DNA Contents in Soil Contaminated with Heavy Metals

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Abstract: The study was performed to show how industrial activity affected soil quality in terms of soil DNA quality and quantity as well as soil characteristics. Soil material originated from an urban area of the Silesia Region (SW Poland). The soil characteristics were estimated: texture, moisture, pH, redox potential (Eh), and total carbon content (TOC), followed by determination of selected heavy metals (Pb, Cd, Zn, Cr, Fe, Cu). The last step was the isolation of soil DNA, its concentration and identification of microorganisms. The results showed that although the studied soil was heavily contaminated with heavy metals, there were still some metal-resistant microorganisms able to sustain soil activity. Moreover, these organisms are not present in the NCBI database, which encourages further studies aimed at identification of new organisms that may be useful in research of metal resistance as well as soil reclamation and remediation.

Keywords: Heavy metal, metal resistant bacteria, soil, t-DNA.

1. INTRODUCTION

Soil constitutes a huge habitat for numerous groups of organisms comprising 10^6-10^9 microorganisms per g of soil [1]. All genetic material found in soil could be defined as soil DNA (s-DNA). This material originates largely from plant material as well as soil microorganisms and fauna [2]. We hypothesized that the qualitative and quantitative estimation of s-DNA could be a valuable indicator of soil quality as soil microbial community is strongly affected by human activity such as agriculture and industry.

In the Silesia region, near Katowice in southern Poland, zinc (Zn), lead (Pb), cadmium (Cd) copper (Cu), chromium (Cr), and iron (Fe) ore mining and metallurgy have been carried out since the 14th century, which has resulted in environmental degradation of the Szopienice area. Generally, a key factor in pollution by metals is the fact that metals are non-biodegradable but can be transformed through sorption, methylation, and complexation, and changes in valence state [3]. These transformations affect the mobility and bioavailability of metals. It is considered that their presence may pose a risk for living organisms [4-6] however, little is known about a positive effect exerted by heavy metals on biological activities, if any. It should also be emphasized that at low concentrations, metals can serve as important components in life processes, often serving important functions in enzyme productivity.

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The activity of soil microorganisms is regulated by several environmental factors, such as soil texture, temperature, moisture (related to aeration status), pH, redox potential (Eh), and the contents and availability of nutrients and carbon. Soil contamination may strongly affect these parameters and eventually all living organisms. Moreover, in the case of chronically contaminated sites, natural selection should result in a predominantly metal-tolerant community [4]. Bacterial communities are known to reflect their micro-environmental conditions by rapid responses at extremely fast rates to environmental and pollution changes. Thus, the estimation of bacterial abundances as well as their genetic diversity under *in situ* conditions is the most fundamental objective of soil microbial ecology [4]. Relatively many bacterial clones have already been identified in different heavy-metal-contaminated environments. These isolates mainly included *Bacillus*, *Arthrobacter*, *Corynebacterium*, *Pseudomonas*, *Alcaligenes*, *Ralstonia, Burkholderia* noted with Ni, Cd, Cu, and Co as contaminants [7], *Actinobacteria* and the subdivision (*α*-, *β*-, *γ*-) of *Proteobacteria* observed in the presence of Ni, Pb, and Zn (Ellis *et al*. 2003), *Acidobacteria*, *Gemma*-timonadetes, Sphingomonas spp., Clostridia in soils contaminated with Cd [8], and *Firmicutes* and *Chloroflexi* [9].

The following study was performed to demonstrate how industrial activity (heavy metal pollution) affected soil biological M. Consequently, our results give new information about bacterial resistance to heavy metals (Pb, Cd, Zn, Cr, Fe, Cu), which are present in the urban soil environment (Polish Silesia Region).

Table 1: Selected Characteristics of the Soil Material (means ± SD). Means Followed by the Same Letters did not Differ Significantly at *p*<0.05 (One-Way ANOVA with Tukey Post Hoc Procedure)

Depth (cm)	Soil moisture (%)	pH	Eh (mV)	TC (%)	t-DNA (μg g^{-1})
0-40	27.32 ± 0.14[a]	6.25 ± 0.03[a]	197.0 ± 1.01[a]	9.08 ± 0.51[a]	465.88 ± 64.11[a]
40-70	15.89 ± 0.06[b]	6.62 ± 0.02[b]	317.2 ± 36.83[b]	3.16 ± 0.45[b]	225.33 ± 6.58[b]
>70	15.75 ± 0.14[b]	6.63 ± 0.02[b]	569.03 ± 3.42[c]	0.25 ± 0.04[c]	187.86 ± 14.41[c]

2. MATERIALS AND METHODS

The examined soil material originated from an urban and most industrialized Polish area (N 50°16'11", E 19°4'55") located ca. 560 m from a non-ferrous metal smelter Szopienice (Katowice, Silesia Region) in the southern part of Poland. Smelter activity continuing for over 150 years had an enormous impact on the diversity of soil minerals and thereby the microbial biodiversity colonizing a given site. For the study, an 80 cm profile was excavated in October 2012 and three soil layers in three replications were distinguished: top 0-40 cm, middle 40-70 cm, and bottom <70 cm.

Under laboratory conditions, the soil samples were dried at 105°C for 24 h (Termaks oven TS8136) and next weighed in order to determine the percentage of soil moisture.

The pH and Eh were determined potentiometrically in fresh soil using a multifunctional potential meter pIONneer 65 (Radiometer Analytical S.A, France), equipped with the following electrodes: a glass electrode (Cartrode pH E16M340) for pH and a combined platinum and Ag/AgCl (reference) electrode (E31M004) for Eh. Each measurement was replicated three times.

Total carbon (TC) was determined using an automatic carbon analyzer TOC-V_{CSH} SSM 5000A (Shimadzu, Japan). The soil sample (200 mg) was combusted at 900°C in a column containing a platinum and cobalt oxide catalyst. Under these conditions, all carbon compounds were converted into the carbon dioxide form and detected by an infrared detector. Each sample was analyzed separately (in three replications). The basic soil profile characteristic is presented in Table **1**.

Before analyses of heavy metals, the soil samples were mineralized. Soil (0.5 g) was digested by a concentrated HNO_3 and HF mixture (4:1, 200°C, 30 min) in a closed microwave system (Ethos One, Milestone). Then were filtrated and the extracts were diluted with distilled water to the volume of 50 ml. The concentrations of metals (Pb, Cd, Zn, Cr, Fe, Cu) were determined by the Atomic Absorption Spectroscopy (AAS) technique (Flame Atomic Absorption Spectroscopy method) with the use of a Z-8200 Hitachi Spectrophotometer (Japan).

Each sample analysis was replicated three times. Total metal concentrations (Table **2**) were expressed as the element content in dry mass of the soil (mg kg^{-1}).

Soil DNA was extracted from soils (450 mg) using the Power Soil DNA Isolation Kit (MoBio) according to the manufacturer's instructions. The kit was designed specifically for rapid isolation of pure, humic-free

Table 2: The Contents of Selected Metals (mg kg^{-1}) in the Studied Soil (means ± SD). Means Followed by the Same Letters did not Differ Significantly at *p*<0.05 (one-way ANOVA with the Tukey *Post Hoc* Procedure)

Metal content (mg kg^{-1})	Depth (cm)		
	0-40	40-70	>70
Pb	3043.99 ± 58.25[a]	726.10 ± 13.92[b]	46.23 ± 1.73[c]
Fe	244.13 ± 33.94[a]	451.54 ± 25.66[b]	619.53 ± 7.03[c]
Cu	114.86 ± 2.12[a]	18.65 ± 1.02[b]	4.59 ± 0.75[c]
Cd	96.83 ± 3.44[a]	20.10 ± 2.93[b]	6.96 ± 0.98[c]
Cr	2.802 ± 0.054[a]	2.334 ± 0.335[ab]	1.837 ± 0.156[b]
Zn	0.05 ± 0.01[a]	45.54 ± 10.48[b]	373.91 ± 3.07[c]

microbial DNA from soil samples, and guaranteed a proper DNA isolation procedure. The DNA concentration was quantified by UV spectroscopy (UV-1800 Shimadzu, Japan) at 230, 260, and 280 nm, and expressed as µg DNA per g^{-1}dry soil.

PCR was performed in 20 µl (total volume) using PCR master Mix (Fermentas), with 4 µl of template DNA, and following universal starters for 16S rRNA: 27f (5'AGAGTTTGATCMTGGCTCAG3') and 1492r (5'GGTTACCTTGTTACGACTT3'). The composition of the PCR Master Mix were as follows: 0.05 U/**µ**L *Taq* DNA polymerase, reaction buffer, 4 mM MgCl2, 0.4 mM of each dNTP (dATP, dCTP, dGT and dTTP). Amplification was performed in a Professional Basic thermal cycler (Biometer) in 30 cycles of 80 s at 94°C, 60 s at 55°C, and 80 s at 72°C with initial denaturation and final extension as described above. Negative controls were included in all experiments by replacing the DNA template with 4 µl of sterile water. PCR products were analyzed by 1% agarose gel electrophoresis, purified with QIAquick PCR Purification Kit (Qiagen, USA). PCR products were visualized with ethidium bromide in a Rad apparatus (ALPHA INNOTECH).

The sequencing processes were performed on the purified product, immediately after the PCR reaction in Genomed SA (Warsaw, Poland). The sequences obtained were compared to the closest relatives in the NCBI Gen-Bank database by the BLAST program.

Data analysis was performed using Statistica 9.0 (Statsoft Inc., USA) software. In order to investigate significant (p<0.05) variations between the tested parameters depending on the depth, one-way ANOVA with the Tukey *post hoc* test was applied. Relationships between the s-DNA content and the environmental factors (moisture, pH, Eh, TOC) and heavy metal concentrations (Pb, Cd, Zn, Cr, Cu, Fe) were determined using a matrix of Pearson r correlation.

3. RESULTS AND DISCUSSION

3.1. Physical and Chemical Soil Profile Characteristics

The physical and chemical soil characteristics are presented in Table **1**. The soil samples had a loamy sand texture (62% and 53%) in the surface (0-20 cm) and subsurface (40-50 cm) horizons, respectively, and silt clay (40%) in the deeper layers (>70 cm). With an increase in the soil depth, the soil moisture decreased from 27.32% to 15.75%, which can be connected with the atmospheric precipitation level and granulometric soil characteristics. Hou *et al*. [6] indicated that when soils or sediments have a higher level of silt they are more compact, which results in the water content being lower. In contrast, sand-dominated soils will have high water content. Thus, the soil moisture estimated in the top layer (0-20 cm) was significantly higher (p<0.001) than in the other horizons. It was observed that the average pH varied between 6.25 – 6.63, indicating slightly acidic conditions. However, the pH of the top layer was significantly lower (p<0.001) in comparison with the deeper soil horizons. Quite surprising is the Eh value noted in the top layer (Table **1**), as the soil was not particularly vulnerable to lack of oxygen. An increase in the Eh value in the subsurface and subsoil was observed, which was consistent with the change in moisture. Zhuang *et al*. [10] reported that the increase in the organic matter level meant higher water content. Even though in the case of the investigated soil the range of TOC constitutes only 0.25 to 9.08% for the subsoil and top horizons, respectively, our results are compatible with this statement as we observed the highest soil moisture percentage in the surface layer - rich in C_{org}. (9.08%), while the lowest moisture in subsoil (>70 cm) characterized by a poor level of C_{org}. (0.25%) was noted.

3.2. DNA Content

The use of the Power Soil DNA Isolation Kit (MoBio) allowed us to extract high concentrations of DNA from soil (ca. 187 - 466 µg g^{-1}). The highest amount of s-DNA was detected in the surface layer. It was connected with well-known spatial distribution of microorganisms and their preferences for occupation of surface layers [11]. With an increase in soil depth, a significant drop in DNA content (p<0.001) by c.a. 60% in comparison to the top horizon was observed. A similar trend for DNA distribution was noted by Wolińska *et al*. [11] in a loess soil profile.

3.3. Heavy metal concentration

The concentration gradient is an important mechanism of metal ions transfer in the contaminated soil environment. The concentrations of the investigated heavy metals (Pb, Fe, Cu, Cd, Cr and Zn) in the soil profile are presented in Table **2**.

For the last decades, metals have been dominant sources of pollution in this area; therefore, high concentrations of Pb, Fe, Cu, and Cd still occur in the soil profile. The contents of heavy metals in the soil

Table 3: Dependence of the s-Dna Concentration on Soil Physicochemical Parameters: Moisture, pH, TOC, and Eh (n=9, *p*<0.01, r Value from the Pearson Correlation)

Factor	Soil moisture (%)	pH	Eh (mV)	TOC (%)
t-DNA (μg g^{-1})	0.98***	0.99***	-0.82**	0.97***

*p< 0.05, **p < 0.01, ***p < 0.001.

profile (from top to subsoil horizons) had the order: Pb>Fe>Cu>Cd>Zn>Cr.

The most striking was the content of Pb; such high values as 3044 and 726 mg kg^{-1} are above the threshold value of 600 mg kg^{-1} for industrial areas according to Polish regulations [12]. Generally, the other investigated metals, e.g. Cu, Cd, and Cr, demonstrated a significant decreasing trend in their concentrations with an increase of the soil depth (p<0.001) and were mostly accumulated in the top horizons. The lowest Cd concentration amounted to 6.96 mg kg^{-1}, which was below the acceptable level (12 mg kg^{-1}), was noted only in the deepest part of the soil profile (>70 cm). However, in the case of Fe and Zn, an opposite trend was found, as we observed a significant increase in their concentration with the depth (p<0.01) and ability for accumulation at the highest doses in the subsoil horizons. The lowest variability of the concentration was found for Cr, which demonstrated a similar concentration ranging from 1.8 to 2.8 mg kg^{-1} in the whole soil profile. Ciarkowska *et al*. [13] reported that soil samples from Olkusz (nearby Katowice, SE Poland) had very high total concentrations of Zn (40.5-10.884 mg kg^{-1}), Pb (959-6661 mg kg^{-1}), and Cd (24.4-174.3 mg kg^{-1}) in their surface horizons, and similar concentrations in their deeper horizons.

3.4. Statistical Analyses

The statistical relationships between s-DNA and some soil physic-chemical parameters (moisture, pH, Eh, TOC) described by correlations coefficient (r) are presented in Table **3**.

A significant impact (p<0.01) of the tested parameters on the s-DNA content was found, which was revealed by the positive relationships between s-DNA and soil moisture, pH, and TOC, and a negative correlation with Eh.

Similarly to the presented data, significant (p<0.05) positive relationships between s-DNA content, moisture, and TOC were indicated by Acosta-Martinez *et al*. [14] and Wolińska *et al*. [11].

However, in the case of loess soil, Wolińska *et al*. [11] noted a positive effect of Eh on DNA concentration (r=0.52**), whilst in the investigated Silesian soil (Table **3**) an opposite trend was found (r=-0.82**) and an optimal Eh level for the s-DNA content was estimated between 190 - 290 mV.

To our knowledge, until recently rather little attention has been paid to the influence of Eh on presence of s-DNA. Consequently, the interpretation of our results has been challenging due to the lack of a sufficient number of reports in available literature with determined r coefficients as goodness of fit. Thus, it is necessary to conduct further investigations and select other soil types in order to explain and confirm the indicated correlation.

Despite the fact that the investigated soil material seemed to be highly contaminated with heavy metals (Table **2**), the presence of the pollutants favorably influenced the s-DNA concentration (Table **4**).

A positive and quite surprising effect on the s-DNA content (p<0.001) was noted for the content of Pb, Cd and Cu, which was confirmed by high values of r coefficients and implied that the increase in each metal mentioned resulted in an increase in the s-DNA content in the investigated soil profile. A negative impact and a

Table 4: Dependence of the S-Dna Concentration on the Heavy Metal Content: Pb, Cd, Cr, Cu, Zn, and Fe (N=9, *P*<0.05, R Value from the Pearson Correlation)

Factor	Metal concentration (mg/kg)					
	Pb	Cd	Cr	Cu	Zn	Fe
t-DNA (μg g^{-1})	0.99***	0.99***	0.51 ns	0.99***	-0.69*	-0.94**

*p< 0.05, **p < 0.01, ***p < 0.001, ns – no significance.

decrease in the s-DNA concentration were noted only in the case of Zn and Fe (p<0.05), whilst in respect of Cr the determined correlation was insignificant (p>0.05), probably because the chromium content differed negligibly throughout the soil profile and Cr was present in low concentrations.

Hou *et al*. [6] observed that Cd posed the greatest potential ecological risk in the soil environment. This is opposite to our results, because we did not find any negative effect exerted by Cd presence on soil biological characteristic – the DNA content. Generally, it is assumed that heavy metals can reduce both microbial abundance and soil enzyme activities [15, 16]. Since dehydrogenase activity is strictly connected with living microbial cells, its activity depends on the same environmental factors, which influence microorganism abundance, activity, and life processes [17]. A study by Pan and Yu [16] carried out on brown soil showed that dehydrogenase activity of soil microorganisms was significantly lower by 37.8% and by 51.1% in Cd and Pb treatments than in control. However, there are also contrasting observations, reporting that Cd had a relatively positive influence on soil microbial diversity, which suggests that the effect of each soil pollutant on soil microbes and their enzymatic activities is strongly specific [18]. We observed that Cd presence at a concentration of 2 mg kg^{-1} had a stimulating effect on the soil dehydrogenase level, and an increase in the enzymatic activity by 8.8% in comparison with the control sample was noted [17]. Abdelatey *et al*. [19] reported that Cd could stimulate the growth of *Staphylococcus aureus*.

3.5. Identification of Soil Microorganisms

The PCR with specific primers for the 16S rRNA gene 1492r and 27f yielded an amplicon of 1500 bp, which confirms the presence of bacteria in our entire material examined.

The sequences obtained were compared with those from the NCBI (National Center for Biotechnology Information) database showing no known microbial strains in the surface and subsurface layer (0-70 cm), which might imply existence of novel taxa or limitation of the data in the NCBI, and several groups in the bottom layer (>70 cm) which are presented in Table **5**.

Microorganisms that can grow in the presence of high metal concentrations are called metalophiles. A

Table 5: Composition of the Microbial Community in the Studied Material Based of References in the NCBI Database

Depth (cm)	Closest relative in NCBI database	Accession number	Similarity (%)	Phylogenetic division
0-40	No references			
40-70	No references			
>70	Bordetella holmesii	AB755629.1	93	β-Proteobacteria
	Bordetella pertussis	HE965805.1	93	β-Proteobacteria
	Bordetella pertussis CS	CP002695.1	93	β-Proteobacteria
	Polaromonas sp. clone N22A02B	JF719332.1	92	β-Proteobacteria
	Acidovorax sp. clone GE7GXPU01CQIRO	HM973020.1	92	β-Proteobacteria
	Rhodoferax sp. clone F5OHPNU07IMNPO	HQ099873.1	92	β-Proteobacteria
	Comamonas sp. clone F5OHPNU07IMNPO	HQ094621.1	92	β-Proteobacteria
	Rhodocyclaceae sp. clone F5OHPNU07H9OOQ	HQ104516.1	91	β-Proteobacteria
	Thauera sp. clone F5OHPNU07H9PAE	HQ085843.1	91	β-Proteobacteria
	Denitratisoma sp. TSA61	AB542411.1	91	β-Proteobacteria
	Alcaligenes sp. HT19	EF608172.1	91	β-Proteobacteria
	Azovibrio sp. BS20-3	AF011349.1	91	β-Proteobacteria
	Azovibrio restrictus BS1-14	AF011348.1	91	β-Proteobacteria
	Chitinimonas sp. clone SM100	FR832314.1	91	β-Proteobacteria

typical feature of these metal-resistant microorganisms is the presence of one or two large megaplasmids that contain genes for multiple heavy-metal resistance [19, 20]. Undoubtedly, all the microorganisms identified by us belonging to the *β-Proteobacteria* division are metalophiles able to grow in an extremely difficult soil environment strongly contaminated with heavy metals.

All the microorganisms identified are Gram-negative; in comparison with Gram-positive bacteria, they are more resistant to antibiotics and environmental contaminants (i.e. heavy metals) despite their thinner peptidoglycan layer. It is known from literature data that *Azovibrio*, *Denitritisoma,* and *Chitinimonas* identified in the current study are perfectly able to survive in heavy-metal contaminated soils [21]. *Bordetella* is a non-motile and non-spore forming coccobacillus bacterium, most of all demonstrating tolerance against Cd [19]. Similar capability also characterized the bacterium from the genus *Acidivorax* [22], which may explain the presence of these species in the soil examined in this study, which contained Cd at the level of 6.9 to 97 mg kg^{-1}. *Polaromonas* is known to be non-spore forming, non-motile coccus capable of growth in the presence of naphthalene and heavy metals and using them as an energy source [23].

Rhodoferax is considered to be a metabolically versatile, Fe (III)-reducing, subsurface microorganism likely to play an important role in the carbon and metal cycles in the subsurface [24], which can be an explanation for our results and the presence of *Rhodoferax* sp. clone in the deep soil profile layers. The *Comamonas* and *Thaurea* are rod-shaped, strictly aerobic, highly motile bacteria with a polar flagellum, which were isolated from soils or sludge contaminated e.g. by phenol, toluene, benzoic acid, and heavy metals [23, 25]. The species mentioned are known to be able to tolerate and degrade very toxic compounds. Some bacteria, like the heavy metal resistant *Alcaligenes* strains are able to promote biomineralization, i.e. biologically induced crystallization of heavy metals. In the presence of heavy metals, this strain may create an alkaline environment in the periplasmic space and outer cell environment appropriate for induction of mechanisms of heavy metals resistance [26].

4. SUMMARY

The studied soil profile located in the vicinity of a non-ferrous metal smelter was characterized by high accumulation of Pb (exceeding the threshold according to Polish regulations), Cd, Cr and Cu, which may be the effect of high binding capacity of the soil sorption complex. The high concentration of s-DNA noted in the two surface and subsurface soil layers (0-40, 40-70 cm) and the absence of known microorganisms (according to the NCBI database) may indicate presence of other, still not known, microbial groups i.e. metal resistant microorganisms able to sustain soil activity. Importantly, a positive and stimulant effect on the s-DNA content was found in the case of Pb, Cd, and Cu, whereas a toxic and inhibitory effect on s-DNA was demonstrated by only two among the investigated metals: Zn and Fe. Based on the 16S rRNA analyses in the subsoil layer, presence of Gram-negative bacteria belonging to the *β-Proteobacteria* division, known to be resistant to heavy metal contamination, was found. Further studies focused on identifying new microorganisms, which may be useful in research of metal resistance as well as soil reclamation and remediation, are recommended. Finally, we assume that the measurement of s-DNA may be a useful tool for fast surveying of soil activity and health.

REFERENCES

[1] Roesch LF, Fulthorpe RR, Riva A, Casella G, Hadwin AK, Kent AD, Daroub SH, *et al.* Pyrosequencing enumerates and contrasts soil microbial diversity. ISME J 2007; 1: 283-290.

[2] Wolińska A, Stępniewska Z, Wołoszyn A, Pytlak A, Dziuba A. Means of molecular nucleic acids analysis in soil investigations. Acta Agrop 2011; 194: 1-62.

[3] Anyanwu CU, Nwankwo SC, Moneke AN. Soil bacteria response to introduced metal stress. Int J Bas Appl Sci 2011; 11: 73-76.

[4] Harichova J, Karelova E, Pangallo D, Ferianc P. Structure analysis of bacterial community and their heavy metal resistance determinants in the heavy metal contaminated soil sample. Biologia 2012; 67: 1038-1048. http://dx.doi.org/10.2478/s11756-012-0123-9

[5] Genthe B, Le Roux WJ, Schachtschneider K, Oberholster PJ, Aneck-Hahn NH, Chamier J. Health risk implications from simultaneous exposure to multiple environmental contaminants. Ecotoxicol Environ Saf 2013; 93: 171-179. http://dx.doi.org/10.1016/j.ecoenv.2013.03.032

[6] Hou D, He J, Lü C, Ren L, Fan Q, Wang J, Xie Z. Distribution characteristic and potential ecological risk assessment of heavy metals (Cu, Pb, Zn, Cd) in water and sediments from Lake Dalinouer, China. Ecotoxicol Environ Saf 2013; 93: 135-144. http://dx.doi.org/10.1016/j.ecoenv.2013.03.012

[7] Kozdroj J, van Elsas JD. Structural diversity of microorganisms in chemically perturbated soil assessed by molecular and cytochemical approaches. J Microbiol Met 2001; 43: 197-212. http://dx.doi.org/10.1016/S0167-7012(00)00197-4

[8] Chien C, Kuo Y, Chen C, Yeh C, Yeh W. Microbial diversity of soil bacteria in agricultural field contaminated with heavy metals. J Environ Sci 2008; 20: 359-363. http://dx.doi.org/10.1016/S1001-0742(08)60056-X

[9] Wu H, Zhao H, Wen C, Guo J, Xu M, Li X. A comparative study of bacterial community structures in the sediments from

brominated flame retardants contaminated river and non-contaminated reservoir. Afr J Microbiol Res 2012; 6: 3248-3260.

[10] Zhuang J, McCarthy JF, Perfect E, Mayer LM, Jastrow JD. Soil water hysteresis in water-stable microaggregates as affected by organic matter. Soil Sci Soc Am J 2008; 72: 212-220. http://dx.doi.org/10.2136/sssaj2007.0001S6

[11] Wolińska A, Stępniewska Z, Wołoszyn A, Rzewuska M. Interrelations between DNA concentration and physicochemical parameters in the less soil profile. Acta Agrop 2012; 19: 437-448.

[12] Polish Regulation for Industrial Areas. Dz U 2002; No 165/1359 (in Polish).

[13] Ciarkowska K, Sołek-Podwika K, Wieczorek J. Enzyme activity as an indicator of soil rehabilitation process at a zincand lead ore mining and processing area. J Environ Manag 2014; 132: 250-256. http://dx.doi.org/10.1016/j.jenvman.2013.10.022

[14] Acosta-Martinez V, Burow G, Zobeck TM, Allen VG. Soil microbial communities and function in alternative systems to continuous cotton. Soil Sci Soc Am J 2010; 74: 1181-1192. http://dx.doi.org/10.2136/sssaj2008.0065

[15] Stępniewska Z, Wolińska A, Ziomek J. Response of soil catalase activity to chromium contamination. J Environ Sci 2009; 21: 1142-1147. http://dx.doi.org/10.1016/S1001-0742(08)62394-3

[16] Pan J, Yu L. Effects of Cd and Pb on soil enzyme activities and microbial community structure. Ecol Eng 2011; 37: 1889-1894. http://dx.doi.org/10.1016/j.ecoleng.2011.07.002

[17] Wolińska A, Stępniewska Z. Dehydrogenases in the soil environment. In: Cantuto R, Ed. Dehydrogenases. Rijeka: In-Tech Publisher 2012; pp. 183-210. http://dx.doi.org/10.5772/48294

[18] Xie W, Zhou J, Wang H, Chen X, Lu Z, Yu J, Chen X. Short-term effects of copper, cadmium and cypermetehrin on dehydrogenase activity and microbial functional diversity in soils after long-term mineral and organic fertilization. Agric Ecosyst Environ 2009; 149: 450-456. http://dx.doi.org/10.1016/j.agee.2008.10.021

[19] Abdelatey LM, Khalil KBK, Ali TH, Mahrous KF. Heavy metal resistance and gene expression analysis of metal resistance gene in gram positive and gram negative bacteria present in Egyptian soil. J Appl Sci Environ Sanit 2011; 6: 201-211.

[20] Gomes J, Steiner W. Extermophiles and extermozymes. Food Technol Biotechnol 2004; 42: 223-235.

[21] Li D, Hendry P, Faiz M. A survey of the microbial populations in some Australian coalbed methane reservoirs. Int J Coal Geol 2008; 76: 14-24. http://dx.doi.org/10.1016/j.coal.2008.04.007

[22] Belimov AA, Hontzeas N, Safronova VI, Demchinskaya SV, Piluzza G, Bullitta S, *et al*. Cadmium-tolerant plant growth-promoting bacteria associated with the roots of Indian mustard (Brassica juncea L. Czern). Soil Biol Biochem 2005; 37: 241-250. http://dx.doi.org/10.1016/j.soilbio.2004.07.033

[23] Van Houdt R, Toussaint A, Ryan MP, Pembroke JT, Mergeay M, Adley CC. The Tn*4371* Ice family of bacterial mobile genetic elements. In: Roberts AP, Mullany P, Eds. Bacterial Integrative Mobile Elements. Austin: Landes Biosciences 2013; pp. 179-200.

[24] Chaudhuri SK, Lovley DR. Electricity generation by direct oxidation of glucose in mediatorless microbial fuel cell. Nat Biotech 2003; 21: 1229-1232. http://dx.doi.org/10.1038/nbt867

[25] Jiang K, Sanseverino J, Chauhan A, Lucas S, Copeland A, Lapidus A, *et al*. Complete genome sequence of Thaurea aminoaromatica strain MZ1T. Standards Genom Sci 2012; 6: 325-335. http://dx.doi.org/10.4056/sigs.2696029

[26] Mahvi AH, Diels L. Biological removal of cadmium by Alacaligenes eutrophus CH34. Int J Environ Sci Tech 2004; 1: 199-204. http://dx.doi.org/10.1007/BF03325833

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