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Abnormal forms in *Pinnularia gibba* (Bacillariophyceae) in a polluted lowland stream from Argentina.

by

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With 5 figures and 4 tables

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Abstract: A study of samples collected in the Don Carlos Stream, a small stream that runs across an urbanised and industrialised area of the Pampean plain, displayed abnormalities in the valves of *Pinnularia gibba* Ehrenberg. Three stations were sampled in different sectors of the stream: station A was located upstream and exposed to agricultural activity, stations B and C were exposed to sewage effluent and to outflows from textile and metallurgical factories, respectively. Acetate sheets placed on bricks, fastened with several staples. were used as artificial substrata and exposed to colonisation during 21 days prior to collection. Diatoms from the artificial substrata were sampled during three weeks. Environmental variables analysed were pH, conductivity. dissolved oxygen. biological oxygen demand, chemical oxygen demand, hardness, total suspended solids and nutrients. Also heavy metals (Cu, Cr, Cd, Zn) were analysed in water and sediment. Two types of abnormalities were found in *P. gibba:* type I had valves exhibiting abnormal stration pattems and/or deformed or interrupted raphe and type II had deformities in the outline of the valves, sometimes linked to anomalies in the striae or raphe. Multiple correlation analysis showed a close relationship between the appearance of the deformations and water quality in the Don Carlos Stream.

Key words: abnormalities; diatoms; lowland stream; *Pinnularia gibba;* pollution; heavy metals.

Introduction

Abnormalities in diatom morphology were reported already by Cox (1890). Van Heurck (1896) and Hustedt (1930a). Deformations in benthic algae have been correlated with physical variables such as temperature, current velocity, flow and rainfall, rather than with chemical variables (Antoine & Benson-Evans 1984). Recent studies (Adshead-Simonsen et al. 1981. Fisher et al. 1981, McFarland et al. 1997. Ruggiu et al. 1998) reported on relationships between anthropogenic stress and abnormalities.

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In this paper we present results from samples collected in a small stream that runs across an urbanised and industrialised area of the Pampean plain. The samples were analysed for abnormalities in the valves of *Pinnularia gibba* Ehrenberg. The aims of the present study were: (1) to describe the types of deformations present in the valves of P. *gibba* in the samples analysed, and (2) to explore the relationship between the occurrence of anomalies in the frustules and environmental conditions in the Don Carlos Stream.

Materials and methods

The Don Carlos Stream is located near La Plata City (34°55'-34°50' S to 58°00'-58°03' W). Argentina, and flows into Río de la Plata (Fig. 1 a). Three sampling stations were chosen in different sectors of the stream: Station A (depth 50 cm, water flow velocity 0.11 m s^{-1}) was located upstream and exposed to agricultural activity; station B (depth 35 cm, water flow velocities 1.8 m s^{-1}) and station C (depth 25 cm. water flow velocities 0.46 m s^{-1}) were exposed to sewage effluent and to outflows from textile and metallurgical factories, respectively (Fig. lb). Downstream from station B. several sites with hydraulic engineering, such as canalisation and modification of the stream bed and banks. were located.
Acetate sheets placed on bricks, fastened with some staples, were used as artificial substrata and exposed to colonisation during 21 days prior to collection. Diatoms from these artificial substrata were sampled on three occasions (25 September, 2 October and 9 October, 2000). For each sampling station the biofilm was removed with toothbrushes from these areas of 160 cm^2 of the acetate sheets (Stevenson & Bahls 1999). The collected material was preserved with formalin (4% v/v final concentration). Subsamples for permanent diatom slides were washed several times to remove the formalin and the organic matter was oxidised with hydrogen peroxide. Clean diatoms were mounted in Naphrax©, and up to 300 frustules were counted from each sample to assess the composition of the diatom assemblage. The percentage of abnormalities in P. *gibba* from a total of 300 valves in each sample was determined usina a light microscope (Olympus BX-50) and photomicrographs were taken using Nomarski DIC optics. The material collected was also examined with a scanning electron microscope (Jeol T-100 Model). Taxonomy follows Hustedt (1930b), Patrick & Reimer (1966. 1975), Krammer & Lange-Bertalot (1986, 1988, 1991 a, 1991 b), and Krammer (1992. 2000).
The physico-chemical variables recorded weekly were= temperature, dissolved oxygen (DO) (Oxymeter 600-ESD), conductivity (Hanna H18633), pH (Cole-Palmer, pH tester 2 waterproof) and water flow velocity (Global Water Flow Meter) were measured with portable meters. Water samples for the analysis of dissolved inorganic nutrients and total suspended solids (TSS) were filtered inmediately through glass fibre filters (Whatman G/FC) and, together with samples for BOD5 and COD, these were stored at 4°C until arrival at the laboratory. Soluble reactive phosphorus, nitrite, nítrate. ammonium. hardness. BOD5 and COD were determined according to Mackereth et al. (1978). Triplícate samples of water and sediment of each sample site were collected, on 2 October 2000, for analysis of Cu, Cr, Cd and Zn and were analysed by atomic absorption spectrophotometry according to APHA (1992) and EPA SW 846 (1986).
Multiple correlation analysis was employed to check the relationship between percentage of anoma-lies and water quality variables (Sokal & Rohlf 1969). Variables that did not display a normal distribution were log-transformed prior to analysis.

Results

The main physical and chemical characteristics of the sampling stations are shown in Table 1. Phosphate and nitrate increased upstream with agricultural activity, while ammonium, COD and BOD_5 increased downstream with urban and industrial activities

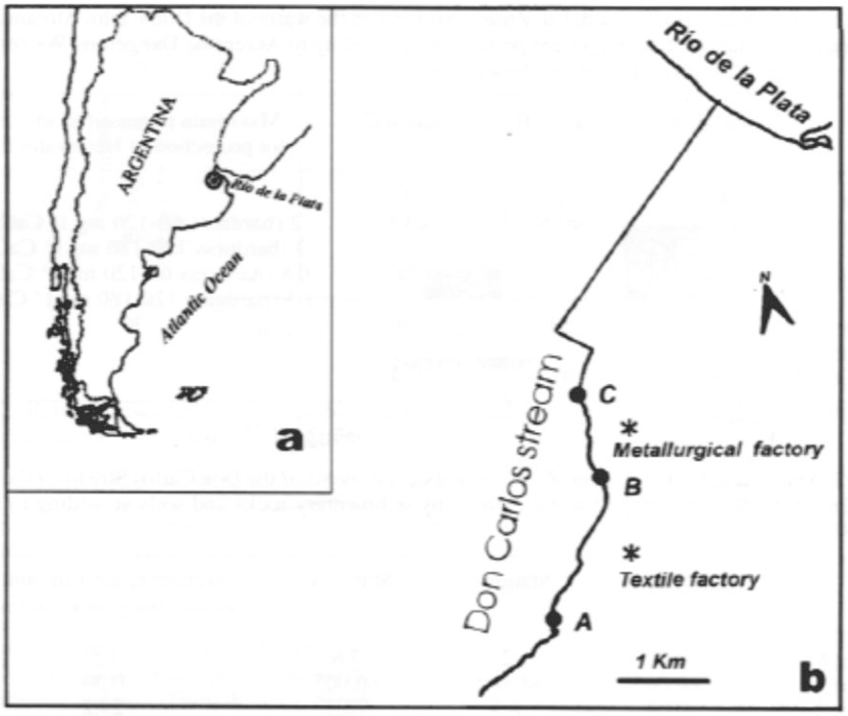

Fig. 1: (a) Study area, (b) location of the sampling stations in the Don Carlos Stream.

Table 1: Environmental variables. average and standard deviations (SD), measured at the three sampling stations in the Don Carlos Stream (n=9).

	Station A	Station B	Station C
Temperature (°C)	14.5 (± 1.2)	19.3 (± 0.3)	16.9 (± 0.93)
Conductivity (µS cm^{-1})	771 (± 124)	990 (± 148)	942 (± 72)
DO (mg 1^{-1})	7.45 (± 1.49)	2.97 (± 1.87)	4.27 (± 0.53)
pH	6.93 (± 0.34)	7.13 (± 0.28)	6.94 (± 0.25)
TSS (mg 1^{-1})	12.4 (± 7.5)	12.3 (± 5.5)	24.4 (± 24.7)
BOD_5 (mg 1^{-1})	4.3 (± 2.1)	34.0 (± 10.6)	21.7 (± 11.7)
COD (mg 1^{-1})	26.7 (± 5.1)	77.0 (± 9.5)	70.3 (± 20.8)
PO_4^{3-} -P (mg 1^{-1})	1.09 (± 0.38)	0.46 (± 0.09)	0.36 (± 0.11)
NO_3 -N (mg 1^{-1})	1.16 (± 0.19)	0.6 (± 0.26)	0.67 (± 0.42)
NO_2^{3-} -N (mg 1^{-1})	0.06 (± 0.03)	0.07 (± 0.05)	0.13 (± 0.05)
NH_4^{2+} -N (mg 1^{-1})	0.05 (± 0.05)	0.73 (± 0.18)	0.82 (± 0.35)

(textile and metallurgical factories). Tables 2 and 3 show heavy metal concentrations measured in water and sediment, respectively. Cu increased downstream, reaching its highest value at station C (59 µg 1^{-1}), while the concentration of Zn was highest at station B (23 µg 1^{-1}). The levels of Cu measured in the Don Carlos Stream exceeded

Table 2: Concentrations or Cr, Cd, Cu, Zn and hardness in the water of the Don Carlos Stream at the three sampling stations and maximum permisible according to Argentine Dangerous Wastes Law N° 24051 (1993) for protection of freshwater life

	Station A	Station B	Station C	Maximum permissible amount for protection of freshwater life
Cr (µg l^{-1})	<2	2	2	2
Cd (µg l^{-1})	<0.5	<0.5	<0.5	2 (hardness 60-120 mg l^{-1} $CaCO_3$)
				3 (hardness 120-180 mg l^{-1} $CaCO_3$)
Cu (µg l^{-1})	47	7	59	0.8 (hardness 60-120 mg l^{-1} $CaCO_3$)
				1.3 (hardness 120-180 mg l^{-1} $CaCO_3$)
Zn (µg l^{-1})	9	23	19	30
Hardness ($CaCO_3$ mg l^{-1})	81	107	114	

Table 3: Amounts of Cr, Cd, Cu and Zn in streambed sediments of the Don Carlos Stream at the three sampling stations and average natural amount by sedimentary rocks and soils according to Frink (1996).

	Station A	Station B	Station C	Average natural amount by sedimentary rocks and soils
Cr (mg kg^{-1})	0.02	2	3.8	129
Cd (mg kg^{-1})	<0.005	<0.005	<0.005	0.34
Cu (mg kg^{-1})	0.07	8.3	75.3	29.2
Zn (mg kg^{-1})	0.19	25.5	41.3	40

the limits specified as the maximum permissible established by the Argentine Dangerous Wastes Law, N° 24051 (1993) for protection of freshwater life (Table 2). Similarly, the level of Cu measured in the bottom sediments of this stream at station C (Table 3) was 2.5 times higher than the average natural amount in sedimentary rocks and soils according to Frink (1996).

Abnormal frustule morphology

The relative abundance of *P. gibba* on artificial substrata in the Don Carlos Stream increased downstream in station B and C (Fig. 2) and the composition of the diatom assemblage also changed (Table 4). The *P. gibba* specimens in the Don Carlos Stream had a mean length of 54.5 µm (range 42.9-65.5 µm), mean width 9.6 µm (8.8-9.8 µm) and 9-10 striae in 10 µm. 41.7% of the specimens observed were smaller (< 50 µm) than the size reported by Krammer (1992).

We classified the anomalies observed in *P. gibba* into two types. Type I had valves exhibiting abnormal patterns of striations and/or a deformed or interrupted raphe (Fig. 3a-h, Fig. 4c-g) and type II included deformities in the outline of the valves, sometimes linked to anomalies in the striae or raphe (Fig. 3i-1, Fig. 4b). Fig. 4a shows a normal specimen of *P. gibba.* No deformities were observed in P. *gibba* at

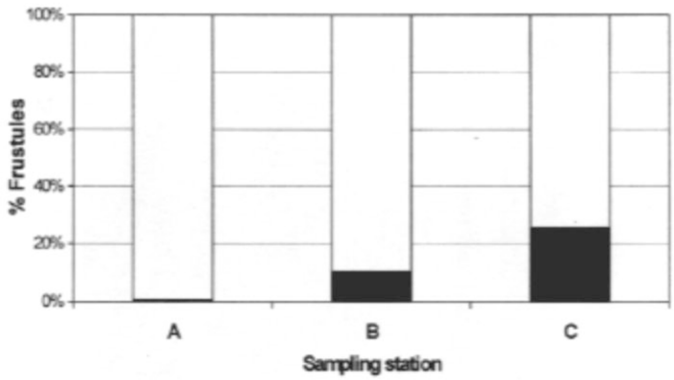

Fig. 2: Relative abundances of *P. gibba* (black bars) and other diatoms (white bars) at the three sampling stations in the Don Carlos Stream.

Table 4: List of the most abundant diatom species found in the Don Carlos Stream and relative abundances at the three sampling stations (*: 0-10%, **: >10-30%, ***: >30%.).

	A	B	C
Achnanthidium minutissimum (Kützing) Czarnecki			*
Amphora copulata (Kützing) Schoeman & Archibald	*		
Craticula accomoda (Hustedt) D.G. Mann	*		*
Craticula cuspidata (Kützing) D.G. Mann		*	*
Encyonema silesiacum (Bleisch in Rabenhorst) D.G. Mann	*		
Eolimna subminuscula (Manguin) Moser, Lange-Bertalot & Metzeltin	*	*	**
Fallacia pygmaea (Kützing) Stickle & D.G. Mann		*	
Gomphonema clavatum Ehrenberg	*	*	
Gomphonema parvulum Kützing	**	*	*
Melosira varians C. Agardh	*		
Navicula angusta Grunow	*	*	
Navicula cryptocephala Kützing	*	*	*
Navicula trivialis Lange-Bertalot	*		
Nitzschia amphibia Grunow	*		
Nitzschia frustulum (Kützing) Grunow	*		
Nitzschia linearis (C. Agardh) W. Smith	*		
Nitzschia palea (Kützing) W. Smith	*	***	***
Nitzschia umbonata (Ehrenberg) Lange-Bertalot	*	*	*
Pinnularia gibba Ehrenberg	*	*	*
Planothidium lanceolatum (Brébisson ex Kützing) Lange-Bertalot	*		
Rhoicosphenia abbreviata (C. Agardh) Lange-Bertalot	*		
Sellaphora pupula (Kützing) Mereschkowsky		*	*
Sellaphora seminulum (Grunow) D.G. Mann			*
Surirella angusta Kützing	*		
Tryblionella hungarica (Grunow) D.G. Mann	*		
Ulnaria ulna (Nitzsch) Compère	***	*	

station A. The most frequently occurring deformity at stations B and C belonged to type I, while type II abnormalities were observed only at station C (Fig. 5). Morphological abnormalities were also observed in *Nitzschia umbonata* and *Ulnaria*

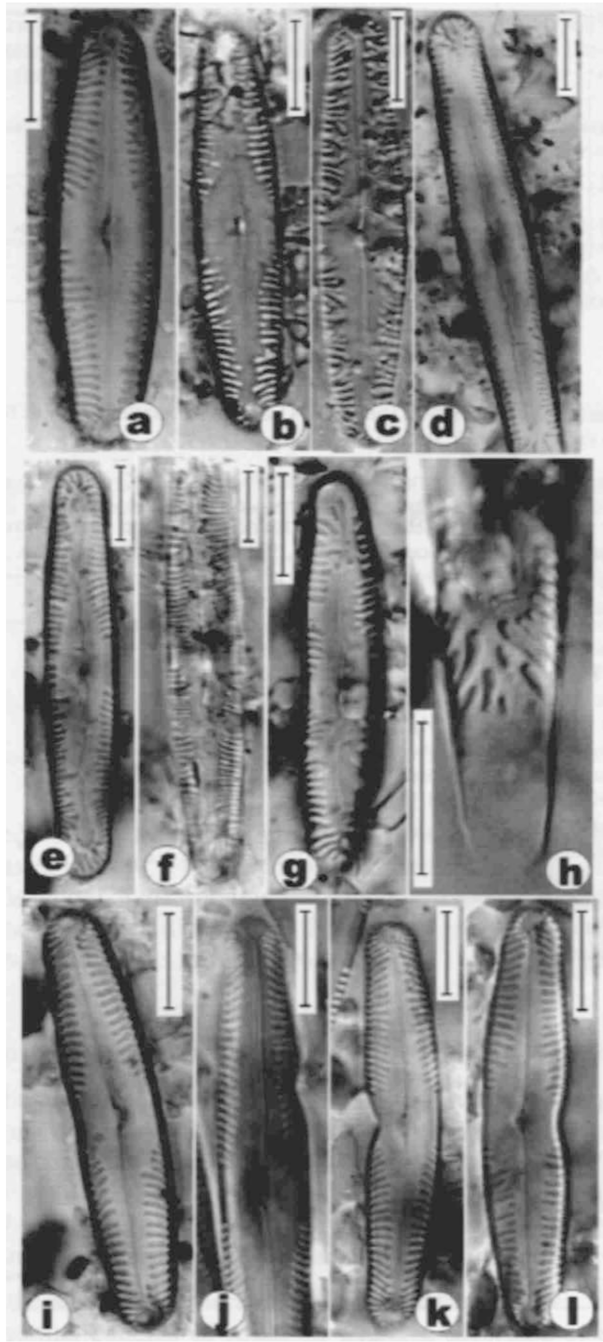

Fig. 3: *Pinnularia gibba* excibiting an abnormal morphology, LM; (a-h) abnormal patterns of striation and deformed or interrupted raphe (Type I); (i-I) deformities in the outline of the valves and abnormal pattern of striation (Type II). Scale bars represent 10 μm.

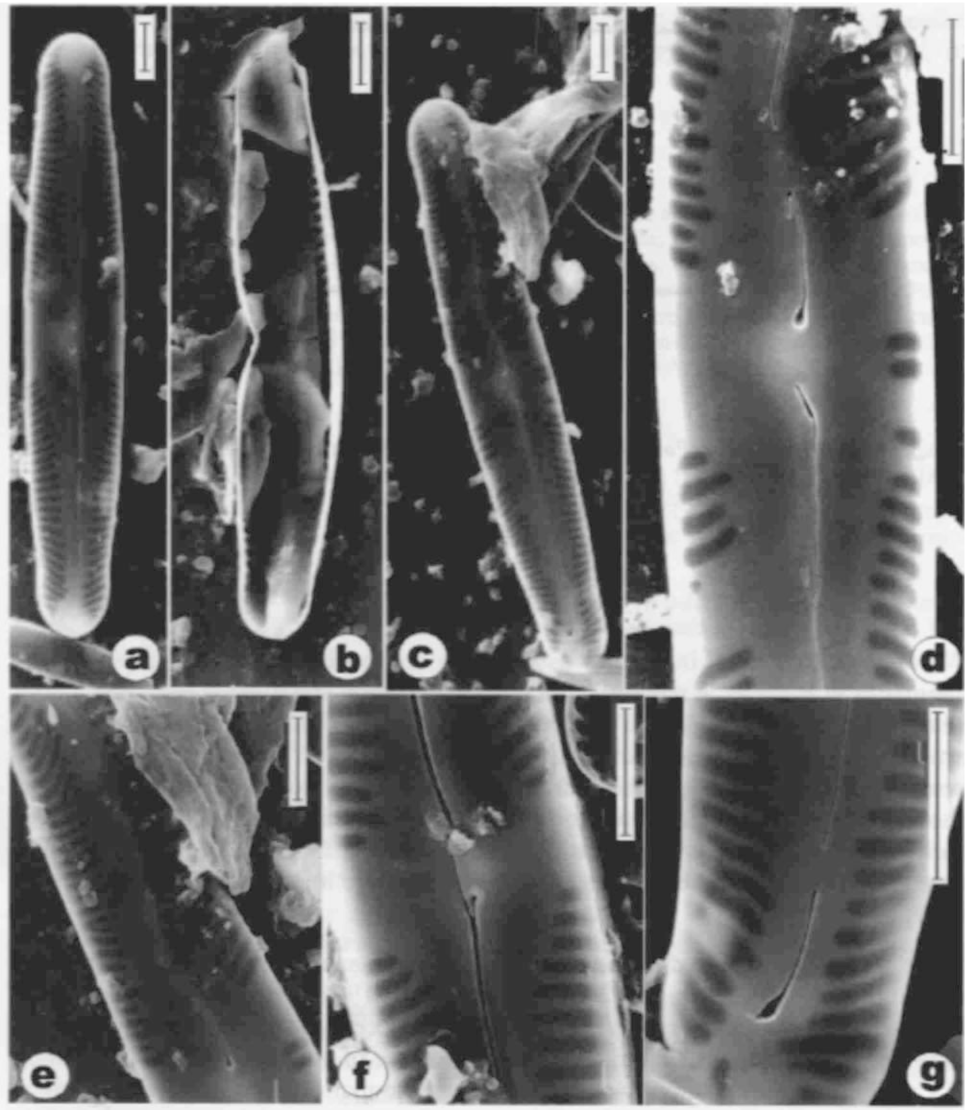

Fig. 4: SEM photomicrograph of *Pinnularia gibba; (a)* normal specimen; (b) with deformity in the outline of the valve (Type II), (c-g) with abnormal patterns of striation and deformed or interrupted raphe (Type 1). Scale bars represent 5 µm.

ulna at station C, although relative to P. *gibba* abnorrnalities were few in these two species.

Multiple correlation demonstrated significant ($p< 0.01$) relationships between the appearance of deformations and changes in water quality (nutrients, BOD5, COD, dissolved oxygen and pH) in the Don Carlos Stream.

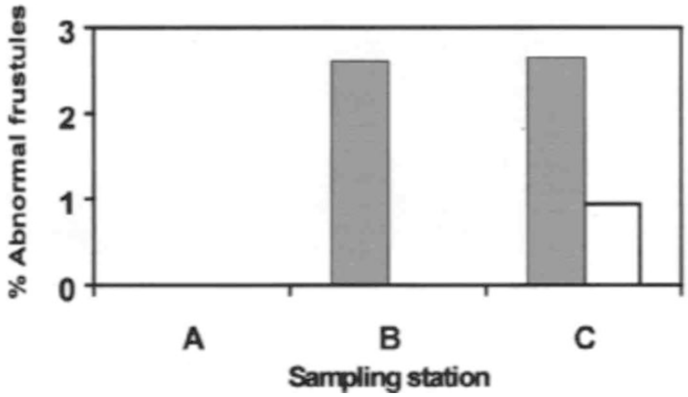

Fig. 5: Mean percentage of *Pinnularia gibba* abnormalities type 1 (grey bars) and type 11 (white bars), in the total number of *P. gibba* observed at the three sampling sites (A, B, C).

Discussion

We observed changes in diatom assemblage composition and the proportion of anomalies in P. *gibba* in relation to increasing pollution and toxic waste, particularly heavy metals (Cu and Zn), in the Don Carlos Stream. Translocations of colonised artificial substrata, carried out in situ in the Don Carlos Stream have previously demonstrated that those transferred from sites less polluted to sites more contaminated showed an increase in the proportion of *P. gibba* and morphological deformations in their frustules one week after translocation (Tolcach & Gómez 2002). Kelly (1998) noted that several members of the genus *Pinnularia* are tolerant of elevated levels of heavy metals.
Some specimens analysed in the Don Carlos Stream samples were smaller than the description by Krammer (1992). Cattaneo et al. (1998) observed a reduction of body size in *Achnanthes minutíssima* Kützing in cores from Lake Orta, in northem Italy and attributed the size reduction to an increase of copper pollution (30-100 μg^{-1} Cu). Furthermore, copper was recognised as the main toxic agent for diatoms in Lake Orta, in studies of palaeoecology and diatom responses to metal pollution (Ruggiu et al. 1998). Gerringa et al. (1995) found that copper stimulates an active production of ligands complexing the metal in diatoms, and this capacity may be enhanced by morphological distortions which result in increases in cell surface. Heavy metal or heavy metals and low pH have been related to deformities in diatoms (Adshead-Simonsen et al. 1981; McFarland et al. 1997). Stevenson & Bahls (1999) proposed that the percentage of aberrant diatoms could be used as a diagnostic measure from which to infer ecological condition. In the Don Carlos Stream probably metal contamination interacts also with other environmental factors, which contributes to the manifestation of diatoms anomalies. More studies should be carried out to clarify these interactions. Hoagland et al. (1996) stress that benthic communities are often

exposed to a mixture, rather than to individual toxicants; few studies have assessed whether such combinations of chemicals act synergistically, additively, or antagonistically.

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