

Study of Mutual Influence of Polycyclic Aromatic Hydrocarbons (Anthracene, Pyrene) and Baikalian Sponges in Model Experiments

OLGA YU. GLYZINA¹, LARISA I. BELYKH², NIKOLAY A. LATYSHEV³, ALEXANDER V. GLYZIN⁴
and ALEXANDER G. GORSHKOV¹

¹Limnological Institute, Siberian Branch of the Russian Academy of Sciences,
Ul. Ulan-Batorskaya 3, Irkutsk 664033 (Russia)

E-mail: info@lin.irk.ru

²Irkutsk State University, Ul. Lenina 3, Irkutsk 664003 (Russia)

³Institute of Marine Biology, Far-Eastern Branch of the Russian Academy of Sciences,
Ul. Palchevskogo 17, Vladivostok 690022 (Russia)

⁴Siberian Institute of Plant Physiology and Biochemistry, Siberian Branch of the Russian Academy of Sciences,
Ul. Lermontova 132, Irkutsk 664033 (Russia)

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Abstract

Model experiments for investigation of mutual influence of anthracene and pyrene (polycyclic aromatic hydrocarbons) on the one hand and sweet-water sponge *Lubomirskia baikalensis* widespread in the Lake Baikal on the other hand have been carried out. The sponge's capacity for active elimination of anthracene and pyrene from water (90–95 % for 36 h), accumulation and conservation of the major part of anthracene and pyrene without their active metabolism, and for reacting to the presence of these ecotoxicants with a specific change of the total fatty acid composition has been demonstrated. Anthracene and pyrene assay was carried out by means of HPLC; the composition of fatty acids was determined by means of GLC.

INTRODUCTION

In the chemical composition of aerosol of the Baikalian region, polycyclic aromatic hydrocarbons (PAH) are characteristic trace components [1]. According to monitoring data, considerable amounts (up to 680 $\mu\text{g}/\text{m}^2$) of PAH are accumulated in the snow cover of the regions of their potential sources on the southern coast of Lake Baikal during winter [2, 3]. In spring, during the period of snow thawing, a burst-like discharge of the accumulated PAH into coastal waters with snow water flows is possible. In summer, the supply of PAH to the aquatory of the lake may be a result of local transfer of contaminating compounds from industrial Pri-Baikalia [4, 5]. At the same time, a very low level of PAH concentration (no more than 0.1 ng/l and 10 ng/l) has been

detected in waters [6] and its benthal sediments [7], respectively.

In the present work, in order to elucidate possible causes of this phenomenon, we studied the mutual influence of PAH and of the sweet-water sponge *Lubomirskia baikalensis* which is widespread in Lake Baikal [8]. For model experiments, polycyclic aromatic hydrocarbons with three rings in their structure were taken, because among PAH identified in the objects of environment in the Pri-Baikalia, compounds having such a structure prevail [2, 3]. *Lubomirskia baikalensis*, just like other Baikalian sponges, belongs to active sedimentator and filtering species [8, 9]. The sponges filter and sedimentate the organic suspension mass from the water layer, providing food to the organisms living in it – insect larvae, Oligochaeta worms and crustaceans which, in their turn,

purify the colony from the excess of the sedimentated suspension. This symbiotic community and its significance for the ecosystem of Lake Baikal has practically not been studied.

EXPERIMENTAL

Sponges *Lubomirskia baicalensis* were collected from the depth of 10–15 m on the southern coast of the Baikal near the settlement Bolshiye Koty in the vicinities of the continuously functioning station of the Limnological Institute, SB RAS. At the station, the sponges were placed into thermostats with running Baikal water supplied from the shore of the lake and having the temperature of 10–15 °C. After adaptation in running-water aquariums for 24 h, for model experiments single young sponges weighing 20–40 g (raw weight) were placed into aquariums filled with water solutions of PAH. Water PAH solutions were prepared by adding 10 ml of anthracene and pyrene solution in ethanol (with the concentration of each compound of 24 µg/ml) to 3 l of Baikal water. The obtained water solution had the initial anthracene and pyrene concentration of 80 µg/l, which corresponded to their maximal solubility in sweet water [10]. The capacity of the aquariums (3 l) ensured optimal conditions for the sponge's life activity. For PAH

concentration assay, 0.2 l water samples were taken from the aquariums at intervals of 6 h. In control experiments (prior to incubation), a sponge was kept in an aquarium with only filtered Baikal water and in an aquarium with addition of an appropriate amount of ethyl alcohol. No influence of the alcohol on the fatty acid composition was found.

PAH assay in water samples was performed by means of HPLC on a chromatograph "Mili-chrom A-02" according to the method described in [2, 11] adapted to anthracene and pyrene assay. Concentrated extracts were separated on columns with sorbent Nucleosil 100-5 C18 using a mixture of acetonitrile with water (volume ratio of 45 : 55) as the mobile phase, with simultaneous detection at the wavelength of 240 and 250 nm. Quantitative anthracene assay was performed by the results of detection at 250 nm, pyrene assay was made by detection at $\lambda = 240$ nm. During analysis of the sponge tissue, the whole mass of the animal was homogenized and extracted with *n*-hexane. In the extracts obtained, PAH assay was carried out according to [11], that of fatty acids as methyl esters according to [12] on a Shimadzu chromatograph with flame ionization detector. Derivates of acids were chromatographed on a capillary column (30 m) with the phase of Carbovax 20M at the thermostat temperature of

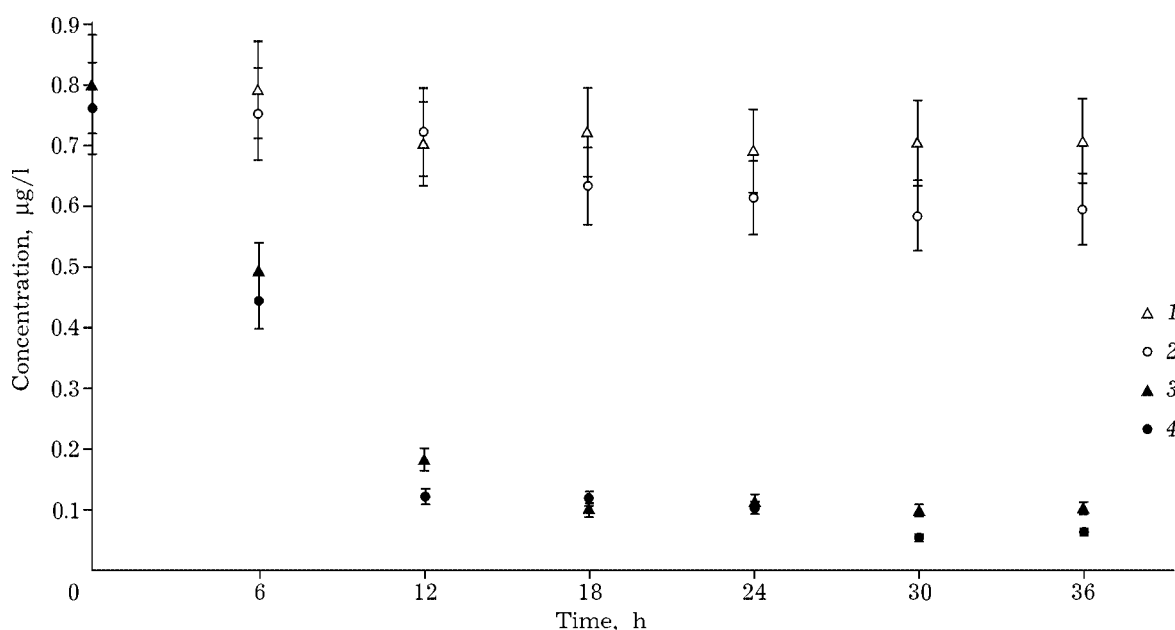


Fig. 1. Anthracene and pyrene concentrations in model (1, 2) and control (3, 4) experiments.

TABLE 1

Fatty acid composition in the sponge *Lubomirskia baicalensis* prior to and after incubation in PAH solution for 36 h, %

Acid	Before incubation	After incubation	Acid	Before incubation	After incubation
14 : 0	1.5	1.9	18 : 3 <i>n</i> -3	8.0	4.2
14 : 1 <i>n</i> -5	0.3	0.2	20 : 0- <i>i</i>	0.1	0.3
15 : 0- <i>i</i>	0.8	0.6	20 : 0- <i>ai</i>	0.2	0.2
15 : 0- <i>ai</i>	0.1	0.1	18 : 4 <i>n</i> -3	1.9	2.8
15 : 0	0.3	0.3	18 : 4 <i>n</i> -1	–	0.1
16 : 0- <i>i</i>	0.1	0.4	20 : 0	0.5	0.6
16 : 0- <i>ai</i>	0.2	0.4	20 : 1 <i>n</i> -9	1.8	0.4
16 : 0	12.0	4.7	20 : 1 <i>n</i> -7	0.2	0.6
16 : 1 <i>n</i> -9	0.5	0.4	–	–	0.3
16 : 1 <i>n</i> -7	1.1	1.3	20 : 2 <i>n</i> -6	0.1	0.6
16 : 1 <i>n</i> -5	–	0.1	20 : 4 <i>n</i> -6	1.1	3.3
17 : 0- <i>i</i>	0.8	1	20 : 3 <i>n</i> -3	0.1	0.4
17 : 0- <i>a</i>	0.2	0.7	20 : 4 <i>n</i> -3	0.2	2.1
16 : 2 <i>n</i> -6	0.5	0.9	20 : 5 <i>n</i> -3	7.2	14.4
X	0.6	0.9	22 : 0	0.2	0.1
16 : 2 <i>n</i> -4	0.7	0.8	22 : 1 <i>n</i> -11	0.3	0.2
17 : 0	0.4	0.2	22 : 1 <i>n</i> -9	0.2	0.1
17 : 1 <i>n</i> -9	0.2	0.1	22 : 1	0.2	0.5
16 : 3 <i>n</i> -3	1.0	0.2	22 : 2	–	–
18 : 0- <i>i</i>	0.1	0.1	–	–	0.2
18 : 0- <i>ai</i>	0.1	0.2	22 : 2 <i>n</i> -9	–	0.1
16 : 4 <i>n</i> -3	2.5	0.8	21 : 5 <i>n</i> -3	–	0.2
18 : 0	3.4	3.5	22 : 4 <i>n</i> -6	–	–
18 : 1 <i>n</i> -9	26.4	28.4	22 : 5 <i>n</i> -6	–	0.5
18 : 1 <i>n</i> -7	0.8	1.4	22 : 4 <i>n</i> -3	–	0.4
18 : 2(5,9)	0.2	0.2	22 : 5 <i>n</i> -3	–	0.3
19 : 0- <i>i</i>	0.3	1.0	22 : 6 <i>n</i> -3	1.2	5.8
18 : 2(5,12)	0.3	0.2	24 : 0	0.2	1.2
18 : 2 <i>n</i> -6	2.2	2.2	24 : 1 <i>n</i> -3	0.3	0.1
18 : 2 <i>n</i> -4	0.2	0.2	–	1.5	3.1
19 : 0	0.1	0.2	26 : 2(5,9)	0.8	0.4
18 : 3 <i>n</i> -6	0.4	0.1	26 : 3 (5,9,19)	13.9	22.8

190 °C and detector temperature of 220 °C, using argon (30 ml/min) as the carrier gas.

In control experiments, PAH were detected in water solutions of aquariums without sponge, and PAH and fatty acids were detected in sponges kept in aquariums without PAH.

RESULTS AND DISCUSSION

The results of model experiments witness to a clear-cut accumulative capacity of the sweet-water sponge *Lubomirskia baicalensis*. As

one can see in Fig. 1, in the presence of the sponge a drastic decrease of PAH concentration in the water of experimental aquariums takes place as compared to control. Therein, during the first 6 h the decrement of anthracene and pyrene concentrations is as large as 60 %, and in 36 h the residual PAH concentration does not exceed 10 %. The PAH concentration decrement in water is practically proportional to the sponge mass. So, an increase of the sponge mass from 20 to 40 g results in an approximately double decrease of PAH concentration for equal time interval (12 h).

According to the data of analysis of extracts of sponge incubated for 36 h in PAH solution, as much as 70 % of accumulated PAH is conserved in unchanged condition. In the studies of the influence of PAH on the sponge, no sharp change of the total composition of fatty acids which are structural elements of cell membranes and a part of the "energy deposit" [13, 14] has been found. There was only a 2–4-fold increase of the content of polyunsaturated acids C20 : 6, C20 : 5, C22 : 6 and C26 : 3 (Table 1). After incubation, a rapid recovery of the initial ratio of the fatty acid pool took place.

CONCLUSIONS

The first results of studies of mutual influence of ecotoxicants – polycyclic aromatic hydrocarbons and Baikalian sponge *Lubomirskia baicalensis* permit making the following conclusions. The capacity of the sweet-water sponge *Lubomirskia baicalensis* for active elimination of PAH from water may be considered as one of mechanisms of self-purification of Baikal waters from contaminants of this class. Accumulation of PAH and their storage by the sponge without active metabolism, the specific change of the total fatty acid composition permit speaking about perspectives of us-

ing *Lubomirskia baicalensis* as a bioindicator in monitoring of aquatic ecosystems.

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