BENZPYRENE BREAKDOWN BY THE SOIL MICROFLORA

A. Ya. Khesina, N. P. Shcherbak,
L. M. Shabad, and I. S. Vostrov

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The soil microflora is able to destroy up to 70% of the benzpyrene contained in the soil. This property is most marked in the microflora of soil contaminated with polycyclic hydrocarbons as a result of industrial waste.

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Earlier reports [1-5] have shown that many microorganisms can decompose aromatic polycyclic hydrocarbons (APH), especially benzpyrene (BP), present in the nutrient medium. The most active in this respect were found to be several strains of bacteria isolated from soil strongly contaminated with APH (soil from the grounds of the "Neftegaz" factory), and also a strain of the soil bacteria Bacillus megaterium mutilate. These microorganisms exhibited the property of metabolizing (apparently oxidizing) BP and several other APH, whether introduced into the culture medium (meat-peptone broth) or present in the soil as a result of "natural" contamination with industrial waste products. In the latter case, the culture medium consisted of a suspension of sterilized soil from the grounds of an oil refinery, to which meat-peptone broth was added. The quantity of BP decomposed by these strains of bacteria during cultivation for 8 days was 90-80% of that originally present in the medium. The small series of experiments in which meat-peptone broth was added to a suspension of unsterile soil from the grounds of the "Neftegaz" factory showed that BP can be destroyed by the soil microflora itself [3, 4].

The object of the present investigation was to determine whether the microflora of various soils can decompose APH, and, in particular, BP under conditions very close to natural.

EXPERIMENTAL METHOD

Experiments were carried out with BP mainly because earlier experiments showed that the ability of microorganisms to metabolize APH is not specific in character and that any hydrocarbon of this series can be used as test object [5], and secondly because BP is of particular interest as an active carcinogen polluting man's external environment, especially the soil of cities and industrial areas.

The following soils were used as test objects: 1) from the grounds of a boarding house near the Klyazma Reservoir—control series (C); 2) from an area in one of the older built-up parts of Moscow—the Monika district (series M); 3) from the grounds of an oil refinery—the "Neftegaz" factory (series N); 4) soil of series C with artificial addition of BP in the same quantities as were present in the soil of series N (series B).

When choosing the soils, the results of previous experiments were taken into account [8], showing that series C can serve as an example of a "pure" soil. Its contamination with BP does not exceed 10 μg/kg soil. Series M is an example of a moderately polluted soil from Moscow. The BP content of the soil in this series in the present experiments was about 550 μg/kg soil. Series M is an example of soil with the highest

Laboratory of Prophylaxis of Carcinogenic Effects, Institute of Experimental and Clinical Oncology, Academy of Medical Sciences of the USSR. Laboratory of Soil Microorganisms, Institute of Microbiology, Academy of Sciences of the USSR, Moscow. Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 68, No. 10, pp. 70-73, October, 1969. Original article submitted January 7, 1969.

TABLE 1. Decomposition of Benzpyrene (BP) by Soil Microflora

Series of experiments	Content of BP (μg/kg soil)				Difference in BP content			
	initial content	after exposure for 1 month	after exposure for 2 months	after exposure for 3 months	A	B	C	D
C	9,4±0,3	7,4±0,4	7,5±0,2	6,3±1,0	2,0±0,5 $t=2,4$ $P<0,05$	0,1±0,2 $t=2,3$ $P>0,05$	1,2±1,0 $t=2,2$ $P>0,05$	3,1±1,0 $t=2,4$ $P<0,05$
M	545±3,5	377±21,8	346±13,6	159±14	168±22 $t=4,3$ $P<0,05$	31±17,3 $t=2,3$ $P>0,05$	159±19 $t=2,2$ $P<0,05$	386±14 $t=2,6$ $P<0,05$
B	29 200±630	29 300±210	29 000±700	30 100±600	In all cases $P \gg 0,05$			
N	28 500±550	16 600±800	16 700±800	13 700±500	11 800±1 000 $t=2,2$ $P<0,05$	100±1 500 $t=2,2$ $P \gg 0,05$	3 000±1 000 $t=2,2$ $P<0,05$	14 800±700 $t=2,2$ $P<0,05$

Note. A) Decrease in BP during first month of exposure (relative to control); B) decrease in BP during second month of exposure (relative to end of first month); C) decrease in BP during third month of exposure (relative to end of second month); D) total decrease in BP during three months of exposure.

degree of contamination with APH (tens of milligrams, i.e., tens of thousands of micrograms per kg soil). Experiments with the soil of series B were carried out to determine to what extent the microflora of soil virtually free from APH can actively decompose these high concentrations of BP which are encountered by the microflora of soil highly contaminated with APH over a period of many years or even decades.

The soil selected from the above districts was first sifted. In series B, the BP was dissolved in a suspension of acetone, water, and bovine serum (to avoid its precipitation as crystals) and added to soil of series C, initially at the rate of 100 mg/kg soil. The soil was brought to an air-dried state by evaporating the liquid during careful mixing. After drying it was mixed with 2 parts of soil of series C, so that the mean final content of BP was 30-33 mg/kg soil.

To determine the BP content, 10 g of dry soil was extracted for 6 h in Soxhlet apparatus in 200 ml of distilled benzene. BP in the extract was determined by a fluorescence spectral method based on the quasi-line luminescence spectra [7] at the temperature of liquid nitrogen, using a method developed previously [6].

The experiments were carried out in vegetation pots each containing 3 kg soil, in an unheated greenhouse (the object was to make the experimental conditions as nearly natural as possible).

The BP content in the soil was determined before the beginning of the experiment and after exposure for 1, 2, and 3 months. During the first month the soil in the vessels was regularly moistened with distilled water, but during the second month no water was added, and during the third month of exposure the soil was again watered. In each series of experiments (C, M, B, N) 6 vegetation pots were used. In two of them, rise straw was added to the soil to stimulate activity of the microflora, in another two plants (mustard) were grown in the soil, and in the last two pots the experiments were carried out in a pure form. However, the changes in BP content in the six pots of each series were not systematic. Because of this, the mean value of the results for all six pots was calculated in each series.

EXPERIMENTAL RESULTS

The results are given in Table 1 (with the results of statistical analysis).

The soil microflora, as the table shows, can actively decompose aromatic hydrocarbons, and especially BP. However, this property is shared to a different degree by the microflora of soil differing in its polution by APH. The experiment showed that microorganisms in each soil can cope with the APH content to which they are usually accustomed. The higher the intensity of soil polution with waste products from industry and automobiles, the greater the amount of APH and, in particular, of BP which can be

destroyed by its microflora. For instance, the microorganisms in the soil of series C could metabolize about 3 μg/kg soil (34% of the initial content of BP in this soil). The cell microflora of series M decomposed about 340 μg BP/kg soil (about 70% of the initial content). Finally, the microorganisms of the soil with the highest contamination with APH (series N) metabolized about 15,000 μg/kg soil (over 50% of the initial content).

Soil microorganisms can thus decompose very different quantities of BP. The process evidently takes place through metabolism of BP by enzymes induced by APH.

In soil from the boarding house by Klyazma Reservoir, virtually free from APH, and contaminated artificially for these experiments (about 30,000 μg BP/kg soil), no appreciable decrease in the BP content took place through bacterial metabolism. This phenomenon may be due to several causes.

The experiments of series C demonstrated that the microflora of this soil can metabolize only a few micrograms of BP per kilogram of soil, but this decrease in the BP content could not be detected by comparison with the 30,000 μg or thereabouts of BP added to the soil. In "pure" soil, under natural conditions, there are evidently too few microorganisms capable of metabolizing BP in large enough quantities.

Finally, the possibility is not ruled out that after artificial addition of BP to the soil, this substance was present in a form inaccessible to the action of microorganisms. This state was evidently not due to the irregular distribution of BP in the soil in series B, because in all 18 samples of soil investigated in this series, the results obtained (within limits of 10% possible error) corresponded to 30,000 μg/kg.

The experiments thus showed that under natural conditions destruction of BP can take place in soil contaminated with this compound, i.e., a form of biological self-purification occurs. This phenomenon is evidently due to the metabolic activity of the soil microorganisms. Evidence of this is given by the decrease in BP content by the end of the second month of the experiment, i.e., without watering of the soil, whereas at the end of the first month, when the soil had been watered, and after the third month, when it had again been watered, a marked decrease in the BP content was observed (the microflora is known to be active only in moist soil). If the decrease observed in the BP content was primarily due not to the metabolic activity of the soil organism, but to other factors decomposing the compound, such as insolation (ultraviolet radiation), the differences found between the series and periods of the experiments would not have been observed. In particular, the breakdown of BP would have been about equal in the soils of series N and B, in which the initial content of BP was about the same and the soil was kept under identical conditions.

Differences in the activity of the microflora at different periods of the experiment (Table 1) were evidently attributable to seasonal changes in activity of the different microorganisms.

LITERATURE CITED

1. M. N. Poglazova, G. E. Fedoseeva, A. Ya. Khesina, et al., Dokl. Akad. Nauk SSSR, Seriya Biol., 169, No. 5, 1174 (1966).
2. M. N. Poglazova, G. E. Fedoseeva, A. Ya. Khesina, et al., Dokl. Akad. Nauk SSSR, Seriya Biol., 176, No. 5 (1967).
3. M. N. Poglazova, G. E. Fedoseeva, A. Ya. Khesina, et al., Life Sci., 6, 1055 (1967).
4. M. N. Poglazova, G. E. Fedoseeva, A. Ya. Khesina, et al., Dokl. Akad. Nauk SSSR, Seriya Biol., 179, No. 6 (1968).
5. G. E. Fedoseeva A. Ya. Khesina, M. N. Poglazova, et al., Dokl. Akad. Nauk SSSR, Seriya Biol., 183, No. 1, 208 (1968).
6. G. E. Fedoseeva and A. Ya. Khesina, Zh. Prikladn. Spektroskopii, 9, No. 2, 282 (1968).
7. É. V. Shpol'skii, A. A. Il'ina, and L. A. Klimova, Dokl. Akad. Nauk SSSR, 87, No. 6, 935 (1952).
8. N. P. Shcherbak, Vopr. Onkol., No. 1, 77 (1967).