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Microbial Load as Ecotoxicological Assessment of Heavy Metals Presence in Soil Samples from the Kazakhstan Part of the Caspian Sea

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Abstract

Soil as a natural resource and a powerful regulator of matter flow, plays crucial role in providing habitat for proper structuring and functioning soil microbial communities. Under the influence of unregulated industrial activities with social-economic co-development, soil and water involved a whole range of changes leading to soil erosion-degradation and pollution of aquatic ecosystems. One of the most promising techniques for determining the total effect of exposure to heavy metals on environmental media is bio-indication (bio-testing), which is based on rapid, robust and cost-effective methods. Acquaintance, with the microbial background of soil is essential to assess the degree of soil pollution with heavy metals. In this study, an eco-toxicological assessment using microbial community characteristics on heavy metals in soil samples from the urban ecosystems of the Kazakhstan part of the Caspian Sea (Atyrau and Mangystau regions) has been discussed. According to the results of the soil toxicity, it has been established that the soil of these residential areas are exposed to increased levels of heavy metals, such as Cr, Co, Ni, Pb, etc. Comparative analysis of bio-indicative systems and chemical techniques for assessing the quality of soils indicated a remarkable similarity of the results and the priority (high speed, cheapness) of the microbiological load assessment of the soil quality.

1. Introduction

A high level of anthropogenic disturbances on terrestrial environments are especially meaningful in urbanized areas. At present, in settlements and rural districts, soil and water differ significantly from those of natural ecosystems. Constantly increasing negative impacts on soil and water lead to the migration of heavy metals into food chains, which poses a threat to the safety and health of the local population. Undoubtedly, anthropogenic pollution of natural environment dictates the need to develop new reliable, short-term and easily reproducible methods for assessing the toxicity of soils and water, which are the most important components of the environment.

The mining and processing industry is well developed in Kazakhstan and the growth rate is expected to be higher in the last few decades. Many industrial facilities and power plants are being integrated, adding to further pollution sources. Furthermore, billion tons of waste have been dumped into the water and soil over many years, creating a toxic time bomb. The bulk of this waste is the result of different industrial production, including ferrous metallurgy, petrochemicals and construction materials. Despite the fact that the government agencies are confronting this problem by developing programs aimed to correct and prevent pollution, the environmental issues in Kazakhstan leaves much to be desired [1].

The Caspian region of Kazakhstan is one of the biggest natural areas in the country with valuable biological resources and huge potential wealth in the form of minerals and natural raw materials.

Especially taking into account the peculiarities of its geographic location, the role of this region is of critical strategic importance not only in the economy, but also in the foreign policy of the republic. In the economic sphere, the region has huge

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prospects for development. However, currently, the Caspian region is experiencing a number of difficulties related to the negative impact of environmental problems, including the effects of sea-level rise, the outstanding issues pertaining to terrestrial and aquatic pollution, the continuing deterioration of the ecosystems, the disastrous reduction in biological diversity and other factors [2–3].

The complication of the ecological situation has a negative influence on the living conditions and the medical demography of the local population. Because of prolific hydrocarbon production and intensive transportation, as well as prospecting and exploration works on the Caspian shelf, the region is at risk of industrial accidents at oil and gas production facilities and the likelihood of large oil spills.

Enhanced environmental pollution due to heavy metals is also adversely affecting local soil ecosystems and population health. The main sources of heavy metal contamination are mining and processing plants, metalworking, chemical plants, vehicles, etc. The greatest risk of heavy metals is not apparent poisoning, but the fact that they are able to specifically accumulate in the cell. Numerous studies on biological systems of different levels of organization – from microorganisms to mammals – show that heavy metals have mutagenic and carcinogenic effects in addition to the general toxic effect [4–6]. In living organisms, heavy metals can persist for a long time and act as accumulative poisons. The concentration of these substances in the organism can be 100–1000 times higher than their concentration in the environment, which adversely affects the synthesis and functions of many biologically active compounds, such as enzymes, vitamins, hormones. Many metals form strong complexes with organic matter and therefore has a very high mobility and rate of uptake [7–9].

The general ecological health in the Caspian region is characterized by a combination of soil, air, surface and underground water pollution. Obviously, the critical environmental changes in the Caspian region threatens not only millions of inhabitants of coastal regions, but also the flora and fauna of the Caspian Sea. This demonstrates the challenge that deserves a more in-depth analysis about environmental protection, conservation, restoration and rational use of the Caspian's geological and biological resources.

The aim of this study is to perform ecotoxicological assessment based on microbial load response for detecting heavy metals in test soils from the Kazakhstan part of the Caspian Sea.

2. Experimental

2.1. Sampling area

The monitoring area covers the territory of the North and Southeastern parts of the Caspian lowland and includes the Mangystau and Atyrau regions (Fig. 1).

The climate of the regions is harsh continental due to the inland position and orography; the main features are the prevalence of anticyclonic conditions, extreme temperature changes throughout the year and day, a hard wind regime and a precipitation deficit. Soils are infertile and often salinized, mostly brown and gray-brown desert soils, takyrs (soil crusts) and desert sands. Most of the territory of Kazakhstan is located in the arid zone, and about 60% of its territory is vulnerable to varying degrees of desertification processes.

Fig. 1. The location and number of soil sampling points.

Table 1
Sampling location and soil type

Map pin	Names of localities	Region	Coordinates	Soil type
1	Atyrau city	Atyrau	47°11’21.2”N 51°54’48.8”E	Arid-steppe brown soil with solonchak (salt marsh)
2	Kulsary city	Atyrau	47°01’18.0”N 54°00’31.8”E	Semi-arid brown soil
3	Aktau city	Mangystau	43°38’29.3”N 51°11’51.2”E	Semi-arid gray and brown soils
4	Zhanaozen city	Mangystau	43°20’44.7”N 52°50’54.1”E	Arid-steppe brown soil
5	Inder district	Atyrau	48°34’29.8”N 51°44’56.2”E	Arid-steppe light brown soil

There is a complex, retrospective nature of pollution, i.e. industrial emissions in the study area, mostly polluted by heavy metals, smoke dispersion and petroleum products. The soil samples were collected from five human settlements in Atyrau and Mangystau regions (Table 1).

3. Materials and methods

The field trips were conducted to the sampling areas. Test sites were selected on the basis of uniform soil and vegetation cover, as well as the economic and technical feasibility. Point soil samples were collected on a test site from one or more layers/horizons, diagonally, so that each sample was part of the soil typical of genetic layers. Sampling of soil was carried out according to the procedure, which meets the requirements of ISO 10381-1:2002 and ISO 10381-5:2005. Point samples were taken with a sterile knife or spatula from the soil drill at a depth of 0–20 cm horizon in three replicates. The combined sample was made by mixing point samples taken at one test site. For the chemical analysis, the combined sample consisted of not less than three-point samples taken from one test site. The mass of the combined sample was not less than 2 kg. Point samples of the soil, intended for the determination of heavy metals, were selected with a tool that does not contain metals.

The determination of heavy metals was made using atomic absorption method (atomic absorption spectrophotometer “VarianSpectr”, model AA220 GTA 120 (Australia)) according to ISO 11047: 2008 (E); Soil quality: Determination of chromium, cobalt, nickel, lead, cadmium, copper, manganese, and zinc in Aqua Regia extract of soil.

For microbiological analysis, according to ISO/TS 29843-2:2011, soil samples were placed on clean polyethylene, where they were thoroughly mixed, freed from large inclusions and mechanical impurities (stones, debris), etc. The initially treated sample, weighing about 500 g, was placed in an aseptic plastic bag. Each sample was provided with a primary label on which the date of selection, place and serial number were indicated. The samples then were transported to the site of further analysis. During transportation and storage processes the samples were carefully handled to avoid secondary pollution.

Provision of statistical significance of the results obtained in the experiment and the value of correlation coefficient of the soil toxicity through microbial indication were implemented using the standard mathematical and statistical software packages, including Microsoft Excel 2016 and Minitab 2017.

4. Results and Discussion

Mainly soil samples from the monitoring settlements of Atyrau and Mangystau regions demonstrate Pb and Cd content within the normal range; however, exceeded MAC (Maximum Permissible Concentration) values for lead is observed in soil samples from the Aktau city. Exceeding MACs for Cr and Ni are typical for all samples, the concentration of Co in the samples is high as well, except for the soil samples from Kulsary city (Table 2).

In particular, soil samples from the Atyrau city show an excess of MPC for Cr (5.5–6.4), Co (1.4–1.9) and Ni (8.8–12.8). Samples of soil from the Kulsary city reveal an excess of MPC for Cr (1.8–2.0) and Ni (3.1–3.5). In soil samples from the Aktau city, an increased content of Cr (3.0–3.1 MPC), Co (1.2–1.4 MAC), Ni (4.6–4.85 MPC) and Pb (1.2–1.3 MPC) is observed. Samples of soil from Zhanaozen show the excess of MPC for Cr (4.1–4.5), Co (1.9–2.3 MAC) and Ni (8.3–8.9). Samples of soil from the nature protection zone (Inder district) are also highly contaminated with heavy metals: exceeding MAC by Cr (5.9–6.8), Co (2–2.1 MAC) and Ni (10.4–11.6).

Table 2
The content of heavy metals in soil samples from the monitoring settlements of Atyrau and Mangystau regions

Soil samples Replicates	Heavy metal concentration, mg/dm^3				
	Cr	Co	Ni	Pb	Cd
MPC	6.0	5.0	4.0	30.0	2.0
Point No.1 (Atyrau city)					
1.1	35.5	9.70	51.0	12.1	0.97
1.2	38.2	6.93	35.2	15.0	0.93
1.3	32.7	7.90	48.1	10.1	0.98
Point No.2 (Kulsary city)					
2.1	10.7	2.80	13.2	3.17	0.75
2.2	12.2	2.50	12.5	2.86	0.72
2.3	11.1	2.75	13.9	3.93	0.89
Point No.3 (Aktau city)					
3.1	18.7	6.53	18.4	39.6	1.76
3.2	18.0	5.78	19.4	35.8	1.34
3.3	18.2	7.03	18.8	39.4	1.70
Point No.4 (Zhanaozen city)					
4.1	24.6	9.62	35.8	18.3	1.38
4.2	27.1	11.44	33.3	21.1	1.34
4.3	24.9	9.65	35.7	18.0	1.39
Point No.5 (Inder district)					
5.1	40.8	10.4	46.4	11.1	1.15
5.2	35.2	10.0	41.8	10.7	1.18
5.3	40.5	10.8	45.9	11.5	1.10

Development and design of microbial test systems for soil quality assessment are conducted in a variety of ways, taking into account the parameters and characteristics of the specific type reaction of test microbes belonging to different physiological and ecological groups. In the set of model experiments [10–11], quantitative changes are observed in the composition of populations of soil microorganisms. The indicator for monitoring the effect of heavy metals is the total microbial number in samples. Particularly sensitive to anthropogenic influences are bacteria of various taxonomic groups. As is known, the response of bacteria to external factors, especially to heavy metals occurs comprehensively, as they are very susceptible to impacts related to various aspects of their life activity, i.e. growth, accumulation of chemical elements, activity of metabolic and physiological processes.

It is also important that the high intensity of the processes of growth and reproduction of microorganisms for assessing the various parameters of their growth and metabolism makes it possible to identify their response to any environmental factor of the changes in dynamics over many years.

The results of studies on the determination of soil microorganisms indicate that viable bacteria have been detected in all samples in a reliable amount (Fig. 2).

When comparing the quantitative composition of microorganisms during their cultivation on different nutrient media, the following peculiar properties are found:

- The number of bacteria in various soil samples ranged from 0.9 to 5.3 × 10^4 CFU/g, while for fungi 3.1–5.2 × 10^3 CFU/g;
- The maximum number of bacteria and fungi is revealed in the soil samples taken from the Kulsary city (Atyrau region);
- The minimum number of bacteria and fungi is observed in the soil samples from the Atyrau and Zhanaozen cities;
- Among the microorganisms, heterotrophic Gram-positive and Gram-negative bacteria dominated.

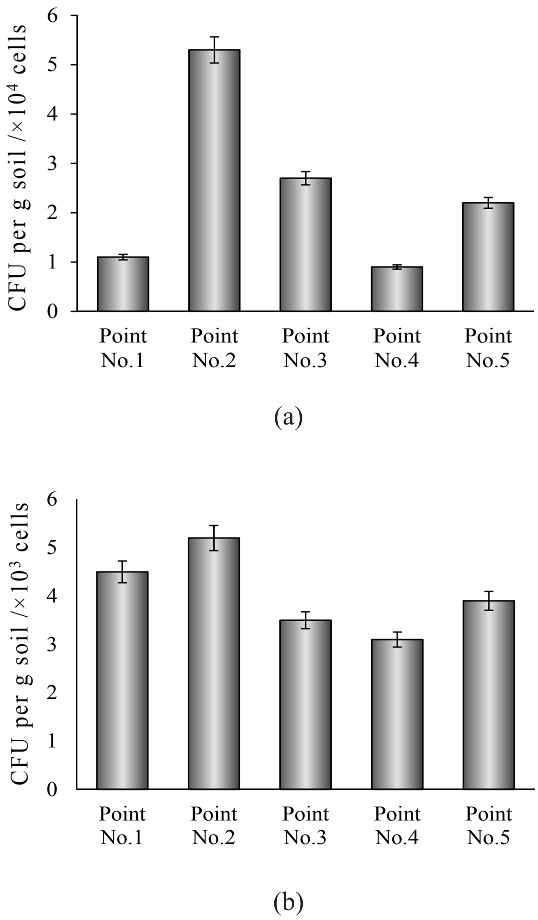

Fig. 2. The total number of microbial groups: (a) – bacteria; (b) –fungi in soil samples (CFU/g soil).

When heavy metals exceed certain levels in the soil the total biomass, as well as the number of individual physiological and taxonomic groups of microorganisms decrease [12]. The most sensitive microbial species are actinomycetes, many saprotrophic bacteria, some ammonifying bacteria, Azotobacter and yeast of the genus Lipomyces. The most resistant to the presence of heavy metal ions among all microorganisms are microscopic fungi, in particular representatives of the genera Mucor and Trichoderma [13, 14]. This specific ability of fungi has been studied by many authors and shown that it is related to their metabolism to accumulate heavy metals through physical and chemical pathways of uptake. In addition, the resistance of fungi to heavy metals is determined by several factors, such as the mechanisms of metal absorption, the role of each metal in normal metabolism, and the presence of genes localized in chromosomes or transposons that control resistance to metal ions [4, 12]. Thus, an increasing number of studies consider the abundance and species composition of microscopic fungi as an adequate indicator system in the environmental monitoring of contaminated soil by heavy metals [14, 15].

The toxic effect of heavy metals on bacterial communities is nonspecific; they are able to combine with proteins, nucleotides, coenzymes, phospholipids, porphyrins, i.e. practically with all types of substances involved in the metabolism of cells. In addition, interacting with groups of the active center or replacing individual ions of microbial enzymes, heavy metals cause inhibition of their activity.

A number of metals are highly toxic in small concentrations (Ag, Pb, Hg, Cd, etc.) and can have a deleterious effect on microorganisms and cause long-lasting changes. The greatest toxic effect on soil biota is established for Cd, the lowest for lead Pb [16]. However, according to our quantitative analysis on the heavy metals, the concentration of Cd in all soil samples was less than MAC, i.e. the adverse impact on the total number of soil microorganisms might be due to the high concentrations of Cr, Co, Ni and Pb.

In the biosphere, in natural nutrient cycling, only those microorganisms that are numerous and exhibit plentiful life activity are of ecological importance. For bacterial groups, the value of at least 1 million cells per gram of substrate (brown soil) is accepted as a conditional criterion for the amount, and only at such a number they can have the main ecological significance. For yeast and fungi, this value is 10 thousand per gram. However, in the tested soil samples, the number of bacteria did not exceed 10^4 CFU/g, which indicates that the microbiota in the studied region is relatively poor in its quantitative and qualitative composition. This, in turn, can negatively affect the processes of natural self-purification of soil ecosystems.

5. Conclusions

The use of indigenous microbial communities in assessing the quality of soils, tracking their vital activity is a promising method. Application this system for biomonitoring of pollution in soil is based on the possibility of assessing their response to the influence of heavy metals in natural and technologically altered conditions by changing the quantitative indices of their vital activity in dynamics. In this regard, the study aimed at determining such indicators of microorganisms have shown the similar results as chemical analysis.

A comparative analysis of microbial community of soil ecosystems in the Atyrau and Mangystau regions showed that the smallest number of the microbiocenosis was in samples from Atyrau and Zhanaozen cities.

In general, according to this chemical and microbiological study ecological situation of soils in the Caspian region is characterized by high pollution with heavy metals, which adversely affects the health and well-being of the human population as well as biodiversity.

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