DOI: 10.2428/ecea.2012.19(08)089 ECOL CHEM ENG A. 2012;19(8):915-921

Iwona KONIECZNA[1], Paulina ŻARNOWIEC[1], Anna ŚWIERCZ[2], Michał GRUSZCZYŃSKI[3], Halina POLKOWSKA-MOTRENKO[4], Ewelina CHEJDUK[4] and Wiesław KACA[1]

QUANTITATIVE ANALYSIS OF AEROBIC CULTIVABLE BACTERIA AND SOIL TOTAL ENZYMATIC ACTIVITY OF KIELCE AND RUDKI URBICENOSE

ANALIZA ILOŚCIOWA TLENOWYCH BAKTERII HODOWALNYCH ORAZ CAŁKOWITEJ AKTYWNOŚCI ENZYMATYCZNEJ GLEBY URBICENOZY KIELC I RUDEK

Abstract: The microbiological and biochemical analysis of two urban soil samples, from Swietokrzyskie province (Kielce, Rudki), were performed. The aim of this study was the quantitative analysis of aerobic bacterial microflora and determination of total enzymatic activity of soil from area with high level of street traffic (Kielce) and from former pyrite mine region (Rudki), where uranium ore were extracted. The commercial media (TSA, LB, MacConkey, M9 and King B agars) and soil extract agars were used for soil microorganisms isolation. The bacteria were cultivated in two temperatures: +25 °C and +4 °C. From Kielce urbicenose, amount of soil bacteria cultivated on commercial media were higher in case of culture in +25 °C (from 10 to 10^8 times more depending on used medium), whereas on soil extract agar almost 2 times more microorganisms were found in +4 °C. For soil contamined by uranium (Rudki), abundant bacterial growth in both temperatures were observed only on soil extract agar. The total ureolytic, proteolytic and lipolytic activities of soil samples were also defined. In case of soil sample from Kielce all from the determinated activities were found, whereas for soil from Rudki only lipolytic activity was noted.

Keywords: soil bacteria, heavy metals, total enzymatic activity.

Pollution of environment by uranium is a serious problem because this element in the oxidized U(VI) form is soluble and highly toxic [1]. Its toxicity concern not only plants

[1] Departament of Microbiology, Institute of Biology, The Jan Kochanowski University in Kielce, ul. Świętokrzyska 15, 25–506 Kielce, Poland, phone: + 48 041 349 63 05, email: iwona.konieczna@ujk.edu.pl

[2] Departament of Soil and Culture Landscape Protection, Chair of Environment Protection and Modelling, The Jan Kochanowski University in Kielce. ul. Świętokrzyska 15, 25–506 Kielce, Poland.

[3] Laboratory of Geology, Institute of Geography, The Jan Kochanowski University in Kielce. ul. Świętokrzyska 15, 25–506 Kielce, Poland.

[4] Institute of Chemstry and Nuclear Technology, Laboratory of Nuclear Analytical Techniques, ul. Dorodna 16, 03–195 Warszawa, Poland.

and animals, but also bacteria [2]. However, there is known, that soil microorganisms are much more sensitive to other heavy metals. In uranium polluted environments, the relatively numerous bacterial microflora, including *Proteobacteria*, *Acidobacteria* and *Bacteroidetes*, were observed [3]. There is known, that microorganisms naturally occurring in such areas influence on migration of heavy metals, including uranium [2].

There is no papers about amount of cultivable bacterial microflora and total biochemical activity of uranium contaminated soils.

The aim of thise work was the quantitative analysis of aerobic cultivable bacterial microflora as well as determination of total enzymatic activity of soil from area with heavy traffic (Kielce) and former piryte mine region (Rudki), where uranium ore was extracted.

Materials and methods

Soil samples – samples from the surface soil layer were collected aseptically into sterile containers by triple puncture with Egner's stick, on depth 20 cm. The research material was collected on November 18th, 2010 and consist of two soil samples: the first was from degraded urban soil from Kielce (marked as G10 sample) while the second was from pyrite mine area in Rudki (marked as G12 sample) (Fig. 1).

Fig. 1. Localization of the soil samples G10 (■) and G12 (●) collection

Chemical analysis of the soil samples – heavy metals content in the sample G12 were determined by ICP-MS, while in sample G10 – by FAAS method.

Soil bacteria isolation – 1 g of soil suspension (G10 or G12, respectively) in 100 cm^3 sterile Winogradsky solution was intensively stirred (20 min 500 rpm). After sedimentation of soil particles solutions were diluted up to 10^{-6} and plated on Petri dishes with media: Luria-Bertani (LB) agar, minimal M9 agar, tryptic-soy agar (TSA), King B agar, MacConkey agar and soil extract agar (SEA). Cultures were incubated at +25 °C up to 7 days and at +4 °C up to two weeks. Amount of bacteria (cfu/1g of wet soil) was determined based on the number of bacterial colonies grown on particular media.

Determination of the total biochemical activity of soil samples – ureolytic activity was determined according to Moreno et al method by detection ammonium ions in phenol-hypochloride assay [4]. Proteolytic activity of soil samples (suspension of 0.5 g soil and 0.4 cm^3 of toluene, incubated 15 min at room temperature) was

determined in azocasein test. Azocasein (20 mg) suspension was incubated with soil samples (G10 or G12, respectively) by 24 h at +25 °C. One unit of proteolytic activity was defined as ability to hydrolyze 1 mg of azocasein per hour [5, 6]. Lipolytic activity was defined according to Margesin et al using *p*-nitrophenyl butyrate (pNPB) as a substrate [7].

Results and discussion

The number of soil microorganisms strictly depends on soil conditions [8]. For the cultivation of aerobic bacteria from two urban soil samples six different microbiological media were used. Those media allow for growth of microorganisms with high as well as low nutritional requirements. The amount of microorganisms cultured from soils was varied. In the case of sample G10 (Kielce), microbial growth on all media was observed, but most abundant was on soil extract agar (SEA). Amount of bacteria ranged from 10^5 to 10^9 cfu/g of soil and was higher (except for MacConkey agar) when the cultivation temperature was +25 °C. In other studies, similar amounts of microorganisms cultured from various urban soils were observed [9, 10]. A completely different situation was observed for the G12 sample (Rudki). In this case, bacteria were grown only on SEA medium (at both temperatures) and on King B agar (only at +25 °C) (Table 1).

Table 1

The quantity of aerobic bacteria (cfu/g of soil) cultivated from G10 (Kielce) and G12 (Rudki) sample, on different microbiological media

Soil sample	Temperature of culture	Microbiological medium					
		TSA	LB	M9	MacConkey	King B	SEA
G10	25 °C	$2 \cdot 10^8$	$2.7 \cdot 10^8$	$1 \cdot 10^7$	$2.2 \cdot 10^6$	$1.2 \cdot 10^8$	$6 \cdot 10^8$
	4 °C	$1 \cdot 10^5$	$2.5 \cdot 10^5$	$7 \cdot 10^5$	$2.2 \cdot 10^6$	$1 \cdot 10^7$	$1 \cdot 10^9$
G12	25 °C	ng	ng	ng	ng	$5 \cdot 10^6$	N
	4 °C	ng	ng	ng	ng	ng	$2.2 \cdot 10^6$

N – unable to count number of microorganisms, ng – no growth.

Investigated soil samples were chemically analysed. In the G12 soil sample the presence of various metals was identified. There were also heavy metals (nickel, copper, zinc, cobalt and others) among them. Attention is paid on high content of iron and manganese ions (Fig. 2).

Presence of the increased concetration of heavy metals in soil from Rudki (G12 sample) probable affects the amount and structure of bacterial microflora occuring in this environment. It is known, that such pollution can reduce intensity of the majority of biochemical reactions in soil [11, 12]. Heavy metals decrease activity of enzymes produced by soil bacteria like: urease, acid and alkaline phosphatase, amidase and nitrate reductase [13].

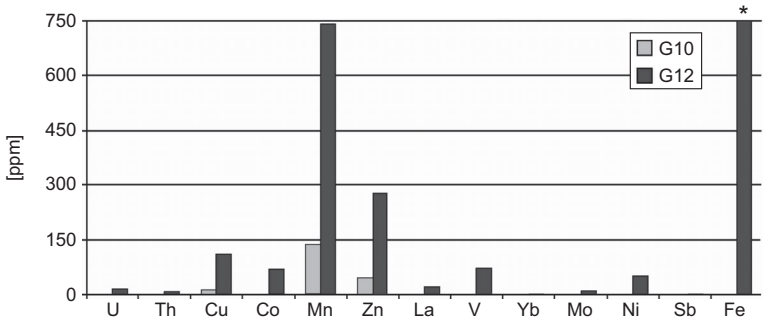

Fig. 2. Chemical analysis of soil samples; * – 156274 ppm.

For the analyzed soil samples the total ureolytic, proteolytic and lipolytic activities were determineted (Fig. 3).

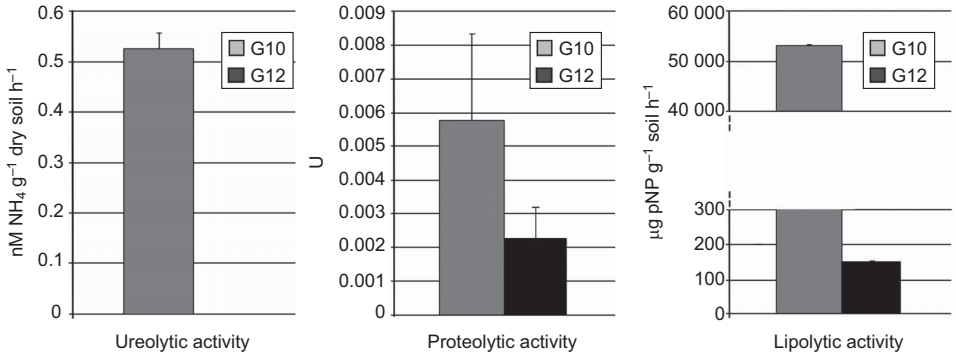

Fig. 3. The total ureolytic, proteolytic and lipolytic activities of G10 and G12 soil samples

Ureolytic activity was totally inhibited in soil highly contaminated by heavy metals (G12 sample). This observation is consistent with previous data [13]. Also Nawaguo *et al* observed, that bacterial urease is especially sensitive to heavy metals presence [14].

In both soils very weak (in G12 at the detection limit) proteolytic activity was noted. Blaszak and Nowak [15] observed the opposite phenomenon. In their studies after soil contamination, only proportions of proteolytic bacteria, which maintained its activity, was varied. However, in this case, in the laboratory conditions only copper ions added to soil samples had toxic activity [15]. In presented studies soil was contaminated by different metals in varying degrees already at the time of samples collection. Therefore, is impose here conclusion, that the presence of various toxic agents is significant. This is confirmed with studies Lopez *et al*, where the inhibitory effect of heavy metals on bacterial proteolytic enzymes activity was also observed [16].

In G10 and G12 samples lipolytic activity was observed, however in G12 sample this activity was significantly lower. In G12 sample, collected from pyrite mine area in Rudki, the presence of cobalt, nickel and zinc was detected. It is known, that presence

of heavy metals inhibits the activity of this enzyme, and cobalt and nickel have a very strong inhibitory effect [17].

There are data, where negative effects of heavy metals on biochemical activity as well as the number and growth of soil bacteria was observed. However, the presence of a relatively rich bacterial microflora (on soil extract agar) allow to hope for the possible bioremediation of these soils.

Conclusions

1. Soil bacteria present in environment highly contaminated by heavy metals have specific nutritional requirements, what causes that they can not be effectively cultured on standard microbiological media. For efficient cultivation of these micro-organisms is necessary to supplement the media with compounds found in their natural habitat.

2. The presence of heavy metals, including uranium, have an inhibitory effect on the general soil biochemical activity.

3. Heavy metals presence abolish urease activity in soil.

Acknowledgements

This work was supported by grant no. NN304044639 from the Ministry of Science and Education, Poland. Some of the experiments were run on apparatus purchased with EU grant "2.2 Innovation Industry".

References

[1] Akob DM, Mills HJ, Gihring TM, Kerkhof L, Stucki JW, Anastácio AS, et al. Appl Environ Microbiol. 2008;74:3159-3170.
[2] Kumar R, Acharya C, Joshi SR. J Microbiol. 2011;49:568-574.
[3] Rastogi G, Osman S, Vaishampayan PA, Andersen GL, Stetler LD, Sani RK. Microb Ecol. 2010;59:94-108.
[4] Moreno JL, García C, Landi L, Falchini L, Pietramellara G, Nannipieri P. Soil Biol Biochem. 2001;33:483-489.
[5] Macura J, Vagnerova K. Rosl Vyroba. 1969;15:173-180.
[6] Stankowska D, Kwinkowski M, Kaca W. J Microbiol Immunol Infect. 2008;41:243-253.
[7] Margesin R, Feller G, Hämmerle M, Stegner U, Schinner F. Biotechnol Let. 2002;24:27-33.
[8] Błaszczyk MK. Mikrobiologia środowisk. Warszawa: PWN; 2010.
[9] Braun B, Böckelmann U, Grohmann E, Szewczyk U. Appl Soil Ecol. 2006;31:267-279.
[10] Johnsen AR, Winding A, Karlson U, Roslev P. Appl Environ Microbiol. 2002;68:6106-6113.
[11] Piotrowska-Seget Z, Kozdrój J. Plant Soil Environ. 2008;54:520-528.
[12] Dziadek K, Wacławek W. Chem Dydakt Ekol Metrol. 2005;1-2:10.
[13] Hemida SK, Omar SA, Abdell-Mallek AY. Earth Environ Sci. 1995;95:13-22.
[14] Nwaugo V, Onyeagba A, Akubugwo E, Ugbogu O. Biokemistri. 2008;20:77-84.
[15] Błaszak M, Nowak A. Ecol Chem Eng A. 2008;15:1-2.
[16] López N, Borrŕs G, Vallespinós F. Scient Mar. 1995;59:149-154.
[17] Gupta R, Gupta N, Rathi P. Appl Microbiol Biotechnol. 2004;64:763-81.

ANALIZA ILOŚCIOWA TLENOWYCH BAKTERII HODOWALNYCH ORAZ CAŁKOWITEJ AKTYWNOŚCI ENZYMATYCZNEJ GLEBY URBICENOZY KIELC I RUDEK

[1] Zakład Mikrobiologii, [2] Zakład Ochrony Gleb i Krajobrazu Kulturowego,
[3] Pracownia Geologii, Uniwersytet Jana Kochanowskiego, Kielce,
[4] Instytut Chemii i Techniki Jądrowej,
Laboratorium Jądrowych Technik Analitycznych, Warszawa

Abstrakt: Wykonano badania mikrobiologiczne i biochemiczne dwóch próbek gleb miejskich województwa świętokrzyskiego (Kielce, Rudki). Celem pracy była analiza ilościowa tlenowej hodowalnej mikroflory bakteryjnej oraz określenie całkowitej aktywności enzymatycznej gleby z okolic o dużym natężeniu ruchu drogowego (Kielce) i terenów byłej kopalni pirytu (Rudki), skąd wydobywano rudę uranową. Do izolacji drobnoustrojów zastosowano pożywki komercyjne (TSA, agar LB, MacConkey'a, M9 i King B) oraz podłoża z ekstraktami glebowymi. Hodowlę prowadzono w dwóch temperaturach: +25 °C i +4 °C. W przypadku gleby urbicenozy Kielc, na pożywkach komercyjnych zaobserwowano większą ilość drobnoustrojów hodowanych w temperaturze +25 °C (od 10 do 10^8 razy więcej w zależności od stosowanej pożywki), natomiast na podłożu z ekstraktem glebowym niemal 2 razy więcej bakterii rosło w temp. +4 °C. W glebie zanieczyszczonej uranem (Rudki) obfity wzrost drobnoustrojów hodowanych w obu temperaturach występował jedynie na agarze z ekstraktem glebowym. Oznaczono również całkowitą aktywność ureolityczną, proteolityczną i lipolityczną próbek glebowych. W przypadku próbek gleby z Kielc obserwowano wszystkie badane aktywności, natomiast dla gleby z Rudek tylko lipolityczną.

Słowa kluczowe: bakterie glebowe, metale ciężkie, całkowita aktywność enzymatyczna