

STUDY ON SANITARY MICROBIOLOGICAL PROPERTIES AND GENOTOXIC PROPERTIES OF DARHAN CITY SOIL

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ABSTRACT

The world population rising very fast and it has resulted in increasing the area of polluted soil, water and air. In order to cater to the demands of the people, the rapid expansion of industries, vehicles, etc. is necessary. But all of these developments make our world, especially urban area, heavily polluted. And many kind of contaminant substances showing bad effects to human health. Our research work aimed to evaluate Darhan city soil contamination using genotoxicological studies.

Genotoxicological effect of soil contaminant substances or ability to cause a mutation to living organisms was determined by Eims test and DNA damaging experiment on test strain Salmonella thyphimurium TA-100 (his G46, rfa B, bio pKm 101) and Escherichia coli (wp, uvr, pol). The result revealed that Darhan city soil samples have inhibition effect to test strain growth rate.

KEY WORDS: Darhan, soil, mutagenic, microorganisms

INTRODUCTION

In the majority of the cases separate chemical compounds are investigated on mutagenicity. However, in nature not individual compounds, but their entire complex is influencing more frequently to the organism. In the natural objects, such as water, soil and air the separate chemical compounds, which relate to different classes, influencing each other, are modifying overall mutagenic potential, strengthening or decreasing it

[1, 3].

The research's goal was to study the mutagenic activity of Darhan City soils. The soil samples were subjected to sanitary microbiological examinations such as number of total bacteria, E.coli titer, Cl.perfringens titer, number of thermophile microorganisms and presence or absence of representatives of genus Proteus.

METHOD ANS RESEARCH MATERIALS

Examined soil samples were taken in May 2012 in Darhan, Mongolia:

1. near roads which have heavy traffic;
2. Darhan factory areas;
3. the yurt suburbs "Mangirt";

4. sewage treatment places;
5. central "Birj" food market.

Sanitary microbiological studies were carried out by envelope method (Dobrovolskii, 1997). Total bacteria were enumerated by viable plate count

technique; for this, 10 g of soil sample was taken into 90 ml of sterile distilled water and mixed well for 2 minutes. The soil suspension was subjected to ten-fold serial dilution, and 100 µl of diluted cells were spread on agar medium (Egorov, 1993). *E.coli* was determined by membrane filter method (Lyubashenko, 1980) using Endo-agar medium; representatives of genus *Proteus* by Shukovish method; *Cl.perfringens* by using selective medium; and thermophile microorganisms by culturing in a high temperature.

In the tests on toxicity and mutagenicity there were investigated the aqueous and ether extracts of selected samples. Examined samples of soils have been dehydrated to the air-dried state at a temperature of 105°C [4]. 1 g of the air-dried soil was extracted in 10 ml of distilled water at room temperature during 12 hours for the purpose to prepare an aqueous extract. The sterilization of samples was carried out by a filtration through the Synpor sterile membrane filters.

The ether extract was obtained by extraction of 5 g of soil by ether. Extract has been evaporated dry at a temperature of 50°C, then organic residue was dissolved in dimethyl sulfoxide ((CH₃)₂SO) and carbon tetrachloride (CCl₄). After dissolution of organic residue, 1 ml DMSO contained the extract

RESULT

In order to evaluate contamination degree of Ulaanbaatar and Erdenet city soils, we had chosen the most contaminated places for sampling and examined total bacterial number, *Cl.perfringens* titer, *E.coli* titer, number of thermophile microorganisms, and presence of representatives of genus *Proteus*.

From the soil samples of Ulaanbaatar city area, we revealed that the most contaminated places are heavy traffic roads, factory areas, scrapheap and yurt suburbs areas. According to *E.coli* titer, "Birj" food market, factory area and scrapheap are contaminated in average scale and sludge of waste water treatment areas are in poor scale.

According to total bacterial number, soil of waste

of 2 g of air-dried soil.

Toxicity in respect of the microorganisms

The toxic properties of extracts were determined by comparing the suppression degree of strain's growth on adequate nutrient medium in the presence of examined extract and same degree of the control strain.

Salmonella/microsome test

Test strain of *Salmonella typhimurium*: TA100 (*his G46, rfa, uvr, bio, pKm101*) auxotrophic on the histidine, which was obtained from the Institute for Chemical Compounds Bioassays (Kupavna), has been used in this work. Experiments were carried out in conformity with Maron and Ames [7] in modification of Fonstein and the co-authors [5].

Determination of free histidine in the samples of waste effluents' sediments

by the thin-layer chromatography method

The increased content of histidine in the samples might be the reason for false-positive results in the Ames test. The histidine dose required to double revertants is equal to 150 nmole/cup (232 µg/ml) [4,6]. A quantity of histidine has been determined by the thin-layer chromatography method.

water treatment areas, scrapheap, and factory area of Darhan city were shown as the most contaminated places. When Darhan and Ulaanbaatar city soils were compared by their bacterial number, Darhan city soil showed higher bacterial number, Ulaanbaatar city soil showed highest *E.coli* titer. Both cities had similar degree of *Cl.perfringens* titer, the number of thermophile microorganisms, and presence of representatives of genus *Proteus*.

The results of evaluating the toxicity of aqueous and organic extracts with respect to the microorganisms are represented in the Chart 1.

Table 1
The results of evaluating the toxicity of aqueous and organic extracts with respect to the microorganisms

Solvent	Survival of test strain, (%) (in comparison with positive control)				
	1	2	3	4	5
H ₂ O	53,1	62,4	73,5	39,4	77,3
DMSO	10,8	17,1	32,9	19,8	46,2
CCl ₄	67,2	23,5	81,2	41,5	35,1

As can be seen from table 1, toxic effect is discovered during extraction of investigated soils by both aqueous and organic solvent. This means that the testing soils contain both inorganic and organic toxic components. Significant toxic effect is registered for soils number 1, 2, 3. And sample 1, which represents an extracted DMSO, showed

maximum toxicity.

The study of mutagenic activity in the experiences without metabolic activation in the Ames test has been shown the absence of mutagenicity in all examined samples and extracts made by different solvents (Chart 2).

Table 2

Mutagenic effect of soil samples

Solvent	Variations	Sample				
		1	2	3	4	5
H ₂ O	-S9	1,7	0,9	1,5	1,8	1,7
	+S9	1,15	23,9	1,4	21,9	1,2
DMSO	-S9	1,02	1,6	0,7	1,2	1,5
	+S9	0,4	2,5	1,8	0,4	1,9
CCl ₄	-S9	1,5	1,4	0,4	1,5	1,3
	+S9	0,5	2,8	1,6	1,7	0,8

Where -S9 – with presence of metabolism activator
+S9 – with absence of metabolism activator

Metabolic activation in vitro by ferments of cells from rats liver is increased the mutagenic activity of aqueous fractions of tests, while almost all organic extracts did not displayed any mutagenicity.

Aqueous extracts of tests number 3 and 5

DISCUSSION

Mutagenicity of urban soil was studied well in high-developed countries, such USA, Italy, Japan, Germany and Russia. American researchers are established that the urban soil is much active in respect of mutagenicity, especially in industrial areas.

La Rossa and his team conducted the genotoxicity study of polluted soils in three Italian cities and in all samples mutated despite of not high content of mutagenic compounds.

When a mutagenicity of Mainz soil (Germany)

displayed a medium mutagenic activity and weak mutagenic potential was registered for test 2.

The soils being investigated contain histidine in quantities less than 7 µg/ml.

was determined using *Salmonella typhimurium* TA100 strain, it was found that mutagenic activity is high in summer, and lowered in spring and winter.

As regards of our country, Dr Ch. Battsetseg has been studied a genotoxicity of Ulaanbaatar soils in 2002 and established genotoxicity of test strains taken near the Ulaanbaatar Treatment Facilities and Shonkhor Food Market. This time we are studied a mutagenicity of Darhan City's soils using the Eims Test.

SUMMARY

1. According to sanitary microbiological experiments, main roads and yurt suburbs soils were shown heavy contamination
2. Water and organic solvent solution of Darhan soils have a high microbial growth inhibition rate. In particular, soil from the near roads which have heavy traffic has shown higher rate.
3. The sample from yurt suburbs and "Birj" food market areas sludge shown the highest DNA damaging effect.
4. Main road and sewage treatment places soils, and the sample from factory areas were shown average mutagenic effect

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