

5-бөлім  
**МИКРОБИОЛОГИЯ**

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Раздел 5  
**МИКРОБИОЛОГИЯ**

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Section 5  
**MICROBIOLOGY**

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**MICROBIAL ANALYSIS OF COAL POLLUTED SOILS  
IN THE REGION OF KARAGANDY, KAZAKHSTAN**

Microorganisms play a major role in ecological biodegradation processes of soil polluted by coal. Here, we assessed the bacterial diversity in coal-contaminated soil samples. The soils samples were taken from four areas for analyses. In present study we conducted the isolation and characterization of microorganisms from Baizhanov mine soil of Karagandy (KCS), State Natural Forest Reserve "Semey Ormany" pinewood soil (SPS), Dmitrievsky forest soil (DFS) and Rudny Altai pinewood soil (APS). Twenty-one species of bacteria were isolated from KCS, SPS, DFS and APS soil samples using serial dilution and spread-plate method. Preliminary identification of microorganisms was carried out by culturing on nutrient agar media and Gram staining. Results of total microbial count and morphology characterization showed that both the KCS and SPS share similar microbial communities. Most of studied microorganisms are Gram-positive bacillus, cocci, streptobacillus and diplobacillus bacteria. These results provide some useful information in biodegradation of coal-contaminated soil for the further studies.

**Key words:** Coal-contaminated soil, forest soil, pine wood soil, bacterial diversity, biodegradation.

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**Қарағанды облысындағы (Қазақстан) көмірмен ластанған  
топырақтың микробиологиялық анализі**

Микроорганизмдер көмірмен ластанған топырақ биодеградациясының экологиялық үдерісінде маңызды рөл атқарады. Бұл зерттеуде Қарағанды облысының шахта маңы аумағының көмірмен ластанған топырақ үлгілеріндегі бактериялардың алуантүрлігіне сараптама жасалды. Микроорганизмдердің алуан түрлілігін зерттеу мақсатында 4 топырақ үлгілері жер бедерінің әртүрлі горизонттарынан алынды. Зерттеу барысында Қарағанды қаласындағы Бижанов көмір шахтасының (KCS), Мемлекеттік табиғи орман резерваты «Семей Орманы» қарағайлы орманының (SPS), Дмитриев орманының (DFS) және Алтай Рудный-қарағайлы орманының (APS) топырақтарынан микроорганизмдері оқшауланып алынды және сипатталды. Дәстүрлі бактериологиялық әдісті пайдалана отырып KCS, SPS, DFS және APS топырақ үлгілерінен 21 түрлі

бактериялардың түрлері идентификацияланды. Кезекті идентификациялау әдістеріне қоректік ортасында өсіру және граммен бояу әдісі жүргізілді. KCS және SPS топырақ үлгілерінің микробтық қауымдастықтарының ұқсастықтары бар екенін көрсетті. Микроорганизмдердің көпшілігі Грамм оң бактериялар, коккалар, стрептобацилдер және диплобацилдер екендігі анықталды. Алынған нәтижелер алдағы уақытта зерттеу жұмыстарын жүргізуге және практикалық қолдануға пайдалы ақпарат береді.

**Түйін сөздер:** Көмірмен ластанған топырақ, орман топырағы, қарағай топырағы микроорганизмдердің алуантүрлілігі, биодegradация.

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### **Микробиологический анализ проб почв, загрязненных углем карагандинского региона, Казахстан**

Микроорганизмы играют важную роль в экологических процессах биодegradации почв, загрязненных углем. В данной работе было проанализировано разнообразие микроорганизмов в пробах почв пришахтных территорий Карагандинской области. Для изучения микробного разнообразия было отобрано 4 образцов почв из различных горизонтов. В результате исследования выделены и проанализированы микроорганизмы из почв Байжановского месторождения г. Караганда (KCS), Государственный Природный Лесной Резерват соснового леса «Семей Орманы» (SPS), леса Дмитриевский (DFS) и Территорий Рудного Алтая соснового леса (APS). Из образцов почвы KCS, SPS, DFS и APS было изолировано 21 вида бактерий с использованием традиционных методов бактериологии. Предварительную идентификацию микроорганизмов проводили путем культивирования на питательных средах и окрашивания по Грамму. Результаты показали, что микробные сообщества территории KCS и SPS имеют сходный характер. Многие микроорганизмы представляют собой грамположительные бактерии, кокки, стрептобациллы и диплобациллы. Полученные результаты содержат полезную информацию для дальнейших исследований и практических работ.

**Ключевые слова:** Почва, загрязненная углем; лесная почва; сосновая почва; разнообразие микроорганизмов; биодegradация.

Surface mining can result in the disturbance of ecological communities throughout the world. Extracting valuable resources through methods such as strip mining can cause devastating effects on the ecosystem. Strip mining is a process in which land is excavated to reach a coal seam. After extraction of coal, the crushed and homogenized overburden is then replaced and covered by topsoil. This leads to decreases in both plant and microbial mass (Poncelet 2013:1917-1929). Until recently, analysis of the land mass recovery and reclamation has been limited to surface examinations which often lead to false conclusion due to the eventual recovery of plant mass at these locations. These studies however do not characterize the possible devastating effect to the subsoil (Mummey 2002:251-259). This approach concludes that visibility of plant communities at the surface is recovered land, but this approach often pays little to no attention to the microorganisms.

Soil is considered to be the most diverse microbial habitat on earth. Soil microorganisms are a very important part of the environmental ecosystems, which could adjust energy flow and cycle of matter by digesting animal, plant other residues, and play a pivotal role in growth and development of agriculture crops, balance of the soil ecosystem, organic matter transfer and bioremediation. Furthermore, the diversity of the microbial community in soil is closely related to the function and structure of its surrounding ecosystem, and is one of the components to maintain soil productivity. However, little is known about how environmental changes affect the microbiota and its functions (Fierer 2007:7059–7066, Liebig 2006:1688–1691). Recently, the relationship between environmental disturbance, biodiversity, and ecosystem function has received much international attention from the research community, yet a universally applicable model has not been achieved

(Barquín 2012:636-646). **Links between disturbances and biodiversity changes** are complicated. The response of bacterial diversity to ecosystem changes has not been systematically addressed with multitudinous research subjects, although many studies have demonstrated that species diversity is closely related to ecosystem function and biogeochemical process (Schwartz 2000:297-305).

Microbes are abundant and ubiquitous in terrestrial ecosystem. For example, the diversity of mycorrhizal fungi affects plant diversity and ecosystem stability and productivity (van der Heijden 1998:69-72). **While microbial diversity in general influences the ability of ecosystems to withstand stress and disturbances** (Sugden 2000:233-235, Johnsen 2001:443-453). **The disturbance effects on soil microorganisms persists until above ground vegetation re-grows and later succession of vegetation can reverse changes in soil properties** (Holden 2013:163). Soil microorganisms are sensitive to environmental change, such as the aforementioned strip-mining (Doran 1994:73-90). **These communities can experience significant degradation in biomass as well as species composition following a disturbance** (Harris 2003:801-808). It is proposed that analysis of microbial communities associated with disturbed land masses may serve as a better microbial indicator of recovery post land mass disturbance (Poncelet 2013:1917-1929). Disturbance of soil ecosystems that impact normal functioning of microbial community structure is potentially detrimental to soil formation, energy transfers, nutrient cycling, plant reestablishment and longterm stability. Significant changes in decades years after the event are expected mainly for soil properties exhibiting a high temporal variability, such as soil moisture and temperature, soluble nutrient contents, soil organism abundance and activity.

The aim of this study was to investigate bacterial communities from the different soil types, (1) comparing the responses of soil microorganisms to different management regimes on disturbed areas, and (2) evaluating the trends in microbial community composition. Such research is expected to improve our understanding of microbial processes recovery after coal disturbance. The area had low human activity, no inputs of fertilizers (except for the manure added by animal activity) and a very low animal influence, which was ideal for testing the effect on soil bacterial communities.

## Materials and methods

*Study Site and Sample Treatment.* The experiment site was situated in the regions of Karagandy,

Kazakhstan. The local soil type is chestnut soil. In this study, the four soil samples were collected from four areas:

1. The coal mine. Baizhanov, Karagandy;
2. GDLR "Semey Ormany", pinewood;
3. The territory of Dmitrievsky forest;
4. The wood territories of Rudny Altai, pine-wood.

Soil samples were collected at depths from the surface down to 30 cm by the five-point sampling method in five plots of each sampling site, which were then pooled and homogenized within the same sterilized plastic bag for detecting soil bacteria diversity on October, 2016. To study the soil samples surface collected by sterile spatula or trowel to a depth of 30 cm. From the individual point shovel site soil dug solid piece. With the sterile knife the soil's top layer of 1,5-2,0 cm was removed and the middle site 500-600 grams of soil was collected by a sterile spoon. Combined sample consisting of five individual soil samples weighed at least 2,5 kg. The samples were placed in a sterile container and transported to the laboratory. The each sample was labeled with the date and sample number. In an accompanying document, the nature of the soil, the location of the sources of pollution, the area of the survey area, data characterizing the climate of the area was noted. In the transport and storage of soil samples, it is necessary to comply with measures to prevent the possibility of secondary pollution. Soil samples were collected from 4 different locations in the sterilized plastic bags stored at 4°C .

## Quality and Quantity analysis

The microorganisms were isolated by serial dilution approach on Nutrient Agar Media (NAM). In this technique, a sample suspension was prepared by adding 1,0 g sample to 9 ml distilled water and mixed well for 15 min and vortexed. Each suspension was serially diluted  $10^{-1}$  to  $10^{-5}$  and repeated three times. 0,1 ml was pipetted onto petri dishes with NAM media, spread with a glass spreader and incubated at 37°C for bacterial observation. The cell density of bacterial communities was **measured simultaneously** using the colony forming units (CFU) method on NAM Petri dishes; the CFU were counted after 2 days of incubation (Dasari and Hwang 2010:5817-5823). Data from triplicate readings were expressed as  $CFU \times g^{-1}$  dry soil.

## Identification of bacteria

The microbial isolates were identified by morphological examination of colonies with morpho-

logical features such as color, diameter, form, elevation, margin, surface, opacity that were typical (Leboffe 2010) and Gram staining technique under light microscope. Prepare the specimen using the heat fixation process. Place a drop of crystal violet stain on the specimen for 1-2 min. Poured into the dye, not washing the smear with water. Apply iodine on the specimen using an eyedropper for 1-2 minutes. The iodine helps the crystal violet stain adhere to the specimen. Iodine is a mordant, which is a chemical that fixes the stain to the specimen. Wash the specimen with an ethanol during 0,5-1 min. Wash the specimen with water to remove the dye. Apply the fuchsine stain to the specimen using an eyedropper. Wash the specimen. Use a paper towel and blot the specimen until the specimen is dry. Gram-negative or positive were examined under microscope (MC-2-ZOOM, 1CR, 2016, Germany),(Gram H.C.1884:185–189).

## Results and Discussion

Table 1 indicate that we analyzed the average total number of cells/g in the soil samples respectively. The coal mine has not been mined for more than 20 years, protected by the local government, thus there are no human activities, and occasionally there will be livestock. The diluted samples were transferred into nutrient agar petri dishes and were incubated. From the observation, these samples take about two days to growth on the petri dishes. Table 2 shows

the growth of the bacteria after 48 h, the different colonies appeared that represent circular, irregular and filamentous in their morphology features, such as, color, diameter, form, elevation, margin, surface, opacity. Figure 1 shows that the microbial species of coal contaminated soil is recovering to the extent consistent with normal soil ( $P < 0.05$ ). Other three soil types belong to chestnut soil. A chestnut soil (US soil classification mollisol, sub-order xeroll), found in more arid grasslands. The xerophytic nature of much of the grassland under which chestnut soils develop retards the development of humus, and there is an accumulation of calcium carbonate in the B horizon.

Isolation of these microorganisms until get single colony had been done by using serial dilution and streaking methods. There are different colonies that represent circular and irregular in their morphology had been isolated from KCS, SPS, DFS and APS. Colonies with morphological features such as color, diameter, form, elevation, margin, surface, opacity that were typical to were observed from the nutrient agar and obtained 21 species, named as AKCS1, AKCS2, AKCS3, AKCS4, ASPS1, APS2, ASPS3, ASPS4, ASPS5, ADFS1, ADFS2, ADFS3, ADFS4, ADFS5, ADFS6, AAPS1, AAPS2, AAPS3, AAPS4, AAPS5, AAPS6 (Table 2) respectively. Four soil samples were analysed with respect to different types of bacteria. Gram staining illustrated that most of microorganisms are gram-positive (Figure 1,2,3,4) respectively.

**Table 1** – The average total number of cells/g in the different place soil samples

No	Soil types	The average of total number, $\times 10^6$ cells $\times$ g <sup>-1</sup>
1	KCS	1,63 $\pm$ 0,08
2	SPS	1,59 $\pm$ 0,08
3	APS	0,78 $\pm$ 0,04
4	DFS	1,14 $\pm$ 0,06

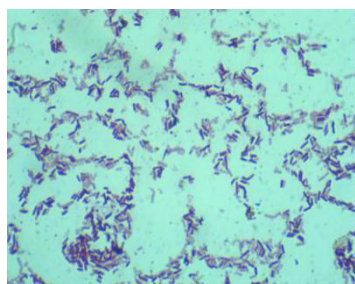
**Table 2** – The morphology characterization of different place soil bacterial colonies

Soil type	Colonies								
	Name	number	color	diameter	form	elevation	margin	Surface	opacity
KCS	AKCS1	83	white	0,2cm	circular	flat	entire	Rugose	opaque
	AKCS2	21	colorless	0,3cm	circular	flat	entire	Smooth	transparent
	AKCS3	7	yellow	0,2cm	circular	convex	entire	Glistening	translucent
	AKCS4	2	beige	0,8cm	circular	flat	entire	Dull	translucent



Continuation of table 3

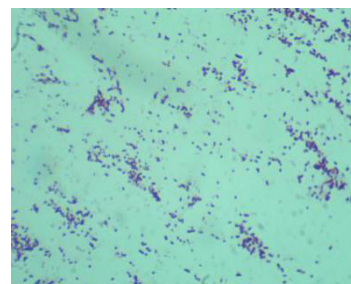
Soil type	Colonies								
SPS	ASPS1	320	white	0,3cm	circular	flat	entire	Smooth	translucent
	ASPS2	100	bull	0,3cm	circular	convex	entire	Glistening	translucent
	ASPS3	13	beige	0,3cm	circular	flat	entire	Smooth	translucent
	ASPS4	8	white	0,4cm	filamentous	flat	filiform	Rough	opaque
	ASPS5	5	white	0,2cm	circular	flat	entire	Smooth	opaque
DFS	ADFS1	1	beige	1,3cm	irregular	raised	undulate	Rough	opaque
	ADFS2	1	beige	0,3cm	circular	raised	entire	Smooth	translucent
	ADFS3	3	colorless	0,4cm	circular	flat	entire	Smooth	transparent
	ADFS4	11	beige	0,2cm	circular	flat	entire	Smooth	transparent
	ADFS5	6	beige	0,4cm	circular	flat	entire	Glistening	translucent
	ADFS6	2	beige	0,5cm	irregular	flat	undulate	Rough	opaque
APS	AAPS1	4	white	0,2cm	Circular	flat	undulate	Rough	opaque
	AAPS2	6	beige	0,1cm	circular	flat	undulate	Rough	translucent
	AAPS3	15	white	0,5cm	irregular	flat	Undulate	dull	opaque
	AAPS4	1	colorless	0,3cm	circular	flat	entire	Smooth	transparent
	AAPS5	1	beige	0,2cm	circular	convex	entire	Rough	translucent
	AAPS6	7	colorless	2,0cm	irregular	flat	Filiform	Rough	translucent



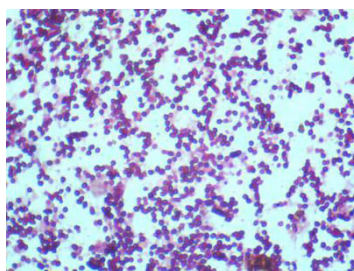
AKCS1 bacilli  
Gram-positive



AKCS2 bacilli  
Gram-positive

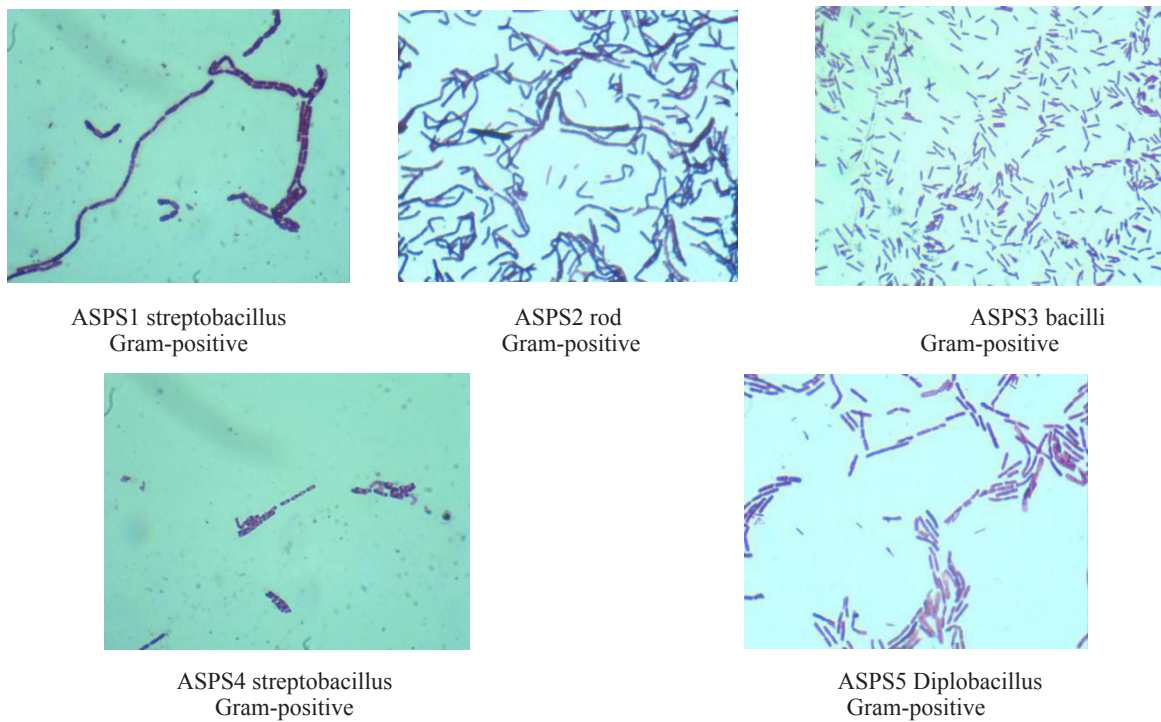


AKCS3 bacilli  
Gram-positive

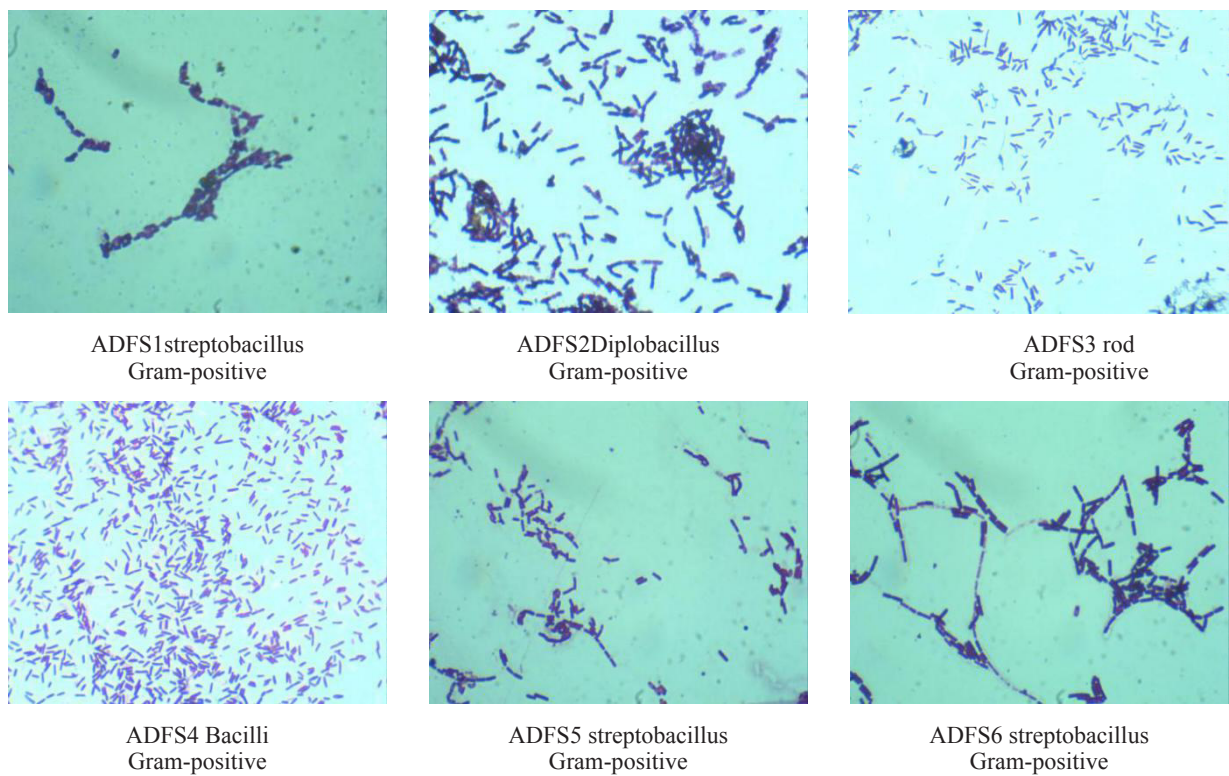


AKCS4 cocci  
Gram-positive

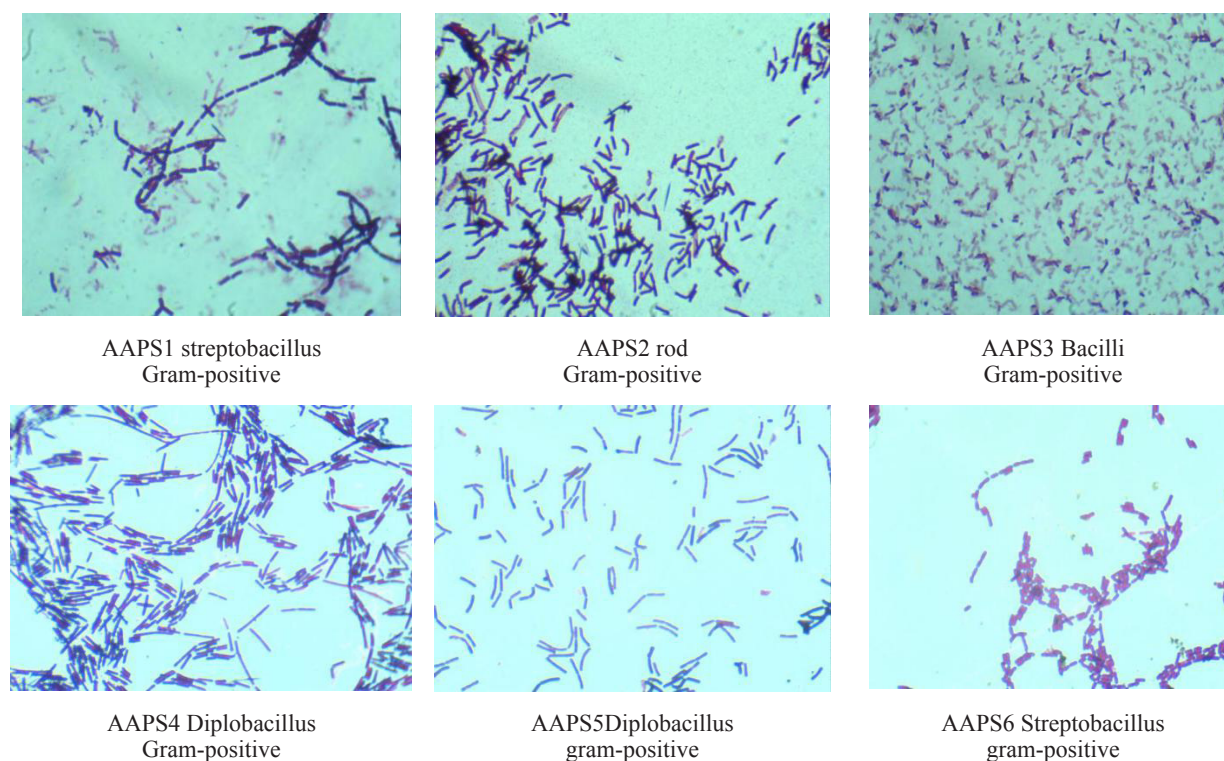
Figure 1 – The Gram staining of AKCS1-4 bacterial isolates, ×100



**Figure 2** – The Gram staining of ASPS1-5 bacterial isolates,  $\times 100$



**Figure 3** – The Gram staining of ADFS1-6 bacterial isolates,  $\times 100$



**Figure 4** – The Gram staining of AAPS1-6 bacterial isolates,  $\times 100$

The coal contaminated soil of microorganisms of Karagandy (KCS) of colonies is described from number, color, diameter, form, elevation, margin, surface, opacity, we discovered the number of colonies  $AKCS1 > AKCS2 > AKCS3 > AKCS4$ , the color of four bacteria is different, the diameter of  $AKCS1$  and  $AKCS3$  is same.  $AKCS2$  and  $AKCS4$  is different, the form of colonies is the same, the elevation of colonies  $AKCS1$ ,  $AKCS2$  and  $AKCS4$  is the same; the margin of colonies is the same; the surface of colonies is different; the opacity of colonies  $AKCS2$ ,  $AKCS3$  and  $AKCS4$  is the same, they are different bacteria. we discovered the number of colonies  $ASPS1 > ASPS2 > ASPS3 > ASPS4 > ASPS5$ , the color of five bacteria is different, the diameter of  $ASPS1$ ,  $ASPS2$  and  $ASPS3$  is the same,  $ASPS4$  and  $ASPS5$  is different, the form of colonies is the same, the elevation of colonies  $ASPS1$ ,  $ASPS3$ ,  $ASPS4$  AND  $ASPS5$  is the same, the margin of colonies  $ASPS1$ ,  $ASPS2$ ,  $ASPS3$ ,  $ASPS5$  is the same. The surface of colonies is different, the opacity of colonies  $ASPS1$ ,  $ASPS2$  and  $ASPS3$  is the same,  $ASPS4$  and  $ASPS5$  is the same. we discovered the number of colonies  $ADFS1 > ADFS2 > ADFS3 > ADFS4 > ADFS5 > ADFS6$ , the different strains color of  $ADFS1$ ,  $ADFS2$ ,  $ADFS4$ ,  $ADFS5$ ,  $ADFS6$  are the same. The diameter of five

bacteria is different, the form of colonies  $ADFS2$ ,  $ADFS3$ ,  $ADFS4$  and  $ADFS5$  are the same,  $ADFS1$  and  $ADFS6$  are the same, the elevation of colonies  $ADFS3$ ,  $ADFS4$ ,  $ADFS5$  and  $ADFS6$  are the same,  $ADFS1$  and  $ADFS2$  are the same. The margin of colonies  $ADFS2$ ,  $ADFS3$ ,  $ADFS4$  and  $ADFS5$  are the same,  $ADFS1$  and  $ADFS6$  are the same; the surface of colonies are different; the opacity of colonies  $ADFS2$ ,  $ADFS3$  and  $ADFS4$  and  $ADFS5$  is the same,  $ADFS1$  and  $ADFS6$  are the same, they are different bacteria. We found the number of colonies  $AAPS3 > AAPS6 > AAPS2 > AAPS1 > AAPS4 > AAPS5$ , the color of six bacteria is different, the diameter of six bacteria is different, the form of colonies  $AAPS1$ ,  $AAPS2$ ,  $AAPS4$  and  $AAPS5$  are the same,  $AAPS1$  and  $AAPS6$  are the same, the elevation of colonies  $AAPS1$ ,  $AAPS2$ ,  $AAPS3$ ,  $AAPS4$  and  $AAPS6$  are the same. The margin of colonies are different; the surface of colonies  $AAPS1$ ,  $AAPS2$ ,  $AAPS5$  and  $AAPS6$  are the same,  $AAPS3$  and  $AAPS4$  are different; the opacity of colonies  $AAPS2$ ,  $AAPS4$  and  $AAPS5$  and  $AAPS6$  is the same,  $AAPS1$  and  $AAPS3$  are the same, they are different bacteria. Soil ecological research over the past two decades has demonstrated extremely high levels of biological diversity belowground, especially in microbial groups.



Microbes exhibit an impressive diversity in their metabolic activities and its diversity is important because it is often regarded as an important index of soil ecosystem health (Entry 2008:146-154). Though of unquestionable importance in regards to the function of terrestrial ecosystems (Conrad 1996:609-640, Whitman 1998: 6578-6583, Copley (2000):452-454), our understanding about the structure of microbial communities, their response to the changing environment and the consequences of alterations in microbial community structure on ecosystem functioning is very little. Microbial diversity describes complexity and variability at different levels of biological organization. It encompasses genetic variability within species, richness, relative evenness of taxons and functional groups in communities (Kozdroj 2001:197-212, Johnsen 2001:443-453). Various parameters like temperature, pH, the changes of carbon resources in electrolyte concentration influence the microbial diversity (Lee K.E CBA International 1991). The effect of soil structure and environmental conditions on microbial diversity has been reported by Torsvik and Ovreas (Torsvik 2002:240-245). It has been reported that, the population composition and the activity of microorganisms are largely regulated by soil physico-chemical properties (Mishra 1996:117-123). Similarly, it has also been reported that changes in soil environment like soil moisture, pH and temperature attributed indirectly by plant characteristics will affect the soil microbial diversity and composition (Angers (1998):55-72, Hooper 2000:1046-1061). Fierer and Jackson (Fierer 2007:7059–7066) have reported the occurrence of high bacterial diversity in neutral soil and lower in acidic soils. Fierer and Jackson (Fierer 2006:626-631) have observed and reported that, the soil pH as a best predictor of bacterial richness. They have also observed some correlation between soil properties including soil moisture, organic carbon content, apart from this; they have also stated the existence of the strong correlation between soil pH and microbial community. In the present study, the least pH value was recorded (pH 6.2) and which lies between the optimal values (6,0-7,5 pH) for microbial growth (Atlas 2005). Soil microbial biomass can be limited by soil moisture under both dry and wet conditions (Rinklebe 2006:2144-2151). Soil microbial biomass C differed seasonally, and this pattern may be attributed to different soil humidity (Silva 2012:257-261) or temperature (Rodrigues 2015:41-48). The information on changes in soil microbial biomass following vegetation removal is valuable, not only because it slower pro-

vides an indication, less easily detects soil organic matter changes (Powlson 1987 :159–164), but also because it represents an important labile pool of plant available nutrients (Jenkinson 1981:415–471) and plays an active role in nutrient conservation in the tropical soils (Singh 1991:869–878) by preventing nutrient leaching (Vitousek 1984:51–52). In this content disturbance is an environmental event that is constrained in time but may have a lasting positive or negative impact on microbes. Full understanding of how soil microbial community abundance and functional relationships is **regulated and it will ultimately require analysis at a multitude of spatial and temporal scales. Understanding of microbial community spatial reorganization after severe disturbance will also require analysis of reclamation sites of different ages.** In addition, spatial relationships of soil biotic and abiotic components in arid soils can change rapidly over time (Mummey 1997:1699–1706), therefore analysis at no single time point can be expected to fully elucidate these relationships. Future studies may examine the site as it continues to develop. Continued microbial testing in the future may lend insight into the dynamics of recovery. While the data indicates recovery of the microbial community, may not depict recovery the entire ecosystem. Measurements of organic carbon, manganese, microbial respiration rates, and plant activity alongside microbial community quantification may depict a full picture of soil recovery.

### Conclusion

The results obtained by other studies indicate that a greater degree of disturbance would be necessary to cause major shifts in microbial diversity and structure for the soil tested in this work. This disturbance may involve changes in soil features, such as physical and chemical degradation, soil pH, nutrient depletion and pollution. The results suggest the prevalence of a resilient microbial community less influenced by plant cover in which the history of land use might influenced present day community structure. In this study we compare microbial community composition and biomass from 4 locations at a regional dominated site at the city of Karagandy, Kazakhstan. This study reported the characterization of microbial communities in the disturbed soils. Richness of microorganisms was relatively higher in coal-contaminated soil than other disturbed soils, i.e. the number of 21 species was obtained from all studied samples. Microbial groups named as AKCS1, AKCS2, ASP1,

ASPS2, ADFS4, ADFS5, AAPS3, and AAPS6 were the dominant species among all the soil samples. This research provides some useful data for bioremediation of coal-contaminated soil using coal-solubilizing bacteria.

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