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THE EFFECT OF HEAVY METALS ON THE GROWTH OF WATERBORNE *Escherichia coli* OF MUNICIPAL LANDFILL ORIGIN

WPŁYW METALI CIĘŻKICH NA WZROST *Escherichia coli* IZOLOWANYCH Z WÓD POCHODZĄCYCH ZE SKŁADOWISKA ODPADÓW KOMUNALNYCH

Abstract: The aim of this study was to assess the sensitivity of *Escherichia coli* isolates originating from a municipal waste landfill to the selected heavy metals. The analyses were conducted using environmental strains, isolated from surface water – a stream flowing along the landfill and from leachate and the observations were compared to the reaction of a reference strain EC ATCC 25922. The growth rate of bacterial cultures was evaluated in the liquid medium supplemented with 0.02; 0.1 and 0.5 mg · dm^{-3} of heavy metal salts: chromium, zinc, cadmium, copper, lead and mercury. The bacterial growth was examined turbidimetrically every 24 hours for 5 days. The performed study showed differences between the examined isolates in their response to the addition of the heavy metals in the liquid medium. Additionally, varied intensity of the heavy metals' effect on bacterial growth was observed, with the weakest growth inhibition being recorded in the case of lead, while chromium and mercury causing the greatest growth inhibition of bacterial strains.

Keywords: municipal waste landfill, *Escherichia coli*, heavy metals, municipal waste

There are many environmental consequences of growing population as well as developing industry, among which there is increasing production of waste, which is accumulated in both industrial and municipal landfills. In 2014 more than 10 thousand Mg of municipal waste was produced with 268 kg of waste produced by an average Polish citizen [1]. However, what is significant, is that an increasing amount of waste is being recycled in various ways, resulting on the other hand in decreasing amount and

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share of waste being deposited in landfills. In 2014, 5.4 thousand Mg of collected waste (*ie* 52.6%) was landfilled, while in 2013 this amount reached almost 6 thousand Mg and constituted 63.1% of the total amount of waste collected [1]. Depending on the amount and composition of waste deposited, there are various components that may be leached out and enter groundwater, including polycyclic aromatic compounds (PAHs) and heavy metals [2].

Heavy metals are among major toxicants of the environment, which are also one of the most persistent pollutants of water. They are both difficult to degrade and they can accumulate in the food chain [3]. Even though some heavy metals, such as copper, iron, manganese, or zinc are essential elements that serve as micronutrients, may function as components of enzymes, catalysts of certain biochemical reactions and stabilize protein structures in bacterial cell walls [4], still the requirements of living organisms for those essential heavy metals are usually very low [5]. On the other hand, many other heavy metals, including cadmium, lead or mercury, have no biological role [4] and may become potentially toxic to living organisms – microorganisms in particular [5]. The toxicity of such metals is manifested among others by the displacement of essential heavy metals from their native binding sites [4]. The excess of both groups of heavy metals may result in damaging cell membranes, altering enzyme specificity, etc. [5]. Common heavy metals that have been identified in polluted water include arsenic, copper, cadmium, lead, chromium, nickel, mercury and zinc [3]. The presence of heavy metals in landfills and landfill leachate is due to different kinds of waste being deposited, such as electronic waste, painting or used batteries [6]. Environmental contamination with heavy metals can cause various alterations to the microbial community of a given environment, including the reduction of microbial biomass or biodiversity [7]. Toxic effects of these contaminants may lead to changes in the microbial community structure and increase the level of physiological adaptation or tolerance, resulting in the selection of heavy metal-reistant species or strains [8, 9].

Microbial survival in contaminated environments depends on their biochemical properties, various adaptation mechanisms, including morphological changes within their cells, which may be associated with chromosomal genes or located on plasmids [10]. Microorganisms developed a wide variety of mechanisms aiming at reducing the impact of these contaminants on their cells, including efflux transporters that excrete the excess metal outside their cells [11].

Another threat to the quality of surface water and groundwater in the neighborhood of landfills is the possibility of their contamination with different groups of microorganisms, including pathogenic and opportunistic bacteria, such as coliforms, *Escherichia coli*, *Staphylococcus* spp. or *Salmonella* spp. [12]. This is due to the fact that the municipal landfills collect various types of mixed waste, including disposable napkins, sanitary towels, hypodermic needles or syringes [13]. Also the presence of large amount of organic matter in landfills may promote an increase in the number of some enteric bacteria [14]. In general, contamination of surface water and groundwater, including increased levels of both chemical compounds and microbial indicators of pollution, is a serious problem throughout the world, as it affects drinking water resources [15]. Landfill leachate may be one of major sources of water contamination – even though landfills have developed their preventive measures in the form of liners, waste had been deposited without proper protection for several years. The quality of landfill leachate is affected by four main factors, including the composition of waste deposited and its size distribution, the age of landfill, its mode of operation and the geometeric parameters [16]. As a result of leachate contamination, municipal landfills may significantly deteriorate the quality of surface water and groundwater quality in their neighborhood [17].

With respect to the previously mentioned issues, a study was undertaken primarily in order to determine the heavy metal contamination and the prevalence of selected microbial indicators of sanitary quality in both leachate and surface water in the vicinity of a municipal landfill. The secondary aim of this study was to assess the effect of some heavy metals on the growth of waterborne strains of *E. coli* isolated from the mentioned landfill.

Material and methods

The object of the field stage of the study were samples of surface water and leachate collected within and in the vicinity of the municipal landfill site in Tarnow, launched in 1985. Surface water samples for microbiological analyses were collected from a small stream flowing along the landfill, while leachate samples were collected directly from the leachate collector located within the landfill. Immediately after collection, the samples were transported to the laboratory of the Department of Microbiology, University of Agriculture in Krakow. The bacteriological analyzes included enumeration of total number of mesophilic bacteria (TS agar, 37^{o}C, 48 h), psychrophilic bacteria (TS agar, 20^{o}C, 72 h), as well as coliforms and *Escherichia coli* (Endo agar, 37^{o}C and 44^{o}C, 48 h). Total number of mesophilic and psychrophilic bacteria was assessed using the serial dilutions method and the results were presented as the number of cfu per 1 cm^3 of water, while the number of coliforms and *E. coli* was analyzed using the filtration method and the results were presented as cfu per 100 cm^3. The results were presented as means from three replicates. The species identification of the *E. coli* isolates was confirmed based on Gram staining and biochemical API tests (BioMerieux, Marcy l'Etoile, France).

Additionally, concentrations of the following heavy metals were evaluated in the leachate and surface water samples: Pb, Cu, Cd, Zn were determined using Inductively Coupled Plasma Mass Spectrometry [18], Cr(VI) – spectrophotometrically [19] and Hg was determined using Atomic Absorption Spectroscopy [20].

Another stage of the study comprised the investigation of the effect of lead, cadmium, copper, zinc, chromium and mercury on three *E. coli* strains – one derived from the surface water sample (E1), the second one derived from leachate (E2) and a reference strain (EC ATCC 25922). Bacteria were cultured on a liquid medium (nutrient broth) in Erlenmeyer flasks protected with gauze tampons. The broth was prepared from dry bullion and distilled water. Aqueous solutions of metal salts were added to the nutrient broth. It was sterilized at 100°C in the Koch apparatus. The following concentrations of metal ions concentration were added to the medium: 0.02, 0.1 and 0.5 mg · dm^{-3}. The cold nutrient broth was inoculated with bacteria and left at 24°C for 5 days (120 h). The growth of bacteria was controlled turbimertrically after each 24 hrs using Shimadzu UV-1201V spectrometer with 520 nm wavelength, referring to McFarland standards. Samples of bullion inoculated with bacteria but without addition of metal salts served as control. The experiment was repeated three times.

Data were analyzed using Statistica v. 10 (StatSoft, USA). Basic descriptive statistics were calculated as well as Pearson correlation coefficient between the prevalence of waterborne bacteria and the concentrations of heavy metals in water samples. One-way ANOVA analysis was employed to verify the significance of differences between the reaction of the tested *E. coli* strains to different heavy metal salts and their concentrations.

Results and discussion

Collection of waste in landfills has been considered as being opposite to sustainability in various aspects, as it is both waste of resources and constitutes health and environmental hazards [21]. For instance, water that enters landfills forms leachate that can carry pollutants to their surroundings, which may result in pollution of groundwater, thus affecting the quality of water used as drinking resources, and can deteriorate surface water quality [22, 23]. However, the concentration of heavy metals in landfill leachate and surface water collected in the direct vicinity of the considered landfill was very low. As compared to the Regulation of the Minister of Environment [24] for the classification of bodies of surface water and environmental quality standards for priority substances, there was no transgression of permissible values, therefore the tested samples were considered clean in terms of heavy metal concentration.

On the other hand, the examined surface water and leachate samples contained large amounts of bacteria, including potential pathogens such as coliforms and *Escherichia coli* (Table 1).

Table 1

The concentration of heavy metals and bacterial abundances in the examined surface water and leachate samples

Parameter	Leachate	Surface water
Pb [mg · dm^{-3}]	0.1	< 0.004
Cd [mg · dm^{-3}]	0.018	< 0.0003
Cu [mg · dm^{-3}]	0.02	0.005
Zn [mg · dm^{-3}]	0.16	< 0.05
Cr VI [mg · dm^{-3}]	0.015	< 0.0004
Hg [mg · dm^{-3}]	< 0.00005	< 0.00005
Psychrophilic bacteria [cfu · cm^{-3}]	725400	86450
Mesophilic bacteria [cfu · cm^{-3}]	398000	70000
Coliforms [cfu · 100 cm^{-3}]	1340	240
Escherichia coli [cfu · cm^{-3}]	490	20

The presence of the latter two groups, *ie* coliforms and *E. coli*, is a commonly used indicator of water contamination with *eg* feces [25]. Comparing the number of coliforms in the tested surface water with the limit values given by the Regulation of the Minister of Environment [26], which divides surface waters into 5 classes of purity, among others depending on the concentration of coliforms, shows that the tested water sample should be qualified as 2nd class of purity, indicating good quality of water, with low anthropogenic impact. What is obvious, much larger numbers of microbial indicators of contamination were observed in leachate from the landfill.

The selected isolates of *E. coli* were tested for their tolerance against different essential and non-essential heavy metals. Figures 1–6 show the changes in the density of liquid bacterial cultures with the addition of the heavy metals selected for the analysis (*ie* lead, chromium, zinc, copper, cadmium and mercury, respectively) at three concentrations – 0.02; 0.1 and 0.5 mg $\cdot$ dm^{-3}. Changes in the density of *E. coli* culture in control liquid medium – without heavy metals – are shown in Fig. 7. It could be observed that the number of bacterial cells decreased in day 5 even in control cultures, but the minimum cell density, recorded for the reference *E. coli* strain (ATCC 25922) was almost 2550 $\cdot$ 10^6 cfu $\cdot$ cm^{-3}. These values were higher in a few cases, *ie* all concentrations of lead (Fig. 1a–c), the smallest concentration of zinc (Fig. 3a) and in the case f reaction of the leachate-derived isolate of *E. coli* to the smallest concentration of chromium (2780 $\cdot$ 10^6 cfu $\cdot$ cm^{-3}, Fig. 2a). As shown in Figures 1–6, the reaction of bacterial cultures to the tested six heavy metals was different, with chromium and mercury having most severe inhibiting effect while lead (all concentrations) and zinc (0.02 and 0.1 mg/dm^3) appeared to be the least toxic to *E. coli* strains. These results are similar to the ones obtained by Mariscal et al [27] in their studies on the toxicity of several heavy metals to *E. coli* measured by fluorescent bioassay, or Spain [28], who observed much higher minimum inhibitory concentrations for lead and zinc (5 and 1 mM, respectively) that for chromium and mercury (0.2 and 0.01 mM, respectively). These observations can be caused b the fact that zinc is among the essential trace elements, for instance it plays a role in forming complexes such as zinc fingers in DNA and acts as a component in cellular enzymes [29]. Also Abskharon et al [5] in their studies on the resistance of *E. coli* strains isolated from wastewater sites to different heavy metals observed that chromium had the greatest inhibiting effect on *E. coli* strains that were tested in their research. It can also be noticed that the density of bacterial cultures gradually decreased with increasing concentration of heavy metals, which is not surprising and in agreement with observations of other researchers [5, 30–32]. Also the reaction of individual isolates to the addition of heavy metals to the liquid medium was statistically significant ($p < 0.05$, F values: leachate 12.54; surface water 14.54 and reference strain 17.37).

Microbial survival in polluted environments depends on their biochemical and structural properties, as well as their adaptability to severe environmental conditions, including morphological changes of cells and modifications of metal speciation [5, 10, 33]. Also the increased resistance of microorganisms to xenobiotics, including heavy metals results among others from exposure to the contaminated environment which causes selection for strains developing the resistance mechanisms [5, 28].

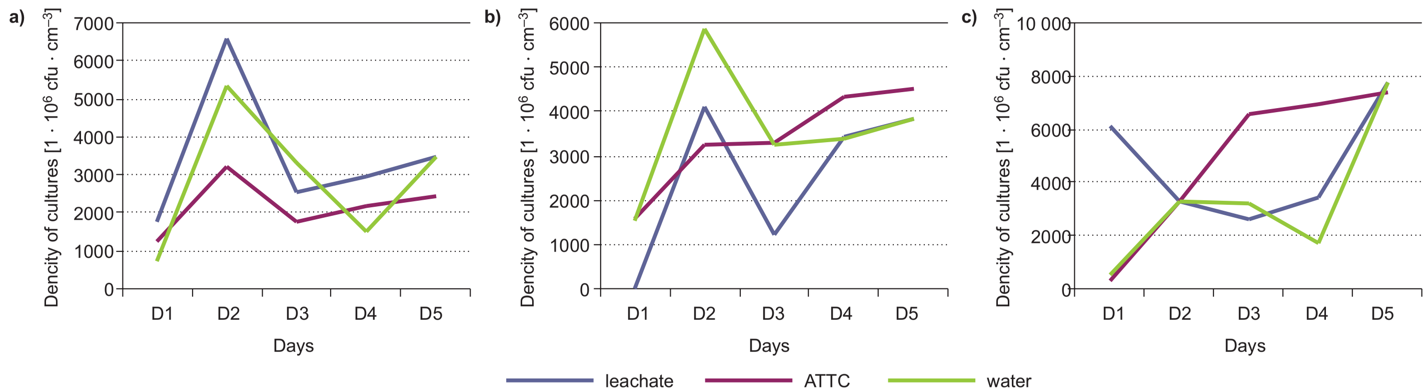

Fig. 1. Changes in the density of bacterial cultures in response to the addition of lead in the concentration of a) 0.02 mg · dm^{-3}; b) 0.1 mg · dm^{-3} and c) 0.5 mg · dm^{-3}

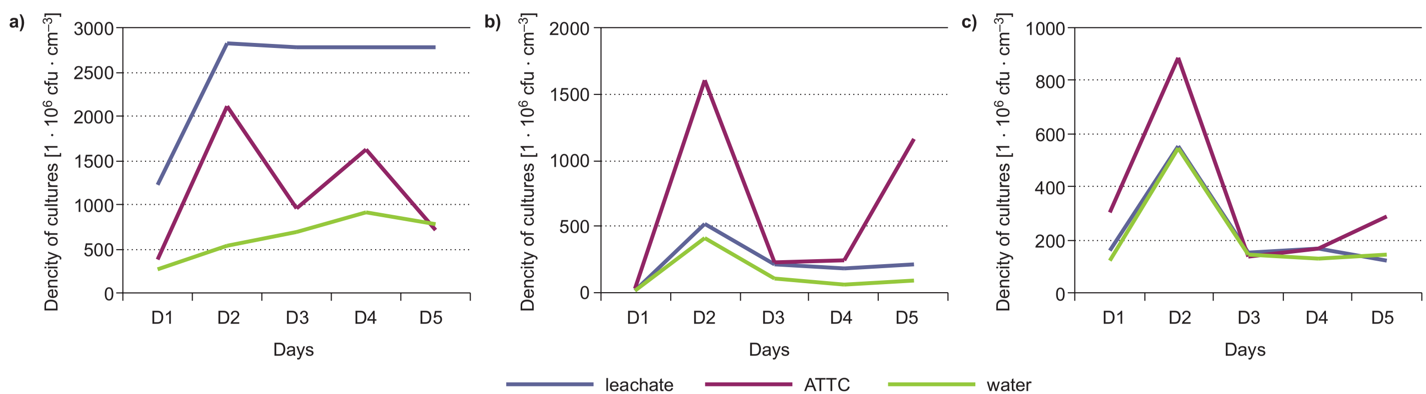

Fig. 2. Changes in the density of bacterial cultures in response to the addition of chromium in the concentration of a) 0.02 mg · dm^{-3}; b) 0.1 mg · dm^{-3} and c) 0.5 mg · dm^{-3}

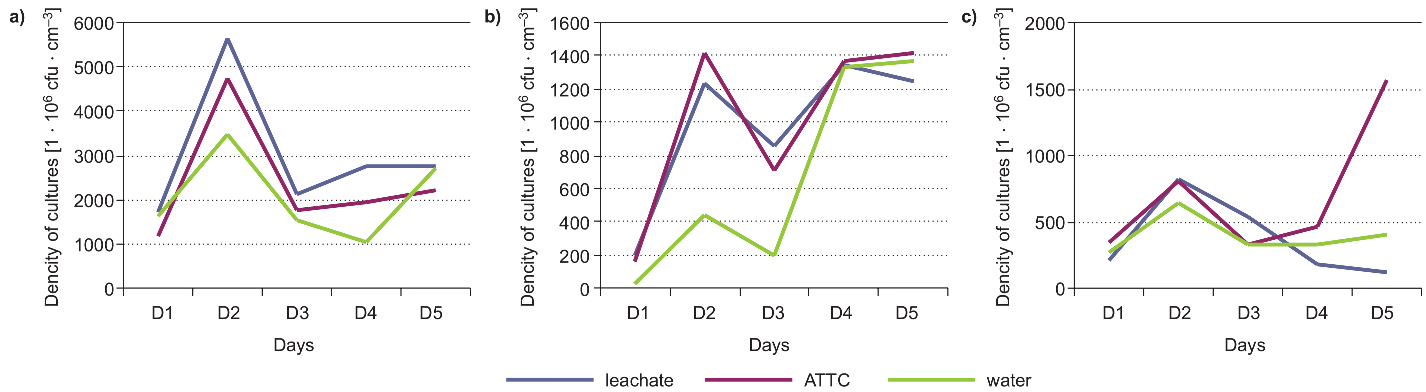

Fig. 3. Changes in the density of bacterial cultures in response to the addition of zinc in the concentration of a) 0.02 mg · dm^{-3}; b) 0.1 mg · dm^{-3} and c) 0.5 mg · dm^{-3}

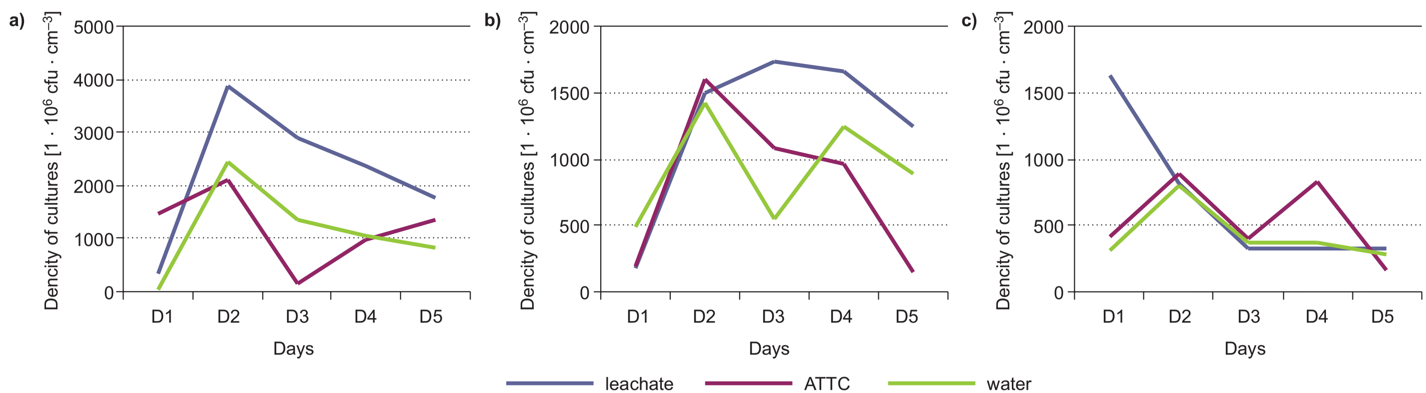

Fig. 4. Changes in the density of bacterial cultures in response to the addition of copper in the concentration of a) 0.02 mg · dm^{-3}; b) 0.1 mg · dm^{-3} and c) 0.5 mg · dm^{-3}

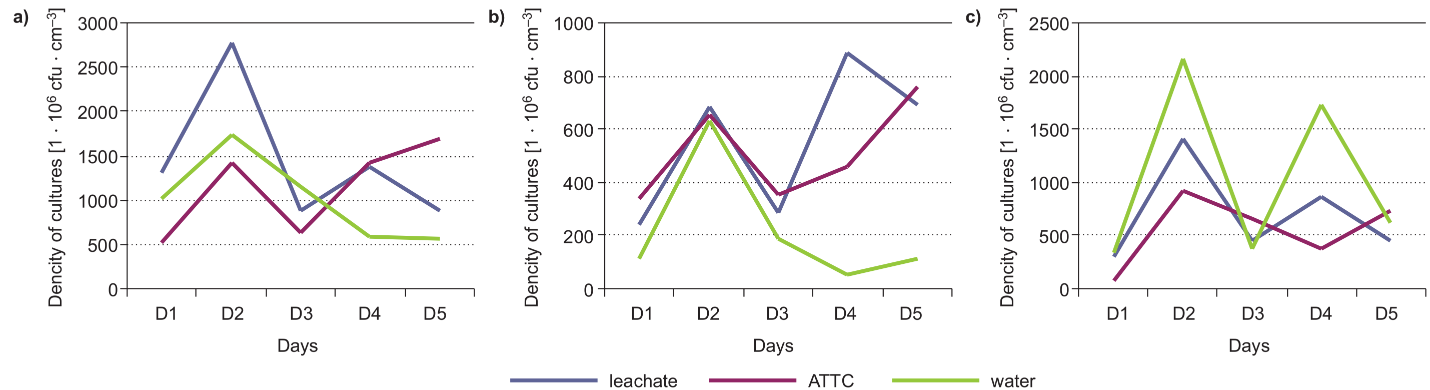

Fig. 5. Changes in the density of bacterial cultures in response to the addition of cadmium in the concentration of a) 0.02 mg · dm^{-3}; b) 0.1 mg · dm^{-3} and c) 0.5 mg · dm^{-3}

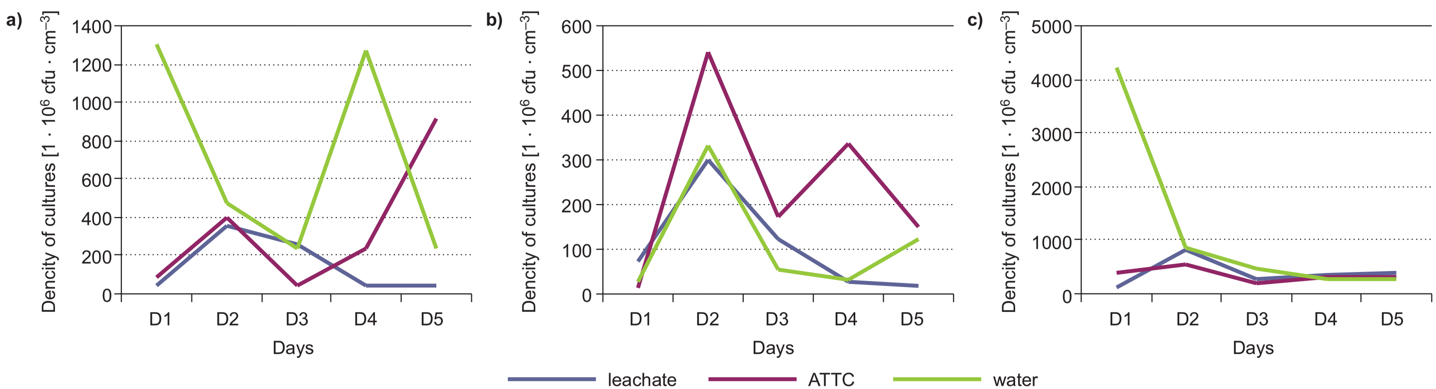

Fig. 6. Changes in the density of bacterial cultures in response to the addition of mercury in the concentration of a) 0.02 mg · dm^{-3}; b) 0.1 mg · dm^{-3} and c) 0.5 mg · dm^{-3}

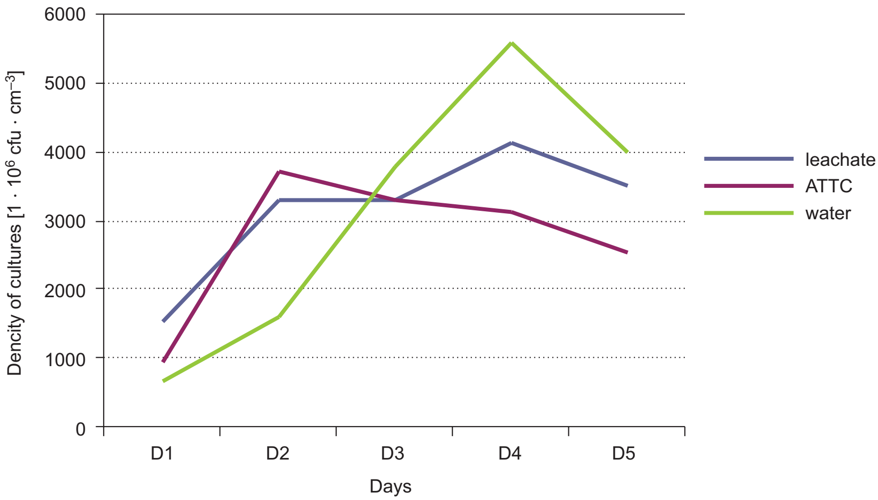

Fig. 7. Changes in the density of bacterial cultures in control cultures

Conclusions

Results of this study show that the values of heavy metal concentrations in both landfill leachate and surface water collected in its direct vicinity meet the environmental standards, so it can be concluded that the current operation mode of the landfill does not result in chemical contamination of the surrounding environment. On the other hand, very high concentrations of bacterial contaminants indicate that the considered landfill may not only pose significant biological threat to the neighboring water resources, but also may have negative heath effect on the landfill workers or residents of nearby areas.

The performed tests on the reaction of waterborne *E. coli* isolates derived from the landfill leachate and the nearby surface water sample showed that the bacterial reaction to the effect of different heavy metals varied strongly. The isolate of *E. coli* derived from the landfill leachate did not show increased resistance to the presence of heavy metals in the liquid medium, except for the smallest concentration of chromium and copper. In general, it can be stated that as compared to the control culture, the tested heavy metals inhibited or decreased the growth rate of *E. coli* strains, and this effect increased with raising concentrations of metals in the medium. Addition of chromium and mercury caused the most severe growth inhibition of tested bacterial strains. Even though some of the tested metals act as important trace elements, most of them have toxic effects on microorganisms.

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WPŁYW METALI CIĘŻKICH NA WZROST *Escherichia coli* IZOLOWANYCH Z WÓD POCHODZĄCYCH ZE SKŁADOWISKA ODPADÓW KOMUNALNYCH

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Abstrakt: Celem pracy była ocena wrażliwości izolatów *Escherichia coli*, pochodzących ze składowiska odpadów komunalnych na działanie wybranych metali ciężkich. Badaniu poddano izolaty środowiskowe, pochodzące z wody powierzchniowej – strumienia płynącego wzdłuż składowiska oraz z odcieków, a także szczep wzorcowy EC ATCC 25922. Ocenie poddano tempo wzrostu kultur bakteryjnych w podłożu płynnym z dodatkiem 0,02; 0,1 oraz 0,5 mg · dm^{-3} soli metali ciężkich: chromu, cynku, kadmu, miedzi, ołowiu i rtęci.

Wzrost bakterii badano turbidymetrycznie w odstępach 24-godzinnych przez okres 5 dni. Na podstawie przeprowadzonych badań stwierdzono różnice pomiędzy badanymi izolatami w ich reakcji na obecność badanych metali ciężkich w podłożu. Zaobserwowano także różną intensywność działania metali, przy czym najsłabsze zahamowanie wzrostu bakterii stwierdzono w przypadku ołowiu, natomiast najsilniejszy efekt hamujący miały chrom i rtęć.

Słowa kluczowe: składowisko odpadów komunalnych, *Escherichia coli*, metale ciężkie, odpady komunalne