastosowano 11 par starterów mikrosatelitarnego DNA do identyfikacji profili genetycznych matki gynogenetycznego potomstwa oraz dawcy nasienia. W wyniku przeprowadzonych analiz molekularnych 108 osobników jesiotra syberyjskiego (spośród grupy liczącej 281 osobników) zidentyfikowano jako gynogenetyczne potomstwo posiadające genotyp odziedziczony wyłącznie po matce. Badania ukazują możliwość identyfikacji poszczególnych osobników w mieszanych stadach ryb za pomocą analizy polimorfizmu mikrosatelitarnego DNA.

Introduction

The Siberian sturgeon, *Acipenser baeri* (Brandt), has been domesticated in Europe and Asia (AKIMOVA 1985, WILLIOT et al. 1991, CHEBANOV and BILLARD 2001). The Siberian sturgeon is one of the most common and most important sturgeon species cultured in Poland. The economic importance of sturgeons together with the fact that they occupy a critical position in understanding vertebrate genome evolution has resulted in many genetic studies in those fishes. Genetic studies of sturgeons are further complicated by the reported tetraploid, octaploid and polyploid nature of those species (BLACKLIDGE and BIDWELL 1993).

DNA fingerprinting has become an important tool for genetic identification in fish breeding as well as wild life management and conservation (O'REILLY and WRIGHT 1995, BEACHAM et al. 2000). In the area of broodstock monitoring and conservation, DNA fingerprinting techniques are used to link individuals found in separate areas, determine migration patterns, estimate gene flow among individuals, profile the genetic diversity and manage captive breeding programs.

In planning for DNA fingerprinting one of the important decisions is the selection of the appropriate marker system and the techniques. Various systems and their related techniques are currently in use and those based on the Polymerase Chain Reaction (PCR). A powerful technique for DNA fingerprinting is based on PCR amplification of tandem repeated sequences, which have long been known to be polymorphic and widespread in plant, animal and human genomes referred to as Simple Sequence Repeat (SSR) or microsatellite DNA (O'REILLY and WRIGHT 1995). Microsatellites occur in many places throughout the genome, but in almost all cases they are in non-coding regions of the DNA (SCHLÖTTERER and TAUTZ 1992). Eukaryotic genomes contain large numbers of microsatellite genetic loci that are widely dispersed along and among chromosomes with known DNA sequence and consist of many tandem

repeats (SCHLÖTTERER 2000). Analysis of these sequences has yielded very high levels of polymorphism. This is due to tandem repeats, presumably resulting from unequal mitotic or meiotic exchanges or by DNA slippage during replication (SCHLÖTTERER 2000). Due to the high variability and abundance of microsatellites throughout the genome, their use as markers for DNA fingerprinting has been found to be a powerful tool for population genetic studies (O'REILLY and WRIGHT 1995, FOPP-BAYAT 2008, FOPP-BAYAT and WOZNICKI 2008) and pedigree reconstruction, specially for communally reared populations (HERBINGER et al. 1995, MOAZAMI-GOUDARZI 1997). These fragments are inherited in a Mendelian fashion and provide a technique suitable for genetic variation studies, forensic and ecological studies, breeding programs, and population genetics (TAUTZ 1989).

The main objective of the present study was genetic separation of two mixed groups of Siberian sturgeon (gynogenetic group and commercial group) based on microsatellite DNA analysis.

Material and Methods

The fin clips were sampled from 281 specimens reared in experimental fish farm Dgal in Pieczarki, Inland Fisheries Institute in Olsztyn, Poland. One part of fish were gynogenetic offspring of Siberian sturgeon breeded in Wasosze Fish farm near Konin, Poland in 2006 year, and the second group were reprsented by individuals of Siberian sturgeon from commercial aquaculture production, breeded in the same farm and at the same time. The procedure of obtaining the gynogenetic offspring of Siberian sturgeon was described by FOPP-BAYAT (2007). The fin clips were also sampled from female used in gynogenesis in 2006 and male – sperm donor. The two groups of fish were mixed and there are not morphological distinctive characters for separate its. Genomic DNA for amplification of eleven microsatellite loci [*Afu-39, Afu-68, AfuB-68,* (MAY et al. 1997), *Spl-104, Spl-105, Spl-113, Spl-163, Spl-168* (MCQUOWN et al. 2000) *Aox-45* (KING et al. 2001) and *AfuG-9*, *Afu-G122* (WELSH and MAY 2006)] was extracted using Chelex 100 (WALSH et al. 1991) – Table 1. All microsatellite loci were amplified for parent of gynogenetic group of fish and three primers pair (*Spl-104*, *Spl-113*, *Spl-168*) were clasified as reliable tool for identification gynogenetic offspring. From the three loci (*Spl-104*, *Spl-113*, *Spl-168*) one *Spl-168* was selected for gynogenetic offspring identification. Reaction mixes for amplification microsatellites were prepared in a total volume of 25 μl with 40 ng DNA template, 1x PCR reaction buffer (50 mM KCl, pH 8.5; Triton X-100), 0.4 mM of each primer, 0.25 mM) of each deoxynucleotide triphosphate (dNTP), 3.3 mM $MgCl_2$ and 0.6 unit Go Taq

Flexi DNA Polymerase (Promega, Madison, WI, USA). Re-distilled water was used to bring the reaction mixture to the desired final volume. Amplification was conducted with a Mastercycler gradient thermocycler (Eppendorf, Germany), with initial denaturation at 94°C for 5 min, followed by 35 amplification cycles (94°C, 1 min; 52–57°C, 30s; 72°C – 30s), and final elongation at 72°C for 5 min. Aliquots containing PCR products and reaction buffer were electrophoresed using 6% polyacrylamide gel, and DNA bands were visualized by the silver staining method (TEGELSTRÖM 1986). Electrophoresis was conducted on a Bio-Rad SequiGen Sequencing Cell-system, and the gel size was 38 x 30 cm. Amplified fragments were sized by comparing migration with two DNA standards: ϕX 174 DNA/*Hinf* I DNA Step Ladder (Promega, Madison, WI, USA) and 25bp DNA Step Ladder (Promega, Madison, WI, USA). Every gel analyzing samples included two lanes containing the appropriate parental microsatellite PCR amplification products. Specific microsatellite profiles for parents were noted and compared to those from analyzed specimens.

Table 1
Primer sequences, annealing temperature and references of studied micxrosatellite loci in Siberian sturgeon (*Acipenser baeri*) specimens

Microsatellite locus	Primer sequence	T °C	References
Afu-39	F..TCCTGAAGTTCACACATTG R..ATGGAGCATTATTGGAAGG	57	MAY et al. 1997
Afu-68	F..TTATTGCATGGTGTAGCTAAAC R..AGCCCAACACAGACAATATC	55	MAY et al. 1997
AfuB-68	F..AACAATATGCAACTCAGCATAA R..AGCCCAACACAGACAATATC	55	WELSH and MAY 2006
Spl-104	F..TTATATGGGTGGGGTGGATG R..TCCTCTTTGGCATTTGTTCC	57	MCQUOWN et al. 2000
Spl-105	F..GCGATTTGATTGGCTCTTGT R..GGCACTGAATAAATGGACCG	57	MCQUOWN et al. 2000
Spl-113	F..TCCCACATGGCTTGTATTGA R..ACCACACCATGCGTCATAAG	57	MCQUOWN et al. 2000
Spl-163	F..TGCTTGTAAACTGCCCCACT R..CCACATGCAGTTTGAGCTGC	57	MCQUOWN et al. 2000
Spl-168	F..CACTGATTCGCTACAACCGT R..AGAAGGACTTGCAGTCCGAA	57	MCQUOWN et al. 2000
Aox-45	F..TTGTCCAATAGTTTCCAACGC R..TGTGCTCCTGCTTTTACTGTC	53	MCQUOWN et al. 2001
AfuG-9	F..CATAATGTAAAGCAAAAGT R..ACCTGAAATGTATGTTATG	52	WELSH and MAY 2006
AfuG-122	F..AACACGACAACAAACTTATTCA R..TGTGTTTCTATGTCTGTCTGTCTA	52	WELSH and MAY 2006

Results and Discussion

In results 108 specimens were verified as gynogenetic offspring while 173 were identified as fish from commercial production using microsatellite locus *Spl-168*. Alleles *212* and *224* bp were observed at locus *Spl-168* in female of Siberian sturgeon (mother of gynogenetic offspring) while allele *184* bp was characteristic for male – sperm donor at the same locus. All verified gynogenetic offspring possesed alleles identical to female used for meiotic gynogenesis. In this group of fish allele *212* and *224* base pairs (bp) were observed. In the second verified group of fish eleven alleles was identified: *248*, *244*, *224*, *220*, *216*, *212*, *208*, *204*, *200*, *112* and *94* bp at studied microsatellite *Spl-168* locus (Figure 1). The alleles identified in gynogenetic group of fish was also observed in the commercial breeding group because the same female was also used to commercial reproduction.

Genetic structure of broodstock analysis of commercially important fish species is essential for optimizing management strategy or stock improvement program. Genetic monitoring is necessary for an effective management strategy because a population can suffer severe genetic erosion for example: bottleneck, genetic drift, inbreeding, founder effect etc. DNA fingerprinting technology with special emphasis on microsatellites is useful in application to fisheries and aquaculture for example in selection and breeding programmes for aquaculture broodstock.

Many applications of microsatellite DNA analysis have been used to investigate stock structure and stock contributions to mixed-stock fisheries. McConnel et al. (1995) were able to discriminate clearly between Canadian and European *Salmo salar*, Ruzzante et al. (1996) differentiated inshore and offshore Atlantic cod, Taylor (1995) described genetic variation among North Pacific populations of steelhead and rainbow trout. Microsatellite markers were also applied for stock identification to management of coho salmon in British Columbia (Beacham et al. 2001).

In sturgeon fishes microsatellite DNA analysis was applied for example in:
- analysis of variation in Atlantic sturgeon (*Acipenser oxyrhinchus oxyrhinchus*) and cross-species amplification in the Acipenseridae (King et al. 2001),
- analysis of genetic variation in the Chinese sturgeon *Acipenser sinensis*; estimating the contribution of artificially produced larvae in a wild population (Zhu et al. 2002),
- genetic identification of black caviar (Jenneckens et al. 2001, Fopp-Bayat 2007, Ludwig 2008),
- estimation of parentage and relatedness in white sturgeon *Acipenser transmontanus* (Rodzen et al. 2004),
- study of genetic population structure of lake sturgeon *Acipenser fluvescens* (DeHaan et al. 2006),

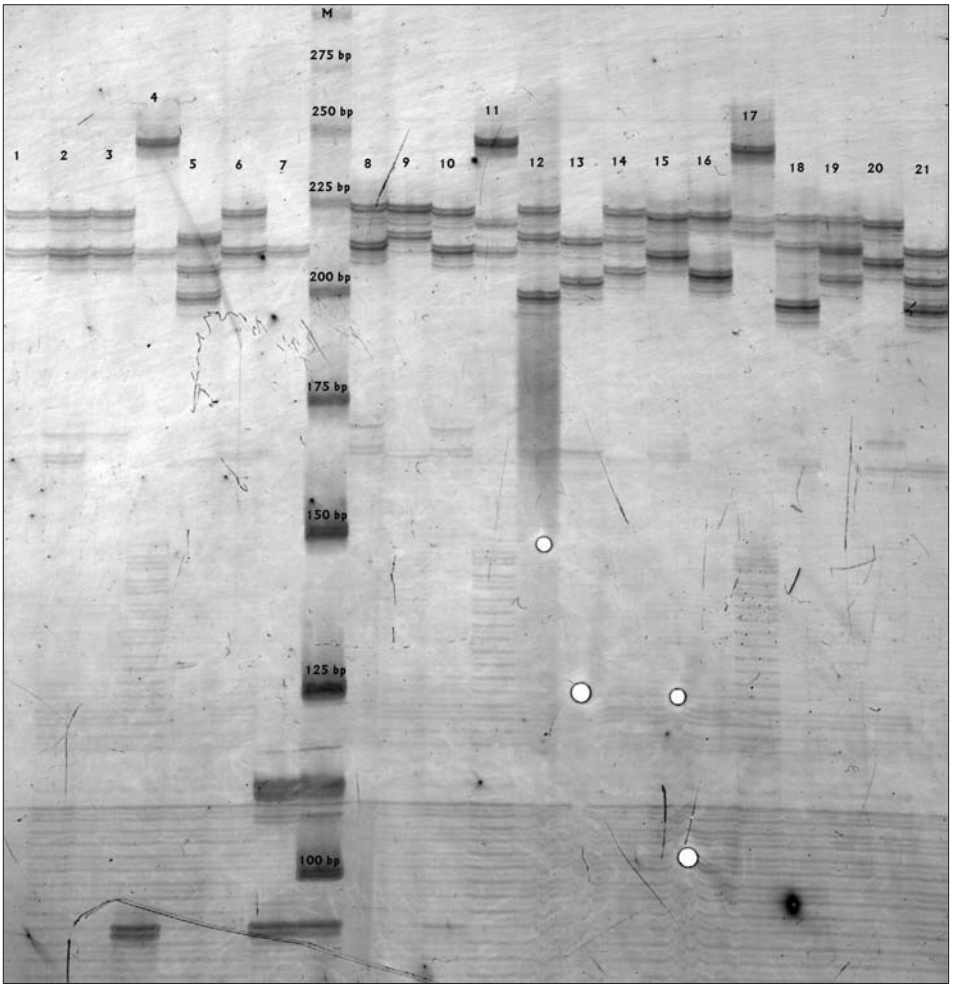

Fig. 1. Allele segregation at microsatellite locus *Spl-168* in Siberian sturgeon (*Acipenser baeri*): M – marker 25 bp (Promega); female of Siberian sturgeon (mother of gynogenetic offspring) – sample 1; gynogenetic offspring – samples: 2, 3, 6, 8, 10, 15, and 20; individuals from comercial production – samples: 4, 5, 7, 9, 11, 12, 13, 14, 16, 17, 18, 19, and 21

– verification of genome manipulation in sterlet *Acipenser ruthenus* and siberian sturgeon *Acipenser baeri* (FOPP-BAYAT et al. 2007, FOPP-BAYAT 2007).

Conclusion

The present study was designed to characterize genetically the progeny of Siberian sturgeon from experimental group mixed with group of fish from commercial production. This method is especially important for genetic monitoring of broodstock condition and it could be applicable in simmilar situations when some unfortunately mixing of fish groups occurred. Moreover identification of polymorphic markers with consistent scorable alleles is a crucial step to generate genetic profile data for spawners.

The present survey reveal enough differentiation between gynogenetic group of fish and group from commercial production. This method could be applied in estimation of broodstock composition in aquaculture or for identification of mixed stock in natural ekosystem.

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THE EFFECT OF ACETIC ACID ON *SALMONELLA* SPP. IN MICROBIOLOGICAL MEDIA AND IN TURKEY CARCASSES

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K e y w o r d s: *Salmonella*, acetic acid, turkey carcasses, microbiological media.

A b s t r a c t

The studies aimed at determining the influence of acetic acid concentrations on *Salmonella* spp. in microbiological media and on turkey carcasses. The average number of bacteria in control samples without the supplement of acetic acid was for *S.* Enteritidis $1.3 \cdot 10^8$, *S.* Anatum $1.9 \cdot 10^8$ and *S.* Typhimurium $2.3 \cdot 10^8$. Acetic acid in agar medium at 0.1% concentration inhibited growth of studies *Salmonella* strains entirely. In case of acetic acid concentration of 0.05% the number of bacteria compared to the controls decreased by 6 logarithmic cycles. In case of 0.03% concentration the number of *S.* Anatum decreased by 5 logarithmic cycles while *S.* Enteritidis and *S.* Typhimurium by 4 logarithmic cycles. In the presence of 0.02% acetic acid *S.* Enteritidis and *S.* Typhimurium grew in numbers that were within the same logarithmic range, only *S.* Anatum decreased in number by one logarithmic cycle as compared to the controls. The results of studies obtained after immersing elements of turkey carcasses in acetic acid indicate that the recovery of *Salmonella* spp. from the samples depends on the inoculum of those bacteria in poultry carcass surface. In case of contamination with 10 colony forming units (cfu) of *Salmonella* spp. on the surface of a turkey carcass element and immersing it for 15 minutes in 1%, 1.5% and 2% acetic acid solutions a decrease in the number of samples from which those microorganisms were recovered as compared to the number of control samples was recorded. In case of contamination with 10^2 cfu on the turkey carcass surface and immersing it in tested 1%, 1.5% and 2% solutions of acetic acid for 15 minutes no influence on detection of *Salmonella* spp. was recorded. The inhibitory influence of acetic acid on *Salmonella* spp. was much more pronounced in case of the microbiological medium than in case of poultry carcasses on which satisfactory elimination of *Salmonella* spp. was not achieved.

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WPŁYW KWASU OCTOWEGO NA PAŁECZKI *SALMONELLA* W PODŁOŻACH MIKROBIOLOGICZNYCH I W TUSZKACH INDYCZYCH

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Słowa kluczowe: *Salmonella*, kwas octowy, tuszki indycze, podłoża mikrobiologiczne.

A b s t r a c t

Celem badań było określenie wpływu stężeń kwasu octowego na pałeczki *Salmonella* w podłożach mikrobiologicznych i na tuszkach drobiowych. Średnie liczby bakterii w próbkach kontrolnych bez dodatku kwasu octowego wynosiły dla *S.* Enteritidis – $1{,}3 \cdot 10^8$, *S.* Anatum – $1{,}9 \cdot 10^8$, *S.* Typhimurium – $2{,}3 \cdot 10^8$. Kwas octowy w podłożu agarowym o stężeniu 0,1% całkowicie hamował wzrost wszystkich badanych szczepów *Salmonella*. Gdy stężenie wynosiło 0,05%, liczba bakterii w porównaniu z kontrolą zmniejszyła się o 6 cykli logarytmicznych. Dla stężenia 0,03% liczba *S.* Anatum zmniejszyła się z kolei o 5 cykli logarytmicznych, a w przypadku *S.* Enteritidis i *S.* Typhimurium – o 4 cykle logarytmiczne. W obecności 0,02% kwasu octowego *S.* Enteritidis i *S.* Typhimurium rosły w liczbach mieszczących się w tym samym przedziale logarytmicznym, jedynie liczba *S.* Anatum w porównaniu z kontrolą zmniejszyła się o jeden cykl logarytmiczny. Wyniki badań uzyskane po zanurzeniu elementów tuszek indyczych w kwasie octowym wskazują, że wykrycie pałeczek *Salmonella* w próbkach zależy od inoculum tych bakterii na powierzchni tuszki drobiowej. Po kontaminacji 10 jednostek tworzących kolonie (jtk) pałeczek *Salmonella* na powierzchni elementu tuszki indyczej i po zanurzeniu jej na 15 minut w wodnych roztworach 1%, 1,5% i 2% kwasu octowego zaobserwowano zmniejszanie się liczby próbek, w których wykryto te drobnoustroje, w stosunku do liczby próbek kontrolnych. Po kontaminacji 10^2 jtk na powierzchni tuszki indyczej i zanurzeniu jej na 15 minut w badanych, wodnych 1-, 1,5- i 2-procentowych roztworach kwasu octowego nie stwierdzono ich wpływu na wykrywalność pałeczek *Salmonella.* Unieszkodliwiające działanie kwasu octowego względem bakterii *Salmonella* było zdecydowanie silniejsze w podłożach bakteryjnych niż w tuszkach drobiowych, na których nie uzyskano skutecznej eliminacji pałeczek *Salmonella.*

Introduction

During the recent years a minor decrease in the number of cases of bacterial food poisoning and infection has been recorded in Poland. In 2004 – 19.872 cases of that type were recorded, in 2005 – 20.065 while in 2006 – 17.264 and in 2007 – 15.241 cases. Nevertheless, cases of food poisoning caused by *Salmonella* spp. still represent the largest group among bacterial food poisoning cases. During the years 2004–2007 they represented in average 77.75% of the total number of bacterial food poisoning cases (*Sytuacja epidemiologiczna...* 2007).

Human and animal infections and contamination of food with *Salmonella* spp. result in high economic losses. They are the consequence of eliminating large numbers of people from work for the duration of the disease and additionally

costs of treatment of people, the costs of treatment of animals and destroying the contaminated batches of foods.

Control of *Salmonella* spp. in the environment is a standing problem for people dealing with prevention and treatment of people as well as animal and food production. Numerous methods concerning, e.g. slaughter, immunisation and application of bactericides have been developed and implemented. None of those methods, however, guarantied full success although all of them improved the hygienic conditions and food health safety.

Elimination of *Salmonella* spp. was tested on various experimental models using a diversity of chemicals, e.g. hexadecylpyridinium chloride (BREEN et al. 1997, WANG et al. 1997, RADKOWSKI and MIKOŁAJCZYK 2004), trisodium phosphate (WANG et al. 1997, XIONG et al. 1998), organic acids (DORSA et al. 1997, TAMBLYN and CONNER 1997, TSAI and INGHAM 1997, SMULDERS and UPMANN 2000, MIKOŁAJCZYK and RADKOWSKI 2002), hydrogen peroxide and sodium bicarbonate (RUSSELL et al. 1993). Not all of those methods proved effective. The medium is considered effective if under its influence the number of specific microorganisms decreases by 2 log (JETTON et al. 1992).

The possibility of applying additional substances in food processing are limited as a consequence of their negative influence on human body as well as difficulties with solubility and possibility of direct application. Care for quality and health standards of food forces application of only those chemicals in food technology that are classified as safe.

Organic acids and their salts, commonly considered safe, are among the preferred additives destroying the bacterial flora on carcasses (NETTON VAN et al. 1994, IZAT et al. 1989).

In Polish food industry application of the following organic acids and their salts is allowed: ascorbic, citric, lactic, acetic and tartaric (*Rozporządzenie Ministra Zdrowia...* 2008).

Acetic acid E 260 (at 4–7% concentration) is defined in food as an additive substance the acceptable daily intake (ADI) of which, according to the opinion of FAO/WHO experts on Food Additives does not require limiting, which means that it does not pose a hazard for consumer health, of course on condition that it satisfies specified purity criteria for that acid. As a consequence, it can be applied in food processing as an additive substance in quantities „quantum satis". Not determining any maximum level of that acid application in food production (including some products for infants and small children), acetic acid as an additive is applied according to the good manufacturing practice in doses not exceeding those necessary for achieving the intended goal on condition that the consumer is not mislead. According to the provisions of the regulation by the Minister of Health of the 18th of September 2008 on allowed additive substances, application of acetic acid is allowed

in some food products as acidity control or stabiliser, e.g. in Feta and Mozzarella cheeses, emulsified sauces, mustards, fish products, fruit and vegetable products excluding products of mushrooms and marinades, cured meat products, bakery products and meal concentrates (*Rozporządzenie Ministra Zdrowia*... 2008). Differentiating between the terms of "acetic acid" and "vinegar", knowing that vinegar is not an additive, manufacturers of food products containing acetic acid are required to mark their products according to the European Union legislation, including the Polish one, i.e. providing the basic information on presence of the above additive substance, specifying its technological function as well as the name and number E 260.

Considering that the acetic acid is legally allowed for application in food industry and understanding the complexity of the problem of search for methods of eliminating *Salmonella* spp. bacteria it was decided to embark on studies aiming at determination of different concentrations of that acid on *Salmonella* spp. in microbiological media and on poultry carcasses.

Materials and Methods

During stage one of the study, the influence of acetic acid in microbiological media on *Salmonella* spp.

The following concentrations of analytically pure acetic acid ($C_2H_4O_2$) in nutritive agar were applied: 0.01%, 0.02%, 0.03%, 0.05%, 0.1%, 0.25%, 0.5%, 1%, 1.5%, 2.0%. Strains: *Salmonella* Enteritidis no. 33/66, *Salmonella* Anatum no. 30/93 and *Salmonella* Typhimurium no. 227/84, obtained from the Museum of bacterial strains of the National Veterinary Research Institute in Puławy were the object of the study.

The acetic acid was sterilised using the Millipore filter (Millex 9P, 022 μ, Bedford), and next added at appropriate concentrations to the medium at the temperature of 50°C.

The studied strains were inoculated into 9 ml of nutritive agar and after 24 hours of incubation at 37°C the starter culture for further studies was obtained. Next ten times dilution of the culture was made and each strain from each dilution was inoculated on the nutritive agar without the chemical (control) and on the nutritive agar supplemented with different quantities of acetic acid. Surface inoculation was applied. Plates were incubated at 37°C for 24 to 48 hours. Tests for each strain was done in ten repetitions and averages for all repetitions were computed.

The second stage of the studies concerned analysis of acetic acid influence on *Salmonella* Enteritidis present in elements of turkey carcasses.

Tests were conducted on 236 samples of turkey breast purchased from

poultry processing plants. After delivery to the laboratory the material was kept in a refrigerator at 4°C, and next used for preparation of 25 g samples for further tests. In the tests for checking the natural presence of *Salmonella* spp. conducted on random samples (20% of all samples prepared for tests from each turkey breast purchased) no *Salmonella* spp. was detected. The other samples were contaminated purposefully. The strain of *Salmonella* Enteritidis no. 33/66 was used for the tests. That strain was first inoculated on nutritive broth and incubated at 37°C for 24 hours and then every sample was inoculated with 0.05 ml of 24-hour broth culture of *S.* Enteritidis diluted to from 10^{-4} to 10^{-8}. The initial inoculum of test samples was determined for each test series. The bacterial suspension was delicately spread with a special wide loop over the widest area possible. After inoculation with the bacteria, each sample was kept for 20 minutes in a refrigerator at 4°C aiming at full drying of the suspension. Next, each sample was transferred to sterile beaker with 250 ml of 1%, 1.5% and 2% solution of acetic acid for 15 minutes. From among the methods recommended for detection of *Salmonella* spp. on poultry carcasses, pluck and products the method given in the regulations (*Microbiology...* ISO 6579 1993, *Mikrobiologia...* PN-ISO 6579 1998) was applied.

Following 15 minutes in acetic acid solution, each sample was moved to a sterile beaker and covered with 225 ml of buffered peptone water (BPW, CM 509, Oxoid Basingstoke Hampshire, UK), and incubated at 37°C for 20 hours. Selective growth was achieved on the SC medium (SC, 0 687-17-1, Difco Laboratories Detroit MI, USA), Müller-Kauffman medium (MK, CM 343, Oxoid Basingstoke Hampshire, UK) and Rappaport-Vassiliadis medium (RV, CM 669, Oxoid Basingstoke Hampshire, UK) while the further culturing was done on brilliantine green and phenol red agar (BGA, CM 329, Oxoid Basingstoke Hampshire, UK) on BSA medium (BSA, 00 73-01-1, Difco Laboratories Detroit MI, USA) and on XLD agar (XLD, CM 469 Oxoid Basingstoke Hampshire, UK). Colonies typical and suspect of belonging to *Salmonella* spp. were identified by serological and biochemical methods. Biochemical characteristics of *Salmonella* spp. were determined using API Test 20 E. Serological types were determined on the basis of the Kauffmann-White classification scheme as proposed by Popoff and Le Minor using the sera produced by the National Salmonella Centre.

Turkey breast samples contaminated with *Salmonella* spp. immersed in sterile water for 15 minutes were the controls.

Each variant of the experiment was done in ten repetitions.

The data collected from the experiment was processed statistically using T – Student test and correlation analysis. Correlation analysis was conducted on log values.

Results and Discussion

The results of studies obtained are presented in Table 1, Table 2 and Figure 1.

Assessment of acetic acid influence on *Salmonella* spp. in microbiological medium is presented in Table 1 and Figure 1.

Table 1

Growth of *Salmonella* spp. on agar medium supplemented with acetic acid ($n=10$)

Salmonella spp. type	Acetic acid concentration (%)										
	0.00	0.01	0.02	0.03	0.05	0.1	0.25	0.5	1	1.5	2
	number of colonies (cfu/ml)										
S. Anatum	$1.9 \cdot 10^8$	$1.6 \cdot 10^8$	$8.0 \cdot 10^7$	$9.3 \cdot 10^3$	$1.0 \cdot 10^2$	0	0	0	0	0	0
S. Enteritidis	$1.3 \cdot 10^8$	$1.6 \cdot 10^8$	$1.7 \cdot 10^8$	$1.0 \cdot 10^4$	$1.0 \cdot 10^2$	0	0	0	0	0	0
S. Typhimurium	$2.3 \cdot 10^8$	$2.3 \cdot 10^8$	$2.0 \cdot 10^8$	$1.0 \cdot 10^4$	$1.0 \cdot 10^2$	0	0	0	0	0	0

Table 2

Number of samples from elements of turkey carcasses treated with acetic acid solution, in which *Salmonella* Enteritidis was detected ($n=10$)

Concentration (%)	Treatment time (minutes)	*Salmonella* Enteritidis no 33/66				
		dilution (inoculum)				
		10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
		number of positive results				
0	15	10	10	10	10	0
1	15	10	10	10	8	0
1.5	15	10	10	10	3	0
2	15	10	10	10	3	0

The data presented in Table 1 indicates that the average numbers of cfu in control samples without acetic acid addition were as follows for *S.* Enteritidis $1.3 \cdot 10^8$, *S.* Anatum $1.9 \cdot 10^8$ and *S.* Typhimurium $2.3 \cdot 10^8$.

Acetic acid in agar medium at the concentration of 0.1% inhibited entirely the growth of all tested strains of *Salmonella*. At the concentration of 0.05% the cfu number decreased by 6 log cycles as compared to the controls. At the concentration of 0.03% the number of *S.* Anatum cfu decreased by 5 log cycles and in case of *S.* Enteritidis and *S.* Typhimurium by 4 log cycles. In presence of 0.02% of acetic acid *S.* Enteritidis and *S.* Typhimurium grew in numbers that were within the same logarithmic range, only the number of *S.* Anatum cfu decreased by 1 log cycle as compared to the controls.

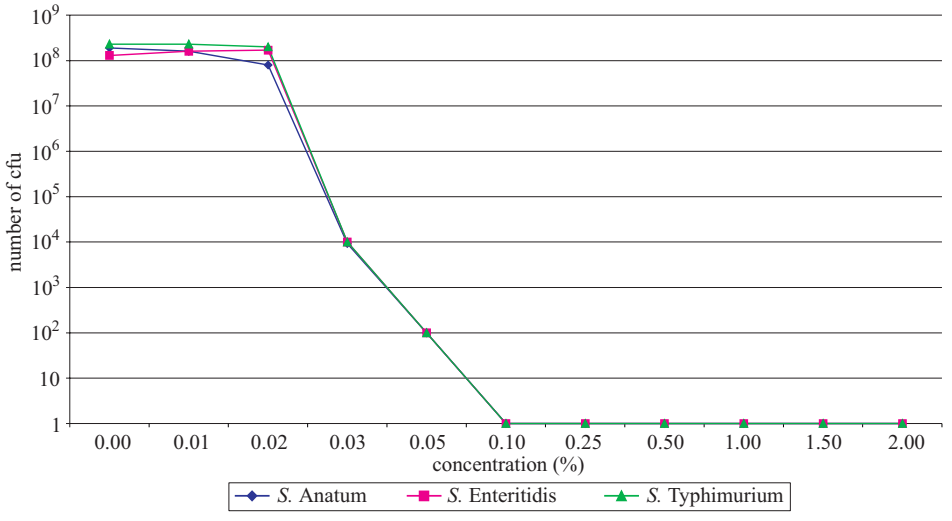

Fig. 1. *Salmonella* spp growth on agar medium supplemented by different acetic acid concentrations

The results of studies on the effect of acetic acid on *Salmonella* Enteritidis on elements of turkey carcasses are presented in Table 2.

Analysing the results of studies obtained after immersing elements of turkey carcasses in acetic acid it is easy to notice that detection of *Salmonella* spp. from samples depends in the inoculum of the bacteria no poultry carcass surface. In case of contamination with 10^1 cfu of *Salmonella* spp. on the turkey carcass surface and immersing it for 15 minutes in 1%, 1,5% and 2% water solutions of acetic acid a decrease in the number of samples in which that microorganism was detected as compared to the controls was observed.

In case of contamination with 10^2 cfu on the turkey carcass surface and immersing it for 15 minutes in tested 1%, 1,5% and 2% water solutions of acetic acid no influence on detection of *Salmonella* spp. was recorded.

The results of own studies show that the effectiveness of anti-bacterial influence of acetic acid at different concentrations on *Salmonella* spp. in microbiological media was high. Unfortunately, in case of applying the tested concentrations of acetic acid against *Salmonella* spp. present on turkey carcasses no effective elimination of *Salmonella* spp. was obtained. Only the number of samples in which the microorganism was detected decreased as compared to the controls.

Obtained results of own studies (Table 2) are not consistent with the data published by other authors (CONNER et al. 1997, DORSA et al. 1997, OKREND et

al. 1986, TAMBLYN and CONNER 1997). Acetic acid at tested concentration showed poor eliminating influence on *Salmonella* spp. present in poultry carcasses.

So far conducted studies (DICKSON et al. 1994, DORSA et al. 1997, LILLARD 1994, OKREND et al. 1986, TAMBLYN and CONNER 1997) on elimination of *Salmonella* spp. from poultry carcasses by chemical means aimed, during the first stage, at liquidating the accompanying microflora, e.g. by means of ultraviolet radiation, and only then contaminating them with *Salmonella* spp. As a result it was possible to apply non-selective media that allowed detection of *Salmonella* spp. damaged by chemical substances (CONNER and BILGILI 1994). All the referred studies show that the experimental model applied in them was far from the actual contamination of carcasses with *Salmonella* spp. In own studies the influence of 1%, 1,5% and 2% of acetic acid on *Salmonella* spp. under conditions closest to the natural, i.e. on carcasses originating directly from processing plants not subjected in the laboratory to any processes aiming at liquidation of accompanying microflora was studied.

In the available literature there is no detailed information on the influence of acetic acid on different initial numbers of *Salmonella* spp. on turkey carcasses (TAMBLYN and CONNER 1997, HWANG and BEUCHAT 1995).

The data obtained from own studies indicate that anti-bacterial effectiveness of acetic acid is diversified and depends on the initial number of *Salmonella* spp. on turkey carcasses.

The *Salmonella* spp. eliminating mechanism in case of acetic acid involves the presence of dissociated particles and low pH of acids (CONNER and BILGILI 1994).

Works by numerous authors (DICKSON 1992, DICKSON and ANDERSON 1992, TAMBLYN and CONNER 1997, SAWAYA et al. 1995) indicate that the higher the concentration of organic acids the higher their effectiveness against *Salmonella* spp. In case of high concentrations of acids in disinfecting solutions organoleptic changes in the carcasses appear (KOTULA and THELAPPURATE 1994). TAMBLYN and CONNER (1997) noticed changes of that type in case of test concentrations of acids equal to or exceeding 2%.

Conclusions

1. Eliminating influence of acetic acid on *Salmonella* spp. was definitely stronger in microbiological media than in poultry carcasses.

2. The inhibitory influence of acetic acid on *Salmonella* spp. in microbiological media was high. The acid in agar medium at 0.1% concentration inhibited growth of all tested strains of *Salmonella* entirely.

3. *Salmonella* spp. present on turkey carcasses showed a relatively low sensitivity to the influence of 1%, 1,5% and 2% concentrations of acetic acid and no effective elimination of those microorganisms was obtained.

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