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Biocatalysis of Matter Transfer in a Microcosm Is Inhibited by a Contaminant: Effects of a Surfactant on *Limnea stagnalis*

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Aquatic invertebrate phytophagous animals excrete large amounts of pellets, which contain some food components because of incomplete digestion. Food assimilability by gastropods varies from 42 to 85% (67.7% on the average) [1]. The excreted pellets settle by gravity to form the bottom detritus, thus contributing to the formation of bottom sediments. This process is important for water ecology; however, the effects of contaminants, including surfactants, on this process have not been studied sufficiently.

Pulmonates (Gastropoda: Pulmonata), including *Limnea stagnalis* (Bassomatophora), are both phyto- and zoophages in water ecosystems. The *L. stagnalis* feeding base comprises plants and other food available in water bodies [1]. The purpose of this study was to detect and analyze the possible disturbances caused by a surfactant in vertical matter transfer in a microcosm (exemplified by *L. stagnalis*) as a model of matter transfer in the entire ecosystem.

The mollusks were collected in a pond in the upper Moskva River in June and placed into jars (five to ten animals per jar). The total biomass of the mollusks per jar was about 10–14 g (on the average, 11.72 g; wet weight with the shell). The mollusks were fed on submerged leaves of *Nuphar lutea* (L.) Smith collected at a depth of 1.5 m in the Moskva River in June. We eliminated the main rib from each leaf and cut the leaf in halves, with one half to be used as a food in the microcosms with a surfactant and the other, in the control microcosms. The surfactant was tetradecyltrimethylammonium bromide (TDTMA). The volume of water in each microcosm was 1 l (settled tap water was used). Four microcosms with the TDTMA solution tested contained 7.04 g of *N. lutea* leaf phytomass (the total wet weight in four jars) and 31 mollusks (the total wet weight was 55.72 g). Four control microcosms contained a total of 6.94 g of phytomass and 20 mollusks with a total wet weight of 38.04 g. A concentrated TDTMA solution (mol wt 336.4) was added to the experimental microcosms to a final concentration of 2 mg/l. The microcosms were incubated at 22.5–24°C for 72 h without stirring. Water was replaced and fresh TDTMA was added daily. Settled tap water preliminarily incubated at room temperature for several days was used in both experimental and control microcosms. The phytomass eaten by the mollusks (Table 1) was estimated from the difference between phytomasses before and after incubation. The total organic nitrogen according to Kjeldal was determined in the phytomass and pellets using mineralization [2, 3]. Phosphorous was determined by the modified phosphomolybdic blue method [4, 5]. The sum of organogenic and soluble (mineral) silicon was determined as described in [4, 5] with modifications; molybdenum [Mo (VI)] contained in silicomolybdic acid was photochemically reduced using riboflavin as a photosensitizer. Absolute dry weight was determined after drying at 105°C for 2–3 h (until the weight became constant).

Exposure to TDTMA decreased the feeding rate of mollusks, which was estimated from the rate of decrease in food, within three days of incubation. The inhibitory effect of TDTMA was observed at the surfactant concentration of 2 mg/l (Table 1). TDTMA inhibited feeding by 27.9–70.9%, depending on the time period. No mollusks died during the first two days of the experiment; after the third day of incubation, the mortality was as low as 9.7% (in the variant with TDTMA).

The decrease in feeding rate was accompanied by a decrease in pellet formation. The pellet production in the four experimental microcosms was less than in the control ones, although the total biomass in experimental microcosms was 1.46 times higher than in the control group. The total production of pellets and their accumulation at the bottom of the jars per unit weight of mollusks in TDTMA-treated microcosms was only 58.3% of the control value (Table 2).

The elemental composition of pellets was also studied (Table 3). In addition to organic carbon, they contained nitrogen, phosphorus, and silicon (2.34–2.58, 0.41–0.44, and 1.11–1.19% of the absolute dry weight,

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Table 1. The effect of TDTMA on the feeding rate of the mollusk L. stagnalis feeding on N. lutea leaves

Time, h	TDTMA dose, mg/l	Temperature, centigrade	Phytomass eaten		Feeding rate inhibition, %
			milligrams of wet phytomass per gram of wet weight of mollusks	percentage of the phytomass eaten by mollusks in the control group	
	0		12.36	100	0
21	2	22.5	3.59	29.1	70.9
	0		19.72	100	0
45	2	22.5	6.82	34.6	65.4
	0		37.85	100	0
72	2	24	27.28	72.1	27.9

Note: Time after the beginning of incubation is indicated.

Table 2. Inhibition of matter transfer via sedimentation of L. stagnalis pellets by TDTMA in the microcosms during 72 h

TDTMA dose, mg/l	Pellet accumulation on the bottom of the microcosms			Inhibition, %
	dry weight (mg) in four microcosms (A)	dry weight (mg) of pellets per gram of wet weight of mollusks (A)	A, %	
0	261	6.86	100.0	0
2	223	4.00	58.3	41.7

respectively). The nitrogen and phosphorus contents were slightly higher than their contents in the *N. lutea* phytomass, whereas the silicon content was at least 30% higher than in the phytomass. Compare these data with our data on the elemental composition of pellets formed by *L. stagnalis* feeding on *Taraxacum officinale* Wigg.: 2.89, 0.48, and 1.87% for nitrogen, phosphorus, and silicon, respectively, versus 2.57, 0.44, and 1.15%, respectively, in the original leaves; in this case, the central rib was also eliminated before the leaves were placed in the microcosms. As far as we know, we were the first to study the elemental composition of *L. stagnalis* pellets. The trend towards a certain change in pellet composition compared to phytomass (especially an increase in silicon) was observed for both plant species studied.

Table 3. Elemental composition of pellets and N. lutea phytomass consumed by L. stagnalis (percentage of dry weight) in the presence and absence of TDTMA

Material	TDTMA dose, mg/l	Nitrogen	Phosphorus	Silicon
Leaves	0	2.19	0.39	0.85
	2	2.11	0.34	0.81
Pellets	0	2.34	0.44	1.11
	2	2.58	0.41	1.19

Note: For analysis of leaves, phytomass that remained in microcosms after 72 h of incubation was used.

Thus, the feeding activity of the mollusks and the related transfer of matter and energy in the food chain phytomass–phytophage–excreted pellets were sometimes suppressed by more than 40%. Note that negative sublethal effects on filtration connected with feeding activity of marine mollusks were earlier observed for other surfactants (sodium dodecylsulfate [6] and Triton X–100 [7]) at similar concentrations. Feeding activity of rotifers, another important group of aquatic invertebrates, is also disturbed by surfactants, as exemplified by the effect of TDTMA on *Brachionus angularis* [8]. The effects of surfactants on feeding rate in invertebrates are important because of the hazard for many ecologically important processes [11], including those involved in water self-purification [9, 10].

Pellet sedimentation means a considerable acceleration of vertical (towards the bottom) matter transfer in an aquatic ecosystem, because *N. lutea* leaves themselves and their fragments are highly buoyant and do not sediment (there was no sedimentation over a period longer than the duration of our experiment).

The disturbance in the mollusk feeding rate may be ecologically important. According to Tsikhon and Lukanina (1965, cited from [1]), the daily ration of gastropods feeding on plants may be as much as 350, 400, and even 424% (*L. stagnalis*, *Radix ovata*, and *Physa fontinalis*, respectively). This indicates that this link of the ecosystem food chain accounts for considerable matter and energy transfer. We may predict that new examples of suppression of pellet production, as well as the detritus formation and matter transfer connected

with it, will be found when effects of various contaminants on invertebrates are studied.

The data obtained allow us to draw the following conclusions.

(1) Pellet excretion by *L. stagnalis* feeding on the phytomass of macrophytes (such as *N. lutea*) may be as high as 4–7 mg absolute dry weight per gram wet weight of mollusks per 72 h.

(2) Pellet sedimentation may contribute to vertical transfer of chemical elements in the ecosystem. The pellet composition varies depending on the species of the plants eaten by mollusks. When *L. stagnalis* feed on *N. lutea*, pellets contain, in addition to organic and inorganic carbon, nitrogen (2.3–2.9%), phosphorus (0.4–0.5%), and silicon (1.1–1.9%). The silicon content may be 30% higher than in the food phytomass.

(3) The surfactant TDTMA and, apparently, other contaminants may suppress pellet production, their accumulation at the bottom of microcosms, and the matter transfer connected with this.

(4) The data obtained demonstrate a new aspect of the ecological hazard due to environmental pollution with TDTMA and other quaternary ammonium compounds at sublethal concentrations.

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