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Microorganisms of the Extreme Zones of the Southern Aral Sea Region

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Abstract

Development of microbial biota of the region of the Southern Aral Sea, which characterized by excessive aridity and salt concentration and presence of pesticides' traces as well, was studied. It was established that analyzed natural microbial biota comprises sporogenous, ammonifying and oligonitrifying microorganisms as well. Nitrifying and denitrifying microorganisms were not revealed. Filamentous fungi belonging to genera Aspergillus, Penicillium, Cladosporium were isolated as well. At the same time, nitrifying and denitrifying microorganisms were isolated from arable soils, where humus content is higher. Strains of Azotobacter, resistant to mixture of pesticides, were isolated as well.

Keywords: Microorganisms of extreme zones; Sensitivity; Salt; Pesticide

Introduction

Microbial diversity of extreme zones attracts attention of many scientists. There are many works devoted to study of microorganisms inhabiting such zones and possible limits of their existence [1-3]. One of such extreme zones is the world known territory of the Aral Sea and its enclosed area. An active aridization and accelerated development of salinization processes continues in the region. Excessive application of pesticides in the ex-USSR agriculture led to pollution of soils and water resources and to their accumulation in the different ecotopes. Despite current considerable decrease of chemical pressure upon hectare of arable land the remaining trace concentrations of pesticides still exert sensible impact on soil biota. High sensitivity, vulnerability of soil surface is stipulated by limited buffer capacity and resistance of soil to impact of forces, uncharacteristic to it in ecological terms [4]. Presence of pesticides in virgin soils is stipulated by their transfer, who is impacted by several factors: composition and properties of soil, humidity, velocity of infiltration, aeolation and so on [4].

The Southern Aral Sea region is a unique natural object, which represents sum of extreme factors including diversity of biotopes with different ecological systems and, at the same time, exists in conditions of the intensive transformation as well. The prevailing here soils are distinguished by extremely low content of organic matter and nitrogen, high content of toxic salts, high alkalinity [5,6]. In these regards, the factor of no small importance for the Aral Sea region is the wind transfer of dust, which also promotes to presence of certain quantities of pesticides in ecotopes located far from arable lands.

Such composition of unfavorable natural and anthropogenic factors led to decreased biological activity, in particular the microbiological one, and seriously troubles rehabilitation of polluted soils [7]. Nevertheless, microorganisms that adapted to high temperature and high concentrations of salts and to presence of certain amount of pesticides inhabit natural substrates of

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the Southern Aral Sea region. Presence of the certain complex of extreme factors creates prerequisites for development of microorganisms-extremophiles, for maintenance of their diversity and for formation of the complex microbiocenoses [8-11]. Such microorganisms are carriers of unique biological features and may represent certain interest for development of the new microbial biotechnologies.

In these regards, isolation, study and preservation of the gene pool of natural microbial populations, and in particular populations of the microorganisms-extremophiles, becomes very important modern task.

Materials and Method

Study of the Southern Aral Sea region was conducted in spring season. Soil samples were collected on the shoreline of the Aral Sea and in suburbs of Nukus (the largest city of the region) and from arable land used for cotton production. The soil samples were collected from 0-10 cm depth. Microbiological study was conducted according to common practice [12,13]. Following groups of microorganisms were recorded: the total number of organotrophic bacteria, ammonifying bacteria, sporogenous bacteria, oligonitrifying bacteria, denitrifying bacteria, nitrifying bacteria, Azotobacter. For this purpose following nutrient media were used: beef-extract agar (BEA), beef-extract broth (BEB), wort agar, Ashby, Giltay, Vinogradski, Czapek-Dox. Determination of microorganisms' quantity present in 1 g (1 ml) of the initial substrate was conducted by method of subsequent dilutions with cultivation on solid and liquid nutrient media [12,13]. Bacteria were identified according to Bergey's manual [14], while micromycetes - according to Litvinov [15]. A mixture of pesticides was used in this study: Chlorpyrifos ($C_9H_{11}Cl_3NO_3PS$)+Cypermethrin ($C_{22}H_{19}NO_3Cl_2$) - 500/50 g/l. Agrochemical analysis of soil samples was done at the chemical lab of the Centre of Hydrometeorological Service at Cabinet of Ministers of the Republic of Uzbekistan (http://meteo.uz/).

Results and Discussion

To determine qualitative and quantitative composition of existing microflora of such specific region, the soil samples were collected from different sites, including former shoreline and bottom of the Aral Sea (Figure 1).

Figure 1: Pictures of some of sample collection sites (former shoreline of the Aral Sea).

Analysis of collected samples from the former shoreline and bottom of the Aral Sea revealed that the highest salt content is observed in samples from the former bottom. By quantity of easily dissolving salts, soils are medium alkaline with chloride/chloride-sulfate salinization and alkali-saline with chloride salinization; pH 7.8-8.0; gypsum content – 0.3-10% (Table 1). Soils are characterized by low level of humus and nitrogen content, which testifies that this zone is specific region where the large part of territory, is subjected to desertification and salinization. Moreover, there was established presence of trace concentrations of DDT (0.1-0.2 maximum permissible concentration – MPC) in studied samples. Soil is an extremely complex medium with conditions often controversial for development of certain groups of microorganisms, where quantitative and qualitative composition of bacteria does not always proportionally correspond to chemical composition. Considerable part of microorganisms inhabiting soil actively participates in transformation of organic matter and hardly accessible forms of elements of mineral nutrition into easily accessible forms for plants. This group of microorganisms does mainly determine the biological activity of soil.

##	**Collection site**	**Chemical composition**					
		Chlorides, mg/kg of soil	**Sulfates, mg/kg of soil**	**Humus, %**	**N-NH_3, mg/kg of soil**	**N-NO_3, mg/kg of soil**	**Mobile phosphorus, (mg of P_2O_5 per 1 kg of**
1.	Former shoreline of the Aral Sea	235.8	136.36	1.0	8.33	27.27	268
2.	Former shoreline of the Aral Sea	288.2	113.64	1.0	10.42	15.91	268
3.	Former bottom of the Aral Sea	419.2	90.91	0.9	10.42	20.45	278
4.	Former bottom of the Aral Sea	4873.2	1204.5	1.1	16.67	5.68	186
5.	Former bottom of the Aral Sea	3930.0	1454.5	0.9	10.42	10.23	54
6.	Soil from the field used for cotton production	230.6	90.21	2.2	25.0	34.09	670

Table 1: Results of agrochemical analysis of selected soil samples.

Results of our study reveal that in saline soils with trace concentrations of pesticides the number of isolated microorganisms, which determine biological activity of soil, is relatively small. Thus, number of sporogenous, ammonifying and oligonitrifying microorganisms considerably low, and denitrifying bacteria and Azotobacter practically are not isolated. This tendency to suppression of these groups of microorganisms occurs at analysis of all studies sites of the Aral Sea region. Nevertheless, the total number of bacteria isolated on BEA is relatively considerable for soils with such composition (Table 2).

##	Number of microorganisms, CFU/g					
	BEA	**Ammonifying**	**Sporogenous**	**Oligonitrifying**	**Denitrifying**	**Azotobacter**
1	7.5×10^5	2.0×10^3	3.4×10^2	3.0×10^2	2.5×10^2	Solitary cells
2	3.8×10	6.0×10^2	2.5×10	2.8×10^2	-	
3	5.0×10^5	6.0×10^2	2.1×10^2	5.6×10^2	-	Solitary cells
4	3.3×10^5	2.5×10^3	6.0×10^2	1.8×10^3	-	
5	2.0×10^5	6.0×10^3	1.8×10^2	3.6×10^2	6.0×10^1	

Table 2: Results of microbiological analysis of soils with trace concentrations of pesticides.

It is known that, as a rule, in soils where processes of mineralization of the organic matter run slowly, sporogenous microorganisms prevail. In studied samples the bacterial biota was mainly represented by sporogenous forms of microorganisms, with dominance of *Bacillus subtilis*, *B. megaterium*, *B. cereus*. Non-sporogenous bacteria were presented by genus Pseudomonas. At the same time, free nitrogen-fixing microorganisms, like Azotobacter, were isolated in extremely small quantities. Obviously, the process of nitrogen fixation in these soils was on account of activity of oligonitrifying bacteria.

In the same region, but in arable soil used for cotton production, a considerable increase of the number of microorganisms of all studied groups is observed. Especially, appearance of nitrifying bacteria and increased number of Azotobacter should be noted, since it testifies presence of positive processes of soil rehabilitation (Figure 2).

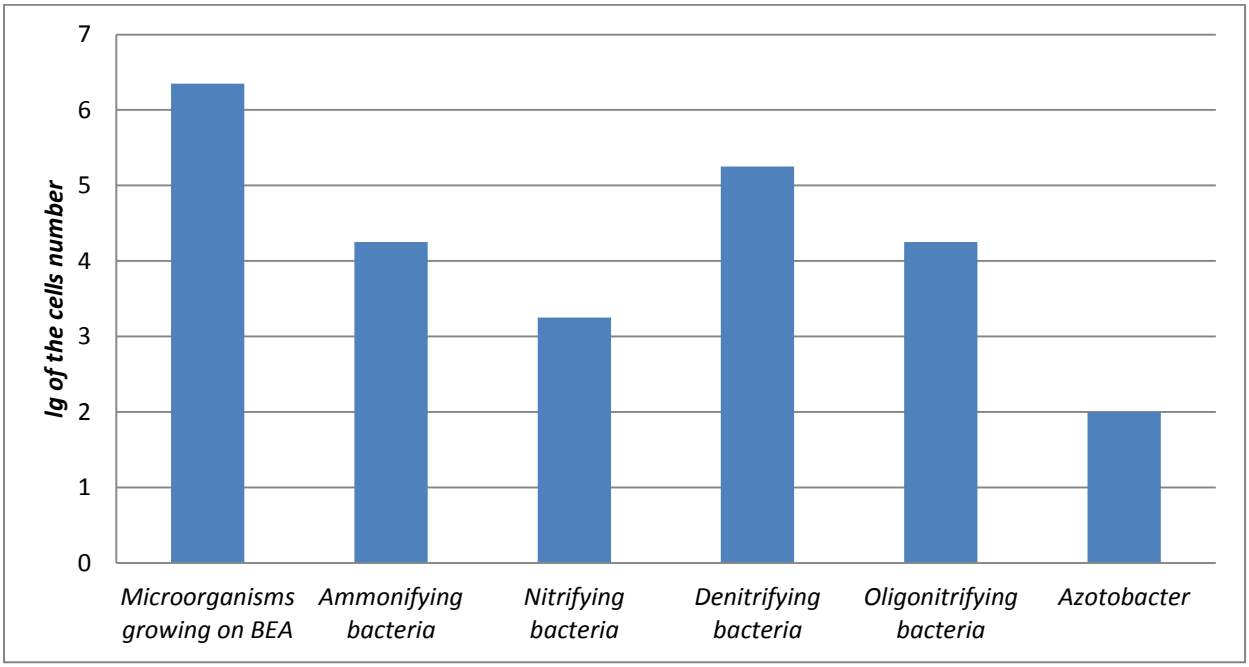
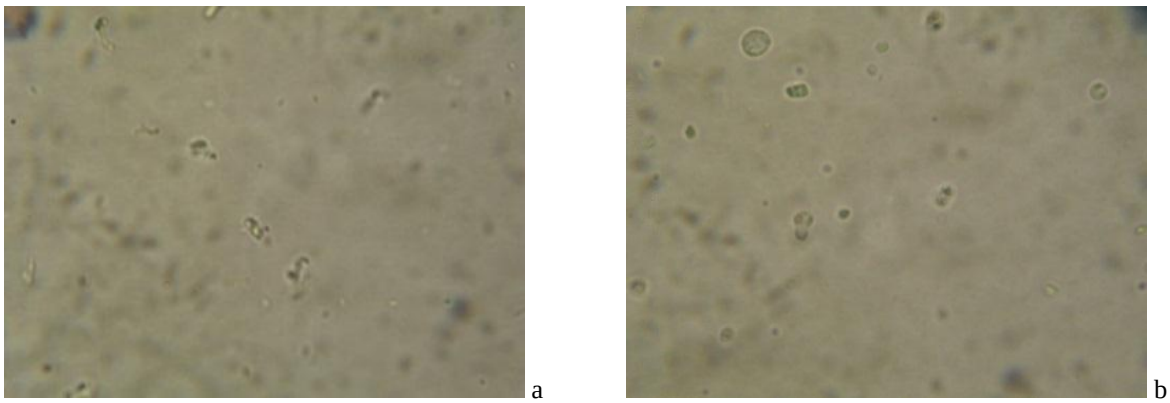


Figure 2: Groups of microorganisms isolated from arable soil.

Using methods of cultivation of soil nubbins we isolated strains of microorganisms, which, after microscopy, study of the growth pattern on solid nutrient media and pigmentation, and determination of some physiological and biochemical properties, were identified as representatives of the genus Azotobacter (Figure 3).



a b

Figure 3: Microscopy of cells of *Azotobacter* sp. (a. 1 day, b. 11 day) (magnification x100).

Young cells of Azotobacter are mobile; formation of cocci forms is observed, which is characteristic for this genus. Azotobacter cells in old cultures cover themselves with thick envelope forming cysts (Figure 3b). They may propagate to give start of the new cells (Figure 3a). Colonies of our isolated strains have color from light brown to dark brown, which is characteristic for *Azotobacter chroococcum*.

Screening among the most active strains of isolated microorganisms resistant to high content of pesticides and salt resulted in determination of a number of strains expressing high levels of resistance towards studied mixture of pesticides (Chlorpyrifos+Cypermethrin; 500/50 g/l). It was established that isolated cultures of Azotobacter (strains 22-M, 13-M, 3M, 1-ZL, 1LO) expressed considerable resistance to these pesticides (Figure 4).

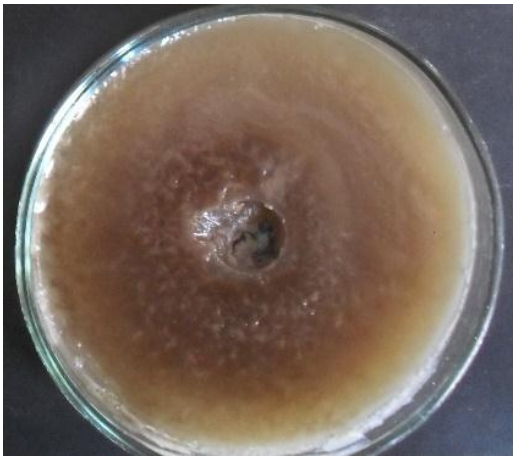


Figure 4: Resistance of the strain *Azotobacter* sp. 1-ZL to mixture of studied pesticides.

It is known from results of other studies that strains of microorganism's resistant to toxic action of pesticides, as a rule, are capable to active biodestruction of these xenobiotics [16].

It is necessary to note that filamentous fungi were isolated from almost in all samples, but in small quantities. It is well known that fungi play significant role in mineralization of organic matter of soil. They may digest not only cellulose, but lignin as well (Basidiomycetes). Activity of fungi is of special importance for formation of acids in soil, which acidify soil solution, which is important for alkali saline soils. We noted that in all studied samples was recorded presence of only several strains of filamentous fungi, which were preliminarily identified as representatives of genera Aspergillus, Penicillium, Cladosporium.

It is reported that in polluted soils diversity of micromycetes considerably decreases. At the same time, isolation of a number of species of filamentous fungi from such soils allows to make a conclusion about adaptation of specific fungal strains to high content of persistent xenobiotics and about possibility of presence of promising species of microorganisms-destructors [17,18].

Conclusion

Thus, it was established that studied soil samples of the Southern Aral Sea region possess low biogenicity stipulated by both the soil type and the level of salinization and presence of trace concentrations of pesticides. Many physiological groups of microorganisms are not present or are present in very low quantities in these soils. The certain tendency about improvement of agrochemical properties of soil, respectively, about intensive development of microbial biota, may be noted only in arable lands of the region. Cosmopolitism of microorganisms does not mean that they are present in considerable quantities everywhere. Obviously, that only certain conditions may cause their abundant development and the certain groups of microorganisms should dominate in the different soils.

Nitrogen fixing microorganisms isolated from extreme natural conditions revealed high level of resistance towards studied mixture of pesticides (Chlorpyrifos+Cypermethrin; 500/50 g/l). Obtained data makes possible development of bacterial preparation combining both pesticide destructive activity and soil improving features.

Considering high adaptive abilities of bacteria, their tight dependence upon environment conditions, one may expect development of forms of microorganisms with a number of valuable properties applicable in biotechnology and agriculture.

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