DE GRUYTER OPEN

INTERNATIONAL
Agrophysics
www.international-agrophysics.org

Int. Agrophys., 2018, 32, 101-109
doi: 10.1515/intag-2016-0096

Community-level physiological profiles of microorganisms inhabiting soil contaminated with heavy metals

Agnieszka Kuźniar[1]*, *Artur Banach*[1], *Zofia Stępniewska*[1], *Magdalena Frąc*[2], *Karolina Oszust*[2], *Agata Gryta*[2], *Marta Kłos*[1], *and Agnieszka Wolińska*[1]

[1]The John Paul II Catholic University of Lublin, Institute of Biotechnology, Department of Biochemistry and Environmental Chemistry, Konstantynów 1 I, 20-708 Lublin, Poland
[2]Institute of Agrophysics, Polish Academy of Sciences, Doświadczalna 4, 20-290 Lublin, Poland

Received December 19, 2016; accepted October 9, 2017

Abstract. The aim of the study was to assess the differences in the bacterial community physiological profiles in soils contaminated with heavy metals *versus* soils without metal contaminations. The study's contaminated soil originated from the surrounding area of the Szopienice non-ferrous metal smelter (Silesia Region, Poland). The control was soil unexposed to heavy metals. Metal concentration was appraised by flame atomic absorption spectrometry, whereas the the community-level physiological profile was determined with the Biolog EcoPlates™ system. The soil microbiological activity in both sites was also assessed *via* dehydrogenase activity. The mean concentrations of metals (Cd and Zn) in contaminated soil samples were in a range from 147.27 to 12265.42 mg kg^{-1}, and the heavy metal contamination brought about a situation where dehydrogenase activity inhibition was observed mostly in the soil surface layers. Our results demonstrated that there is diversity in the physiological profiles of microorganisms inhabiting contaminated and colntrol soils; therefore, for assessment purposes, these were treated as two clusters. Cluster I included colntrol soil samples in which microbial communities utilised most of the available substrates. Cluster II incorporated contaminated soil samples in which a smaller number of the tested substrates was utilised by the contained microorganisms. The physiological profiles of microorganisms inhabiting the contaminated and the colntrol soils are distinctly different.

Keywords: Biolog ECO plates, community-level physiological profiles, heavy metals

INTRODUCTION

Microorganisms hold a key role in soil, and can directly or indirectly modulate the amount of heavy metal content (Xie *et al.*, 2016). Heavy metals are naturally present in soils; however, anthropogenic human activities lead to increases of these elements concentrations to amounts that are harmful to microorganisms, plants and animals (Chibuike and Obiora, 2014; Gosh and Das, 2017; Hu *et al.*, 2013; Stępniewska *et al.*, 2009). The anthropogenic sources of metal contamination can be divided into five main groups:

– metalliferous mining and smelting (arsenic, cadmium, lead and mercury);
– industrial activity (arsenic, cadmium, chromium, cobalt, copper, mercury, nickel, zinc);
– atmospheric deposition (arsenic, cadmium, chromium, copper, lead, mercury, uranium);
– agriculture (arsenic, cadmium, copper, lead, selenium, uranium, zinc); and
– waste disposal (arsenic, cadmium, chromium, copper, lead, mercury, zinc).

Heavy metals have long been known to generate disruption in ecosystem structure and function. Furthermore, toxic concentrations of heavy metals may induce enzyme damage and, consequently their inactivation, as the enzymes associated with metals can be inhibited by its toxic effect (Lenart-Boroń and Boroń, 2014; Stępniewska *et al.*, 2009). The results by Juwarkar *et al.* (2007) indicate that the microbial populations are much less abundant in contaminated soils (Cd and Pb) than in non-contaminated. Xie *et al.* (2016) suggest that the levels of heavy metals in soils had significant impacts not only on the population size, but also the physiological activity of soil microbial communities. Lenart-Boroń and Boroń (2014) suggested that changes in the physiological state of bacteria may cause changes in the community structure. In addition, long-term

*Corresponding author e-mail: agawoloszyn@kul.pl

© 2018 Institute of Agrophysics, Polish Academy of Sciences

metal stress within soil microbial communities can lead to changes of the genetic structure of the microbiome, and decrease its diversity (Giller *et al.,* 1998; Kozdrój and van Elsas, 2001).

Soil bacteria generally can be described as metabolically heterogeneous (Lenart-Boroń and Wolny-Koładka, 2015). At least 150 diverse bacterial metabolic pathways and 900 different bacterial reactions have been revealed (Haferburg and Kothe, 2012; Scheffer and Schachtschabel 2010). Therefore, from an ecological perspective, it is important to determine the metabolic microbial profile in respect to contaminated soils. One of the tools appropriate for the analysis of the functional diversity of microbial communities is the community level physiological profile (CLPP) technique, also known as the BIOLOG system (Frąc *et al.*, 2012; Garland, 1999; Rutgers *et al.,* 2016).

Other tests are connected with soil enzymatic activities, and, in the literature, the dehydrogenase activity (DHA) assay is mostly recommended (Oliveira and Pampulha, 2006; Swędrzyńska and Grześ 2015; Wolińska *et al.*, 2013, 2014). These authors note that the DHA assay is a sensitive assay for determining the effect of heavy metals on physiologically active soil microorganisms. Among many types of soil enzymes, dehydrogenases (EC 1.1.1.) are underlined as being important because they exist only inside viable microbial cells, and, thus, may provide reliable information about soil biology, fertility and productivity (Swędrzyńska and Grześ, 2015; Wolińska *et al.*, 2015). It is known that dehydrogenases are present in the cells of some aerobic microbiota; however, this enzyme is more effectively produced by anaerobes (Brzezińska *et al.*, 1998; Włodarczyk, 2000). This simple test on soil DHA performed under laboratory conditions is considered as a sensitive marker of overall microbial activities (Frąc and Jezierska-Tys, 2011; Wolińska *et al.*, 2015; Yuan and Yue, 2012).

In the literature database, there are many studies in which the CLPP method has been effectively applied to discover physiologically active bacteria in heavy-metal-contaminated sites (Margesin *et al.,* 2011; Stefanowicz, 2006; Xie *et al.*, 2016). The application of the BIOLOG system is an indirect technique for profiling the changes in the carbon metabolism of a microbial community during a remediation operation (Alisi *et al.,* 2009; Miller and Rhoden, 1991).

Consequently, the goal of this study was to assess the differences in the physiological profiles of the bacterial communities inhabiting soil contaminated with heavy metals (Pb, Cd and Zn).

MATERIAL AND METHODS

The surveyed soil material originated from an urban area (N 50° 16' 11", E 19° 4' 55") located *ca*. 560 m from the Szopienice non-ferrous metal smelter (Katowice, Silesia Region). in the past this smelter processed zinc, lead, cadmium, copper and brass. A preliminary study revealed strong soil contamination (CT) with these metals, thus, this situation encouraged us to investigate further. In order to compare the studied material with more natural soils not exposed to heavy metals, non-contaminated soil (C) from the reference location was selected. Based on wind directions in Katowice, an area located ca. 20 km from the smelter (Imielin, N 50° 8'43", E 19° 12' 12") was chosen. Its more natural state was confirmed by the neighbouring presence (1730 m) of the 'Dziećkowice' drinking water basin. Both soils were not agriculturally utilised.

The chosen soils were sampled on 12 April 2014. For the current study the soil profile divided into layers of 0-20, 20-40, 40-60, and 60-80 cm were excavated from both locations (*ca*. 1 kg). Soil material was collected into black plastic bags and transported to the laboratory where it was shortly stored (up to 3 days) at 4°C until further analyses. The sampling sites, C – 50°8'43"N;19°12'12"E, CT – 50°23'33"N;18°54'34"E. The obtained soil material was used for determining the physicochemical characteristics of soil texture, moisture, pH, redox potential (Eh), total carbon (TC) and heavy metal content (Pb, Zn, Cd, Ni).

Soil texture was determined based on the Bouyoucos areometric method (Plaster, 1997), using a Soil Texture Diagram and water content, by drying soil samples at 105°C for 24 h (Termaks oven TS8136).

Soil pH and Eh was measured within 24 h after soil sampling directly in fresh material. For both measurements, a sensIon™ 156 (Hach Lange, USA) multifunctional meter and appropriate electrodes (sensIon™ 51940 for pH and Pt-Ag/AgCl MC3187Pt Radiometer, France for Eh) were used.

The total carbon content (%) was determined by the TOC-V_{CSH} Carbon Analyser with the SSM-5000 module (Shimadzu, Japan), in dried soil material.

The total content of the selected heavy metals were estimated after the destruction of a dry soil sample in a mixture of 3 65% HNO_3, HF and HCl (3:3:1), using a microwave ETHOS One (Milestone Srl, Italy) digestion system. The obtained samples were analysed *via* the Flame Atomic Absorption Spectrometry (FAAS) method, by means of a Hitachi Z-8200 Polarised Zeeman Atom Absorption Spectrophotometer (Hitachi, Japan).

Soil dehydrogenase activity was determined using 2.3.5-triphenyltetrazolium chloride (TTC), according to the protocol of Casida *et al.* (1964), by applying the optimal TTC dose for the investigated soil type, as described by Wolińska *et al.* (2016). The soil sample (6 g) was mixed with 120 mg $CaCO_3$ and 1 ml 2% (w/v) TTC, as well as 4 ml of distilled water, and incubated for 20 h at 30 ± 1°C (Heraens Instruments). Enzymatic activity was quantified by reference to a calibration curve constructed with data obtained by incubating TTC standards under the same conditions described above. The results were expressed in µg TPF g^{-1} min^{-2} (Wolińska *et al.*, 2013).

Table 1. Physicochemical characteristics of the studied soils

Soil	Depth (cm)	Redox potential (mV)	pH	H_2O (%)	Total carbon (%)
CT	0-20	679.60(±5.29)a	7.91(±0.03)a	31.02(±0.13)d	12.450(±0.60)d
	20-40	502.77(±48.14)bc	6.37(±0.03)c	23.94(±0.14)e	8.309(±0.53)c
	40-60	679.30(±1.08)a	6.37(±0.02)c	20.34(±0.05)f	5.833(±0.29)b
	60-80	549.33(±15.12)b	6.41(±0.01)c	20.39(±0.99)f	0.035(±0.02)a
C	0-20	459.30(±5.53)c	6.39(±0.02)c	5.34(±0.02)a	0.072(±0.04)a
	20-40	464.47(±11.80)c	6.70(±0.03)b	7.62(±0.04)b	<0.01a
	40-60	453.33(±0.67)c	6.38(±0.07)c	3.70(±0.04)c	<0.01a
	60-80	469.13(±6.49)c	6.33(±0.02)c	6.86(±0.02)b	<0.01a

Mean values marked with the same letter do not differ significantly at $\alpha = 0.05$ for a given variable (mean values ± SD, CT – contaminated, C – non-contaminated soil).

CLPP was analysed by applying the Biolog EcoPlate™ system (Biolog Inc., Hayward, CA, USA). Each of 96-well plate contained the three replicates, with 31 different sole carbon sources and water (as a blank sample). The carbon substrates were subdivided into five groups of substrates: carbohydrates (Cs), carboxylic and ketonic acids (CK), amines and amides (AD), amino acids (AA), and polymers (P). The soil samples (1 g) were shaken in 99 ml of sterile water for 30 min before incubating (20 min, 4°C). Then, 120 µL of the sample suspension was inoculated into each well of the Biolog EcoPlates™ and incubated at 26°C. The utilisation rate was indicated by the reduction of tetrazolium violet, a redox indicator dye that changes from colourless to purple (Islam *et al.*, 2011; Stefanowicz, 2006). The data were recorded at 490 nm every 24 h for up to 216 h. Next, the absorbance readings of the individual wells of the EcoPlates™ while under incubation were used to determine the average well colour development (AWCD). From the obtained AWCD data, the substrate richness was calculated (Garland, 1999, Gomez *et al.*, 2006). Additionally, the percentages of the carbon sources that were used by each group were evaluated. The collected data were analysed statistically by means of Statistica 12PL (StatSoft, USA). Prior to the analyses, the normality of data and homogeneity of variances were assessed using W Shapiro-Wilk and Levene tests, respectively. If the data normality condition was not met, a ln(x+1) transformation was applied to all data.

In order to assess differences among the studied soil parameters, a two way ANOVA with Tukey as *post hoc* test was applied. Significance was accepted at $p<0.05$. All data are presented as mean, non-transferred data, along with their standard deviation statistic (±SD).

RESULTS

The chemical and physical characteristics of the two soil sample (CT and C) profiles are shown in Table 1.

In terms of aeration status, both soils were quite well aerated (Eh>400 mV), but the CT soil displayed a quite higher Eh level (502.77-679.6 mV). There were also differences in the soil moisture ($p<0.001$), and the CT soil was characterised by higher moisture levels (>20%) than the C soil. pH was slightly alkaline in the top layer of the CT soil, in comparison to the C soil horizons (7.9 *vs* 6.4). The control soil profiles were carbon-poor, only in the top layer TC achieved a level of 0.07%, but in the deeper part of the C soil profile, TC content was less than 0.01%. In contrast, in the CT soil profile, TC varied from 0.04 to 12.5%, achieving maximum values in the surface part (0-20 cm) and showing a decreasing tendency with a growth of the soil profile depth.

The CT soil was characterised as a sandy loam (68% sand, 30% silt, 8% clay), with higher silt and clay fractions in the deeper layers (both up to 40%), resulting in a silty clay layer of 60-80 cm. In the case of the C soil, the sand fraction dominated (93%), with 2% of silt and 5% of clay (sandy soil); the deepest layer was more clayey (77% sand, 6% silt and 17% clay) – indicating a sandy loam type.

The CT soil profile was characterised by its very high content of Pb, Zn and Cd (Table 2). In contrast, the C soil showed much lower (more than a hundred times, $p<0.001$) content of the studied metals. The average content in the top CT layer was 6867.87, 12265.42 and 147.27 mg kg^{-1} DW, for Pb, Zn and Cd, respectively. Such levels were extremely high and significantly exceeded the maximum permissible concentrations of potentially toxic elements in soils (Directive 86/278/EEC). The CT soil also showed a decreasing tendency of metal content with a growth of the soil profile depth. This decline was even 1/6 of the initial

Table 2. The selected heavy metal content in the studied soil profiles

Metal (mg kg^{-1})	Soil	Depth (cm)			
		0-20	20-40	40-60	60-80
Pb	CT	6867.87(±17.82)d	2302.74 (±43.64)c	1409.91(±22.01)b	<1.00a
	C	12.50(±1.14)a	<1.00a	15.63(±3.13)a	3.13(±0.64)a
Zn	CT	12265.42(±78.51)a	11625.41 (±59.22)b	10017.64(±236.29)c	979.58 (±77.59)d
	C	43.59(±2.65)e	30.85(±1.97)e	28.87(±1.63)e	66.83(±1.29)e
Cd	CT	147.27(±2.62)a	96.36(±5.744)b	48.37(±5.31)c	<1.00d
	C	0.19(±0.10)d	1.03(±0.49)d	0.52(±0.30)d	0.84(±0.49)d

Explanations as in Table 1.

levels, in comparison to the top soil values. In the case of Pb and Cd, no metal presence in the layer of 60-80 cm was noted (limit of detection < 1 ppm).

In the C soil profile, the levels of Zn ranged between 29 and 67 mg kg^{-1} DW – with no significant changes between soil layers; Pb was recorded at the level of 13-16 mg kg^{-1} DW, but only in the two layers: 40-60 and 60-80 cm, respectively (Table 2); Cd was present at very low levels within the range of detection limits of the FAAS method (ca. 1 mg·kg^{-1} DW). Changes in DHA level at different soil profile depths and heavy metals contamination are presented in Fig. 1.

We found that in C soils, DHA decreased with an increase of the soil depth, equalling 4.61 µg TPF g^{-1} min^{-1} in the surface layer (0-20 cm), while in the deepest part of the soil profile (60-80 cm) DHA was reduced by 74% and amounted 1.19 µg TPF g^{-1} min^{-1}.

An opposite trend was observed in the case of the CT soil profile (Fig. 1). Inhibition of DHA (*ca*. by 18%) as a consequence of heavy metals contamination was noted only in the surface layer (0-20 cm). In the deeper parts of the investigated profile, a heavy metal content stimulation effect on DHA was observed (by 56, 34, and 37% – for the 20-40, 40-60, and 60-80 cm profiles, respectively). Still, from the 20-40 cm soil profile, a linear decrease of DHA with soil depth was recorded. The different ability of microorganisms to metabolise the 31 substrates is shown in Fig. 2. The utilisation of carbon sources began to increase after 48 h of incubation regardless of the various sampling, with higher values noted in the non-contaminated soil samples.

There are significant differences (F=287.22; p≤0.05) in AWCD between the CT and C soils. The AWCD values were lower in CT soil samples than in C samples (Fig. 3A). Our results suggest that the substrate utilisation rate by soil microbial communities inhabiting the CT soil was always lower than in the C; however, no significant differences (F=2.531; p≥0.05) in substrate richness were observed between CT and C sites (Fig. 3B).

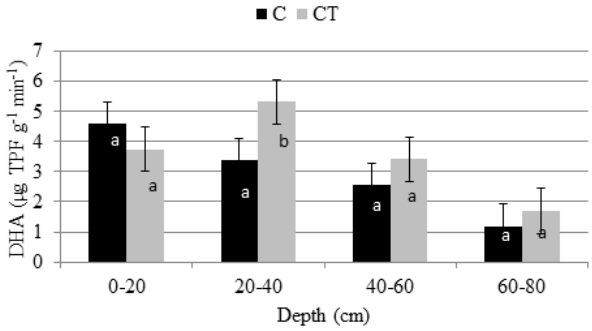

Fig. 1. Dehydrogenase activity at different depths of the control (C) and contaminated (CT) soil profiles. Explanations as in Table 1.

From the available substrates, the carbohydrates (pyruvic acid methyl ester, N-acetyl-D-glucosamine), carboxylic and ketonic acids (γ-hydroxybutyric acid and D-malic acid) were most frequently absorbed by the contained soil microorganisms. An amino acid (L-Threonine) was preferred only by the microbial community inhabiting the C soils (Fig. 4).

The microbiome of the CT soil was characterised by its ability to utilise specific C-substrates: putrescine amines/amides and Tween 80 polymers. It was observed that certain common C-substrate sources were utilised similarly by both of the two microbial communities (C and CT). Among the following carbon sources: carbohydrates (D-mannitol), carboxylic and ketonic (itaconic acid, 4-hydroxy benzoic acid, D-galacturonic acid, D-glucosaminic acid), acids amino acids (L-Arginine, L-Asparagine, L-Serine, glycyl-L-glutamic acid), amines/amides (phenylethylamine) as well as polymers (Tween 40) were utilised in the same way by the microbiome of the C and CT soil (Fig. 4). Cluster analysis revealed two main distinct cluster groups. They included non-contaminated soil samples (C) collected from layers of 40, 60 and 80 cm (Cluster I) in which microbial communities utilised most of the tested substrates and

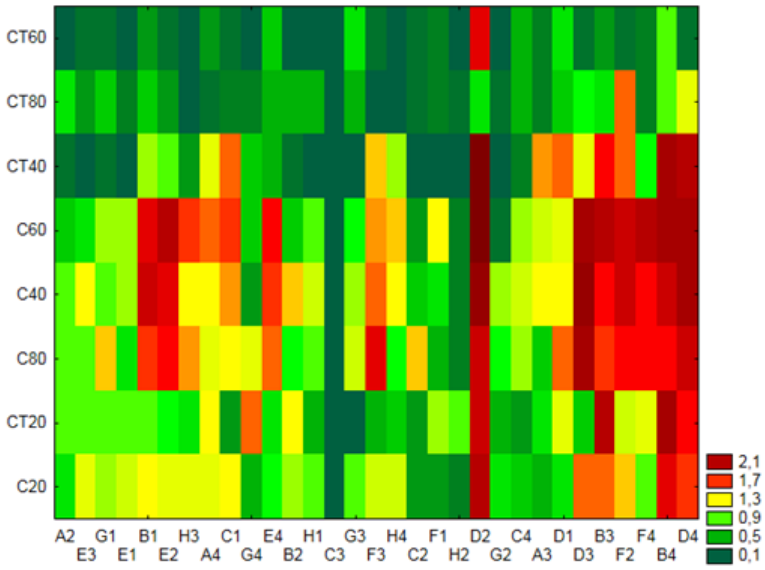

Fig. 2. Categorised substrate utilisation patterns by CT and C soil microbial communities after 216 h of incubation. Error bars indicate the standard errors of the mean (n=3).

contaminated soil samples (with one exception of C20) (Cluster II) in which a lower number of the tested substrates was utilised (Fig. 5). Cluster II was subdivided into the other two sub-groups (IIA, IIB), among which sub-cluster IIA and IIB contained contaminated subsurface soils (20 and 40 cm depths), the two soil samples collected from the deepest soil layers. In these two samples the substrates were utilised with a weak intensity .

DISCUSSION

Our results indicated that the heavy metals imposed a significant negative effect on the CLPP of microorganisms inhabiting the CT soil. Statistical analysis confirmed this fact, as there are significant differences ($F=287.22$; $p\leq0.05$) in the AWCD between the CT and C soils (Fig. 4A). A similar trend was observed by Xie *et al.* (2016), who determined that the total bioactivity of microorganisms decreased with increasing heavy metal concentration, because microorganisms differ in their sensitivity to heavy metals toxicity. Muñiz *et al.* (2014) noted that Cd in an exposed microbial community modified the physiological profile of the microorganisms. Klimek *et al.* (2016) reported the negative relationship between total petroleum hydrocarbons (TPH) and functional soil bacterial diversity (CLPP). The effect is indicative of a significantly decreased or increased rate of cell proliferations resulting from the short- or long-term contact of bacteria with the metals (Piotrowska-Seget and Kozdrój, 2008). If soil is contaminated with high metal concentrations (Cu – up to 2500 mg kg^{-1} and Cd – up to 1500 mg kg^{-1}), bacterial numbers decreased significantly (Piotrowska-Seget and Kozdrój, 2008). This trend is associated with the time-related dominance of the populations represented by slow-growing *K*-strategists in the structures of soil bacterial communities with time (Krzyżak *et al.*, 2013; Piotrowska-Seget and Kozdrój, 2008). Our results also indicate that there are differences in functional diversity between bacterial communities inhabiting CT and C soils. This tendency is confirmed by the cluster analysis (Fig. 5), where the two main distinct cluster groups were revealed. Cluster I included non-contaminated soil samples collected from layers of 40, 60 and 80 cm. Furthermore, the mentioned groups of microorganisms utilised most of the available substrates (Fig. 2). Cluster II was formed by a bacterial community inhabiting the contaminated soil samples (with one exception of C20). This branch was represented by microorganisms which utilise smaller numbers of tested substrates (Fig. 2). However, the statistical analysis did not reveal significant differences ($F=2.531$; $p\geq0.05$) in substrate utilisation rate. Such a result may have occurred because the number of microorganisms can increase during incubation time. The effect could also have resulted from the development of a metal-resistant microbial population (Piotrowska-Seget and Kozdrój, 2008; Xie *et al.*, 2016). Results from the present study indicate that the bacterial community of the CT soil utilised all five categories (Cs, CK, AD, AA, P) of carbon sources. However, the rate of utilisation averaged lower in the CT soil than in the C soil, especially in the deepest soil layers (Fig. 3B). The amines/amides and the amino acids groups were the most intensively metabolised by the CT soil microbiome, while in the C soil, the carbohydrates and the polymers were preferred nutrient sources.

Numerous studies indicate that long-term heavy metal contamination decreases biodiversity or disturbs the community structure (Lenart-Boroń and Boroń, 2014). What is more, metal exposure may lead to the creation of tolerant microbial populations. These are often the Gram-positive

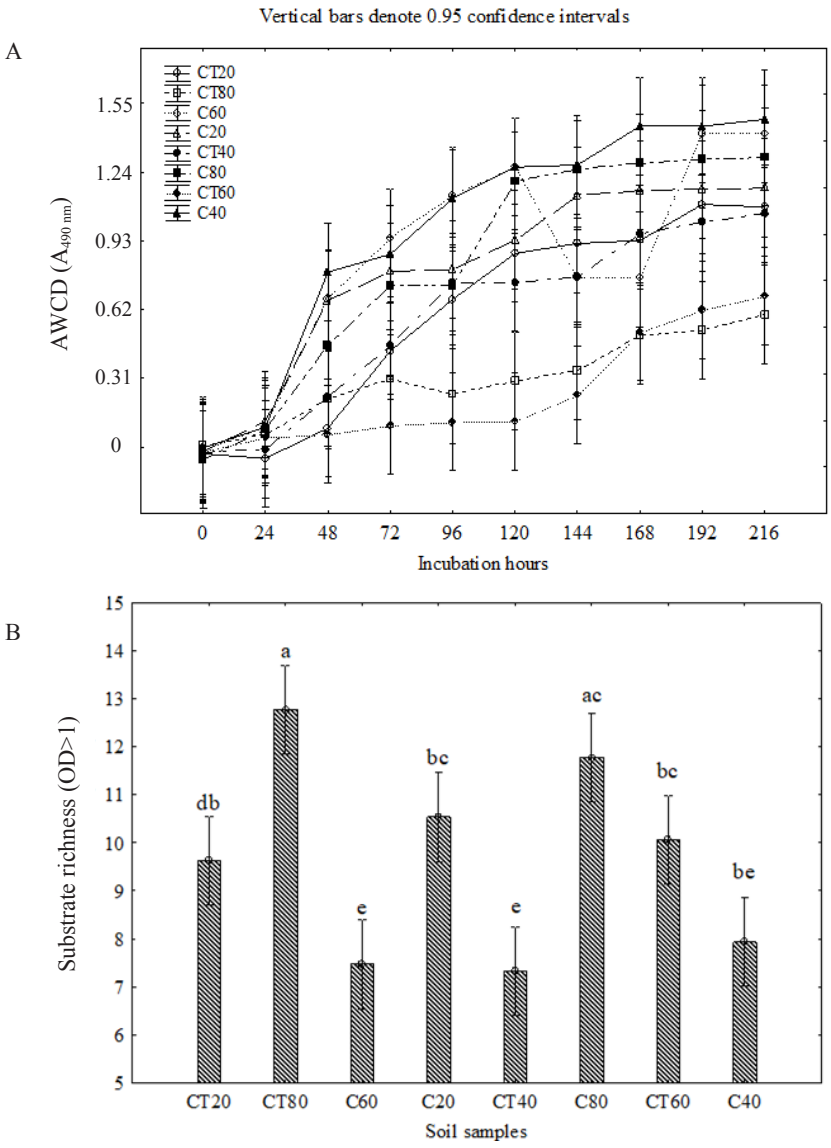

Fig. 3. Utilisation of carbon substrates by C and CT soil microbial communities (A) and the richness of the soil microbial community diversity (B).

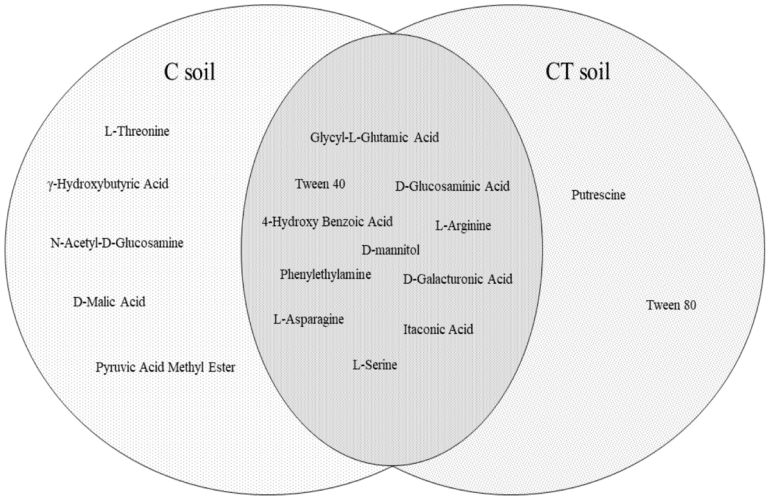

Fig. 4. Comparison of C and CT soil microbial community utilisation of carbon substrates at the end of the incubation period (216 h).

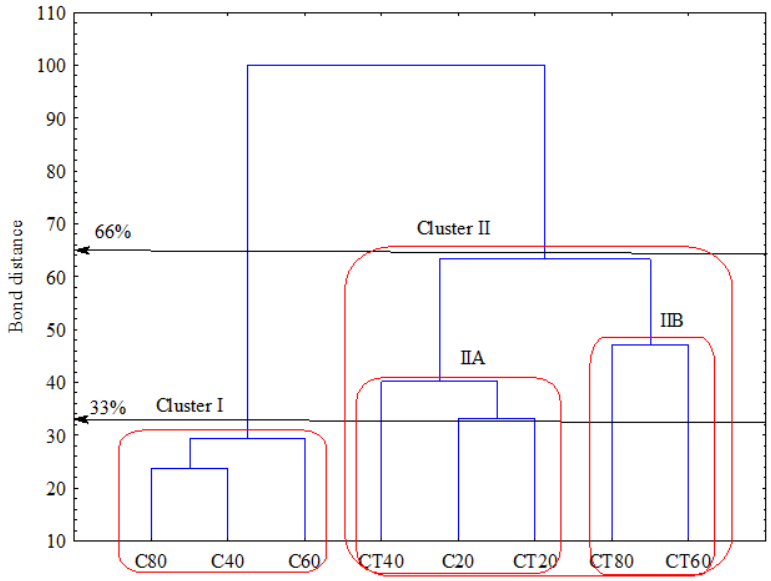

Fig. 5. Cluster analysis – based on a dendrogram showing correlations between tested soil samples in relation to the utilisation of carbon sources.

genera: *Bacillus*, *Arthrobacter*, *Corynebacterium* or the Gram-negatives: *Pseudomonas*, *Alcaligenes*, *Ralstonia* or *Burkholderia* (Lenart-Boroń and Boroń, 2014). For example, Elsilk *et al.* (2014) isolated *Bacillus anthracis* PS2010, which has variable resistance to heavy metals such as Cd, Cu, Co, Zn and Pb, while Banerjee *et al*. (2015), utilising the serial dilution technique in nutrient medium, isolated *B. cereus* and *B. subtilis*. These isolates are resistant to a number of metals – Pb, Hg, Ni, Co, Cu and M (Banerjee *et al.*, 2015). Then, Ansari *et al.* (2016), in their study, isolated from soil six bacteria: *Alcaligenes* sp. HM-7, *Alcaligenes* sp. HM-24, *Alcaligenes* sp. HM-27, *Alcaligenes* sp. HM-51, *Bacillus cereus* HM-85, *Bacillus cereus* HM-6. In these, the highest Cd, Pb and As tolerance were found in *Alcaligenes* HM-7 – 62.52% and *Alcaligenes* sp. HM-24 – 82.56%. The aforementioned authors suggest that soil bacteria accumulated heavy metal in nutrient rich as well as in nutrient deficient environments, and could be potentially used in the bioremediation of heavy-metal-contaminated environment (Ansari *et al.*, 2016). Our results reveal that microorganisms accumulated heavy metals in the CT soil because they are adapted to a very high content of heavy metals. Heavy metals contamination may cause changes in the composition and activity of soil bacterial communities (Giller *et al.*, 1998b; Piotrowska-Seget and Kozdrój, 2008; Stępniewska *et al.*, 2009; Xie *et al.*, 2016). What is more, the sites contaminated with heavy metals may develop increasing numbers of microorganisms, and even a dominance of metal-tolerant populations (Kozdrój, 2001; Xie *et al.*, 2014, 2016).

Soil DHA is the most frequently used approach for determining the influence of various pollutants (*i.e.* heavy metals) on the microbiological quality of soils (Stępniewska *et al.*, 2009; Tejada *et al.*, 2010). Generally, it is assumed that heavy metals can reduce enzyme activity by interacting with the enzyme-substrate complex (Pan and Yu, 2011). A study by Pan and Yu (2011) undertaken with brown soil showed that DHA was significantly lower by 37.8 and by 51.1% in Cd and Pb contaminated soil than in the control. On the contrary, our earlier study (Wolińska and Stępniewska, 2012) resulted in the observation that the presence of Cd at a concentration of 2 mg kg^{-1} had a stimulating effect on soil DHA level, and enhanced DHA by 8.8% in comparison with the control. The negative effect of heavy metals on DHA was also reported by Kizilkaya *et al.* (2004), who arranged this inhibition in the following order: Cu > Cd > Co. In the current study, we observed a reduction in DHA with a growth of the soil depth, both in CT, as well as in C soils. This trend remains in agreement with the knowledge about the stratification of soil microorganisms and its preference for colonising the soil surface layers (Levyk *et al.*, 2007; Wolińska, 2010). The depth of the soil profile is one of the best researched environmental factors reducing soil DHA level (Wolińska and Stępniewska, 2012; Xiang *et al.*, 2008).

Biernacka and Małuszyński (2006) determined the concentration of heavy metals (Cd, Pb) using AAS, in the surface layer (0-20 cm) of soils located in areas under different anthropopressure impacts. These studies indicated that the concentration of Cd and Pb were higher in soils under strong anthropogenic impact than in soils from a region regarded as unpolluted. Pb concentration in soils under strong anthropopressure ranged from 392 to 1568 mg kg^{-1} (Biernacka and Małuszyński, 2006). We suggested a similar conclusion that the CT soil has a significant heavy metal content in comparison to the C soil. The Pb content

in respect to the CT soil was at levels of 6 867.87±17.82 mg kg^{-1}. Our results evidenced a four times higher concentration of Pb in soils situated *ca*. 560 m from the Szopienice non-ferrous metal smelter (Katowice, Silesia Region) than in soils located within five kilometres from the 'Huta Katowice Steelworks' (Biernacka and Małuszyński, 2006). Similarly, the Cd concentration in soils from the region of the 'Huta Katowice Steelworks' was twofold lower than that in the soils sampled close to the Szopienice non-ferrous metal smelter (Biernacka and Małuszyński, 2006). Nazir *et al.* (2015) detected concentrations of Cd in all their collected soil samples (Tanda Dam region, Kohat, Pakistan). These ranged from 0.029 to 0.328 mg kg^{-1}, while the content of Pb in their soil samples ranged between 0.061 and 0.461mg kg^{-1} (Nazir *et al.*, 2015). However, the Pb and Cd concentrations in the studied soil (CT) were significantly higher (1 409.91 – 6 887.87 mg kg^{-1} – Pb; 1-147.27 mg kg^{-1} – Cd). Klimek *et al.* (2016) suggest that the metal concentration in soils from the Upper Silesian region (Olkusz and Miasteczko Śląskie) are 1 577 mg Pb kg^{-1} and 5437 mg Zn kg^{-1}. In conclusion, the concentration of heavy metals in the studied soils was higher in comparison to the earlier studies carried out in the Upper Silesian region (Biernacka and Małuszyński, 2006; Klimek *et al.*, 2016).

CONCLUSIONS

1. The higher heavy metal content, the more negative effect on the average well colour development values.

2. There were no differences in substrate richness between the contaminated and the control sites. This suggests that soil microorganisms inhabiting the contaminated soil can adapt to very high heavy metal content.

3. The physiological profiles of microorganisms inhabiting the contaminated and non-contaminated control soil are distinctly different.

4. The contaminated sites likely contained metal-resistant microbial communities that are able to make the most intensive use of carbon and nitrogen from the amines/amides and the amino acids groups.

Conflict of interest: The Authors do not declare conflict of interest.

REFERENCES

Alisi C., Musella R., Tasso F., Ubaldi C., Manzo S., Cremisini C., and Sprocati A.R., 2009. Bioremediation of diesel oil in a contaminated soil by bioaugmentation with a microbial formula tailored with native strains selected for heavy metals resistance. Sci. Total Environ., 407, 3024-3032.

Ansari R.A., Qureshi A.A., and Ramteke D.S., 2016. Isolation and characterization of heavy-metal resistant microbes from Industrial soil. Int. J. Environ. Sci., 6(5), 670-680.

Banerjee S., Gothalwal R., Sahu P.K., and Sao S., 2015. Microbial observation in bioaccumulation of heavy metals from the Ash Dyke of thermal power plants of Chhattisgarh, India. Advances Biosci. Biotechnol., 6, 131-138.

Biernacka E. and Małuszyński M.J., 2006. The content of cadmium, lead and selenium in soils from selected sites in Poland. Polish J. Environ. Studies, 15(2a), 7-9.

Brzezińska M., Stępniewska Z., and Stępniewski W., 1998. Soil oxygen status and dehydrogenase activity. Soil Biol. Biochem., 30(13), 1783-1790.

Casida L., Klein D., and Santoro T., 1964. Soil dehydrogenase activity. Soil Sci., 98, 371-376.

Chibuike G.U. and Obiora S.C., 2014. Heavy metal polluted soils: effect on plants and bioremediation methods. Appl. Environ. Soil Sci., 2014, 1-12.

Elsilk S.E., El-Shanshoury A.E.R., and Ateya P.S., 2014. The bacteria Bacillus anthracis PS2010 have variable resistant to heavy metals such as Cd, Cu, Co, Zn and Pb. African J. Microbiol. Res., 8(12), 1266-1276.

Frąc M. and Jezierska-Tys S., 2011. Agricultural utilisation of dairy sewage sludge: its effect on enzymatic activity and microorganisms of the soil environment. African J. Microbiol. Res., 5(14), 1755-1762.

Frąc M., Oszust K., and Lipiec J., 2012. Community level physiological profiles (CLPP), characterization and microbial activity of soil amended with dairy sewage sludge. Sensors, 12, 3253-3268.

Garland J.L., 1999. Potential and limitations of BIOLOG for microbial community analysis (Eds C.R. Bell, M. Brylinsky, P. Johnson-Green). Microbial Biosystems: New Frontiers Proc. 8th Int. Symp. Microbial Ecology, August 9-12, Halifax, Canada.

Ghosh S. and Das A.P., 2017. Bioleaching of manganese from mining waste residues using Acinetobacter sp. Geology, Ecol. Landscapes, 1(2), 77-83.

Giller K.E., Witter E., and McGrath S.P., 1998. Toxicity of heavy metals to microorganism and microbial processes in agricultural soils: A review. Soil Biol. Biochem., 30(10-11), 1389-1414.

Gomez E., Ferreras L., and Toresani S., 2006. Soil bacterial functional diversity as influenced by organic amendment application. Bioresource Technol., 97, 1484-1489.

Haferburg G. and Koth E., 2012. Chapter 2 Biogeosciences in Heavy Metal-Contaminated Soils. Springer-Verlag Berlin Heidelberg, 17-34.

Hu J., Liu X., Bai J., Shih K., Zeng E. Y., and Cheng H., 2013. Assessing heavy metal pollution in the surface soils of a region that had undergone three decades of intense industrialization and urbanization. Environ. Sci. Pollution Res., 20, 6150-6159.

Islam M.R., Chauhan P.S., Kim Y., Kim M., and Sa T., 2011. Community level functional diversity and enzyme activities in paddy soils under different long-term fertilizer management practices. Biol. Fertility Soils, 47, 599-604.

Juwarkar A.A., Nair A., Dubey K.V., Singh S.K., and Devotta S., 2007. Biosurfactant technology for remediation of cadmium and lead contaminated soils. Chemosphere, 68(10), 1996-2002.

Kizilkaya R., Askin T., Bayrakli B., and Saglam M., 2004. Microbiological characteristics of soils contaminated with heavy metals. European J. Soil Biol., 40, 95-102.

Klimek B., Sitarz A., Choczyński M., and Niklińska M., 2016. The Effects of Heavy Metals and Total Petroleum

Hydrocarbons on Soil Bacterial Activity and Functional Diversity in the Upper Silesia Industrial Region (Poland). Water, Air, & Soil Pollution, 227-265.

Kozdrój J., 2001. Microbial reaction to soil contamination with Cd (II) at different temperatures. Microbiol. Res., 155, 285-290.

Kozdrój J. and van Elsas J.D., 2001. Structural diversity of microorganisms in chemically perturbed soil assessed by molecular and cytochemical approaches. J. Microbiol. Methods, 43(3), 197-212.

Krzyżak J., Wasilkowski D., Płaza G. A., Mrozik A., Brigmon R.L., and Pogrzeba M., 2013. Culture methods as indicators of the biological quality of phytostabilized heavy metal-contaminated soil. Environ. Biotechnol., 9(1), 6-13.

Lenart-Boroń A. and Boroń P., 2014. The Effect of Industrial Heavy Metal Pollution on Microbial Abundance and Diversity in Soils - A Review. Intech Chapter 26: Environmental Risk Assessment of Soil Contamination, 760-783.

Lenart-Boroń A. and Wolny-Koładka K., 2015. Heavy metal concentration and the occurrence of selected microorganisms in soils of a steelworks area in Poland. Plant, Soil and Environ., 61(6), 273-278.

Levyk V., Maryskevych O., Brzezińska M., and Włodarczyk T., 2007. Dehydrogenase activity of technogenic soils of former sulphur mines (Yvaoriv and Nemyriv, Ukraine). Int. Agrophysics, 21, 255-260.

Margesin R., Płaza G., and Kasenbacher S., 2011. Characterization of bacterial communities at heavy-metal-contaminated sites. Chemosphere, 82, 1583-1588.

Miller J.M. and Rhoen D.L., 1991. Preliminary evaluation of Biolog, a carbon source utilization method for bacterial identification. J. Clinical Microbiol., 29(6), 1143-1147.

Muñiz S., Lacarta J., Pata M.P., Jiménez J.J., Navarro E., 2014. Analysis of the diversity of substrate utilisation of soil bacteria exposed to Cd and earthworm activity using generalised additive models. PLOS ONE, 9(1), 1-10.

Nazir R., Khan M., Masab M., Ur Rehman H., Ur Rauf N., Shahab S., Ameer N., Sajed M., Ullah M., Rafeeq M., and Shaheen Z., 2015. Accumulation of heavy metals (Ni, Cu, Cd, Cr, Pb, Zn, Fe) in the soil, water and plants and analysis of physico-chemical parameters of soil and water Collected from Tanda Dam kohat. J. Pharmaceutical Sci., 7(3), 89-97.

Oliveira A. and Pampulha E.M., 2006. Effects of long-term heavy metal contamination on soil microbial characteristics. J. Biosci. Bioeng., 102, 157-161.

Pan J. and Yu L., 2011. Effects of Cd or/and Pb on soil enzyme activities and microbial community structure. Ecological Eng., 37, 1889-1894.

Piotrowska-Seget Z. and Kozdrój J., 2008. Changes in culturable bacterial community of soil treated with high dosages of Cu or Cd. Plant, Soil Environ., 54(12), 520-528.

Rutgers M., Wouterse M., Drost S.M., Breure A.M., Mulder C., Stone D., Creamer R.E., Winding A., and Bloem J., 2016. Monitoring soil bacteria with community-level physiological profiles using Biolog ECO-plates in the Netherlands and Europe. Applied Soil Ecol., 97, 23-35.

Scheffer F. and Schachtschabel P., 2010. Bodenmikroflora. In: Lehrbuchder Bodenkunde (in German) (Eds F. Scheffer, P. Schachtschabel). Spektrum Akademischer Verlag, Heidelberg, 84-99.

Stefanowicz A., 2006. The Biolog Plates Technique as a Tool in Ecological Studies of Microbial Communities. Polish J. Environ. Studies, 15(5), 669-676.

Stępniewska Z., Wolińska A., and Klin R., 2009. Heavy metals release from eroded loess soils. Int. Agrophys., 23, 377-382.

Swędrzyńska D. and Grześ S., 2015. Microbiological parameters of soil under sugar beet as a response to the long-term application of different tillage systems. Polish J. Environ. Studies, 24(1), 285-294.

Tejada M., Garcia-Martinez A., Gomez I., and Parrado J., 2010. Application of MCPA herbicide on soils amended with biostimulants: short-time effects on soil biological properties. Chemosphere, 80, 1088-1094.

Włodarczyk T., 2000. Some of aspects of dehydrogenase activity in soils. Int. Agrophysics, 14(3), 365-376.

Wolińska A., 2010. Dehydrogenase activity of soil microorganisms and oxygen availability during reoxidation process of selected mineral soils from Poland (in Polish). Acta Agrophysica, 180, 1-88.

Wolińska A. and Stępniewska Z., 2012. Dehydrogenase activity in the soil environment. In: Dehydrogenases (Ed. Rosa Angela Canuto), INTECH Publisher, 8, 183-210.

Wolińska A., Stępniewska Z., and Pytlak A., 2015. The effect of environmental factors on total soil DNA content and dehydrogenase activity. Archives of Biological Sciences, 67(2), 493-501.

Wolińska A., Stępniewska Z., and Szymańska E., 2013. Dehydrogenase activity of soil microorganisms and the total DNA level in soil of different use. J. Agric. Sci. Technol. B, 3, 613-622.

Wolińska A., Szafranek-Nakonieczna A., Banach A., Rekosz-Burlaga H., Goryluk-Salmonowicz A., Błaszczyk M., Stępniewska Z., and Górski A., 2014. Biological degradation of agricultural soils from Lublin region (SE Poland). Int. J. Current Microbiol. Appl. Sci., 3(11), 558-571.

Wolińska A., Zapasek M., Stępniewska Z., 2016. The optimal TTC dose and its chemical reduction level during soil dehydrogenase activity assay. Acta Agrophysica, 23(2), 303-314.

Xiang S., Doyle A., Holden P., and Schimel J., 2008. Drying and rewetting effects nn C and N mineralization and microbial activity in surface and subsurface California grassland soils. Soil Biol. Biochemistry, 40, 2281-2289.

Xie Y., Fan J., Zhu W., Amombo E., Lou Y., Chen L., and Fu J., 2016. Effect of heavy metals pollution on soil microbial diversity and bermudagrass genetic variation. Frontiers Plant Sci., 7, 1-12.

Xie Y., Luo H., Du Z., Hu L., and Fu J., 2014. Identification of cadmium-resistant fungi related to Cd transportation in bermudagrass [*Cynodon dactylon* (L.) Pers.]. Chemosphere, 117, 786-792.

Yuan B. and Yue D., 2012. Soil microbial and enzymatic activities across a chronosequence of Chinese pine plantation development on the loess plateau of China. Pedosphere, 22, 1-12.