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MICROBIOLOGICAL RESEARCH ON IRON MINE SPOILS SUBMITTED TO BIOREMEDIATION

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Abstract: Microbiological and physico-chemical analyses were carried out on the mine spoils resulted from the mining of iron ore in Iara, Cluj county, in order to establish the toxicity of the spoils. The decreasing order of the metal concentration in the raw spoils is: Fe > Ca > Mg > Cr > Pb > Zn > Cu > Ni. The raw spoils have an alkaline pH and contain only traces of organic matter (3-4 mg $\bullet g^{-1}$ dry matter spoil). The following 5 ecophysiological bacterial groups have been studied: aerobic mesophilic heterotrophs, ammonifiers, denitrifiers, iron-reducers and desulphofiers. Based on the number of bacteria belonging to each ecophysiological groups, the bacterial indicators of spoil/soil quality (BISQs) were calculated. A strong positive correlation statistically very significant has been established between the BISQ and the organic matter content. Between the most abundant metals (Fe, Ca, Mg, Zn, Cu) on one hand and the BISQ and organic matter, respectively, on the other hand, a negative correlation, statistically very significant was also established. Based on the results obtained, a biological recultivation experiment was initiated on the spoil dump. 26 experimental plots were installed on the spoil dump, submitted to different treatments, and cultivated with herbaceous species. The same microbiological analyses were carried out on the experimental plots for establishing their bacterial potential at the beginning of the recultivation experiment. The number of bacteria decrease in the order: aerobic mesophilic heterotrophs (10^5-10^6) > ammonifiers (10^2-10^4) > denitrifiers and iron-reducers (10^2-10^3) > desulphofiers (10^1-10^2 cells$\bullet g^{-1}$ dry matter spoil/soil). The desulphofiers were present only in 9 plots, all of them covered with soil. The plots covered with soil have higher values of the BISQ than those uncovered.

Introduction

The development of mining and other industries results in increasing amounts of wastes. It is estimated that up to 1980, about 1.6×10^{12} m^3 of mine spoils accumulated on the earth. This amount increases yearly by about 40×10^9 m^3, approximately three times more than the amount of soil affected yearly by the water erosion (about 13×10^9 m^3) [9]. Acoording to a report of the Ministry of Waters and Environment Protection, in the year 2000, 251 spoil dumps were registered in Romania, covering a total surface of 5,932 ha. These wastes constitute an important source of environmental pollution. That is why, the recultivation of wastelands becomes, first of all a problem of environment protection, as well as a major economic necessity.

The aim of the biological recultivation of wastelands is to transform them into technogenic soils, proper for agriculture, forestry or other purposes, like fitting out of parks, sports fields etc. At the same time, the biological recultivation of the spoil dumps results in reduction or elimination of the polluting effects of wastes on the environment. In the last years, good results were obtained by Romanian researchers in recultivation of the spoils resulted from the underground mining of lead and zinc ores in Rodna (Bistriţa-Năsăud county) [4-8, 11, 12, 14-16]. The research was focused especially on the enzymatic aspects of the spoil recultivation. In this paper we present the initiation of the experiment for bioremediation of the spoils resulted from the underground mining of iron in Iara (Cluj county), emphasizing the microbiological aspects of the experiment. 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.

Materials and Methods

Microbiological and physico-chemical analyses were carried out on the mine spoils resulted from the mining of iron ore in Iara, Cluj county, sampled on the 15.09.2004. Two samples were analysed for each of the 5 terraces of the spoil dump (4-21-years old), one for East aspect, and one for South aspect, respectively. Two soils were also analysed: one soil supposed to be used for covering the experimental plots we intended to install on the spoil dump, and one, as control, sampled from the next vicinity of the spoil (Tab. 1). 26 experimental plots were installed on the terraces of the mine spoil (Tab. 2), and microbiological analyses were carried out for establishing the bacterial potential of the experimental plots at the beginning of the bioremediation experiments.

The following 5 ecophysiological bacterial groups have been studied: aerobic mesophilic heterotrophs (agar plates) [3], ammonifiers (peptone medium), denitrifiers (De Barjac culture medium) [18]), iron-reducers (Ottow modified medium) [13, 17], 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]. The concentration of the heavy metals (Fe, Zn, Cu, Cr, Pb, Ni, Ca, Mg) in the spoils was determined by atomic absorbance spectroscopy.

Results and Discussion

The results of the physico-chemical analyses carried out on the mine spoils in the autumn of 2004 are presented in tab. 1. The raw spoils have an alkaline pH. In all of the samples, the pH exceeds the value 8. The soil which was supposed to be used for fitting out the experimental plots had also an alkaline pH. As we can see in the table 1, the raw spoils contain only traces of organic matter (3-4 mg •g^{-1} dry matter spoil). Even the control soils analyzed have a low level of organic matter (under 1%); they can be framed in the class of mineral materials with high content of humus.

Table 1: Results of the physico-chemical analyses carried out on the spoils and control soils
OM = organic matter (mg·g^{-1} dry matter soil)

No.	Terrace	Aspect	pH	OM	Metals (ppm)							
					Fe	Zn	Cu	Cr	Pb	Ni	Ca	Mg
1	I	South	8.11	3.45	46025	401	240	4054	505	12	13515	9552
2	II	South	8.15	3.23	45032	409	226	3461	514	11	14948	10331
3	III	South	8.32	3.60	45906	595	285	3489	621	13	16726	10714
4	IV	South	8.25	3.69	58538	499	339	10638	887	14	15967	10373
5	V	South	8.31	3.16	61090	658	329	5593	1060	11	19352	9920
6	I	East	8.20	3.44	46027	402	241	4055	508	11	13514	9549
7	II	East	8.16	3.23	45033	409	228	3462	516	10	14946	10328
8	III	East	8.15	3.60	45907	595	287	3489	624	12	16724	10711
9	IV	East	8.25	3.11	58540	499	341	10638	890	13	15966	10369
10	V	East	8.21	3.76	61091	659	330	5594	1063	11	19350	9917
11	Covering Soil		7.81	89.23	12187	26	24	796	12	26	2445	3251
12	Soil in vicinity		5.97	92.18	13757	39	25	994	26	24	177	2236

The aim of the chemical analyses was to determine the toxic charge of the spoil, in order to establish the technology of bioremediation. The raw spoils reaches high concentration of the heavy metals analyzed. Naturally, the highest concentration is that of the iron: 4.5-6.1% in dry matter. One can also notice the high concentration levels of calcium, manganese and chrome.

The decreasing order of the metal concentration in the raw spoils is: Fe (10^4) > Ca (10^4) > Mg (10^3-10^4) > Cr (10^3-10^4) > Pb (10^2-10^3) > Zn (10^2) > Cu (10^2) > Ni (10^1) (order of magnitude – ppm – in parantheses). The high level of metal concentration explains the toxicity of the raw spoils, the lack of any vegetation, even on the first terrace of 21-years old. No statistically significant difference was registered either between terraces, or between aspects.

The toxicity of the raw spoil is well illustrated by the result of the microbiological analyses (Fig. 1). All the five ecophysiological groups of bacteria were detected in the two soils. The desulphofiers were not detected in any of the spoil samples. The mesophilic heterotrophic bacteria are the best represented. According to the criterion of cell abundance, follow the ammonifiers, iron-reducers and denitrifiers. A great difference between the number of bacteria in the two soils, on one hand, and the spoil on the other is obvious. The difference is of 2-3 orders of magnitude in the soil advantage.

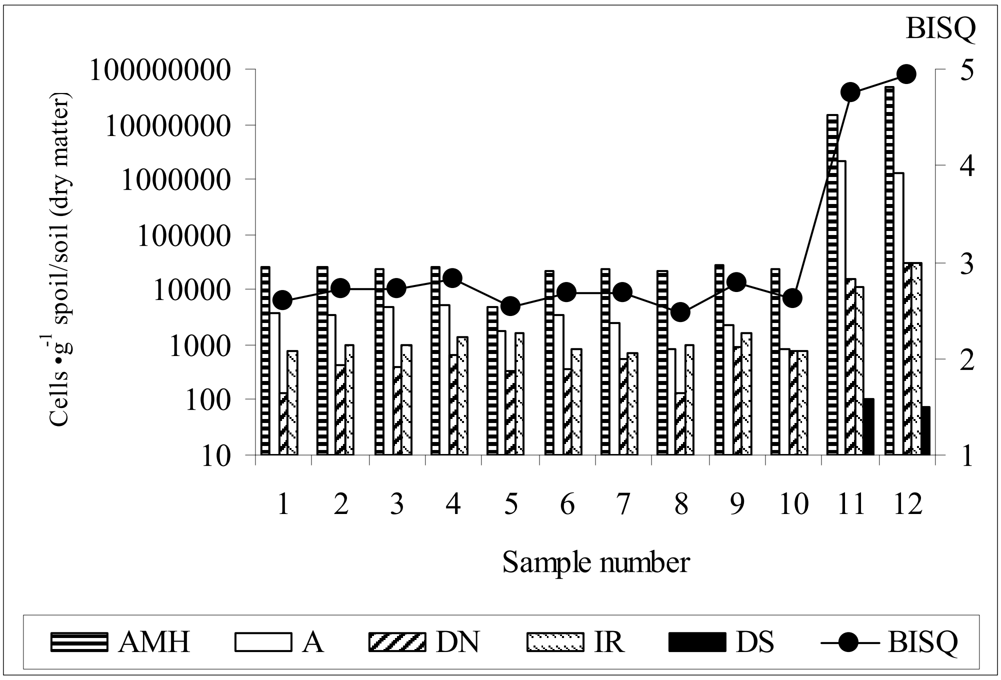

Fig. 1: Results of the microbiological analyses carried out on the spoils and control soils. AMH = aerobic mesophilic heterotrophs; A = ammonifiers; DN = denitrifiers; DS = desulphofiers; IR = iron-reducers; BISQ = bacterial indicator of spoil/soil quality.

Based on the number of bacteria belonging to each ecophysiological groups, the bacterial indicators of spoil/soil quality (BISQ) were calculated according to the formula proposed by Muntean [10]. One can notice the difference between the two soils on one hand, and the spoil samples on the other, as regard the bacterial potential as it is defined by the BISQ value. The BISQ values of the spoils are only half from those registered in the raw spoils.

The correlation between the analyzed parameters was calculated. A strong positive correlation (r = +0.993), statistically very significant ($p<0.001$) has been established between the BISQ and the organic matter content. Between the most abundant metals (Fe, Ca, Mg, Zn, Cu) on one hand and the BISQ and organic matter, respectively, on the other hand, a strong negative correlation, statistically very significant ($p<0.001$) was also established. No significant correlation was noticed between the two biological indicators, on one hand, and chrome and nickel, on the other.

Based on the results previously presented, a biological recultivation experiment was initiated on the spoil dump in Iara, in the 11-13.05.2005 period. 26 experimental plots, each of 10 m^2, were installed on the spoil dump, submitted to different treatments, and cultivated with the herbaceous species mentioned in table 2. The odd plots were covered with a 10-cm layer of soil analyzed previously, and the even plots were not. Both types of plots were fertilized with 300 g NPK (300 kg·ha^{-1}) and 200 g NH_4NO_3 (200 kg·ha^{-1}). The quantities of seeds used for cultivation were as follows: *Festuca rubra* – 100 g, *Dactylis glomerata* – 60 g, *Lolium perenne* – 60 g, *Onobrychis viciifolia* – 100 g, *Trifolium repens* – 30 g, *Trifolium pratense* – 30 g, *Lotus corniculatus* – 40 g, *Medicago sativa* – 40 g, the grass mixture (*Lolium perenne* - 55% + *Festuca arundinacea* - 40% + *Festuca rubra* - 5%) – 100 g. The quantities were by 50% higher than those calculated according to the 72/1981 STAS, because of the very improper conditions in the spoils.

Table 2: Experimental plots installed on the spoil dump.

Experimental plot	Aspect	Terrace	Soil	Species
1	South	II	+	*Festuca rubra*
2			-	
3		III	+	*Onobrychis viciifolia*
4			-	
5		IV	+	*Dactylis glomerata*
6			-	
7		V	+	*Trifolium repens*
8			-	
9		V	+	*Lolium perenne*
10			-	
11		Surface	+	*Lolium perenne* (55%) + *Festuca arundinacea* (40%) + *Festuca rubra* (5%)
12			-	
13		Surface	+	*Festuca rubra* (50%) + *Trifolium pratense* (50%)
14			-	
15	East	II	+	*Dactylis glomerata*
16			-	
17		III	+	*Onobrychis viciifolia*
18			-	
19		IV	+	*Lolium perenne*
20			-	
21		V	+	*Lotus corniculatus*
22			-	
23	North	V	+	*Trifolium pratense*
24			-	
25		Surface	+	*Medicago sativa*
26			-	

The same microbiological analyses were carried out on the experimental plots for establishing their bacterial potential at the beginning of the recultivation experiment. The sampling depth was 30 cm. The results are presented in figure 2. The aerobic mesophilic heterotrophs, ammonifiers, denitrifiers and iron-reducers were present in all the experimental plots. The desulphofiers were present only in 9 plots, all of them covered with soil. The number of bacteria decrease 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 (10^1-10^2 cells•g^{-1} dry matter soil).

The number of bacteria is low, and, consequently, the values of the bacterial indicators of spoil/soil quality are also low: the maximum value – 3.229 (plot 25, covered with soil and cultivated with *Medicago sativa*); the minimum value – 2.443 (spoil control). Even the difference between the maximum and the minimum values is lower than 1. We notice than in the soil used for covering the experimental plots, the BISQ was 4.747.

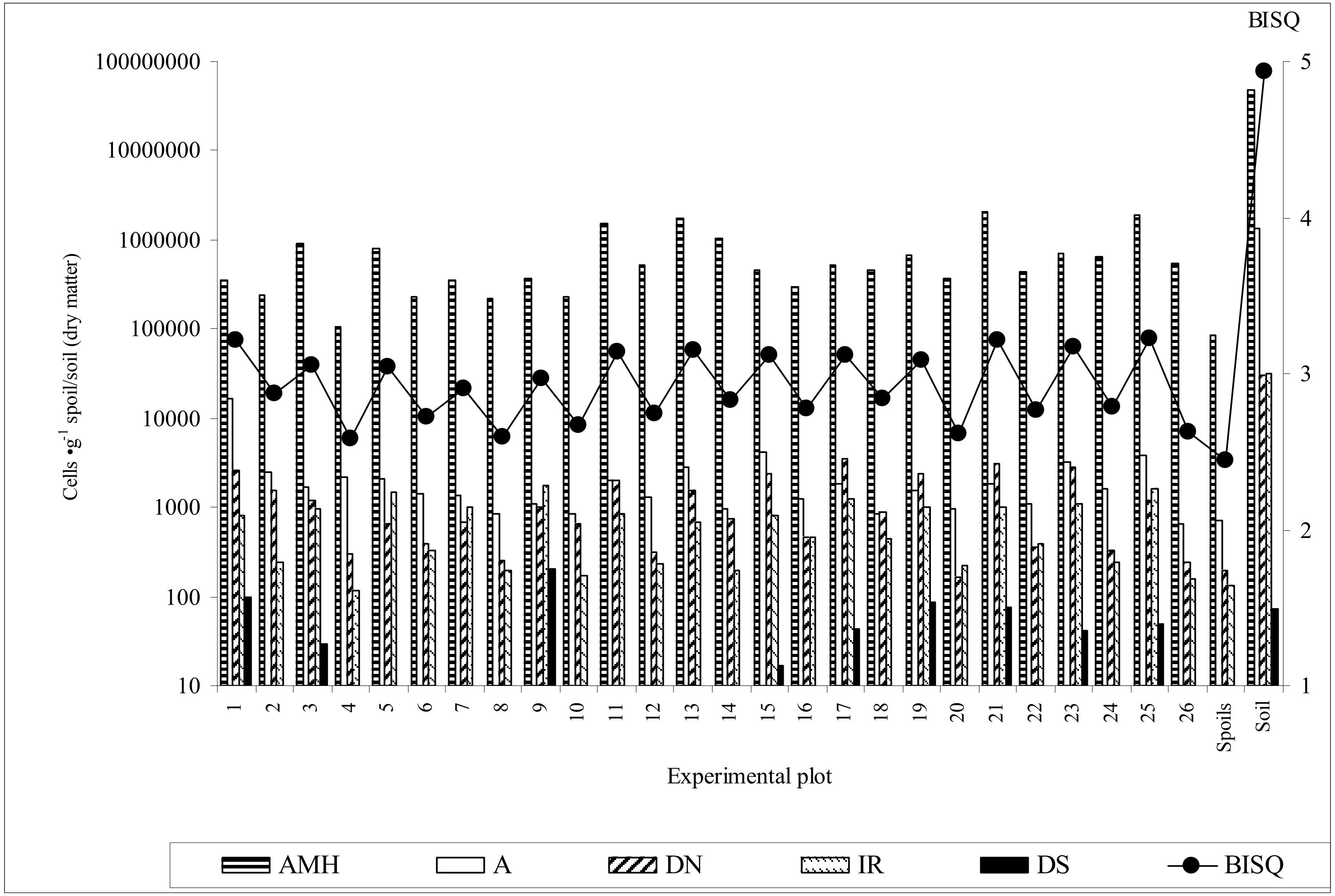

Fig. 2: Results of the microbiological analyses carried out on the experimental plots installed on the mine spoil.
AMH = aerobic mesophilic heterotrophs; A = ammonifiers; DN = denitrifiers; DS = desulphofiers; IR = iron-reducers; BISQ = bacterial indicator of spoil/soil quality.

The most obvious remark is the zig-zag-like trajectory of the BISQ curve: 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 sampled from the vicinity of the spoil dump (control) and the experimental plots, as regard the biological potential, as it is defined by the BISQ values. The two controls have the BISQ of 2.443 (raw spoil), and 4.932 (native soil), respectively. We only mention that all the experimental plots had a good evolution over the summer, as illustrated in photo 1. The evolution of the bacterial potential in the experimental plots is monitored seasonally.

Photo 1: Experimental plots 5 (covered with soil) and 6 (uncovered with soil), cultivated with *Dactylis glomerata*, three months after the initiation of the recultivation experiment

Conclusions

The chemical analyses carried out on the raw spoils in Iara have pointed out the high concentration of the heavy metals analyzed. The decreasing order of the metal concentration in the raw spoils was: Fe > Ca > Mg > Cr > Pb > Zn > Cu > Ni. The raw spoils contain only traces of organic matter (3-4 mg $\bullet g^{-1}$ dry matter spoil). No statistically significant difference was registered either between terraces, or between aspects.

All the five ecophysiological groups of bacteria studied were detected in the two control soils. The desulphofiers were not detected in any of the spoil samples. The mesophilic heterotrophic bacteria are the best represented. According to the criterion of cell abundance, followed the ammonifiers, iron-reducers and denitrifiers. A difference of 2-3 orders of magnitude, in the soil advantage, has been detected between the number of bacteria in the two soils, on one hand, and the spoils on the other. The bacterial indicators of spoil/soil quality (BISQ), calculated on the base of the number bacteria belonging to each ecophysiological groups, had the values almost twofold higher in the control soils than in the raw spoils.

A strong positive correlation ($r = +0.993$), statistically very significant ($p<0.001$) has been established between the BISQ and the organic matter content. Between the most abundant metals (Fe, Ca, Mg, Zn, Cu) on one hand and the BISQ and organic matter, respectively, on the other hand, a strong negative correlation, statistically very significant ($p<0.001$) was also established.

26 experimental plots were installed on the spoil dump, submitted to different treatments, and cultivated with herbaceous species, in May 2005. The same microbiological analyses were carried out on the experimental plots for establishing their bacterial potential at the beginning of the recultivation experiment. The aerobic mesophilic heterotrophs, ammonifiers, denitrifiers and iron-reducers were present in all the experimental plots. The desulphofiers were present only in 9

plots, all of them covered with soil. The number of bacteria decrease 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 (10^1-10^2 cells•g^{-1} dry matter soil).

The values of the bacterial indicators of soil quality are also low: the maximum value – 3.229 (plot 25, covered with soil and cultivated with *Medicago sativa*); the minimum value – 2.443 (spoil control). The odd plots (covered with soil) have higher values of the BISQ than the even ones (uncovered with soil). The experimental plots had a good evolution over the summer.

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CERCETĂRI MICROBIOLOGICE ASUPRA UNEI HALDE DE STERIL DE LA O MINĂ DE FIER, SUPUSĂ BIOREMEDIERII

(Rezumat)

Lucrarea prezintă rezultatele analizelor microbiologice şi fizico-chimice asupra haldei de steril rezultată de la mina de fier din Iara (jud. Cluj) şi iniţierea experimentului de bioremediere a acesteia. Au fost determinate: concentraţia metalelor grele în sterilul haldei, conţinutul în substanţă organică şi prezenţa a 5 grupe ecofiziologice de bacterii: heterotrofe mezofile aerobe, amonificatoare, denitrificatoare, fier-reducătoare şi desulfoficatoare. Pe baza numărului de bacterii s-a calculat indicatorul bacterian al calităţii sterilului (BISQ). Sterilul se caracterizează prin prezenţa doar a unor urme de substanţă organică (3-4 mg •g^{-1} steril substanţă uscată) şi printr-o concentraţie mare de metale, care descreşte în ordinea: Fe > Ca > Mg > Cr > Pb > Zn > Cu > Ni. Valorile indicatorilor bacterieni ai calităţii în sterilul analizat sunt de numai jumătate faţă de cele consemnate în cazul celor două soluri studiate comparativ. Între concentraţia metalelor pe de o parte şi indicatorii bacterieni ai calităţii, respectiv conţinutul în substanţă organică, pe de altă parte, s-a stabilit o corelaţie negativă statistic foarte semnificativă. Pe baza rezultatelor obţinute, a fost iniţiat un experiment de bioremediere a haldei. Au fost amenajate 26 de parcele experimentale, 13 acoperite cu un strat de 10 cm dintr-unul din solurile analizate, celelalte nefiind copertate. Parcelele au fost tratate cu NPK şi NH_4NO_3 şi cultivate, individual sau în diverse combinaţii, cu următoarele specii ierboase din familiile **Poaceae**, respectiv **Fabaceae**: *Festuca rubra*, *Festuca arundinacea, Dactylis glomerata*, *Lolium perenne*, respectiv, *Onobrychis viciifolia*, *Trifolium repens*, *Trifolium pratense*, *Lotus corniculatus* şi *Medicago sativa*. A fost determinat potenţialul bacterian al sterilului/solului parcelelor experimentale în momentul iniţierii experimentului de bioremediere, prin calcularea indicatorului bacterian al calităţii, pe baza numărului de bacterii din cele 5 grupe ecofiziologice prezentate. Rezultatele obţinute şi evoluţia parcelelor în primele 3 luni relevă efectul benefic al copertării parcelelor cu sol.