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DIVERSIDADE MICROBIANA DE SOLOS CONTAMINADOS NOS CAMPOS PETRÓLÍFEROS DO CAZAQUISTÃO

MICROBIAL DIVERSITY OF THE CONTAMINATED SOILS IN KAZAKHSTAN OILFIELDS

МИКРОБНОЕ РАЗНООБРАЗИЕ НЕФТЕЗАГРЯЗНЕННЫХ ПОЧВ МЕСТОРОЖДЕНИЙ КАЗАХСТАНА

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ABSTRACT

O petróleo e seus derivados afetam adversamente a biodiversidade dos microorganismos e a função do solo. Em solos contaminados com óleo, desenvolvem-se comunidades bacterianas únicas, adaptadas à poluição. Neste trabalho, a estrutura bacteriana e a diversidade da comunidade microbiana foram estudadas em amostras de solos contaminados com óleo nos depósitos do Cazaquistão usando o seqüenciador Illumina MiSeq. Os resultados do estudo mostraram que os representantes dos seguintes filos bacterianos dominaram nas amostras de solo selecionadas: Proteobactérias, predominantes em solos contaminados com óleo (até 48%), Actinobactérias (até 29,33%), Firmicutes (até 25,74%), Bacteroidetes (até 33,28%). Os representantes dos filos de Planctomycetes, Verrucomicrobia, Chloroflexi (0,76% -4,62%) foram encontrados em menor quantidade. Todos os solos não contaminados foram dominados pelas famílias Micrococcaceae, Flexibacteraceae, Sphingomonadaceae, Planococcaceae, Flavobacteriaceae, contaminados pelas famílias Halomonadaceae, Flavobacteriaceae, Alteromonadaceae, Dietziaceae, Pseudomonadaceae, Bacillaceae, Peptomonadáceae, Bacillaceae. No nível de gênero, amostras de solos não contaminados e contaminados também demonstraram diversidade significativa. Os gêneros bacterianos dominantes nas amostras do solo não contaminado foram Hymenobacter, Arthrobacter, Gillisia. Em solos contaminados de três depósitos, os microrganismos dos gêneros Halomonas, Marinobacter, Pseudomonas (principalmente em amostras de solo 2KO), Bellilinea e Mycobacterium (principalmente amostra Md) se espalharam mais amplamente; e uma população muito grande de microrganismos do gênero Halomonas foi encontrada na amostra de solo contaminado da região de Atyrau. Uma comparação da estrutura taxonômica das comunidades microbianas de solos contaminados com óleo indica que a composição da população microbiana muda dependendo do grau de poluição por óleo. Amostras de solos não contaminados foram caracterizadas por maior diversidade bacteriana do que amostras de solos contaminados. Os microrganismos pertencentes aos filos dominantes foram principalmente associados à decomposição de hidrocarbonetos oleosos. A caracterização das comunidades bacterianas que vivem nos solos contaminados e a avaliação de sua capacidade de decompor o óleo podem ser potencialmente um guia para a biorremediação de solos contaminados.

Keywords: *metagenômica, solo contaminado com óleo, comunidade microbiana.*

ABSTRACT

Oil and oil products adversely affect both the biodiversity of the microorganisms and the soil function. In oil-contaminated soils, unique bacterial communities develop that are adapted to pollution. In this work, the bacterial structure and diversity of the microbial community have been studied in samples of oil-contaminated soils in Kazakhstan deposits using the Illumina MiSeq sequencer. The results of the study showed that the representatives of the following bacterial phyla dominated in the selected soil samples: Proteobacteria, prevailing in oil-contaminated soils (up to 48%), Actinobacteria (up to 29.33%), Firmicutes (up to 25.74%), Bacteroidetes (up to 33.28 %). The representatives of Planctomycetes, Verrucomicrobia, Chloroflexi (0.76% -4.62%) phyla were found in smaller amounts. All the uncontaminated soils were dominated by Micrococcaceae, Flexibacteraceae, Sphingomonadaceae, Planococcaceae, Flavobacteriaceae families, contaminated ones – by Halomonadaceae, Flavobacteriaceae, Alteromonadaceae, Dietziaceae, Pseudomonadaceae, Bacillaceae, Xanthomonadaceae, Anaerolinaceae, Mycobacteriaceae and Peptococcaceae families. At the genus level, samples of uncontaminated

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and contaminated soils also demonstrated significant diversity. The dominant bacterial genera in the samples of the uncontaminated soil were Hymenobacter, Arthrobacter, Gillisia. In contaminated soils of three deposits the microorganisms of the Halomonas, Marinobacter, Pseudomonas (mostly in 2KO soil sample), Bellilinea and Mycobacterium (mostly Md sample) genera were spread more widely; and a very large population of the microorganisms of the Halomonas genus was found in the contaminated soil sample from the Atyrau region. A comparison of the taxonomic structure of microbial communities of oil-contaminated soils indicates that the composition of the microbial population changes depending on the degree of oil pollution. Samples of uncontaminated background soils were characterized by higher bacterial diversity than samples of contaminated soils. The microorganisms belonging to the dominant phyla were mostly associated with the decomposition of oil hydrocarbons. The characterization of the bacterial communities living in the contaminated soils and the assessment of their ability to decompose oil can potentially be a guide for bioremediation of contaminated soils.

Keywords: *metagenomics, oil-contaminated soil, microbial community.*

АННОТАЦИЯ

Нефть и нефтепродукты отрицательно влияют как на биоразнообразие микроорганизмов, так и на функцию почвы. В нефтезагрязненных почвах развиваются уникальные бактериальные сообщества, адаптированные к загрязнениям. В данной работе исследована бактериальная структура и разнообразие микробного сообщества в образцах нефтезагрязненных почв месторождений Казахстана методом секвенирования основанного на технологии Illumina с использованием прибора MiSeq. Результаты исследований показали, что в отобранных образцах почв доминировали представители типов: *Proteobacteria,* преобладающие в загрязненных нефтью почвах (до 48%), *Actinobacteria* (до 29,33%), *Firmicutes* (до 25,74%), *Bacteroidetes* (до 33,28%). В меньшем количестве были обнаружены типы *Planctomycetes, Verrucomicrobia*, *Chloroflexi* (0,76%-4,62%). *И*зучение микробоценоза на уровне семейства и рода показало, что в не загрязненных почвах доминировали семейства *Micrococcaceae*, *Flexibacteraceae*, *Sphingomonadaceae*, *Planococcaceae*, *Flavobacteriaceae,* в загрязненных же почвах - *Halomonadaceae, Flavobacteriaceae, Alteromonadaceae, Dietziaceae, Pseudomonadaceae, Bacillaceae, Xanthomonadaceae, Anaerolinaceae, Mycobacteriaceae u Peptococcaceae.* На уровне рода в образцах незагрязненной и загрязненной почв разнообразие значительно отличалось. Доминантными родами в образцах чистой почвы были представители *Hymenobacter*, *Arthrobacter*, *Gillisia*. В загрязненных почвах трех месторождений более широкое распространение в образце почвы 2KO получили микроорганизмы родов: *Halomonas*, *Marinobacter*, *Pseudomonas*; в образце Md: *Bellilinea*, *Mycobacterium* и в образце At - *Halomonas*. Сравнение таксономической структуры микробных сообществ нефтезагрязненных почв указывает на то, что в зависимости от степени загрязнения нефтью изменяется состав микробной популяции. Более высокое бактериальное разнообразие наблюдалось в незагрязненных фоновых почвах по сравнению с загрязненными образцами. Микроорганизмы, относящиеся к доминирующим типам, связаны в большинстве своем с разложением углеводородов нефти. Характеристика бактериальных сообществ, живущих в загрязненных нефтью почвах, и оценка их способности разлагать нефть могут потенциально служить ориентиром для биоремедиации загрязненных сред.

Keywords: *метагеномика, нефтезагрязненная почва, микробное сообщество*

1. INTRODUCTION:

Oil is the most valuable raw material, without which modern civilization is impossible. However, the processes of its development, transportation, storage, and processing very often become the sources of environmental contamination and acquire a catastrophic scale. The share of hydrocarbon fuel is 2/3 of the world's energy consumption. The existing demand for oil and oil products annually increases on average by 8 %, and production — by 5.5 % (Diarov *et al*., 2006). The increasing contamination of the environment with oil and oil products leads to severe disruption in the natural ecosystems, biological balance, and biodiversity. The studies showed that soil properties, such as pH, the presence of water, air, and nutrients, the redox potential, the presence of organic substances affected the structure of bacterial communities (Wakelin *et al*., 2008). The abundance and diversity of the microbial communities are indicators of soil quality (Mathew *et al*., 2012).

Thus, the problem of soil contamination with oil poses a serious environmental threat to the environment and leads to disruption in the ecological balance, to degradation, and even to the desertification of these territories. Emergency oil spills often lead to the formation of technogeneous deserts, where the process of self-healing lasts for 10 – 25 years (Parkhomenko, Soprunova, 2008; Polyanskaya, Zvyagintsev, 2005). The oil biodegradation management should be, first of all, aimed at activating microbial

communities and creating the optimal conditions for their existence. In this regard, studying the microbial complexes in the contaminated soils is of scientific and practical interest.

In Kazakhstan, oil production is concentrated in the Caspian region, in the Atyrau and Mangistau regions, and in the south in the Kyzylorda region. The climate in these regions is sharply continental and arid. The soils are characterized as highly saline gray-brown soils, salt marches, and solonetzes. The development of oilfields leads to contamination of large areas and creates environmental problems that pose a threat to public safety.

Today, in terms of proven oil reserves, Kazakhstan is among the 15 leading countries in the world, with 3% of the world's oil reserves. Oil and gas regions occupy 62% of the country's area and have 172 oilfields, of which more than 80 are under development. More than 90% of oil reserves are concentrated in the 15 largest fields - Kumkol, Tengiz, Kashagan, Karachaganak, Uzen, Zhetybai, Zhanazhol, Kalamkas, Kenkiyak, Karazhanbas, Northern Buzachi, Alibekmola, Central and Eastern Prorva, Kenbai, Korolevskoye. Deposits are located in six of the 14 regions of Kazakhstan. These are Aktobe, Atyrau, West Kazakhstan, Karaganda, Kyzylorda, and Mangystau regions. At the same time, approximately 70% of hydrocarbon reserves are concentrated in the west of Kazakhstan. It should be noted that the Aktas deposit (Mangistau region) and the Zhanatalap deposit (Atyrau region) are not the largest oil and gas deposits.

The Atyrau region possesses the most explored oil reserves. On its territory, more than 75 oilfields with industrial reserves of 930 mln t are discovered. The largest oilfield in the region is Tengiz (initial recoverable reserves are 781.1 mln t). The region ranks first in oil production in the Republic. The Zhanatalap oil and gas deposit was discovered in 1968 and is located in the Isataevsky district of the Atyrau region, 85 km west of Atyrau. The field is located in a very dry, hot, agroclimatic area. The climate is sharply continental, arid, with manifested large annual and daily amplitudes of air temperatures. Low-sulfur oil constitutes 0.12-0.38%, low-paraffin oil – 1.31-1.01%. The operating mode of the reservoir is water driven. Oilfield water contains calcium chloride, has a density of 1,177-1,184 kg/m^3 and mineralization of 277.4-289.2 g/l.

Kumkol is an oil and gas deposit in the Kyzylorda region of Kazakhstan. The total area of the Kumkol field is 23,143 ha. This field referred to the Turanian oil and gas province and was discovered in February 1984. Kumkol is located 150-170 km north of the city of Kyzylorda. Hydrocarbon deposits are located at a depth of 0.9 - 1.4 km. The initial flow rate of the wells is 20 - 130 t/day. The density of oil is 0.81-0.83 g/cm^3, the sulfur content is 0.11 - 0.52%, paraffins constitute 10.8-15.5%, asphaltenes content ranges from 0.11-0.92% to 5.7%, resins content – from 4.8% to 19.8%. Recoverable oil reserves at Kumkol field amount to 130 mln t; that of gas – to 15 bln m^3 (Bulekbaev *et al.,* 1996).

In the Mangistau region, over 70 fields have been discovered with recoverable oil reserves of 725 mln t, as well as reserves of condensate equaling to 5.6 mln t. Less than half of the fields are in operation. Most of them are in the late stages of development. The vast majority of residual reserves are classified as hard to recover. The largest deposits are Aktas, Uzen, Zhetybai, Kalamkas, Karazhanbas. The Aktas oil and gas condensate field is located in the Mangistau region, 85 km southeast of Aktau. The oils of productive horizons are heavy (horizons IV, X, XI) and very heavy (horizon III), with a density of 0.87-0.915 g/cm3, low content of sulfur (0.2%) and high content of paraffin (20% to 26.14%). The asphaltenes content varies from 2.33 to 5.6%, and silica gel resins content is within the range of 4.54 - 6.8% (Portnov, Petrov, Talerchik, 2015).

The research methods based on microbial cultivation does not provide complete comprehensive knowledge of the microbial community since most microorganisms do not grow on conventional culture media, and researchers deal only with a small fraction of the real soil microbiota (Wolińska, 2019). The widespread use of the molecular methods in studying the ecology of the microorganisms in the contaminated areas provides extraordinary opportunities for new bioremediation strategies. With the introduction of the new-generation sequencing methods in molecular ecology, it has become possible to increase the number of identified types of microorganisms (Ma *et al*., 2015; Chakraborty *et al*., 2014).

To comprehensively assess the potential of biodegradation in the contaminated area and the changes in the structure and functional activity of the microbial communities involved in bioremediation, we used the Illumina MiSeq method of high-throughput sequencing. This allowed obtaining a more comprehensive and updated vision of the microbial community in the studied samples.

The present work was aimed at studying the bacterial composition of the contaminated soils in the oilfields of Kazakhstan, compared to the uncontaminated soils, for understanding the dynamics of the bacterial community development using the new generation high-throughput sequencing.

2. MATERIALS AND METHODS:

2.1. Experimental site

Six soil samples from three regions of Kazakhstan were selected in sterile plastic containers for the analyses according to state standards 17.4.4.02-2017 (GOST 17.4.4.02-2017, 2017). Soil samples were taken sterile layer by layer from the depth of 0-5 and 5-20 cm by the envelope method. Each combined sample consisted of three-point samples weighing from 200 to 250 g of soil each. Samples were taken under aseptic conditions: they were taken with a sterile metal spatula and placed in sterile plastic containers. Then the samples were packed to the cooler bags at 4 ºC and immediately delivered to the laboratory.

Two samples were taken from the Atyrau region, the Zhanatalap oilfield: sample **At** — contaminated with oil, sample **Pl2** — from the clean area (following the WGS84 system): latitude: 44° 51' 10" N (44.8528), longitude: 65° 30' 33" E (65.5092). Two samples were taken from the Kyzylorda region, the **Kumkol** oilfield: **1KO** — uncontaminated, **2KO** — from the contaminated area (WGS84): latitude: 44° 51' 10" N (44.8528), longitude: 65° 30' 33" E (65.5092). Two samples were taken from the Mangistau region, the Aktas oilfield**: MCl** — uncontaminated, **Md** — from the contaminated area (WGS84): latitude: 43° 39' 0" N (43.65), longitude: 51° 12' 0" E (51.2).

In the area of each oilfield, soil samples were taken at various distances from the oil production zone. Directly from the oilfield, a sample was taken for obtaining soil with a high degree of contamination, and for control (clean, uncontaminated soil, background), a sample was taken from the territory of a settlement 7 km away from oil production. The soil samples were taken from the depth of 20 cm using the method of sampling from five points in three repetitions. One part of the soil was combined in a sterile bag and delivered to the laboratory with a refrigerant for studying the bacterial diversity. The other part was used for determining the oil content. Prior to the analysis, the delivered soil samples had been stored at -20°C. Before the analysis, all samples had passed through a 2 mm sterile sieve for removing large particles and foreign objects.

2.2. Determination of oil content in the soil samples

The quantitative content of oil in the soil was determined by the gravimetric method (Lurie, 1984). For this purpose, 10 g weighed samples of the soil were made. They were put into funnels with filtering paper and poured with chloroform until the extract became colorless. The chloroform oil extract was collected into tared vials and naturally evaporated in a fume hood for 24 hours. The residue was weighed on an Explorer analytical weigher (Ohaus, USA). For each soil sample, three weighed samples were made.

2.3. Parameters of the oil from the Kumkol, Zhanatalap, and Aktas oilfields

The specific weight of the oil from the Zhanatalap oilfield was 0.87 g/cm^3, the density at 20°C was 0.8437 g/cm^3, the viscosity at 0 ºC was 36.4 - Pa • c. The content of paraffin was 1.31 – 1.01 %, of sulfur — 0.12 %, of asphaltenes — 0.02 %, of silica gel resins — 5.62 %, and of sulfate resins — 6 %. The oilfield water was calcium-chloride with a density of 1,177 – 1,184 kg/m^3 and the mineralization of 277.4 – 289.2 g/l. The oil in the productive horizons of the Aktas oilfield was viscous, with a density of 0.87 – 0.91 g/cm^3; it was low-sulfur (0.2 %), and high-paraffin (20 – 26.14 %). The content of asphaltenes was 2.33 – 5.6 %, and of silica gel resins — 4.54 to 6.8 %. The Kumkol oil was low-viscosity and low-sulfur, relatively light (0.81 – 0.83 g/cm^2), contained many light fractions, and was distinguished by the absence of harmful impurities. The content of paraffin was 15 %, of sulfur — 0.27 %, of asphaltenes — 5.4 %, and of silica gel resins — 19.2 %. The mineralization of water (calcium chloride) in the horizons was 49.7 – 84 g/l (Nadirov, 1995).

2.4. Parameters of the soils in the oilfields

The soils of the Kumkol deposit are characterized as gray-brown desert sandy-clay soils, the soils of Aktas deposit of the Mangystau region are gray-brown solonetzic soils, and the soils of Zhanatalap deposit of the Atyrau region are coastal meadow solonchak soils. The amount of humus in the upper soil horizon of Kumkol deposit is 0.9%, of Zhanatalap deposit -1.7%, of Aktas deposit - 1.1%. The soils of all deposits are characterized by a sulfate-chloride type of salinization. The establishment of a taxonomic affiliation (type, subtype, genus) of soils was carried out by the employees of the U.U. Uspanov

Kazakh Research Institute of Soil Science and Agrochemistry in accordance with the methods (Gavrilyuk, 1959) and the regulatory requirements approved for Kazakhstan (Scientific and methodological guidelines for monitoring the land of the Republic of Kazakhstan, 1994). The research was based on the comparative geographical method, which consisted of comparing some soils with others taking into account the conditions of soil formation. A method of obtaining the actual material was field soil studies with subsequent processing and generalization of the results of laboratory chemical analysis of the selected samples.

At the stage of conducting field studies, morphological methods were used (Rozanov, 2004), which ensured the reliability and validity of field soil diagnostics and characteristics of the main morphological properties of soils. The location of the soil section for the characterization of background soils was selected, taking into account the most typical natural conditions. Field soil studies included the laying of soil sections, then their description was made, and soil samples were taken. The location of the soil section for the characterization of background soils was selected, taking into account the most typical natural conditions. The depth of soil sections was determined by the depth of soil formation processes.

The description of the soil section included:

- location and number;
- characteristics of the terrain and underlying rocks;
- the position of the section relative to the macro-, meso- and microrelief;
- the depth of boiling, the form of carbonate emissions;
- characteristics of the soil surface;
- description of genetic horizons.

The selection and description of genetic horizons were carried out according to the following morphological indicators (Gavrilyuk, 1959; Rozanov, 2004):

- horizon thickness;
- color and character of coloring;
- moisturizing;
- structure;
- mechanical composition;
- density;
- porosity;
- allocation of water-soluble salts and other newly formed soil structures;
- the presence and type of inclusions;
- development of the root system of plants;
- the nature of the transition of one horizon to another.

Samples for analysis were taken at the most typical sections characterizing the soil cover of the territory to establish the basic chemical and physicochemical properties of soils. Sampling was carried out over genetic horizons, in a 10-cm layer in their center.

The taxonomic determination of the soil was made on the basis of the description of the soil sections in accordance with the generally accepted classification (Egorov, Ivanova, Friland, 1977) and the systematic list of soils previously developed for the study area (Soils of the Kazakh SSR: Guryev region, 1970; Soils of the Kazakh SSR: Soils of the Kyzylorda region, 1983).

The involvement of regional classifications was due to the fact that they were developed according to the results of extensive systematic soil studies of Kazakhstan; monographs were published for each region, accompanied by soil maps with the 1:300,000 scale where soil diagnostic indicators (morphological and chemical) were determined (Soils of the Kazakh SSR: Guryev region, 1970; Soils of the Kazakh SSR: Soils of the Kyzylorda region, 1983).

To obtain the actual material, field soil studies were carried out with subsequent processing and generalization of the results of laboratory chemical analysis of the selected samples. Organic matter (humus) was determined by the method of Tyurin. The method is based on the oxidation of organic matter by a solution of potassium dichromate in sulfuric acid and the subsequent determination of trivalent chromium equivalent to the content of organic matter. To assess soil salinization, anions (CO32-; HCO3 -; Cl-; SO42-) and cations (Ca2 +; Mg2 +; Na +; K +) of highly soluble salts were determined. The degree of soil salinization was estimated by the total content of highly soluble salts in the water extract of the soil (GOST 26213-91, 1991). It is known that saline soils include soils in which the concentration of highly soluble salts in soil solutions exceeds 5-7 g/l, i.e., soils containing 0.05-1.15% of highly soluble salts depending on their composition.

2.5. DNA isolation, amplification, and sequencing on MiSeq Illumina

Two hundred and fifty milligrams of the obtained soil samples were taken for DNA isolation. The general genomic DNA was isolated using a NucleoSpin® Soil kit (Macherey-Nagel GmbH & Co.KG, Duren, Germany), following the manufacturer's guidelines. The DNA concentration was measured on a Qubit® 2.0 fluorimeter using a Qubit™ds DNA HS Assay Kit (Life Technologies, Oregon, USA).

The genetic libraries were prepared for sequencing following the *16S Metagenomic Sequencing Library Preparation* guide (part no. 15044223 rev. A). Each DNA sample was amplified using the KAPA HiFi Hot Star Ready Mix (KAPA Biosystems, Cape Town, South Africa). The PCR amplification was made in Eppendorf Master ProS thermal cycler (Eppendorf AG, Hamburg, Germany). The V3 and V4 regions of the 16Sr RNA gene were amplified using universal bacterial primers with the addition of the Illumina adapters and contained the following sequences of the nucleotide pairs: 5′-TCGTCGGCAGC GTCAGATGTGTATAAGAGACAGCCTACGGGN GGWGCAG-3′ for the forward primer, and 5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGA CAGGACTACHVGGGTATCTAATCC-3′ for the reverse primer (Klindworth *et al*., 2013).

The reaction mixture consisted of 2.5 µl of the DNA template, 5 µl of each primer in the concentration of 1 µM, and 12.5 µl of KAPA HiFi Hot Start Ready Mix in the 2X concentration. The amplification cycles were performed according to the following program: one cycle at 95 °C for three minutes, followed by 25 amplification cycles at 95 °C for 30 seconds, one cycle at 55 °C for 30 seconds, one cycle at 72 °C for 30 seconds, and one cycle at 72 °C for five minutes. The PCR product was purified using an Agencourt AM Pure PCR purification kit (Beckman Coulter Inc. Beverly, Massachusetts, USA). Nextera XT Index primer adapters (Illumina Inc., San Diego, CA, USA) were added to each sample through the amplification in the reaction mixture containing 12.5 µl of KAPA HiFi Hot Start Ready Mix, 5 µl of each index primer, 10 µl of water, and 5 µl of the PCR product. The amplification program included one cycle at 95 °C for three minutes, followed by eight cycles of amplification at 95 °C for 30 seconds, one cycle at 55 °C for 30 seconds, one cycle at 72 °C for 30 seconds, and one cycle at 72 °C for five minutes. The PCR product with added indices was also purified using an Agencourt AM Pure PCR purification kit (Beckman Coulter Inc. Beverly, Massachusetts, USA).

At each stage of preparing the libraries after the amplification, the concentration and the size of the obtained PCR products were determined through their detection in the agarose gel and on Agilent 2100 bioanalyzer (Waldbronn, Germany) using an Agilent DNA 1000 Kit (Agilent Technologies, Waldbronn, Germany). Each sample was diluted to a concentration of 4 nM and combined into a single pool. The pooled libraries were denatured with NaOH and diluted with the hybridization buffer. The pooled libraries were sequenced on Illumina MiSeq device using a 600-cycle MiSeq® Reagent Kit v3 (Illumina Inc., San Diego, CA, USA), following the manufacturer's recommendations.

3. RESULTS AND DISCUSSION:

3.1. Oil contents in the soil samples

The analysis showed that the oil content in the contaminated soil from the Kumkol oilfield was 7.5 %, from the Zhanatalap oilfield — 7.3 %, and from the Aktas oilfield — 7.8 %.

3.2. The 16S rRNA metagenomic analysis of the soils from the oilfields in Kazakhstan

3.2.1. The changes in the composition of the bacterial communities at the phylum level

Microorganisms in the soil play a key role in various biogeochemical processes, and some functionally significant microbial groups can be especially vulnerable to oil spills (Urakawa *et al*., 2012; Rodriguez *et al*., 2015). Petroleum hydrocarbons can directly influence the microorganisms, exerting a toxic effect (especially the polyaromatic ones), or changing the physicochemical properties of the soil (reducing the availability of mineral nutrition elements, deteriorating the water and air conditions, deterioration of the nitrogen regime of soils and a decrease in the content of mobile forms of phosphorus) (Voevodina, Rusanov, Vasilchenko, 2015; Liao, Wang, Huang, 2015). As a result of oil ingress, the total population and the structure of the microbial community change. Its composition and the degree of diversity depend both on the type, concentration, and duration of exposure to the pollutant, and on the type of the soil and the state of the microbiocenosis before the ingress of the pollutant (Kirienko, Imranova, 2015; Liao *et al*., 2015).

The structure of the microbial community was analyzed in three taxonomic categories — the

phyla, the families, and the genus. The structure of bacteriocenosis at the level of phylotypes allowed characterizing the processes in the contaminated samples. As a result of the studies, in considering the taxonomic structure of the soil microbiomes at the three deposits, the dominance of seven bacterial phyla was detected: *Proteobacteria, Actinobacteria, Bacteroidetes, Firmicutes*, *Planctomycetes, Verrucomicrobia,* and *Chloroffexi* (Tables 1, 2). All soil samples were dominated by the *Proteobacteria* (21.22 – 44.48 %)*, Actinobacteria* (9.66 – 29.33 %), *Bacteroidetes* (3.9 – 33.28 %), and *Firmicutes* (12.44 – 25.79 %) phyla. These are the phylotypes that almost always dominate both in the contaminated and uncontaminated soils (dos Santos *et al*., 2011). *Proteobacteria* are the largest group of bacteria that play an important role in the cycles of biogenic elements in nature, and are typical dominants in the contaminated soils, especially at the early stages of hydrocarbon degradation. Numerous studies showed that proteobacteria were the dominant group that decomposed PAHs (Winding, Hund-Rinke, Rutgers, 2005; Wu *et al*., 2016). The greatest number of the microorganisms of this phylogroup was found in the contaminated soil in the Atyrau (At — 44.48 %) and the Kyzylorda regions (2KO — 42.49 %) (Table 2). Their population was two times higher compared to the soils in the Mangistau region. The results were similar to those of other studies, where the *Proteobacteria* were the dominant type decomposing the PAHs found in the contaminated samples (Dörr de Quadros *et al*., 2016; Yadav *et al*., 2015).

The representatives of the *Actinobacteria* phylum were the next prevailing group in the analyzed samples. Actinobacteria are widely spread in the soil and water contaminated with oil, and can actively decompose diesel oil, n-alkanes, phenols, and PAHs (Zhang, Mortelmaier, Margesin, 2012).

As a rule, the microorganisms belonging to this phylum are well adapted to the environment with limited resources and do not show significant changes due to contamination with xenobiotics. The *Actinobacteria* phyla prevailed in the soil from the Mangistau region (MCl — 29.33 %, and Md — 23.67 %) (Tables 1, 2). According to some researchers, the *Actinobacteria* perform anaerobic degradation of cyclic and aromatic hydrocarbons at the later stages of oil biodegradation (Pineda-Flores *et al*., 2004). The next group in terms of the population were the microorganisms that were part of two phyla — *Bacteroidetes*, which prevailed in the soil samples from the Kyzylorda region: 1KO — 14.78 %, 2KO — 12.20 %, and in the sample of the uncontaminated soil Pl 2 (Atyrau region) — 33.28 %, and *Firmicutes*, the largest number of which was found in the contaminated soil from the Atyrau region and amounted to 25.79 % (Table 2). The representatives of these two phyla belong to the common component of the soil microorganisms and are present in all types of soils that are resistant to drying; therefore, in arid climatic conditions, they can maintain their population. As a rule, the bacteria in this group have a wide range of physiological adaptations, which allows them to occupy various ecological niches, while multi-enzyme systems ensure the utilization of various substrates as the sources of carbon and energy (Matishov *et al*., 2013). The largest number of bacteria of the *Chloroflexi* phylum (Md — 19.42 %) was noted in the contaminated soil from the Aktas oilfield in the Mangistau region. In other soil samples, their content was quite low and ranged from 0.68 % to 0.91 %. The representatives of the *Verrucomicrobia* (3.33 % – 3.88 %) and *Planctomycetes* (0.76 % – 4.62 %) phyla were present in the uncontaminated soils from all three oilfields (Table 1). *Verrucomicrobia* are extremely widespread in nature; they amount to 1 – 10 % of the soil microbiota and have an important ecological value, while the *Planctomycetes* phylum plays an important role in the nitrogen cycle, methanogenesis, and methylotrophy (Fuchsman *et al*., 2012). The content of other representatives of the phyla in the soil samples amounted to small fractions in the range of 0.26 – 1.05 %. The presence of the microorganisms belonging to the *Euryachaeota* phylum in the soils from the Zhanatalap oilfield and in the contaminated soil from the Aktas oilfield raised interest. Moreover, the highest content was found in the contaminated soil from the Zhanatalap oilfield (5.56 %); besides, the representatives of the *Synergistetes* phylum were found in the contaminated soil from the Aktas oilfield in the Mangistau region (6.06 %). It is known that the representatives of the *Euryachaeota* phylum are chemoautotrophic unicell organisms that play a significant role in the carbon and nitrogen cycles, and the *Synergistetes* phylum includes the gram-negative anaerobic microorganisms that are found in the soils from the areas of oil production.

3.2.2. The changes in the composition of the bacterial communities at the family level

At the family level, a complex community structure was observed (Fig. 1).

The comparison of the diversity of the families in the uncontaminated and contaminated samples from the three deposits showed that the contaminated soil from the Kumkol oilfield in the Kyzylorda region (2KO) was dominated by the *Halomonadaceae* (9.45 %), *Flavobacteriaceae* (8.94 %), *Alteromonadaceae* (5.37 %), *Dietziaceae* (3.59 %), and *Pseudomonadaceae* (5.04 %) families. The soil from the Aktas oilfield in the Mangistau region (Md) was dominated by the *Xanthomonadaceae* (4.90 %), *Anaerolinaceae* (18.85 %), *Mycobacteriaceae* (8.73 %), *Peptococcaceae* (5.27 %), and *Hydrogenophilaceae* (4.30 %) families. In the contaminated soil of the Zhanatalap oilfield in the Atyrau region (At), the *Halomonadaceae* (21.39 %), *Bacillaceae* (12.73 %), *Halobacteriaceae* (5.55 %), and *Alteromonadaceae* (4.15 %) families were identified.

It should be noted that the contaminated soils from all the oilfields differed in the diversity of the families. All the microorganisms belonging to the families named above were active oil destructors. In our opinion, the difference among the families in all the samples of the contaminated soils could be explained by the different fractional composition of the oils at all three oilfields. For example, the oil from the Aktas oilfield in the Mangistau region was heavy, with the density of 0.872 – 0.915 g/cm^3; the paraffin content in this oil was high (20 – 26.14 %), while the oil from the Zhanatalap and the Kumkol oilfields was slightly viscous, low-sulfur, and relatively light.

All the uncontaminated soils were dominated by the *Micrococcaceaee* (3.41 – 8.21 %), *Flexibacteraceae* (3.07 – 7.52 %), *Sphingomonadaceae* (4.75 – 4.82 %), and *Planococcaceae* (3.24 – 9.77 %) families. The last family was not found in the uncontaminated soil from the Kumkol oilfield; however, other families were found in this sample, such as the *Oxalobacteraceae* (3.72 %), *Isosphaeraceae* (3.58 %), and *Chitinophagaceae* (3.01 %) families. The *Halomonadaceae, Flavobacteriaceae, Rhodobacteraceae,* and *Verrucomicrobiaceae* families were identified in the uncontaminated soil from the Zhanatalap oilfield in the Atyrau region. The diversity of the families was higher in the uncontaminated background soils, both in terms of the qualitative and the quantitative composition. Most representatives of the *Pseudomonadaceae, Micrococcaceae, Mycobacteriaceae, Halomonadaceae, Flavobacteriaceae, Dietziaceae, Pseudomonadaceae, Rhodobacteraceae, Verrucomicrobiaceae, Bacillaceae,* and other families are directly involved in oil and petroleum hydrocarbons biodegradation. These families include hydrocarbon-oxidizing bacteria, such as *Arthrobacter, Pseudomonas, Mycobacterium, Rhodococcus*, and others, the species of which are active oil destructors in various soil types (Bogan *et al*., 2003; Nopcharoenkul, Netsakulnee, Pinyakong, 2013).

3.2.3. Changes in the composition of the bacterial communities at the genus level

The diversity of the microbial community at the genus level in the soils of all the oilfields revealed a large share of unclassified microorganisms (13.69 % – 26.87 %). According to the data in Figure 2, the dominant bacterial genera in the samples of the uncontaminated soil were *Hymenobacter* (1KO — 3.63 %), *Arthrobacter* (MCL — 6.43 %), and *Gillisia* (P2I — 16.4 %).

In the contaminated soils from all the oilfields, the microorganisms of the *Halomonas* (2KO — 8.11 %), *Marinobacter* (2KO — 5.26 %), *Bellilinea* (Md — 13.69 %), and *Mycobacterium* (Md — 8.73 %) genera were spread more widely, and a very large population of the microorganisms of the *Halomonas* genus (At — 20.32 %) was found in the contaminated soil from the Atyrau region. The increased content of the microorganisms of the *Halomonas* genus (20.32 % and 8.11 %), which dominated in the contaminated soils from the Atyrau and Kyzylorda regions, could be explained by the fact that the soils of these biotopes were salt marshes and solonetzes with a fairly high level of salinity. The representatives of this genus are characterized by growth in the presence of 5 – 25 % of NaCl.

The soils of the Aktas oilfield in the Mangistau region are dominated by the microorganisms of the *Bellilinea* genus (Md — 13.69 %), which belong to the *Chloroflexi* phylum, and by the representatives of the *Mycobacterium* genus (Md — 8.73 %). These microorganisms are relatively resistant to moisture deficiency and are widely spread in dry soils. They have a rich enzymatic apparatus, which allows mineralization of the poorly soluble organic substances, and are actively involved in the decomposition of nitrogen-containing and nitrogen-free organic substances in the soil. According to S. Y. Zhang, mycobacteria can efficiently decompose tetracyclic and pentacyclic aromatic hydrocarbons (Zhang, Wang, Xie, 2012).

The analysis of the microbial composition showed that at the genus level, the diversity in the samples of the uncontaminated and contaminated soils was significantly different. The microbial community was more diverse in the samples of the uncontaminated soil. It should be noted that there were more unclassified microorganisms in the uncontaminated soils compared to the contaminated soils. In considering the dominant genera in the contaminated samples, it is noteworthy that, along with the presence of the *Halomonas, Bellilinea, Pseudomonas, Marinobacter, and Mycobacterium* genera, which are active oil destructors in various soil types, representatives of the *Planomicrobium, Agromyces, Thiobacillus*, and other genera were also found in the samples.

4. DISCUSSION

Currently, there are many studies available on various bacterial communities and the diversity of the soils contaminated with oil at a different time (Yadav *et al*., 2015; Abed *et al*., 2014). The abundance and the diversity of the microbial communities are indicators of soil quality (Mathew *et al*., 2012; García-Orenes *et al*., 2013). Oil and oil products disrupt the ecological state of the soil cover and deform the structure of the biocenoses. Therefore, restoring the soil microbial communities is important because they are responsible for the physiological and metabolic processes that are of great importance for soil quality (Rutgers *et al*., 2016). Changing the species composition of some groups of microorganisms reveals a more definite relationship with the degree and the composition of oil pollution. A high degree of oil pollution may cause complete suppression of the growth and development of microorganisms. Upon the ingress of oil and oil products into the soil, microorganisms are involved in the process of their transformation, which should bring the soil system into equilibrium. The changes in the soil microflora may be used for biomonitoring and bioindication of oil pollution (Kolesnikov *et al*., 2007).

The metagenomic analysis showed that it was mainly formed by the representatives of seven bacterial phyla: *Proteobacteria, Actinobacteria, Bacteroidetes, Firmicutes*, *Planctomycetes, Verrucomicrobia,* and *Chloroffexi*, with *Proteobacteria* and *Actinobacteria* dominating in almost all microbiomes. The microorganisms belonging to the listed dominant types are mostly associated with the decomposition of oil hydrocarbons. An important indicator of soil health is the *Proteobacteria* phylum, the population of which increases markedly in disturbed soils. In analyzing heterogeneous extremal soil habitats, such as desert soils, permafrost, soils contaminated with oil products and other pollutants, these microorganisms, which, most likely, make up the core or the main part of the soil metagenome, may be detected. The absolute champions among such microorganisms are bacteria of the *Pseudomonas (Gammaproteobacteria), Arthrobacter (Actinobacteria), Sphingomonas (Alphaproteobacteria), Bacillus* (*Firmicutes*), *Rhodococcus* (*Actinobacteria*), *Flavobacterium* (*Bacteroidetes*), and some other genera (Chernov, 2016). All of them are common not only in extremal but also in all other types of soils.

Without enough information, it is difficult to comprehensively understand the structural characteristics of the soil bacterial community and the nature of the changes in its diversity. Therefore, the categories of family and genus were also considered in the analysis of the microbocenosis.

An analysis of the data about the diversity of the families in the contaminated soil from all oilfields showed that they were dominated by the *Halomonadaceae*, *Flavobacteriaceae*, *Alteromonadaceae*, *Pseudomonadaceae*, *Xanthomonadaceae*, *Anaerolinaceae, Mycobacteriaceae*, *Halomonadaceae*, *Bacillaceae, Halobacteriaceae*, and *Alteromonadaceae* families. All uncontaminated soils were dominated by the *Micrococcaceae*, *Flexibacteraceae*, *Sphingomonadaceae, Planococcaceae, Oxalobacteraceae, Isosphaeraceae, Chitinophagaceae*, *Halomonadaceae, Flavobacteriaceae, Rhodobacteraceae,* and *Verrucomicrobiaceae* families.

The above-listed microorganisms are widespread in the environment. Most representatives of the *Pseudomonadaceae, Mycobacteriaceae, Halomonadaceae, Flavobacteriaceae, Dietziaceae, Pseudomonadaceae, Rhodobacteraceae, Verrucomicrobiaceae, Bacillaceae*, and other families are directly involved in oil and petroleum hydrocarbons biodegradation. These families include hydrocarbon-oxidizing bacteria, such as *Arthrobacter, Pseudomonas, Mycobacterium, Rhodococcus,* and others, the species of which are active oil destructors in various soil types. Most of them are halotolerant.

As noted earlier, studying the

microbocenosis at the genus level showed a huge number of unidentified microorganisms. The comparative analysis showed that in the uncontaminated soils, the microbial community was more diverse than in the contaminated ones. While the contaminated soils were dominated by the bacteria of the *Halomonas, Marinobacter, Mycobacterium, Bellilinea* genera, in the uncontaminated soils from the oilfields, microorganisms of such genera as *Hymenobacter*, *Arthrobacter, Gillisia, Salinimicrobium, Planomicrobium, Planococcus,* and *Halomonas* were found. These data correlate with the studies of Lu Gan *et al*. (2018) and Sutton N.B. *et al*. (2013), who found that the presence of oil pollution had a significant effect on the structure and diversity of the bacterial communities, regardless of the type of the soil matrix. Moreover, it was proven that the uncontaminated soil samples were more diverse than the contaminated ones. Other authors, for example, Mu Peng *et al*. (2015) have shown an inverse correlation, when the microbial diversity is higher in the contaminated soils (Peng, Zi, Wang, 2015). Most likely, these kinds of discrepancies in interpreting the diversity of the microbial coenosis depend on many environmental factors (climatic conditions, soil type, temperature, pH, pollutant concentration).

A change in the population of various groups of microorganisms indicated that the soil microbiota was significantly modified upon contamination with oil hydrocarbons, and various groups of microorganisms reacted differently. The population of some of them increased, the population of some of them decreased, and that of the others remained almost constant. At present, it may be taken as the fact that oil pollution causes changes in the functioning of the soil microbiocenosis. The determination of the bacterial structure and the function in the contaminated soil is the basis for further studies aimed at identifying active bacterial strains in bioremediation.

5. CONCLUSIONS:

In this work, it was analyzed the bacterial diversity in the contaminated and uncontaminated soil from three oilfields in Kazakhstan, and it was presented that high bacterial diversity is observed in the uncontaminated background soils.

The characterization of the bacterial communities living in the contaminated soils and the assessment of their ability to decompose oil can potentially be a guide for bioremediation of the contaminated soils. Future studies are required for choosing the bacterial strains that are active in the degradation of various oil fractions, especially PAHs, which may be used for active management of the bioremediation processes.

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Table 1. *Comparative characteristics of the microbial composition of the uncontaminated soils (phyla)*

No.	Phylum	Kyzylorda		Mangistau		Atyrau	
		1KO		MCl		P2 I	
		%	Number of Reads	%	Number of Reads	%	Number of Reads
1	*Unclassified*	9.26	63.732	8.52	32.445	3.35	11.522
2	*Proteobacteria*	31.78	218.664	26.3	100.103	6.2	90.182
3	*Actinobacteria*	18.05	124.184	29.33	111.642	13.87	47.754
4	*Bacteroidetes*	14.78	101.681	10.39	39.555	33.28	114.563
5	*Firmicutes*	12.44	85.611	15.13	57.581	16.08	55.342
6	*Planctomycetes*	4.62	31.766	2.14	8.148	0.76	2.624
7	*Verrucomicrobia*	3.88	26.706	3.25	12.363	3.33	11.455
8	*Chloroflexi*	0.91	6.252	-	-	-	-
9	*Tenericutes*	-	-	1.05	4.010	-	-
10	*Euryachaeota*	-	-	-	-	1.13	3.885
11	*Synergistetes*	-	-	-	-	-	-

Table 2. *Comparative characteristics of the microbial composition of the contaminated soils (phyla)*

No.	Phylum	Kyzylorda		Mangistau		Atyrau	
		2KO (contaminated)		Md (contaminated)		At (contaminated)	
		%	Number of Reads	%	Number of Reads	%	Number of Reads
1	Unclassified	5.47	13.819	8.51	14.099	6.08	3.313
2	Proteobacteria	42.49	107.397	21.22	35.140	44.48	178.082
3	Actinobacteria	20.37	51.482	23.67	39.202	9.66	38.683
4	Bacteroidetes	12.20	30.841	3.9	6.458	3.99	15.963
5	Firmicutes	13.91	35.152	14.74	24.410	25.79	103.253
6	Planctomycetes	1.43	3.613	-	-	-	3.313
7	Verrucomicrobia	1.29	3.250	-	-	-	-
8	Chloroflexi	0.85	2.143	19.42	32.157	0.84	3.382
9	Tenericutes	-	-	-		-	-
10	Euryachaeota	-	-	1.25	2.071	5.56	-
11	Synergistetes	-	-	6.06	10.038	-	-

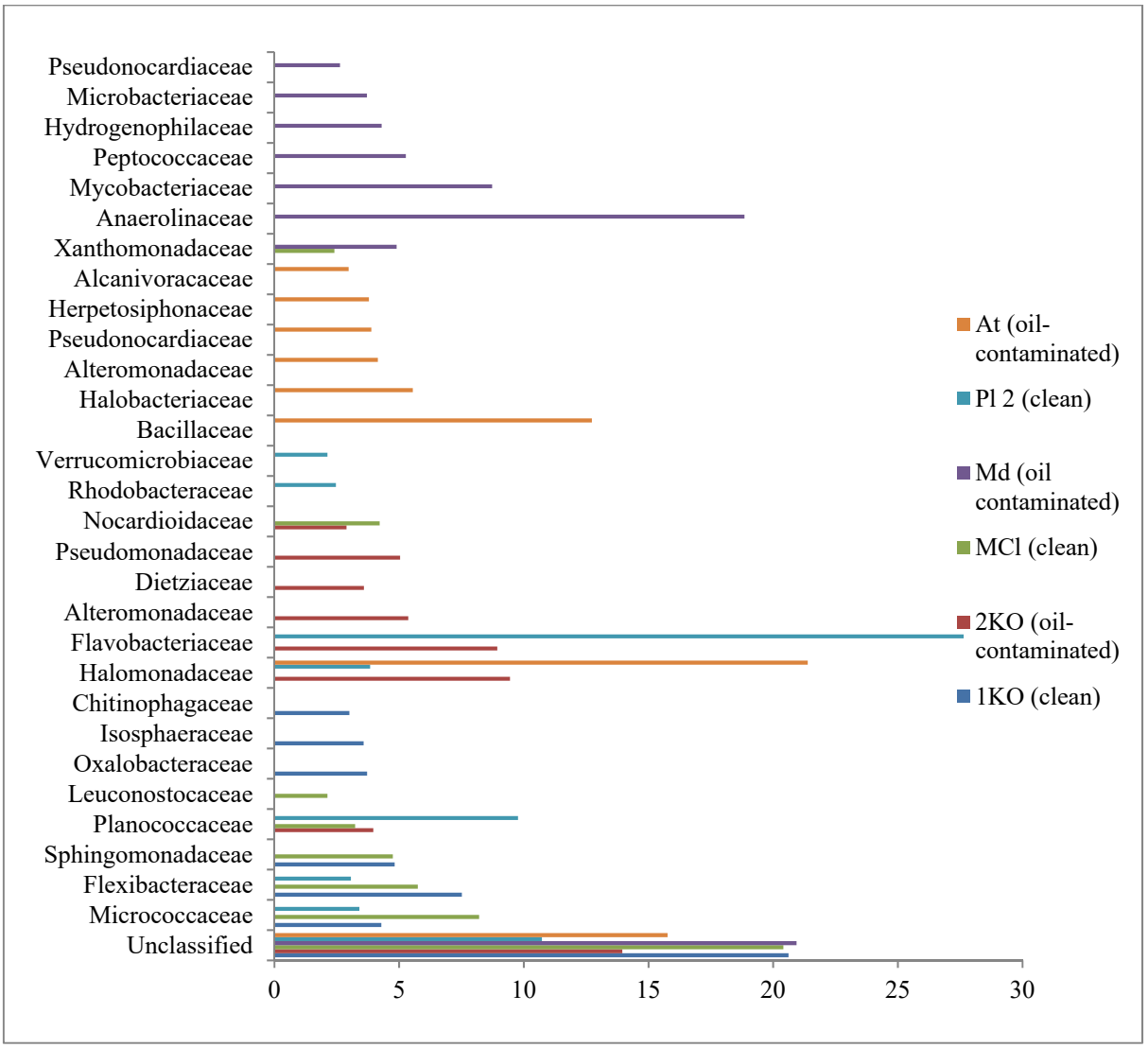

Figure 1. *Comparison of the relative population (% of the total sequence) of the main bacterial families found in the most dominant bacterial classes in the samples of the contaminated and uncontaminated soil*

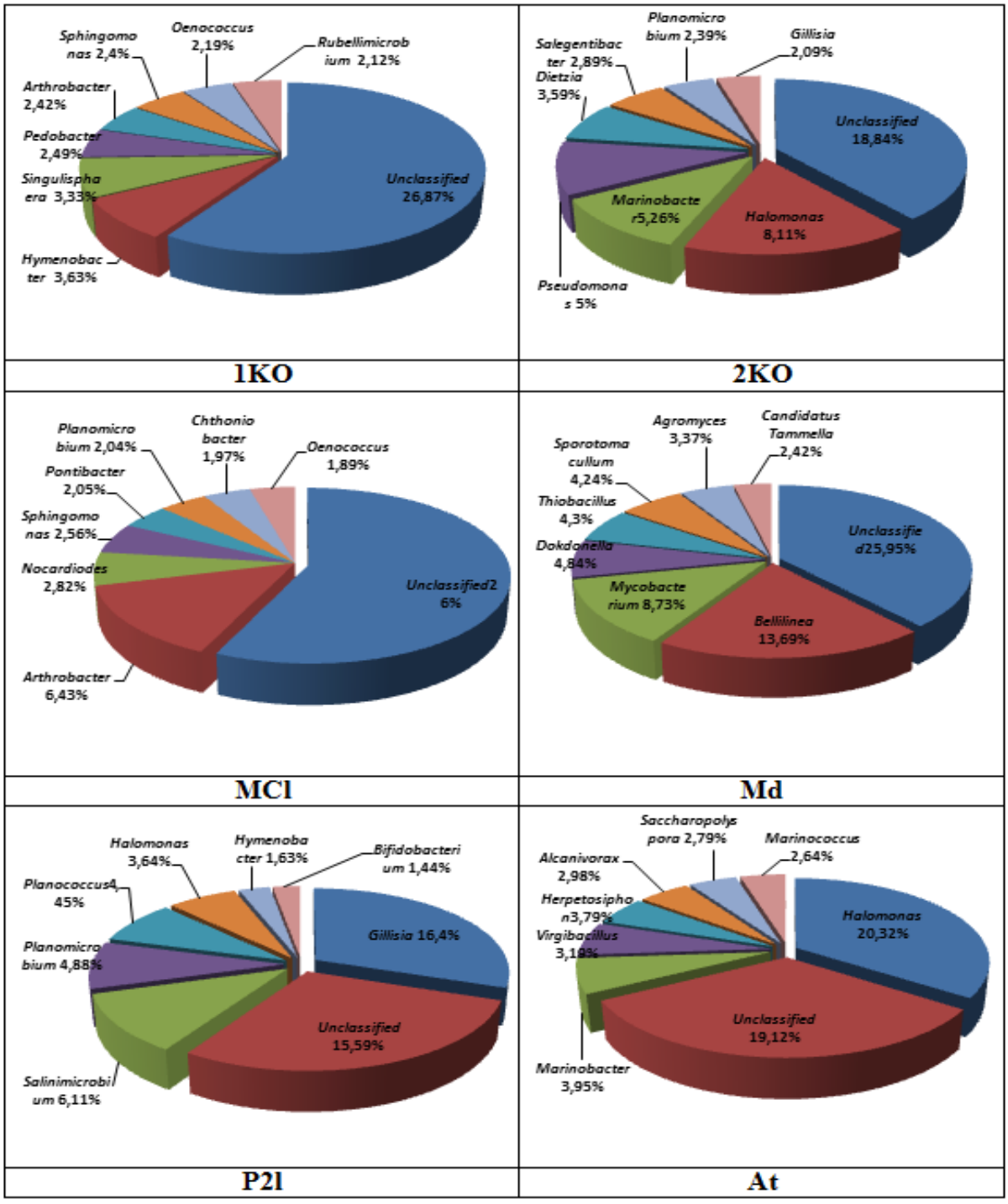

Figure 2. *The microbial diversity at the genus level.* ***1KO, MCl, P2L*** *are samples of the uncontaminated soil;* ***2KO, Md, At*** *are samples of the contaminated soil*