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Short research contribution

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IMPACT OF HUMIC SUBSTANCES ON BACTERIOPLANKTON IN EUTROPHIC LAKE

ABSTRACT: This paper presents the results of research on impact of humic substances (HS) on bacterioplankton in eutrophic Lake Jeziorak (north-eastern Poland). In cultures of natural bacteria from the lake water, enriched with 0, 5, 10, 25 and 50 mg dm⁻³ of HS (natural HS – isolated from lake water or commercial HS – sodium salt of humic substance; Sigma-Aldrich), were determined the following parameters: total number of bacteria (TNB), number of heterotrophic bacteria (CFU), bacterial production (BP) (measured with [³H]methyl thymidine method) and general activity of esterases. Natural HS had a more positive impact on bacterial growth, bacterial production and activity of esterases than the commercial preparation HS. The highest TNB and CFU was observed when the water was enriched with 25 mg dm⁻³ of natural HS. All concentrations of the natural HS stimulated bacterial production. The activity of hydrolytic enzymes increased with rising concentrations of natural HS.

KEY WORDS: humic substances, bacterioplankton, heterotrophic bacteria, eutrophic lake

Soluble humic matter constitutes the majority (50–70%) of the total content of DOC in lacustrine waters (Münster and Chróst 1990, Górniak 2004). Humic substances (HS) greatly affect aquatic mi-

croorganisms both indirectly, by changing the physico-chemical properties of water and determining the bioavailability of nutrients (Hillbricht-Ilkowska *et al.* 1998, Steinberg 2003, Górniak 2004), and directly, by involvement in their metabolism (Burkowska and Donderski 2003). Therefore, these substances may have a considerable impact on the trophic composition of aquatic communities.

Most of the previous studies that have addressed the impact of HS on bacterio- and phytoplankton (Hessen 1992, Jones 1992, Klug 2002) were conducted on bacterial populations found in lakes with extreme humus content (highly humic and oligotrophic lakes). However, the eutrophic lakes dominate in temperate, lowland lake-lands, like in Poland. Therefore, the purpose of this study was to determine the impact of HS on the number and activity of bacteria from a typical eutrophic lake.

The eutrophic Lake Jeziorak, located in the Drwęca-Wisła river basin (53°42'N, 19°37'E), was the object of the study. The Lake Jeziorak is a postglacial channel lake situated longitudinally. From the western side, the lake is surrounded by broadleaf and mixed pine – beech forests. The 13 km

section of the eastern shoreline to the north of the town of Iława borders meadows and cultivated land; beyond that, the shore is covered with coniferous and mixed forests. The Lake Jeziorak is an eutrophic lake. The water is characterized by low transparency, yellow-green color and alkaline conditions (Table 1).

Sampling was carried out on the summer (25th July) of 2005 in the pelagic zone of Lake Jeziorak. The samples were taken with Ruttner sampler at 1 m depth and transferred to sterile glass flask.

Water from the lake of 500 cm³ volume samples were poured into sterile Erlenmeyer flasks (1 dm³) following filtration through sterile filter paper (MN 617) in order to eliminate the larger particles. Five, 10, 25, and 50 mg dm⁻³ of natural humic substances, which were isolated from water following the method designed by Thurman and Malcolm (1981), were added to four flasks. The same quantities of commercial humic substance (sodium salt of humic substance; Sigma-Aldrich) were added to a second set of four flasks. Lacustrine

Table 1. Morphometric and trophic characteristics of eutrophic Lake Jeziorak (north-eastern Poland) (Jańczak 1997).

Characteristic	Value
Area (km ²)	32.2
Maximal depth (m)	12.0
Mean depth (m)	4.1
Length of shore line (m)	117700
pH ⁽¹⁾	7.8–8.3
Electrolytic conductivity (μS cm ⁻¹) ⁽¹⁾	461–485
Water transparency (m) ⁽¹⁾	0.61–0.98
Total phosphorus (mg dm ⁻³) ⁽²⁾	0.20
Total nitrogen (mg dm ⁻³) ⁽²⁾	4.62
Chlorophyll <i>a</i> (μg dm ⁻³) ⁽¹⁾	42.8–67.3

⁽¹⁾ data supplied by Department of Environmental Microbiology and Biotechnology, Nicolaus Copernicus University (mean for epilimnion, spring, summer and autumn 2005)

⁽²⁾ data supplied by Department of Hydrobiology, Nicolaus Copernicus University (mean for epilimnion, spring and summer 2001)

Table 2. Effect of humic substances (HS) isolated from lake water (natural HS) and commercial preparation (commercial HS) on the bacterial production (BP). Values in shadow indicate stimulation of bacterial production (BP).

	Concentration of HS (mg dm ⁻³)	BP	
		(μg C dm ⁻³ h ⁻¹)	
natural HS	0	1.5	±0.10
	5	11.71	±2.04
	10	5.74	±1.16
	25	6.05	±1.61
	50	7.27	±1.82
commercial HS	5	2.03	±0.27
	10	3.29	±0.84
	25	1.43	±0.39
	50	1.01	±0.26

water without humic additives constituted the control sample. All cultures were incubated at 20°C for 7 days.

The following parameters in cultures of natural bacteria from the lake were determined using the following methods:

a) total number of bacteria (TNB) – using the direct counting of bacteria on membranes, following method of Zimmerman (1981). Bacteria were placed on a filter and dyed with a 100 mg dm⁻³ water solution of acridine orange. Dyed bacteria were counted under the epifluorescent microscope “Jenalummar” equipped with a set of inducing filters 2 × KP 490 + B 229, lens “Planachromat fl” with a magnification of 1 × 100, aperture 1.30, and an eyepiece with a magnification of 1 × 10,

b) number of heterotrophic bacteria (CFU) – using Koch’s plate technique with a spread plates method on an iron-peptone agar medium (IPA) according to Ferrer *et al.* (1963),

c) bacterial production (BP) – by determining the incorporation of radioactive [³H]-methyl thymidine by bacteria cells following Fuhrman and Azam (1982) method. Bacterial production was calculated based on the assumption that production of 1.24 × 10⁹ cells corresponds to incorporation of 1 nm of thymidine into DNA (Chróst *et al.* 1988). Bacterial production, expressed as the quantity of carbon incorporated into the cells, was calculated using the conversion factor of 19.8 fg of C per cell (Lee and Fuhrman 1987),

d) general activity of esterases – by measuring the rate of fluorescein released from fluorescein diacetate (FDA) (Gillian and Duncan 2001). The quantity of released fluorescein was measured using a Hitachi f-2500 spectrofluorometer at an excitation wave of 480 nm and emission wavelength of 505 nm.

The addition of 5–50 mg dm⁻³ of either natural or commercial HS to the Lake Jeziorak water stimulated bacterial growth. The highest total number of bacteria (TNB) was observed when the water was enriched with 25 mg dm⁻³ of HS (Fig. 1).

All concentrations of natural humic substances stimulated the growth of heterotrophic bacteria (CFU) from the Lake Jeziorak. The addition of 25 mg dm⁻³ of natural humic substances to the water had the most favorable impact on the growth of heterotrophic bacteria. The commercial preparation of HS stimulated the growth of heterotrophic bacteria when its quantity equaled 10 mg dm⁻³, while the addition of 50 mg dm⁻³ of commercial HS insignificantly inhibited the growth of heterotrophic bacteria (Fig. 2).

All concentrations of the natural humic substances stimulated bacterial production (BP) (Table 2). Addition of 5 mg dm⁻³ to the lacustrine water caused almost an 8-fold increase in bacterial production in comparison to the water without HS additives. The impact of the HS commercial preparation on bacterial production was much weaker and depended on the concentration of HS. Enrichment of the lacustrine water with 5 and

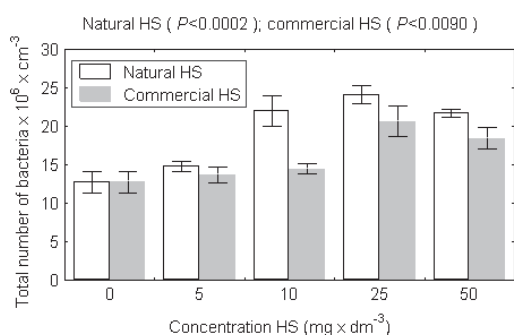


Fig. 1. Impact of humic substances HS on total number of bacteria (TNB) in the lake water (natural HS – isolated from lake water, commercial HS – sodium salt of humic substance, Sigma-Aldrich; *P* – significance level).

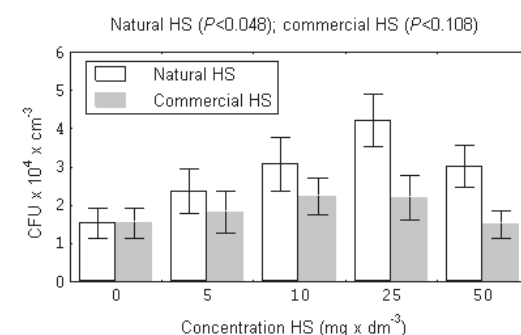


Fig. 2. Impact of humic substances (HS) on the numbers of heterotrophic bacteria (CFU) in the lake water (natural HS – isolated from lake water, commercial HS – sodium salt of humic substance, Sigma-Aldrich; *P* – significance level).

10 mg dm⁻³ of the HS commercial preparation stimulated bacterial production (almost twice in case of 10 mg dm⁻³). However, the addition of 25 and 50 mg dm⁻³ of commercial HS to the water insignificantly hampered the rate of thymidine incorporation and thus, bacterial production.

Increased activity of esterases was observed in the water samples enriched with natural humic substances. The activity of hydrolytic enzymes increased with rising concentrations of natural humic substances. When HS concentration reached 50 mg dm⁻³, the activity of esterases was 9.5 higher than in the control sample. Water enrichment with the commercial preparation HS caused only insignificant shifts in esterase activity (Fig. 3)

Tranvik and Höfle (1987) observed higher numbers of bacteria on substrate enriched with HS than on substrate without HS. Tranvik and Sieburth (1989) state that the addition of dissolved HS to lacustrine water caused a five-fold increase in bacterial production in relation to the control sample. This phenomenon was confirmed by culturing bacteria from both highly humic lakes and oligotrophic lakes with low concentrations of humus. The results of this study demonstrate that the presence of HS may also increase the abundance of bacterioplankton in eutrophic lakes.

Many researchers have concluded that the positive impact of humus on micro-organisms is a result of the fact that this sub-

stance contains growth stimulators. Compounds similar to that of growth-promoting substances, for example phytosterol and ergosterol, vitamin D precursors, have been found in humic preparations. A high similarity of fulvic acids to ascorbic acid has also been established (Grabińska-Łoniewska *et al.* 2002). Visser (1985) suggested that HS also increase the permeability of cytoplasmic membranes and, in this way, facilitate penetration of poorly-dissolving substrates to the cells' interior. Also, some nutrient compounds, primarily iron and phosphorus, are taken up more easily from chelate compounds with humus compounds than from non-organic compounds (Grabińska-Łoniewska *et al.* 2002).

Determination of threshold concentrations of HS, above which the microbiological processes become inhibited, is essential. The conducted studies demonstrate that commercial HS may impede development of bacterial production if their concentrations range between 25–50 mg dm⁻³. Studies by Visser (1984) and Fujimura *et al.* (1994) demonstrated that growth of the majority of soil bacteria, which are naturally adapted to the presence of higher concentrations of humic substances, was hindered by a humus concentration of 100 mg dm⁻³. According to Pempkowiak (1997), some fractions of humic substances isolated from seawater may even possess antibiotic properties.

Generally, the concentration of humic substances in the majority of water bodies does not exceed 10 mg dm⁻³ (Górniak 1996). In water bodies located in agricultural areas, this concentration may be even higher, reaching *ca.* 30 mg dm⁻³ (Szpakowska 1999), and in underground water or polyhumic rivers may reach values of over 50 mg dm⁻³ (Zieliński 2004). James (1991) demonstrated that DOC content in highly humic lakes with a humus concentration not exceeding 20 mg dm⁻³ has a positive impact on the abundance of bacterial cells. However, at concentrations above 20 mg dm⁻³, he observed a toxic effect caused by excessive quantities of such substances as phenols and unspecified growth inhibitors.

Experiments of Fujimura *et al.* (1994) show that the bacterio-static effect of HS is

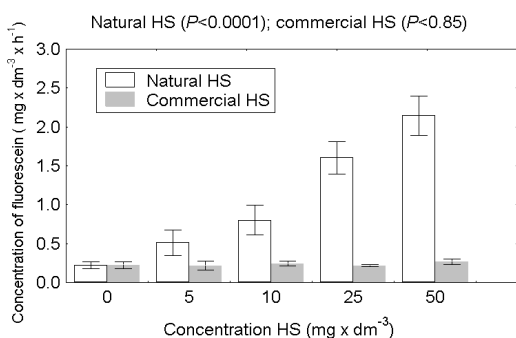


Fig. 3. Impact of humic substances (HS) on the esterase activity measured as concentration of fluorescein (natural HS – isolated from lake water, commercial HS – sodium salt of humic substance, Sigma-Aldrich; *P* – significance level).

related to the occurrence of free hydroxyl radicals, which according to Senesi (1990) are stable in HS solutions and remain constant for a long time.

Inhibition of growth of microorganisms in the presence of high concentrations of HS can also be associated with a different mechanism. Dissolved HS has the ability to form complexes with the compounds of phosphorus and ammonia, as well as metal cations. This effect is responsible for the "biological deactivation" of these compounds (i.e., it makes some nutrients inaccessible to microorganisms). If the HS load dominates over the nutrient load in the solution, nearly the entire nutrient pool may be bonded in humus complexes, and as a result becomes biologically inaccessible. Under natural conditions, the above process may be responsible for dystrophication of a lake (Wojciechowski 1999).

The changes in the total numbers and production of bacteria depend on the concentration and source of the HS. Natural HS, which was isolated from lake water using a method of sorption on Amberlit XAD-8 resin, had a more positive impact on bacterial growth than the commercial preparation (made by Sigma-Aldrich). Donderski and Burkowska (2000) observed that the supply of natural HS made the bacteria more active in respiration than when they are supplied with the commercial HS. That phenomenon may have been caused by the fact that, depending on its origin, the HS are not homogenous and are composed of various fractions occurring in different proportions. The availability of these fractions to bacteria is differentiated (Grabińska-Łoniewska *et al.* 2002). The extraction techniques used are also of some importance to HS compliance. The extraction procedures used may either cause its partial split and make it prone to microbiological decomposition or make it totally unavailable and not assimilable.

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