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Iwona KONIECZNA[1], Paulina ŻARNOWIEC[1],
Anna ŚWIERCZ[2] and Wiesław KACA[1]

CHARACTERIZATION OF THE AEROBIC CULTIVABLE BACTERIA ISOLATED FROM SOILS: HEAVY METALS CONTAMINATED (BIALOGON, KIELCE) AND ARABLE (KLONOWSKIE RIDGE)

CHARAKTERYSTYKA TLENOWYCH BAKTERII HODOWALNYCH IZOLOWANYCH Z GLEBY SKAŻONEJ METALAMI CIĘŻKIMI (BIAŁOGON, KIELCE) I GLEBY UPRAWNEJ (PASMO KLONOWSKIE)

Abstract: The aim of this study was quantitative and enzymological analysis of aerobic bacterial microflora isolated from heavy metals contaminated soil and unpolluted arable soil. The amounts of haevy metals: zinc, copper, cadmium, lead, chromium in soil samples were determined. The commercial media and soils extracts media were used for soil microorganisms isolation. Amount of soil bacteria cultivated on commercial media were significant higher (3 to 10^6 times) in case of heavy metals contaminated soil, whereas during growth on soil extract agar more microorganisms were found in arable soil. The Gram-negative bacteria dominated among strains isolated from both soil samples. For isolated microorganisms the ureolytic and proteolytic activity, as well as the ability for nitrification and denitrification were determined. The total ureolytic and proteolytic activity of soil samples were also defined. It was revealed, that only few bacterial strains isolated from polluted soil indicated analyzed properties (27 % – urea hydrolysis, 13 % – skim milk hydrolysis, 19 % – nitrification and 27 % – denitrification) in comparison with isolates from arable soil (65 %, 35 %, 30 % and 35 % respectively). In heavy metals contaminated soil the total ureolytic activity was much lower than in unpolluted soil sample. The presence of heavy metals has inhibitory effect on appearance of microorganisms participating in nitrogen circulation.

Keywords: soil bacteria, heavy metals, biochemical properties.

Soil is environment where dwells a giant amount of microorganisms. The composition, distribution, growth and development of soil bacteria affect various factors *eg* temperature, pH, nutrients and toxic substances [1]. To these latter heavy metals are included. Small amounts of metals (zinc, copper, nickel, manganese and other) have a

[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, Faculty of Environment Protection and Modelling, The Jan Kochanowski University in Kielce, Poland.

positive effect on bacterial metabolism by mediating in enzymatic reactions [2]. However, increase of their concentration result in toxic effects against microbes. On the other hand, metal ions such as mercury, cadmium or lead are harmful even at low concentrations [3]. Contamination of soil by such ions contributing in reduction of most biochemical reactions intensity and have also toxic influence on bacterial microflora amount and structure [4, 5]. It has been proven, that heavy metals decrease the activity of urease, acid and alkaline phosphatase, amidase, nitrate reductase, produced by soil bacteria [6].

The aim of this study was to quantitative and enzymologic analysis of aerobic bacterial microflora isolated from arable soil and soil contaminated by heavy metal ions.

Materials and methods

Soil samples were collected aseptically into sterile containers using Egner's stick, from the surface soil layer. Two samples were collected on November 18th, 2010: first was a brown arable soil, from the ecological area of Klonowskie Ridge (designated as G1 sample), while the second was from the outer basin of dried Bialogonski pond in Kielce (designated as G2 sample) (Fig. 1).

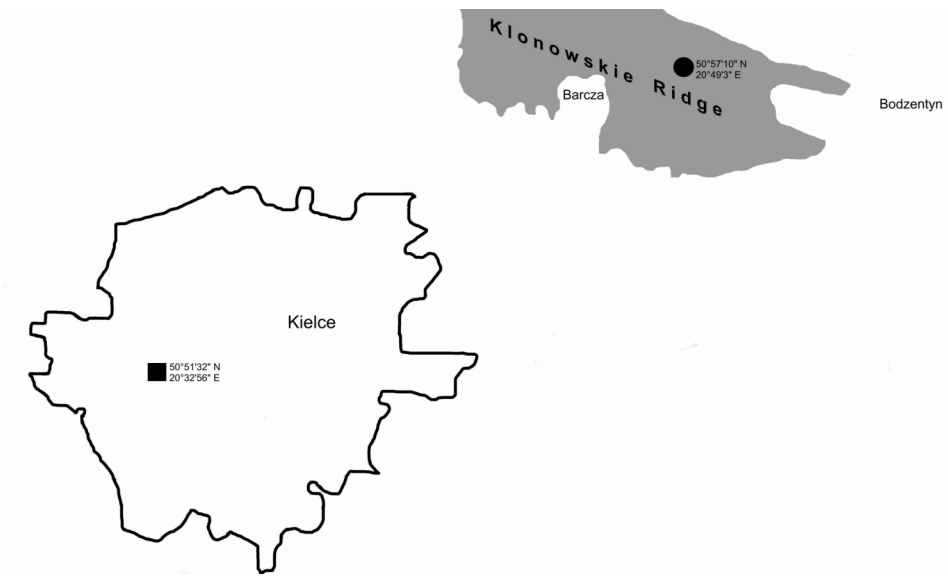

Fig. 1. Location of soil (● – G1; ■ – G2) sampling points

Chemical analysis of the soil samples – content of heavy metals: zinc, copper, cadmium, lead, chromium was determined by FAAS. The content of organic carbon and organic nitrogen were determined by titration and titration-destillation method, respectively. All analysis was performed at the Regional Agricultural-Chemical Station in Kielce.

Isolation and biochemical properties of soil bacteria – suspensions of 1 g of soil samples (G1 and G2, respectively) in 100 cm^3 of sterile Winogradsky solution were made and well stirred (20 min ok. 500 rpm). After soil particles sedimentation, solutions were serially diluted up to 10^{-6} and plated on Petri plates with media: Luria-Bertani (LB) agar, minimal M9 agar, tryptic-soy agar (TSA), KingB agar, soil extract agar (SEA). Cultures were incubated at 25 °C up to 7 days. Quantity bacteria (cfu/1g of wet soil) was determined based on the number of bacterial colonies grown on the individual media. For further studies, colonies with different macroscopic morphology were isolated. Isolated bacterial strains were stained according to Gram's method. Proteolytic activity on the medium with 2 % skimmed milk was determined, ureolytic activity – by spectrophotometric method (wavelength λ = 560 nm) on the Christensen in Maslen modification liquid medium (results were differences between culture and control absorbances), nitrification and denitrification – spectrophotometric method (wavelength λ = 520 nm) on the liquid mineral medium according to Winogradsky or medium with potassium nitrate (absorbance were calculated on the amount of nitrite ions), respectively. In spectrophotometric methods all results were related to the non-inoculated control media.

Determination of the soil samples total biochemical activity – ureolytic and proteolytic activities were defined according to previous works [7, 8].

Results and discussion

In soil sample collected from the former Bialogonski pond basin in Kielce (G2 sample), against to the arable soil from ecological area of Klonowskie Ridge (G1 sample), a higher content of heavy metals: cadmium, copper, lead, zinc and chromium was noted, where the quantity of zinc was repeatedly (almost 38 times) higher. Also content of organic carbon and nitrogen was higher in soil G2 (Table 1).

Table 1

Chemical analysis of soil samples

Amount / Soil sample	C_{org} [%]	N_{tot} [g/kg]	Cd [mg/kg]	Cu [mg/kg]	Pb [mg/kg]	Zn [mg/kg]	Cr [mg/100 g]
G1	1.56	1.46	0.190	6.45	16.2	43.5	9.42
G2	25.77	17.02	3.85	14.8	47.0	1646	22.4

This contamination has historical reason. Soil sampling station (the former Bialogonski pond basin) during the nineteenth century was the center of exploitation of copper and lead ores as well as silver melting. The earlier analysis also showed a high heavy metals (lead, cadmium, silver) contamination [9, 10].

There are evidences, that heavy metals (*eg* cadmium, chromium, copper, mercury, lead, zinc) have toxic effects on microbes [11]. In this study a quantitative analysis of aerobic bacteria isolated from soil contaminated by heavy metals (soil G2) was performed. The reference point were results obtained for arable soil from ecological

areas (soil G1). Quantity of microorganisms cultivated from two soil samples varied depending on the type of culture medium. On the commercial media, more of microorganisms was cultured from G2 soil sample, with the most abundant microbial growth on TSA and KingB was occurred. In case of soil G1, amount of microorganisms cultivated on these media was much lower. The opposite situation occurred in the case of the soil extract agar (SEA). On this medium, significantly more organisms were grown from the sample G1 (Table 2).

Table 2

The quantity of aerobic bacteria [cfu/1 g soil] cultured from G1 (arable soil) and G2 (Bialogonski pond basin soil) samples, on various microbiological media

Soil sample	Microbiological medium				
	TSA	LB	M9	KingB	SEA
G1	$2 \cdot 10^7$	$1.5 \cdot 10^7$	$2.1 \cdot 10^6$	$1 \cdot 10^7$	*
G2	$6 \cdot 10^9$	$3.5 \cdot 10^7$	$1 \cdot 10^7$	$6.5 \cdot 10^9$	$3.6 \cdot 10^8$

* – unable to count number of microorganisms.

These results may be surprising in light of earlier reports of heavy metals toxic effects on soil bacteria number and growth [12]. However, there are also studies demonstrating, that in the soils of this type numerous bacteria, including actinomycetes are present [1, 4, 13–16]. In this work, among the isolated strains, actinomycetes were also observed (based on macro- and microscopic morphology) and the percentage participation of particular morphological forms was similar to the distribution recived for G1 sample (Table 3).

Table 3

Percentage participation of bacterial strains with different microscopic morphology, isolated from various soils

Soil sample	Amount of isolate strains	Microscopic morphology [%]				
		Gram-negative	Gram-positive			
		rods	cocci	bacilli	rods	actinomycetes
G1	20	60	10	15	10	5
G2	37	48	11	11	22	8

Shentu *et al* showed a toxic effect of cadmium on the soil bacterial microflora structure [12]. In this work, the participation of individual morphological forms among the isolated bacterial strains was similar in both soil samples (heavy metals contaminated, including cadmium and uncontaminated).

These results are consistent with other work on the biodiversity of heavy metals contaminated soil microflora, where both Gram-negative as well as Gram-positive bacteria were observed [3, 4, 13]. Above this, it was demonstrated, that shortly after

contamination total amount of soil microbes rapidly decreasing. However with time, the number of isolated microorganisms growing and among them are often Gram--negative rods [4]. Contamination of G2 station was quite distant in time, so it is possible, that there was a reconstruction (at least partially) bacterial microflora size and structure.

For the 57 examined bacterial strains, their ability to participate in nitrogen cycle various stages were analyzed. Nitrification, denitrification, proteolytic and ureolytic activities were studied. The isolated bacterial strains demonstrating varied biochemical properties. The level of nitrification in analysed strains was low and does not exceed (with the exception of strain G2/18) 10 μM of nitrite(III) ions (Fig. 2A). Denitrification activity was much more diverse (Fig. 2B). Single strains possesed activity much higher than average *ie* G2/18 or G1/19 for nitrification and denitrification activities, respectively (Fig. 2).

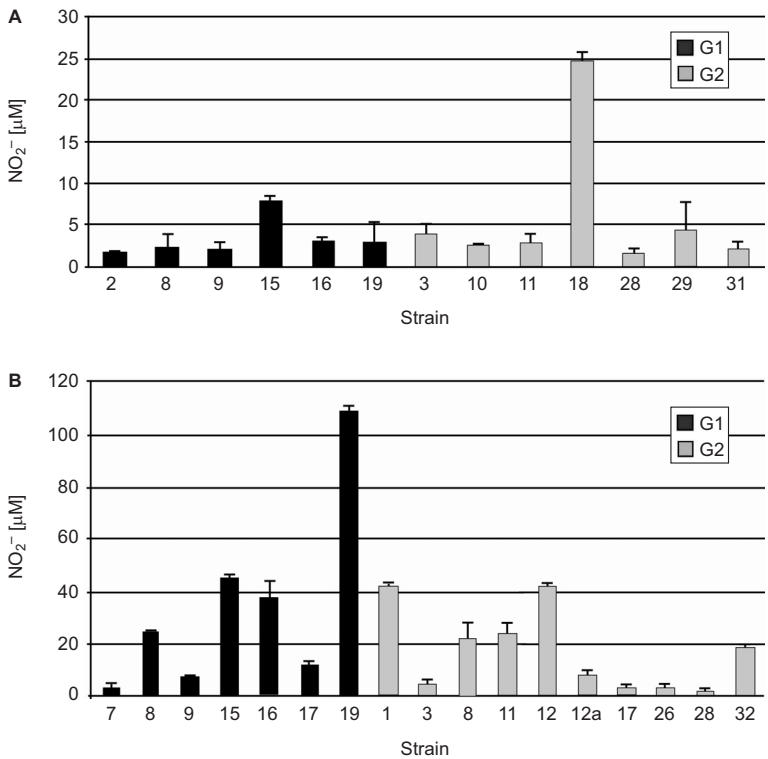

Fig. 2. Nitrification (A) and denitrification (B) of bacterial strains isolated from soil G1 and G2

Relatively large diversity were also observed in case of ureolytic and proteolytic activities, when stronger activity possesed strains isolated from arable soil (Table 4).

Analysis of the total ureolytic activity in examined soil samples showed, that soil G2 is characterized by a much lower level of this activity.

Table 4

The quantity of bacterial strains with ureolytic and/or proteolytic activities, isolated from different soils

Soil sample	Ureolytic activity				Proteolytic activity			
	–	+	++	+++	–	+	++	+++
G1	1	7	3	2	6	3	3	1
G2	8	7	1	2	13	4	1	0

– no activity; + weak activity (Δ of absorbance below 0.5 for the ureolytic activity and zone of milk hydrolysis less than 15 mm for the proteolytic activity); ++ moderate activity (Δ of absorbance from 0.5 to 1 for the ureolytic activity and zone of milk hydrolysis from 15 to 20 mm for the proteolytic activity); +++ strong activity (Δ of absorbance over 1 for the ureolytic activity and zone of milk hydrolysis over 20 mm for the proteolytic activity).

There are data, where negative effects of heavy metals on the biochemical activity of soil bacteria was observed [12]. Toxic influence of some metals (copper and lithium) on the proteolytic activity of isolated from soil *Bacillus cereus* was demonstrated [17]. Heavy metals are also nitrification inhibitors. It was shown, that chromium, nickel, copper, zinc, cadmium and lead may inhibit nitrification at each stage [18]. Nawaguo et al observed, that also urease is especially sensitive to the presence of heavy metals [19].

Conclusions

The presence of heavy metals significantly reduce biochemical activity of soil as well as bacterial strains isolated from soil. The structure of morphological forms of microorganisms isolated from contaminated soil does not differ significantly to the distribution of isolates from unpolluted soil, which may be caused by slowly (over one century) reconstruction of microflora in the area contaminated for more than 100 years.

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References

[1] Błaszczyk MK. Mikrobiologia środowisk. Warszawa: PWN; 2010.
[2] Gremion F. PhD disertation, Swiss Federal Institute of Technology Lausanne, ENAC, ISTE-Laboratory of Soil Science (LPE), Lozanna, Szwajcaria; 2003.
[3] Rathnayake IVN, Megharaj M, Bolan N, Naidu R. World Acad Sci Eng Technol. 2009;53:1185-1189.
[4] Piotrowska- Seget Z, Kozdrój J. Plant Soil Environ. 2008;54:520-528.
[5] Dziadek K, Wacławek W. Chem Dydakt Ekol Metrol. 2005;1-2:10.
[6] Hemida SK, Omar SA, Abdell-Mallek AY. Earth Environ Sci. 1995;95:13-22.
[7] Macura J, Vagnerova K. Rosl Vyroba. 1969;15:173-180.
[8] Moreno JL, Garcõa C, Landi L, Falchini L, Pietramellara G, Nannipieri P. Soil Biol Biochem. 2001;33:483-489.
[9] http://www.um.kielce.pl/srodowisko/pliki/oszal021.pdf

[10] http://www.um.kielce.pl/srodowisko/pliki/rap02.pdf
[11] Pradhan AA, Levine AD. Sci Total Environ. 1995;170:209-220.
[12] Shentu J, He Z, Yang X, Li T. J Zhejiang Univ Sci B. 2008;9:250-260.
[13] Ellis RJ, Morgan P, Weightman AJ, Fry JC. Appl Environ Microbiol. 2003;69:3223-3230.
[14] Chien Ch, Kuo Y, Chen Ch, Hung Ch, Yech H, Yech W. J Environ Sci. 2008;20:350-363.
[15] Al-Yemeni MN, Hashem AR. Saudi J Biol Sci. 2006;13:129-133.
[16] Wyszkowska J, Kucharski J, Borowik A, Boros E. J Elementol. 2008;13:443-453.
[17] Shafee N, Aris SN, Abd Rahman RNZ, Basri M, Salleh AB. J Appl Sci Res. 2005;1:1-8.
[18] Peng Y, Zhu G. Appl Microbiol Biotechnol. 2006;73:15-26.
[19] Nwaugo V, Onyeagba A, Akubugwo E, Ugbogu O. Biokemistri. 2008;20:77-84.

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[1] Zakład Mikrobiologii, [2] Zakład Ochrony Gleb i Krajobrazu Kulturowego,
Uniwersytet Jana Kochanowskiego, Kielce

Abstrakt: Celem pracy była analiza ilościowa i enzymologiczna tlenowej mikroflory bakteryjnej izolowanej z gleby zanieczyszczonej metalami ciężkimi oraz gleby uprawnej terenów czystych ekologicznie. Próbki zbadano pod względem zawartości metali ciężkich: cynku, miedzi, kadmu, ołowiu, chromu. Do izolacji drobnoustrojów z próbek glebowych zastosowano pożywki komercyjne oraz podłoża z ekstraktami glebowymi. Ilość wyhodowanych mikroorganizmów na pożywkach komercyjnych była znacznie większa (od 3 do 10^5 razy) w przypadku gleby skażonej metalami ciężkimi, natomiast na podłożu zawierającym ekstrakt glebowy obserwowano bogatszy wzrost bakterii dla gleby uprawnej. Wśród szczepów bakterii wyizolowanych z obu próbek dominowały drobnoustroje Gram-ujemne. Dla wyizolowanych mikroorganizmów określono aktywność ureolityczną, proteolityczną oraz zdolność do przeprowadzania procesów nitryfikacji i denitryfikacji. Oznaczono również całkowitą aktywność ureolityczną i proteolityczną próbek glebowych. Wykazano, że tylko nieliczne szczepy bakterii izolowanych z gleby zdegradowanej wykazywały badane właściwości (27 % – hydroliza mocznika, 13 % – hydroliza białek mleka, 19 % – nitryfikacja i 27 % – denitryfikacja) w porównaniu do izolatów z gleby uprawnej (odpowiednio: 65 %, 35 %, 30 % i 35 %). W glebie zanieczyszczonej metalami ciężkimi całkowita aktywność ureolityczna była znacznie mniejsza w porównaniu do próbki gleby czystej. Obecność metali ciężkich wpływa hamująco na występowanie drobnoustrojów uczestniczących w obiegu azotu.

Słowa kluczowe: bakterie glebowe, metale ciężkie, właściwości biochemiczne