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Influence of Chromium Ions on Effectiveness Degradation of Low-molecule PAHs in Sewage Sludges

Wpływ chromu na efektywność degradacji małocząsteczkowych WWA w osadach ściekowych

The aim of the study was to determine the chromium ions influence on removal efficiency of selected polycyclic aromatic hydrocarbons (PAHs) from sewage sludges under aerobic conditions. The studies were carried out using dewatered and biochemically stabilized sewage sludges taken from a small municipal wastewater treatment plant (WWTP). The changes in the concentration of PAHs were conducted in three series: in sludge samples taken after filter press (biotic samples-control samples), in sewage sludge samples amended with added chromium ions and in sewage sludge samples with added sodium azide (abiotic samples). The chromium ions were added into the sludges samples as a solution of chromium(III) chloride. The sewage sludge samples were incubated for 90 days. Determination of PAHs in sewage sludge samples was made at the beginning of the experiment (the initial concentration) and then six times over 15 days. The gas chromatography-mass spectrometry (GC-MS) was used to qualify and quantify PAHs. Six PAHs (2-ring and 3-ring of hydrocarbons) listed by EPA were analysed: naphthalene Naph, acenaphtylene Acyl, acenaphtene Ac, fluorene Flu, phenanthrene Phen, anthracene Antr. In sewage sludges taken from a municipal treatment plant the average PAHs concentration were in the range of 242÷296 μg/kg d.m. The effectiveness of naphthalene was 79 and 81% in the control samples and 78 and 65% in samples amended with chromium chloride carried out in two series, respectively. In abiotic samples the effectiveness removal of naphtalene was 24 and 62% in series, respectively. The average effectiveness of 3-ring PAHs removal ranged 73% in control sewage sludge samples and 56% in sewage sludge amended with chromium(III) chloride, respectively. The effectiveness of removal hydrocarbons in sewage sludge amended with sodium azide (abiotic samples) was 41.5% on average.

Keywords: **biotic sewage sludge, biotic sewage sludge with chromium ions, abiotic sewage sludge, naphthalene, acenaphtylene, acenaphtene, fluorene, phenanthrene, anthracene, GC-MS**

Introduction

There are reports in the literary sources of PAHs content in sewage sludge. The presence of these compounds in sewage sludge is associated with the sorption onto organic matter particles during wastewater treatment processes [1-3]. During digestion processes the changes in the concentration of PAHs in sewage sludge were observed. In the sewage sludge from large wastewater treatment plant, other inorganic micropollutants such as PAHs, heavy metals were identified [4-6]. This limits the use of sewage sludge in agriculture. In Polish legislation the application of sewage sludge in agriculture is limited by permissible heavy metals (Zn, Pb, Cu, Cr, Hg, Ni, Cd) concentrations (Table 1) and pathogens (*Salmonella sp., Ascaris sp., Trichuris sp., Toxocara sp.*) [7]. The proposed changes of EU Directive demand the control of selected toxic organic micropollutants in sewage sludge applied in agriculture including PAHs, PCBs, PCDD, PCDF, DEHP, NPE, AOX and LAS [8]. Sewage sludge originating from municipal wastewater treatment plants is usually loaded with both heavy metals and organic micropollutans, especially PAHs. Therefore, it is important to investigate the behaviour of hydrocarbons in the presence of metals when sewage sludge is stored.

PAHs are regarded as persistence compounds to the decomposition process that depends on both abiotic factors and the presence of microorganisms [9-11]. The level of decomposition depends on physical-chemical hydrocarbon properties and the environment conditions [12, 13]. There have only been a handful of investigations made concerning the impact of the occurrence or addition of some heavy metals on PAHs fates. Maliszewska-Kordybach et al. described the investigations into the effect of selected metals on PAHs persistence in soil environment [14, 15]. Lazzari et al. found a correlation between the presence of PAHs and mercury, cadmium, zinc and copper in composted sewage sludge [16]. In both studies it reported that the presence of metals inhibited the PAHs degradation. This was due to the lower biological activity of bacteria responsible for the decomposition of these compounds. The obtained results confirm the differential dependency between the concentration of individual hydrocarbons and the studied metals (Cu, Cd, Zn, Pb). It was found that the intensity of the degradation processes depends on the number of rings in the molecule [14-18]. Stability of PAHs is defined as half-life of their decomposition. The available investigations take into account mainly soils or the mixture of soil and sewage sludge. It was found that half-lives of PAH were in the range of 3-3111 days and of 15-408 days in mixture of soil with sewage sludge and in soil, respectively [14, 15]. In the earlier investigation, in biotic samples the half-time of 16 hydrocarbons was in the range of 7-961 days. Half-life of these compounds in abiotic sewage sludges was in the range of 8-2961 days [19]. The objective of this work was to find out the impact of the addition of chromium chloride (in concentration exceeding permissible levels) on selected PAHs in sewage sludge stored under aerobic conditions.

1. Experimental procedure

1.1. Materials

The investigations using dewatered sewage sludge taken from a small municipal wastewater treatment plant (WWTP) were carried out. The sewage sludge was stabilized in anerobically digestion process. The sewage sludge was primary analysed for: hydratation, contents of organic compounds and contents of chromium. Initial PAH contents was also determined. The sewage sludge originating from WWTP had a low water contents (81÷83%). Organic matter contents of 52÷54% proved that the sewage sludge was well digested. Comparison of the concentration of chromium ions in sewage sludge and in: the Polish and EU directive limits for municipal sludge in agriculture is presented in Table 1. The sewage sludge included low chromium concentration (122÷126 mg/kg d.m.) and was below permissible levels for sewage sludge applied in the agriculture and recultivation areas assigned for agricultural purposes [7, 8].

Table 1. **Concentration of PAHs in sewage sludge and limits of metals concentration used in agriculture in Polish and EU legislations, mg/kg d.m.**

Metal	Sewage sludge taken from WWTP	Use of sewage sludge Polish legislation [7]			EU Directive 86/278/EWG [20]	Proposal of changes of EU Directive [8]
		in agriculture	recultivation	other		
Chromium	122.0÷126.0	500	1000	2500	–	1000

1.2. Methods

Sewage sludge samples were mixed and representative samples were selected. In the series, forty two samples of 10 g each were prepared, and they were protected from photodegradation and volatilization process. A chromium(III) chloride solution (500 mg of Cr/kg d.m.) including original Cr concentration was added to seven sludge samples. The sodium azide was added to seven samples in order to inhibit microbial activity (abiotic samples). The influence of added sodium azide on the microorganisms activity was measured with the use of TTC test previously. In the sample with the added sodium azide the dehydrogenase activity was equal to zero. The samples without chromium salt were treated as the control samples. All the samples were incubated for 90 days at 20°C in the dark in order to limit photovolitalization process (laboratory condition). Hydratation was kept during the experiment. The whole volume of each sewage sludge sample (10 g) was used in order to determine PAHs. PAH samples were taken at the beginning of the experiment (the initial concentration) and six times at two weeks intervals (after 15, 30, 45, 60, 75 and 90 days). The samples in duplicate were prepared. The following investigations were carried out:

– determination of changes in the PAH concentrations in sewage sludge samples taken from WWTP (control samples),
– determination of changes in the PAH concentrations in sewage sludge samples taken from WWTP with the added sodium azide (abiotic samples),
– determination of changes in the PAH concentrations in sewage sludge samples with added chromium(III) chloride (biotic samples with Cr).

1.3. PAHs analysis

The gas chromatography-mass spectrometry (GC-MS) for qualification and quantification of PAHs was used. The extraction process for samples was carried out in the ultrasonic bath with cyclohexane (20 mL) and dichloromethane (4 mL) mixture as solvents. Prepared extracts were primarily concentrated under the nitrogen stream. Then the extracts were purified using silica gel in vacuum conditions. Subsequently those extracts were concentrated again to the volume 1 mL. GC 8000 gas chromatograph Fisons equipped with a mass spectrometric detector MD 800 for chromatographic determination of individual PAHs was used. The parameters of chromatographic analysis were as follows: carrier gas - helium 70 kPa, temperature program 40÷120°C (40°C/min) to 280°C (5°C/min) and 280°C for 20 min, volume injection - 1 μL, injection system - on column injector, interface temperature - 280°C, column DB-5 (30 m; 0.25 mm; 0.25 μm), integration system - MassLab. The following PAHs, according to US EPA list were analyzed [21]: 2-ring hydrocarbons: naphthalene (Naph) and 3-ring of hydrocarbons: acenaphtylene (Acyl), acenaphtene (Ac), fluorene (Flu), phenanthrene (Phen) and anthracene (Antr).

The samples with determined concentration of hydrocarbons in order to verify the method of preparation of samples for qualification and quantification of PAHs control were prepared. The Standard mixture of PAHs in the amount 10 mg/kg d.m. (Accu Standard Inc. USA - PAH Mix) in benzene and dichloromethane (v/v 1:1) was added into sewage sludge samples. The standard mixture was added to samples before adding the solvents and extraction process. Then, the samples were prepared and analyzed for PAHs content according to the procedure described above. The recoveries of PAHs standard mixture for concentrations in sewage sludges taken from WWTP varied from 65 to 95%. The average value was equal to 85% which corresponds and confirms the previously obtained data found in the literary sources [5, 14]. Comparison of effectivities of PAHs degradation in the sewage sludge amended with (or not) chromium chloride and between the PAHs concentration in biotic and abiotic samples were calculated according to a *Student* test. The critical value was read from tables for specified degree of freedom (n – 2) and at a confidence level of 95%. Theoretical value of decomposition t_d ranged 4.303 [22].

2. Results

The concentration of PAHs in sewage sludge were different in series and different value for the individual compounds. The average concentration of PAHs in sludges taken from WWTP did not exceed 300 μg/kg d.m. which corresponds with the results of other authors as well as our own experience [4, 5, 19]. The concentration of naphthalene were in the range 119÷125 μg/kg d.m. in series, respectively. The dynamic of concentration changes of naphthalene in the control samples, samples with added chromium and in abiotic samples is given in Figure 1a-c.

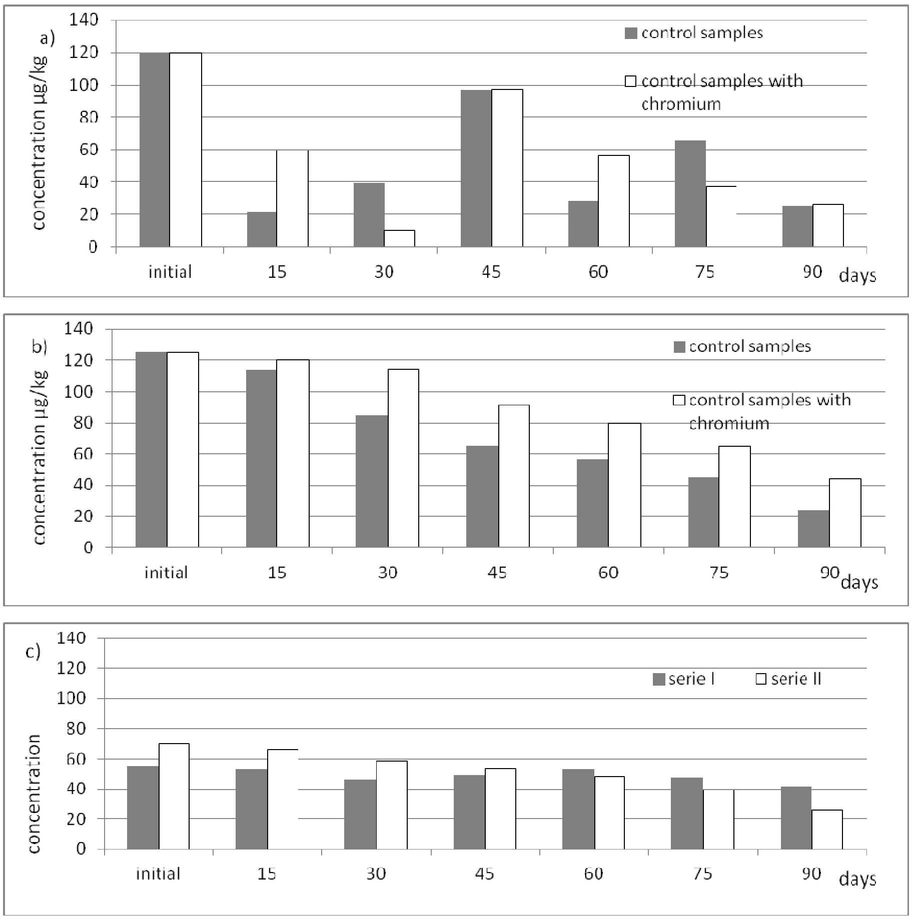

Fig. 1. The changes in the concentration of naphthalene in sewage sludge samples: a) serie I, b) serie II, c) abiotic samples

The percentage decrease in the concentration of naphthalene varied from 72 up to 74% in the control samples and were in the range of 45÷60% in sewage sludge samples with the added chromium. In Figure 1c the changes in the naphthalene concentration in abiotic samples are presented. The losses of naphthalene in abiotic sewage sludge during 90 days of incubation were in the range of 40÷43%. The concentration of 3-ring hydrocarbons was in the series 122 and 171 µg/kg d.m., respectively. The dynamic of concentration changes of individual hydrocarbon in the control samples as well as those with added chromium(III) chloride and in the sewage sludge samples with sodium azide is presented in Figures 2-6, respectively.

At the end of experiment the concentration of acenaphtylene (Fig. 2) was lower by 88 and 81% than initial concentration in sewage sludge (control samples) in the series, respectively. In the samples amended with chromium(III) chloride the decrease of acenaphtylene concentration was 62% in average. In abiotic samples the losses of acenaphtylene were in the range of 48÷56%.

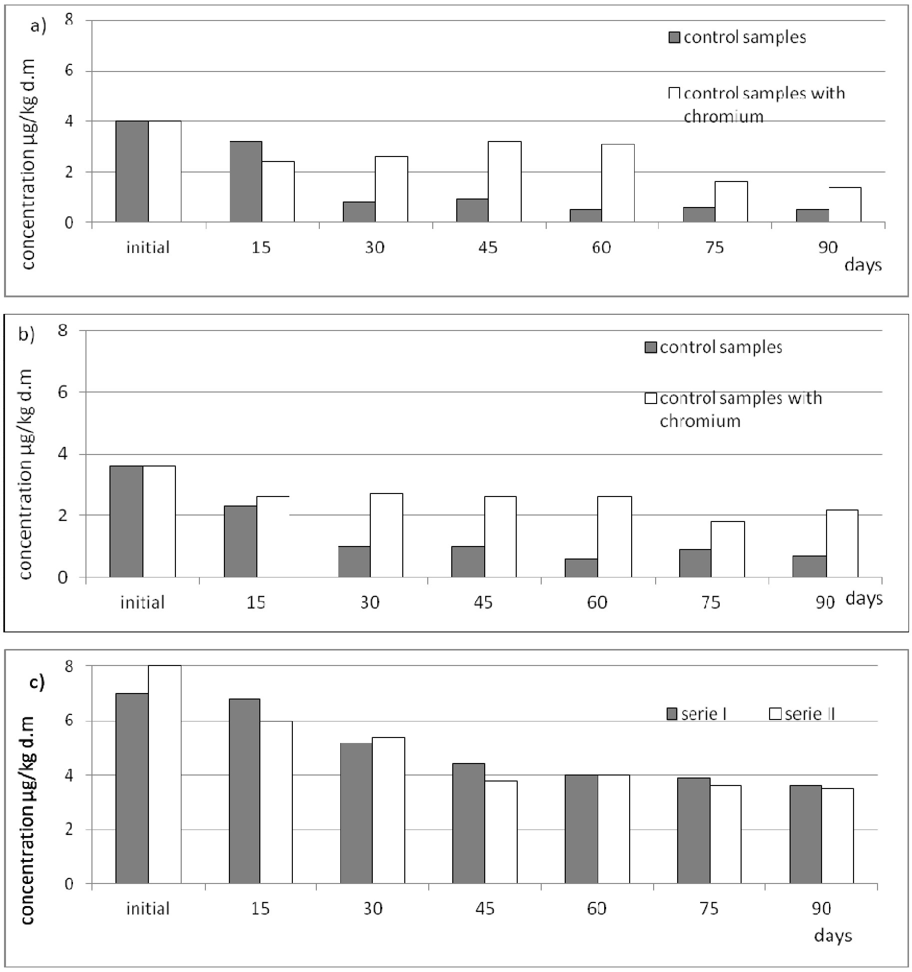

Fig. 2. The changes in the concentration of acenaphtylene in sewage sludge: a) serie I, b) serie II, c) abiotic samples

In Figure 3 the concentrations of acenapthene in sewage sludge samples were given. The changes of PAHs concentration during incubation of biotic and abiotic sewage sludge (with sodium azide) were significant (t_d was equal 5.21 and was higher than the critical value of t-*Student* $t_d = 4.303$). In the control samples the concentration of acenaphtene after 90 days of incubation were 88 and 70% lower than initial content in series, respectively. The decrease of acenaphtene contents was lower in sewage sludge amended with chromium ions than in the control samples. The percentage losses of hydrocarbons were 60 and 63% in the series, respectively. In the abiotic sewage sludge samples with sodium azide after 2 and after 3 months the acenaphtene concentrations were at the same level as in those 1 month of incubation. At the end of experiment the concentration of acenaphtene was lower than initial content and ranges the values of 38÷40%.

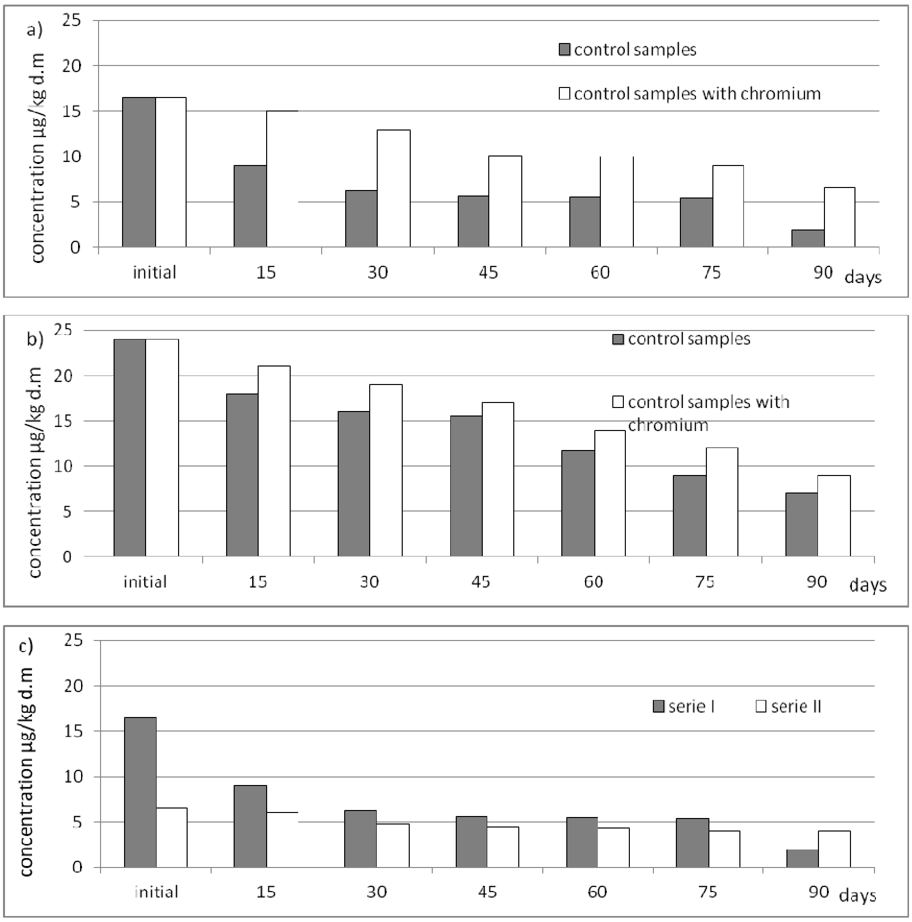

Fig. 3. The changes in the concentration of acenapthene in sewage sludge: a) serie I, b) serie II, c) abiotic samples

In Figure 4 the changes of fluorene concentration in sewage sludge samples during 90 days of incubation are presented. The initial concentration of fluorine was in the range of 20÷28 µg/kg d.m. in series, respectively. The average fluorine concentration at the end of experiment in control samples was 71.5% lower than initial content of this compounds. The effectiveness of fluorine degradation in sewage sludge amended with chromium(III) chloride was lower than in the control samples and did not exceed 54% (50-54%). The losses of fluorine in abiotic sewage sludge samples did not exceed 43%.

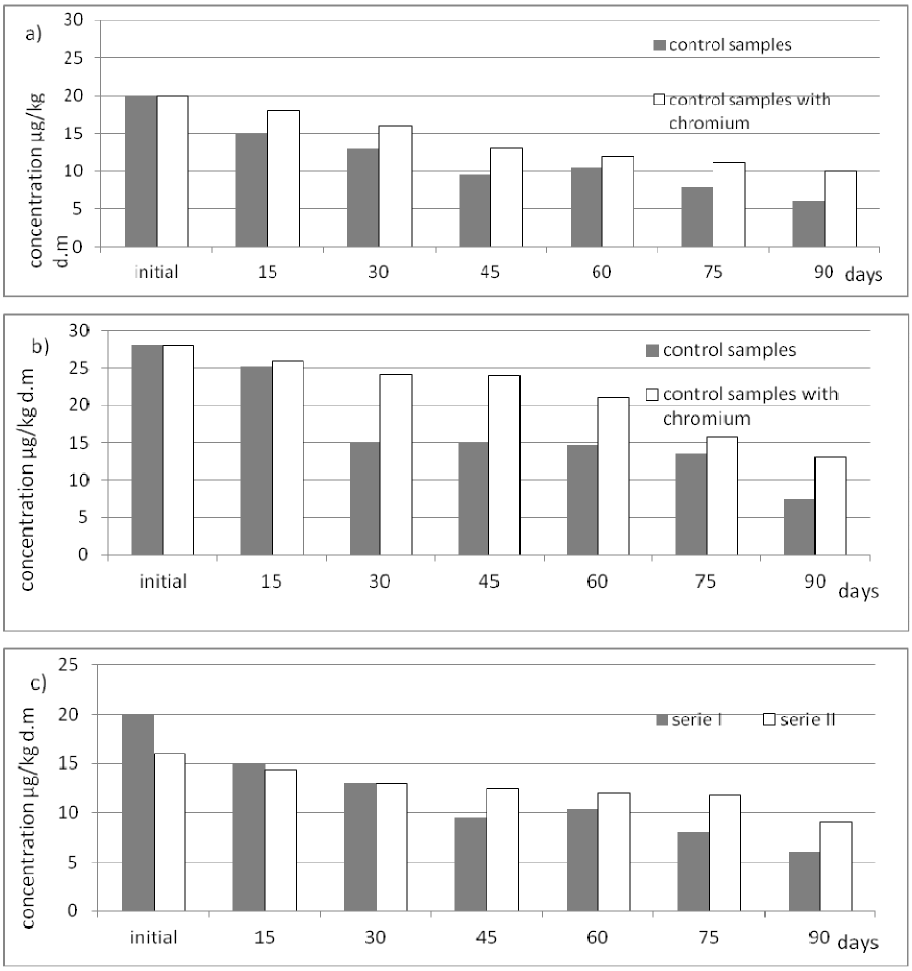

Fig. 4. The changes of fluorene concentration in samples: a) serie I, b) serie II, c) abiotic samples

The changes in the phenanthrene concentration in sewage sludge samples are given in Figure 5. The decreases in the concentration of this hydrocarbons were similar in abiotic sewage sludge (38÷42%) and in sewage sludge amended with chromium ions (42÷49%). In sewage sludge taken from wastewater treatment plant (control samples) the effectiveness of phenanthrene degradation were in the range 62÷67%.

The changes of anthracene in sewage sludge samples are given in Figure 6. The initial concentration of this hydrocarbons was equal to 36 and 57 µg/kg d.m. in biotic samples in the series, respectively. In abiotic samples the initial content of anthracene were 13 and 10 µg/kg d.m. in the series, respectively. In the control samples the decrease in the anthracene concentration were 69÷88%. In sewage sludge amended with chromium(III) chloride the inhibition of anthracene was observed. The effectiveness of this compounds degradation was 32.5% in average.

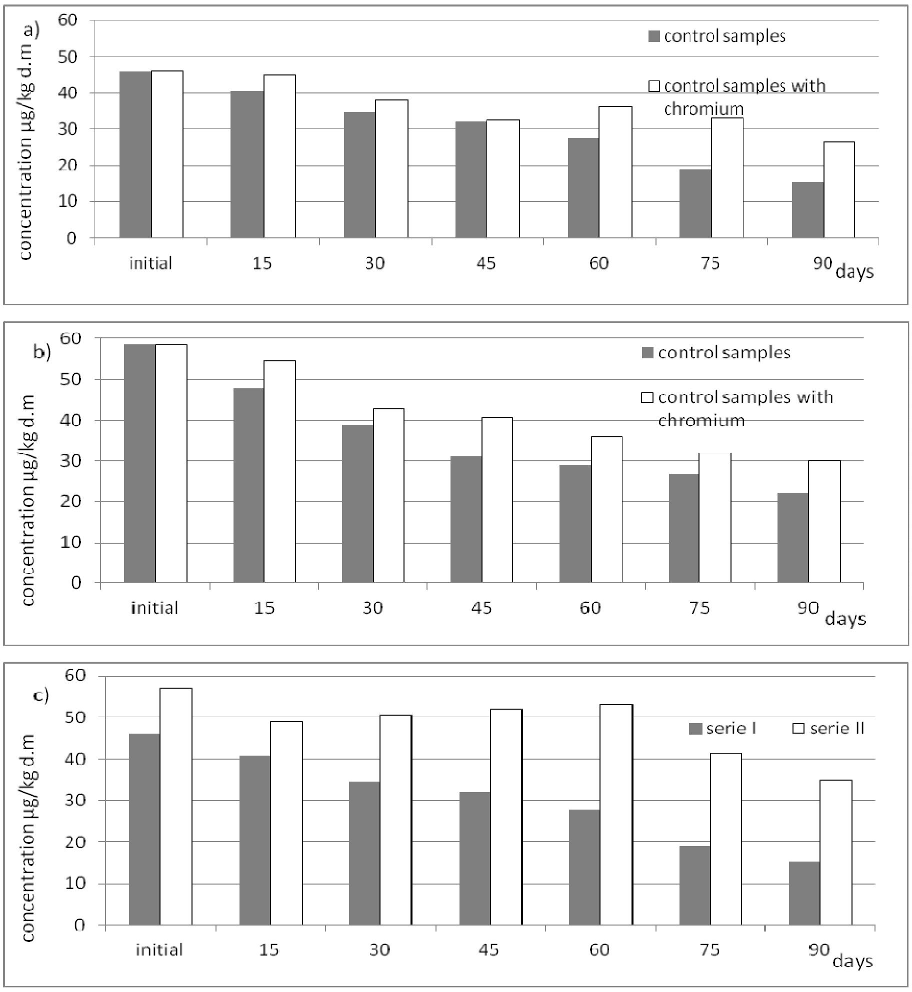

Fig. 5. The changes in the concentration of phenanthrene in sewage sludge: a) serie I, b) serie II, c) abiotic samples

Significantly lower PAH concentrations were found during incubation time, mainly in the control sludges (t_d was equal 8.23). At the end of sewage sludge incubation the total concentrations of 3-ring hydrocarbons were 73 and 56% lower than the initial one in the control samples and in samples with the added chromium(III) chloride, respectively. A *Student* t-test was used in order to assess the statistically significant differences between the initial and final PAH concentrations as well as in order to estimate the statistically significance presence of chromium(III) chloride in the sewage sludge after 90 days of incubation. It was estimated that changes in the concentrations of naphthalene and 3-ring hydrocarbons between the initial and final concentration differed significantly in the control sewage sludges and in the sewage sludges with added chromium chloride (t_d was equal 9.73).

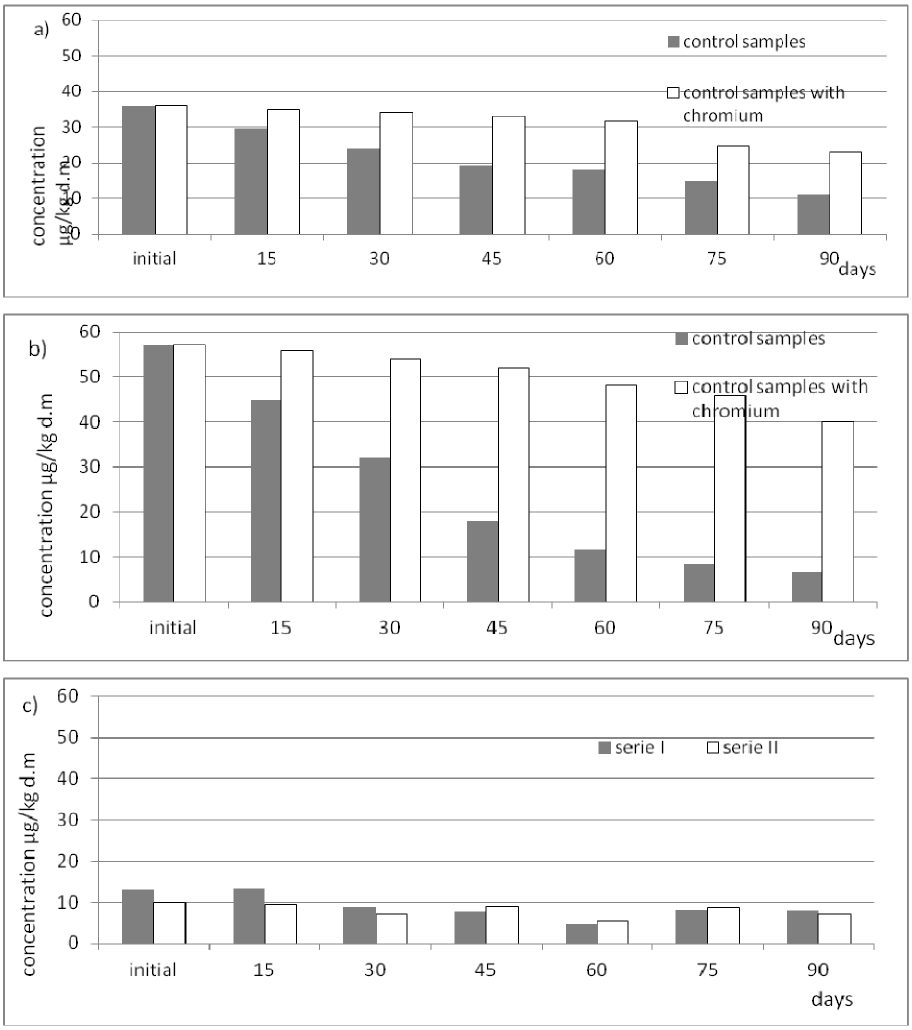

Fig. 6. The changes in the concentration of anthracene in sewage sludge: a) serie I, b) serie II, c) abiotic samples

In the abiotic sludge samples with sodium azide the concentration of naphthalene and 3-rings of PAHs were 43 and 42% lower than initial ones, respectively. Comparing the loss of individual PAHs in sewage sludge under biotic and abiotic conditions it can be concluded that during the incubation which was recorded by analyzing the concentrations at fixed intervals, significant losses of these compounds were associated with physical and chemical transformations (t_d was equal 7.44). Among the physico-chemical processes, the most significant are chemical reaction of hydrocarbons with other compounds of sewage sludges, volatilization of hydrocarbons and sorption onto solid particles. Sorption of PAHs on the particles may be so strong that it hinders the extraction of these compounds to organic solvents during separation of samples. The reactions with other components of sewage sludge: oxidation and substitution are mentioned. The biodegradability of these compounds was also observed. Biological changes are the results of enzymatic degradation of hydrocarbons in liquid phase during incubation [11, 21, 23, 24]. The statistically significant differences between the initial and final PAH concentrations as well as in order to estimate the statistically significance presence of microorganisms in sludges were found (t_d was equal 7.11). It was estimated that changes in the concentrations of naphthalene and 3-ring hydrocarbons between the initial and final concentration different significantly in the abiotic sewage sludges (t_d was equal 6.54). In Table 2 percentages of PAHs removal from sewage sludge are presented.

Table 2. **Percentage of PAHs removal from sewage sludge, %**

	Sewage sludge - control (biotic) samples		Sewage sludge - biotic samples with $CrCl_3$		Sewage sludge with sodium azide - abiotic samples	
	Serie I	Serie II	Serie I	Serie II	Serie I	Serie II
Naphtalene	79	81	78	65	24	62
Acenaphtylene	88	81	65	38	48	56
Acenaphtene	88	70	60	63	40	38
Fluorene	70	73	50	54	32	43
Phenanthrene	67	62	42	49	46	38
Anthracene	69	88	36	29	38	30
3-rings of PAHs	72	74	45	44	43	40
Total PAHs	76	77	61	53	36	49

The presence of chromium ions had a significant impact on the studied hydrocarbons and on the changes in the total PAHs contents after 90 days of incubation. This might have been due to the selective limitation of microorganisms activity that causes decomposition of individual hydrocarbons. The results are similar to the literary sources [14-18] concerning the behaviour of PAHs in soil contaminated with heavy metals as well as in the sewage sludge composting process. It is stated that the stability of individual hydrocarbons could vary and the dynamic of changes of concentrations during incubation of studied materials could be irregular [25, 26].

Conclusions

Based on the results of the experiments it can be concluded:

- Significant differences between initial concentrations and final concentrations for naphthalene and 3-ring PAHs in the control samples and in the sewage sludge samples with added chromium were observed.
- The inhibition of 3-rings of PAH degradation in sewage sludges supplemented with chromium(III) chloride was found (the final average PAH concentration

in sewage sludge modified with chromium(III) chloride was two times higher than in the control sample).
– The presence of microorganisms have a statistically significant impact on the degradation of naphthalene and 3-rings of hydrocarbons in the sewage sludges.

Acknowledgements

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Streszczenie

Celem badań było określenie wpływu obecności jonów chromu na efektywność degradacji wybranych wielopierścieniowych węglowodorów aromatycznych WWA w warunkach tlenowych. Badania prowadzono z wykorzystaniem biologicznie ustabilizowanych osadów ściekowych pobranych z miejskiej oczyszczalni ścieków. Zmiany stężenia WWA w osadach były prowadzone w dwóch seriach: w osadach pobranych z pras filtracyjnych (próbki osadów biotyczne - kontrolne), w osadach ściekowych, do których wprowadzono jony chromu oraz w osadach z dodatkiem azydku sodu (próbki abiotyczne). Jony chromu wprowadzono do osadów w postaci roztworu chlorku chromu(III). Osady były inkubowane przez 90 dób. Oznaczanie WWA w osadach ściekowych prowadzono na początku badań oraz 6-krotnie w odstępach 15-dobowych. Oznaczanie ilościowo-jakościowe WWA prowadzono z wykorzystaniem układu chromatograf gazowy-spektrometr masowy (GC-MS). Oznaczono sześć związków zaliczanych do listy EPA: 2-pierścieniowy naftalen i 3-pierścieniowe: acenaftylen Acyl, acenaften Ac, fluoren Flu, fenantren Fen, antracen Antr. W osadach ściekowych pobranych z oczyszczalni ścieków średnie stężenie WWA było w zakresie 242÷296 µg/kg s.m. Efektywność usuwania naftalenu wynosiła odpowiednio 79 i 81% w osadach kontrolnych i 78 i 65% w osadach, do których wprowadzono roztwór chlorku chromu(III) w dwóch seriach. W osadach abiotycznych efektywność usuwania naftalenu wynosiła w serii I 24% oraz w serii II 62%. Średnia efektywność degradacji 3-pierścieniowych węglowodorów wynosiła 73 i 56% odpowiednio w osadach kontrolnych i osadach z dodatkiem jonów chromu. Efektywność usuwania tych związków w osadach abiotycznych wynosiła średnio 41,5%.

Słowa kluczowe: **osady ściekowe biotyczne, osady ściekowe z dodatkiem jonów chromu, osady ściekowe abiotyczne, naftalen, acenaftylen, acenaften, fluoren, fenantren, antracen, GC-MS**