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# BACTERIAL POTENTIAL OF THE EXPERIMENTAL PLOTS INSTALLED ON THE IRON MINE SPOILS IN IARA

***Vasile MUNTEAN*[1], *Gheorghe GROZA*[2]**
[1] Universitatea "Babeş-Bolyai", Facultatea de Biologie şi Geologie, Catedra de Biologie Experimentală
str. M. Kogălniceanu, nr. 1, **RO-400084 Cluj-Napoca**;
[2] Universitatea de Științe Agricole şi Medicină Veterinară, Facultatea de Agricultură, Calea Mănăştur, nr. 3,
**RO-400372 Cluj-Napoca**;
**e-mail:** vamutiro@yahoo.co.uk; ghgroza@yahoo.com

**Abstract:** The paper presents the evolution of the bacterial potential in the soils of the experimental plots, in the frame of a bioremediation experiment carried out on iron mine spoils. The bacterial potential was appreciate on the base of the bacterial indicators of soil quality (BISQ), calculated taking into account the number of bacteria which belong to the following 5 ecophysiological groups: aerobic mesophilic heterotrophs, ammonifiers, denitrifiers, iron-reducers and desulphofiers. Seasonal analyses were carried out, and annual BISQs have been calculated too. Both the seasonal and the annual BISQs follow a zig-zag-like trajectory: the plots covered with soil have higher values of the BISQ than the uncovered ones. As compared to the control native soil, in the moment of the experiment initiation, the BISQs had values lower with untill 50%. After the vegetation period, an increase of the BISQ values was noticed: at all the covered plots, the annual BISQs were >60% towards soil and >125% towards spoil; at all the uncovered plots, the BISQs were <60% towards soil and <120% towards spoil. The best evolution along the first year of vegetation was registered in the plot 25, covered with soil and cultivated with Medicago sativa. A positive correlation has been established between the bacterial and the enzymatic indicators of soil quality. The both synthetic indicators reflect the good evolution of the experimental plots along the first year of vegetation.

**Keywords:** spoils, iron mine, bacteria, bacterial indicator of soil quality, bioremediation, microbial potential, evolution, experimental plots

## Introduction

The research is focused on the microbiological aspects of a bioremediation experiment carried out on the mine spoils resulted from the mining of iron ore in Iara, Cluj county. The work was carried out with the financial support of the Romanian Ministry of Education and Research, in the frame of the national research programme Biotech, the project: Biotechnologies for remediation of spoils resulted from mining. The spoil in Iara covers approximately 29 ha, has approximately two millions $m^3$, and it is one of the 251 spoil dumps registered in Romania, acoording to a report of the Ministry of Waters and Environment Protection, in the year 2000. Recultivation of wastelands is a very important problem of environment protection, because the wastes from mining constitute an important source of environmental pollution.

Based on the good results obtained in recultivation of the spoils resulted from the underground mining of lead and zinc ores in Rodna (Bistrița-Năsăud county) [4-8, 11, 12, 15-17], in the autumn of 2004 we initiated a complex experiment for recultivation of the spoils from Iara. In the spring of 2005, 26 experimental plots, each of 10 $m^2$, were installed on the spoil dump, submitted to different treatments, and cultivated with 9 herbaceous species: *Festuca rubra, Festuca arundinacea, Dactylis glomerata, Lolium perenne (Poaceae), Onobrychis viciifolia, Trifolium repens, Trifolium pratense, Lotus corniculatus* and *Medicago sativa* (**Fabaceae**) [13]. The odd plots were covered with a 10-cm layer of native soil, and the even plots were not. Both types of plots were fertilized with NPK and $NH_4NO_3$. In this paper we present the evolution of the microbial potential in the soil of the experimental plots along the first year of vegetation.

## Materials and Methods

Seasonal analyses were carried out: in May – when the experiment was initiated, in August and November (2005), and in January (2006). We analyzed the soils of the 26 experimental plots comparatively with two controls: the raw spoil and a native soil sampled from the next vicinity of the spoil dump.

The bacterial potential was appreciate on the base of the bacterial indicators of soil quality (BISQ) values, calculated according with Muntean (1995-1996) [9]. The indicators were calculated taking into account the number of bacteria which belong to the following 5 ecophysiological groups: aerobic mesophilic heterotrophs (agar plates) [3], ammonifiers (peptone medium), denitrifiers (De Barjac culture medium) [19]), iron-reducers (Ottow modified medium) [14, 18], and desulphofiers (Van Delden medium) [2]. With the exception of the aerobic mesophilic heterotrophs (where we used the method of successive dilutions), the most probable number of bacteria was calculated according to the statistical table of Alexander [1].

## Results and Discussion

The aerobic mesophilic heterotrophs, ammonifiers, denitrifiers and iron-reducers were present in soils of all the experimental plots, in all the seasons. The presence of desulphofiers was never registered in the control spoils, or in the plots uncovered with soil; they were only present in 9 plots (the same) at the beginning of the experiments (spring) and in winter, as well as in all the plots covered with soil (summer and autumn).

The number of bacteria decreases in the order: aerobic mesophilic heterotrophs ($10^5$-$10^6$ cells•$g^{-1}$ dry matter soil) > ammonifiers ($10^2$-$10^4$ cells•$g^{-1}$ dry matter soil) > denitrifiers and iron-reducers ($10^2$-$10^3$ cells•$g^{-1}$ dry matter soil) > desulphofiers (0-$10^2$ cells•$g^{-1}$ dry matter soil). The number of analysed bacteria were always lower in the raw spoil, and higher by an order of magnitude in the native control soil. With few exceptions, the highest values were registered in autumn, and the lowest ones in winter.

The bacterial indicators of soil quality (BISQ) were calculated based on the number of bacteria belonging to each ecophysiological group. The BISQs faithfully reflect the actual bacterial potential of the analyzed soils. We calculated the seasonal BISQs, as well as the annual ones (Tab. 1).

Both the seasonal and the annual BISQs follow a zig-zag-like trajectory: the odd plots (covered with soil) have higher values of the BISQ than the even ones (uncovered with soil). We notice that the samples of the odd plots resulted by mixing the upper soil layer (10 cm) with the covered spoil (until 30 cm depth). It is also obvious the great difference between the native soil (higher values of the BISQs) and the experimental plots.

The values of the BISQs of the experimental plots are included between 2.502 (minimum, in winter, plot 8 cultivated with *Trifolium repens*), and 3.694 (maximum, in autumn, plot 25 cultivated with *Medicago sativa*). Actually, the plot 25, situated on the surface of the spoil dump, has the highest annual mean value of this indicator (3.581). By comparison, the highest value was registered in control soil, in autumn (BISQ = 5.140), and the lowest one in the raw spoil control, in winter (BISQ = 2.254).

The seasonal and annual hierarchy of the experimental plots, on the base of the BISQs values are presented in Tab. 2.

The analyses carried out seasonally reflect a low constancy of the BISQs values: the highest values have been registered as follows:

- spring – plot 1 (*Festuca rubra*);
- summer – plot 5 (*Dactylis glomerata*);

- autumn – plot 19 (*Lolium perenne*);
- winter – plot 25 (*Medicago sativa*);
- annual – plot 25 (*Medicago sativa*).

**Table 1: The values of the seasonal and annual indicators of soil quality**

| Experimental plot | Bacterial indicator of soil quality | | | | |
|---|---|---|---|---|---|
| | Spring | Summer | Autumn | Winter | Annual |
| 1 | 3.609 | 3.531 | 3.762 | 3.402 | 3.576 |
| 2 | 2.869 | 2.978 | 3.031 | 2.784 | 2.916 |
| 3 | 3.343 | 3.414 | 3.493 | 3.145 | 3.349 |
| 4 | 2.581 | 2.763 | 2.808 | 2.487 | 2.660 |
| 5 | 3.042 | 3.333 | 3.450 | 2.914 | 3.185 |
| 6 | 2.725 | 2.842 | 2.902 | 2.646 | 2.779 |
| 7 | 2.903 | 3.243 | 3.367 | 2.806 | 3.080 |
| 8 | 2.596 | 2.708 | 2.817 | 2.502 | 2.656 |
| 9 | 3.434 | 3.371 | 3.621 | 3.316 | 3.435 |
| 10 | 2.673 | 2.778 | 2.826 | 2.585 | 2.715 |
| 11 | 3.144 | 3.622 | 3.694 | 3.041 | 3.375 |
| 12 | 2.741 | 2.787 | 2.830 | 2.593 | 2.738 |
| 13 | 3.145 | 3.383 | 3.458 | 2.997 | 3.246 |
| 14 | 2.831 | 2.812 | 2.850 | 2.668 | 2.790 |
| 15 | 3.358 | 3.702 | 3.587 | 3.228 | 3.469 |
| 16 | 2.779 | 2.874 | 2.931 | 2.715 | 2.825 |
| 17 | 3.450 | 3.546 | 3.614 | 3.327 | 3.484 |
| 18 | 2.838 | 2.889 | 2.868 | 2.660 | 2.814 |
| 19 | 3.470 | 3.697 | 3.793 | 3.358 | 3.580 |
| 20 | 2.619 | 2.687 | 2.727 | 2.516 | 2.637 |
| 21 | 3.591 | 3.388 | 3.475 | 3.324 | 3.444 |
| 22 | 2.761 | 2.813 | 2.741 | 2.662 | 2.744 |
| 23 | 3.491 | 3.604 | 3.688 | 3.384 | 3.542 |
| 24 | 2.785 | 2.813 | 2.853 | 2.685 | 2.784 |
| 25 | 3.569 | 3.652 | 3.694 | 3.409 | 3.581 |
| 26 | 2.626 | 2.681 | 2.795 | 2.527 | 2.657 |
| Spoil | 2.443 | 2.536 | 2.578 | 2.254 | 2.453 |
| Soil | 4.537 | 5.044 | 5.140 | 4.790 | 4.878 |

The odd plots (covered with) soil which have the highest values of the seasonal and annual BISQs [1, 15, 19, 25], and the corresponding even ones (uncovered with soil: 2, 16, 20, 26) have been selected in order to illustrate the seasonal evolution of bacterial potential (Fig. 1). The figure also includes the plot 7 (cultivated with *Trifolium repens*), which has the lowest values of the BISQs between the covered plots in all the seasons, and its uncovered correspondent [8], as well as the two controls: raw spoil and native soil.

As compared to the control native soil, in the moment of the experiment initiation, the BISQs had values lower with untill 50%. After the vegetation period, an obvious increase of the BISQ values was noticed. The percent of the annual BISQs of each experimental plots related to the BISQs of the native soil and the raw spoil, respectively, reflects the degree of this increase (Tab. 3).

Fig. 2 illustrates the hierachy of the experimental plots based on the annual BISQ values. We can see the clear difference between the plots covered with soil and the uncovered ones. The correspondent plots (covered – odd numbers, and uncovered with soil – even numbers), situated on the same terrace, on the same aspect of the spoil dump, and cultivated with the same species, don't have a parallel evolution of the microbial potential.

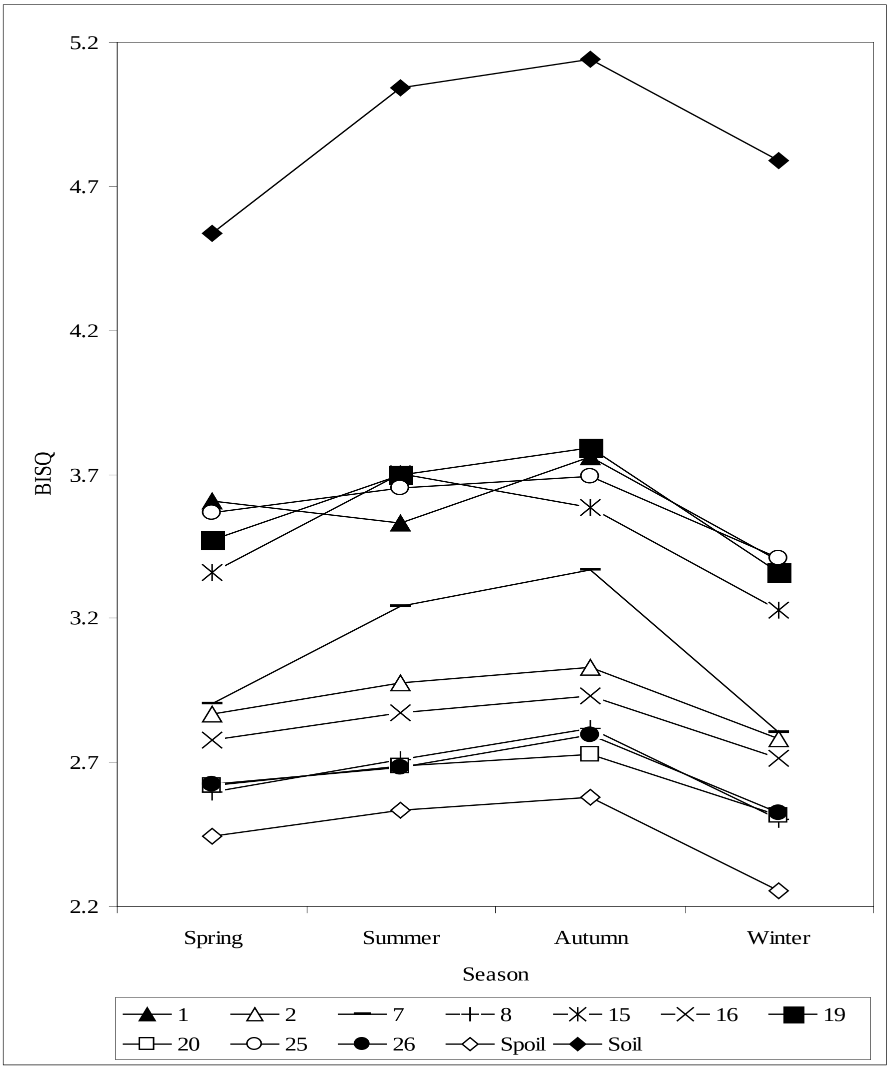


**Fig. 1: Seasonal evolution of the microbial potential of the experimental plots covered with soil, where the highest values of the bacterial indicators of soil quality (BISQ) have been registered, comparatively with the correspondent plots, uncovered with soil.**

As one can see, the annual BISQ value of the control soil is twofold higher than that of the spoil control. The annual BISQ values show a difference between bacterial potential of the experimental plots, as follows:

- at all the covered plots – BISQ >60% towards soil; >125% towards spoil;
- at 8 from the covered plots – BISQ >70% towards soil; >140% towards spoil;
- at all the uncovered plots – BISQ <60% towards soil; <120% towards spoil;
- at 4 from the uncovered plots – BISQ <55% towards soil; <110% towards spoil.

**Table 2: Hierarchy of the experimental plots, based on the BISQ values.**

| Position | Spring | Summer | Autumn | Winter | Annual |
|---|---|---|---|---|---|
| 1 | 1 | 15 | 19 | 25 | 25 |
| 2 | 21 | 19 | 1 | 1 | 19 |
| 3 | 25 | 25 | 11 | 23 | 1 |
| 4 | 23 | 11 | 25 | 19 | 23 |
| 5 | 19 | 23 | 23 | 17 | 17 |
| 6 | 17 | 17 | 9 | 21 | 15 |
| 7 | 9 | 1 | 17 | 9 | 21 |
| 8 | 15 | 3 | 15 | 15 | 9 |
| 9 | 3 | 21 | 3 | 3 | 11 |

| | | | | | |
|---|---|---|---|---|---|
| 10 | 13 | 13 | 21 | 11 | 3 |
| 11 | 11 | 9 | 13 | 13 | 13 |
| 12 | 5 | 5 | 5 | 5 | 5 |
| 13 | 7 | 7 | 7 | 7 | 7 |
| 14 | 2 | 2 | 2 | 2 | 2 |
| 15 | 18 | 18 | 16 | 16 | 16 |
| 16 | 14 | 16 | 6 | 24 | 18 |
| 17 | 24 | 6 | 18 | 14 | 14 |
| 18 | 16 | 22 | 24 | 22 | 24 |
| 19 | 22 | 24 | 14 | 18 | 6 |
| 20 | 12 | 14 | 12 | 6 | 22 |
| 21 | 6 | 12 | 10 | 12 | 12 |
| 22 | 10 | 10 | 8 | 10 | 10 |
| 23 | 26 | 4 | 4 | 26 | 4 |
| 24 | 20 | 8 | 26 | 20 | 26 |
| 25 | 8 | 20 | 22 | 8 | 8 |
| 26 | 4 | 26 | 20 | 4 | 20 |

**Table 3: The percentage of the annual BISQs of each experimental plot** towards **the BISQs of the native soil and of the raw spoil.**

| Position | Plot | % BISQ towards soil | % BISQ towards spoil |
|---|---|---|---|
| Soil | | 100 | 200 |
| 1 | 25 | 73 | 146 |
| 2 | 19 | 73 | 146 |
| 3 | 1 | 73 | 146 |
| 4 | 23 | 73 | 144 |
| 5 | 17 | 71 | 142 |
| 6 | 15 | 71 | 141 |
| 7 | 21 | 71 | 140 |
| 8 | 9 | 70 | 140 |
| 9 | 11 | 69 | 138 |
| 10 | 3 | 69 | 137 |
| 11 | 13 | 67 | 132 |
| 12 | 5 | 65 | 130 |
| 13 | 7 | 63 | 126 |
| 14 | 2 | 60 | 119 |
| 15 | 16 | 58 | 115 |
| 16 | 18 | 58 | 115 |
| 17 | 14 | 57 | 114 |
| 18 | 24 | 57 | 114 |
| 19 | 6 | 57 | 113 |
| 20 | 22 | 56 | 112 |
| 21 | 12 | 56 | 112 |
| 22 | 10 | 56 | 111 |
| 23 | 4 | 55 | 108 |
| 24 | 26 | 54 | 108 |
| 25 | 8 | 54 | 108 |
| 26 | 20 | 54 | 108 |
| Spoil | | 50 | 100 |

Enzymological analyses has also been carried out on the soil of the experimental plots (paper in press). We only use here the values of the enzymatic indicators of soil quality [10], calculated on the base of the following enzymes: catalase, actual and potential dehydrogenase, phosphatase and saccharase. A strong positive correlation, statistically very significant ($p<0.001$) has been established between the bacterial and the enzymatic indicators of soil quality: $r = +0,931$ (spring), $r = +0,960$ (summer), $r = +0,964$ (autumn), $r = +0,929$ (winter), $r = +0,974$ (annual).

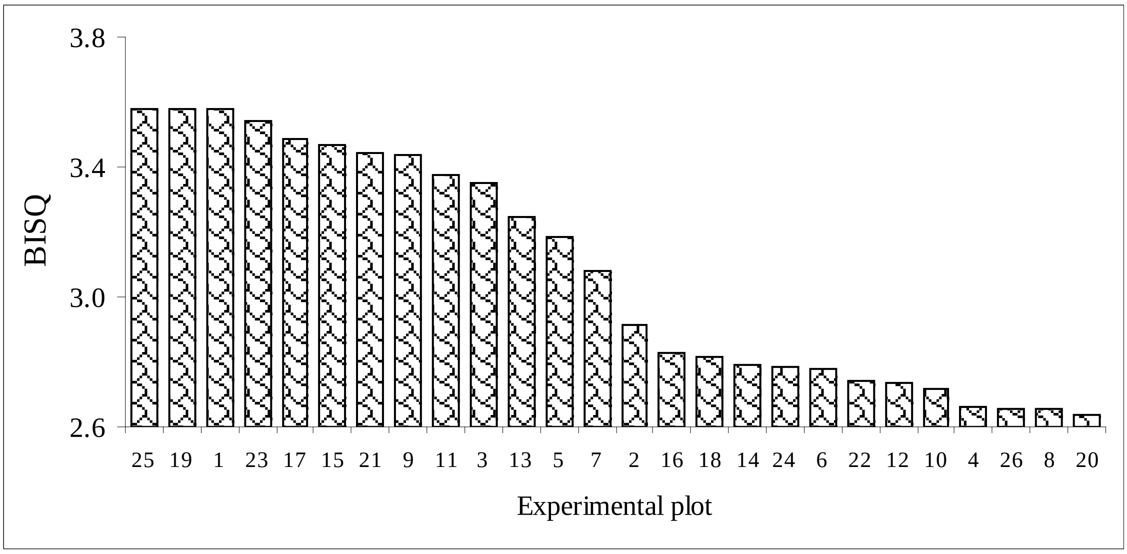


**Fig. 2: Hierachy of the experimental plots based on the values of the annual bacterial indicators of soil quality (BISQ).**

## Conclusions

The number of bacteria belonging to the five ecophysiological groups studied in the experimental plots installed on the iron mine spoils decreases in the order: aerobic mesophilic heterotrophs ($10^5$-$10^6$ cells•$g^{-1}$ dry matter soil) > ammonifiers ($10^2$-$10^4$ cells•$g^{-1}$ dry matter soil) > denitrifiers and iron-reducers ($10^2$-$10^3$ cells•$g^{-1}$ dry matter soil) > desulphofiers (0-$10^2$ cells•$g^{-1}$ dry matter soil).

The bacterial indicators have a seasonal evolution, with the highest values in autumn. The values of the annual BISQs show the better evolution of the bacterial potential in the plots covered with soil, as compared to the uncovered ones: at all the covered plots, the BISQs were >60% towards soil and >125% towards spoil; at all the uncovered plots, the BISQs were <60% towards soil and <120% towards spoil. The best evolution along the first year of vegetation was registered in the plot 25, covered with soil and cultivated with *Medicago sativa*.

A strong positive correlation, statistically very significant, has been established between the bacterial and the enzymatic indicators of soil quality. The both synthetic indicators reflect the good evolution of the experimental plots along the first year of vegetation.

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## POTENȚIALUL BACTERIAN AL PARCELELOR EXPERIMENTALE INSTALATE PE HALDA DE STERIL DE LA MINA DE FIER DIN IARA

### (Rezumat)


În primăvara anului 2005, pe halda de steril rezultată de la mina de fier din localitatea Iara (județul Cluj), au fost amplasate 26 de parcele experimentale: 13 au fost acoperite cu un strat de 10 cm de sol clinohidromorf, celelalte nefiind copertate. Parcelele au fost cultivate cu următoarele specii ierboase: *Festuca rubra, Festuca arundinacea, Dactylis glomerata, Lolium perenne* (familia **Poaceae**), *Onobrychis viciifolia, Trifolium repens, Trifolium pratense, Lotus corniculatus* şi *Medicago sativa* (familia **Fabaceae**). Lucrarea de față prezintă evoluția potențialului bacterian în solul parcelelor experimentale pe parcursul anului scurs de la instalarea experimentului de bioremediere a haldei. Potențialul bacterian este apreciat pe baza valorilor indicatorilor bacterieni ai calității solului (IBCS). Indicatorii au fost calculați luând în considerare numărul de bacterii care aparțin următoarelor grupe ecofiziologice: heterotrofe mezofile aerobe, amonificatoare, denitrificatoare, fier-reducătoare şi desulfoficatoare. Determinările au fost efectuate sezonier. Comparativ cu solul martor, în momentul inițierii experimentului, indicatorii bacterieni ai calității solului/sterilului parcelelor experimentale au valori mai mici cu până la 50%. La trei luni de la inițierea experimentului, se constată deja o uşoară creştere a valorilor acestor indicatori. Creşterea este şi mai evidentă după alte trei luni de vegetație. În sezonul de iarnă se constată o scădere a potențialului bacterian, atât în solurile parcelelor experimentale cât şi în solul, respectiv sterilul care au servit drept martori. Pe ansamblul primului an de vegetație, potențialul bacterian al solurilor parcelelor experimentale, reflectat de valorile indicatorului bacterian anual al calității solului, a crescut, mai mult în parcelele copertate cu sol decât în corespondentele lor, necopertate, dar cultivate cu aceleaşi specii. Astfel, în toate parcelele copertate, IBCS au avut valori de cel puțin 60% față de solul martor, respectiv de 125% față de steril; în niciuna dintre parcelele necopertate cu sol, IBCS anuali nu depăşesc 60% față de sol, respectiv 120% față de steril Cea mai bună evoluție de-a lungul primului an de vegetație a înregistra-o parcela 25, copertată cu sol şi însămânțată cu *Medicago sativa*. Se poate aprecia că pe parcursul perioadei de vegetație, plantele au avut o evoluție bună, afirmație valabilă şi pentru potențialul bacterian al solului parcelelor experimentale.