Inter-annual depth-dependent toxicity and bioaccumulation of cadmium in marine benthic protist communities*

OCEANOLOGIA, 49 (3), 2007.
pp. 305–336.

KEYWORDS
Cadmium toxicity
Bioaccumulation
Protist communities
Density
Number of species
Biomass
Depth levels

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Received 16 May 2007, revised 17 July 2007, accepted 7 September 2007.

Abstract

The toxicity and bioaccumulation of cadmium in a marine benthic protist community were examined at different depths within the sediment. For this purpose, sediment-water microcosms with 1000 μg Cd dm^{-3} of the pollutant were used in two assays. The addition of cadmium caused a significant reduction in protist density, number of species and biomass. There was also a decrease in these three parameters with depth. During the treatment the density of protist groups was strongly depth-dependent. The dominant groups of protists at the different

* This study was supported by a grant (BOS2001-1334) of the CICYT (MCYT, Spain).

depths during the assay were also considered. The most dominant protist group in terms of density were the heterotrophic flagellates, both in the control and in the treatment with cadmium. In the treatments with cadmium, these were followed by ciliates and by dinoflagellates in both assays. In the control, all protist groups were present during the assay, whereas in the treatments with cadmium, autotrophic flagellates, diatoms and sarcodines were found in reduced proportion or not at all. Cadmium bioaccumulation increased towards the end of the assay. At any time during the assay, the proportion of cadmium bioaccumulated was an increasing function of depth.

1. Introduction

Some natural components of marine sediments could be regarded as pollutants at concentrations high enough to cause adverse biological effects. These are: nutrients, including phosphorus, and nitrogen compounds such as ammonia; metals, including iron, manganese, lead, cadmium, zinc and mercury; and metalloids such as arsenic and selenium. These contaminants do not ordinarily biomagnify in marine food webs, but at extremely high concentrations they are able to travel up the food chain. All have been linked to health problems in humans. Cadmium is a relatively rare element that acts as a minor nutrient for plants at low concentrations (Price & Morel 1990, Lee et al. 1995, Lane & Morel 2000), but is toxic to aquatic life at only slightly higher concentrations. Typical maximum cadmium concentrations in bays and estuaries range from 0.3 to 862 ppm (Förstner & Wittmann 1983), although the typical Cd concentration range for relatively uncontaminated marine sediments lies between 0.1 to 0.6 ppm (Warren 1981). Cadmium can enter the environment from various anthropogenic sources: by-products of zinc refining, coal combustion, mine wastes, electroplating processes, iron and steel production, as well as the pigment, metal, plastics, battery, fertiliser and pesticide industries (Hutton 1983, Clark, 1992, EPA 2001, Rubinelli et al. 2002). The impact of cadmium on aquatic organisms depends on its occurrence among different chemical forms (Callahan et al. 1979), which can have different toxicities and bioconcentration factors. In saltwaters with salinities from about 10 to 35 g kg^{-1}, cadmium chloride complexes predominate. Because of chloride complexation, the adsorption of cadmium to particles decreases with increasing salinity (Li et al. 1984). In both fresh and saltwaters, particulate matter and dissolved organic material may bind a substantial portion of the cadmium, and under these conditions cadmium may not be bioavailable as a result of this binding (Callahan et al. 1979, Kramer et al. 1997). In fact, up to about 80%, but usually less, of the cadmium in coastal seawater is complexed with dissolved or colloidal organic matter (Muller 1996, 1998).

Protozoa have been used to analyse cadmium toxicity in the marine environment because of their physiological and ecological characteristics: they are eukaryotic; their biology is well-known; the physiology and biochemistry of several species has been studied extensively; they are easily maintained in the laboratory and in large numbers; life-cycles and generation times are short; they are able to accumulate high levels of metals in their organelles; they are conveniently handled in the laboratory (Nilsson 1979, Fisher et al. 1983, 1995, Dive & Persoone 1984, Fernandez-Leborans & Novillo 1995). Several authors have described the effects of certain heavy metals, cadmium among them (Fernandez-Leborans & Novillo 1994), using species such as *Tetrahymena pyriformis* and *Acanthamoeba castellani* (Krawczynska et al. 1989), or *Uronema marinum* (Coppelloti 1994). In addition to the biochemical and physiological effects of cadmium on protozoans, its toxicity has also been determined at the community level (Fernandez-Leborans & Olalla-Herrero 1999). In sandy bottom coastal areas, heavy metals are subject to enrichment processes, and the concentration of cadmium in several areas decreases from the surface to deeper levels (Erlenkeuser et al. 1974). A number of studies have focused on the mobilisation of heavy metals from marine sediments. Microorganisms, algae, annelids, molluscs, echinoderms and other organisms have been implicated in cadmium removal (Förstner & Wittmann 1983, EPA 2001, Perez-Rama et al. 2002). However, there are no studies on the behaviour of protist communities at different depths of the sediment after the addition of a toxic metal such as cadmium. For this reason, the effects of Cd on these communities with respect to their density, biomass and species diversity were analysed in the present study. One key finding was that protist communities responded differently, depending on the depth within the sediment at which they were found.

2. Material and methods

Sample collection. In October 2002 and February 2004 samples were obtained from Da Seras beach (42°41′N, 9°02′W) (Coruña, Galicia, Spain), a beach open to the Atlantic Ocean, close to the southern entrance to the Muros estuary. Near-shore sediment samples were taken with a drag at a depth of 1 m. Samples were carried to the laboratory in 5 dm^3 polyurethane containers with the least possible disturbance of the sediment, adding seawater over the upper surface of the sediment. The sediment profile was preserved as far as possible during sample collection and transport.

Microcosms. In the laboratory, the samples were stored in 20 dm^3 microcosms (80% of sandy sediment and 20% of seawater). Enrichment was

with sterilised wheat grains (4 grains per dm^3). After an acclimatisation period of 7–9 days, the benthic community of protists exhibited a high density.

Bioassay. After the acclimatisation period, cadmium was added as cadmium acetate from a stock solution of 1 g dm^{-3}. Two sets of experiments were carried out in the presence and absence of cadmium:

(1) control without cadmium;

(2) nominal concentration of 1000 μg Cd dm^{-3} at time 0.

Three replicate samples were taken from the control as well as from the cadmium treatment.

Every 24 h the following variables were measured:

– density, number of species and biomass of protists;

– physicochemical parameters: temperature (°C), pH, oxidation-reduction potential (mV), salinity (PSU), conductivity (mS cm^{-1}), and mean grain size.

These variables were measured at the following depths: 0, 4, 8 and 12 cm from the surface of the sediment.

A high concentration of cadmium was added in order to ensure rapid diffusion to the deeper layers of the sediment.

Extraction of subsamples. The subsamples (5 cm^3) from the different depths of the sediment were obtained using a sucking pipette with a closing device at the end introduced within the sediment. Subsamples were obtained so as to minimise sediment disturbance.

Protist count. This was carried out with a Lackey Drop Microtransect (APHA 1989), using 100 μl subsamples. Dilutions were used for high densities of microorganisms. A minimum of three subsamples per depth were examined.

Abundance. This was expressed as the number of cells per cm^3. A minimum of three subsamples per depth in each microcosm were examined.

Classification of protists. This was done by light microscopy (X100, X1000) in bright field and in phase contrast. Additionally, an Image Analysis System (KS300 Zeiss) was used to support identification. Various fixation and staining techniques were used:

a) Silver impregnation techniques for the observation of ciliate morphology: protargol and silver carbonate techniques (Truffau 1967, Fernandez-Galiano 1976).

b) Lugol, acridine orange, cresyl blue and Noland's technique for flagellate identification.
c) Methyl green (Gabe 1968).

Biomass. This was obtained from the biovolume. Cell dimensions were measured using a micrometer eye-piece and the biovolume was obtained from the most appropriate formula for its geometrical shape. Volumes were converted into biomass using only one conversion factor: 0.15 pgC μm^{-3}. A number of conversion factors have been proposed for different protist groups, but this factor represents a well-documented standard value (Baldock et al. 1983, Baldock & Sleigh 1988, Montagnes et al. 1988, Butler & Rogerson 1996). The biomass is expressed in mg m^{-3} of dry weight.

Functional groups. The protists were classified into the following groups to represent the diversity of marine protist communities: heterotrophic flagellates (HFLA), autotrophic flagellates (AFLA), dinoflagellates (DIN), diatoms (DIAT), ciliates (CIL) and sarcodines (SARC).

Physicochemical parameter analysis. The pH and oxidation-reduction potential were measured using a pH meter (Crison 507) with a calomel electrode. The temperature, conductivity and salinity were measured with a conductivity meter (WTW LF196). All chemical products used were of a high degree of purity (proanalysis). A vibrating screen was used for the granulometric analysis of the sand, which was passed through a series of sieves (dry sieving), according to the Wentworth scale (Giere 1993). The mean grain size was calculated from the phi value (Krumbein 1939). A minimum of three subsamples per depth in each microcosm were examined.

Cadmium bioaccumulation. Pore water samples free of sediment (achieved with the help of a stereoscopic microscope and a pipette) were filtered (1.2 pore diameter) to harvest the protists. Filters were used that had been treated with nitric acid in a Millestone digestor. The cadmium bioaccumulated on the protists was measured using atomic absorption spectrophotometry (Perkin Elmer 4100 ZL with automatic sampler-injector AS70) coupled to a graphite furnace corrected for the Zeeman effect at 229 nm, using palladium as matrix modifier.

Statistical analysis of data. This was performed using the Statgraphics, SPSS and Multicua (Cuadras et al. 1991) programs.

3. Results

Physicochemical variables. In general, there were few significant differences between the control and microcosms with cadmium in either year (Tables 1 and 2). In October 2002, there were significant differences with

Table 1. Physico-chemical parameters during the 2002 assay

Mean ± standard deviation (above, treatments with cadmium; below, control)

Depth [cm]	Hours	Temperature [C°]	Potential [mV]	Salinity [PSU]	Conductivity [mS cm^{-1}]	pH
	0	20.5 ± 02	–31.2 ± 05	39.8 ± 05	54.1 ± 05	7.5 ± 05
		16.7 ± 02	–27.4 ± 05	40.1 ± 05	54.2 ± 05	7.5 ± 01
	24	15.6 ± 05	2.6 ± 02	33.2 ± 05	45.9 ± 01	6.5 ± 02
		16.8 ± 04	81.6 ± 05	40.8 ± 05	55.0 ± 02	5.6 ± 01
0	48	16.0 ± 05	5.8 ± 05	32.6 ± 01	45.2 ± 01	7.0 ± 05
		16.8 ± 00	36.9 ± 05	40.0 ± 05	54.0 ± 05	6.5 ± 06
	72	15.5 ± 00	14.9 ± 01	32.6 ± 05	45.3 ± 01	6.8 ± 03
		15.0 ± 04	32.0 ± 05	39.8 ± 05	54.0 ± 05	6.5 ± 01
	96	16.9 ± 04	55.5 ± 00	33.0 ± 05	45.5 ± 05	5.7 ± 05
		16.4 ± 04	43.5 ± 00	38.9 ± 01	52.7 ± 05	6.3 ± 05
	0	16.4 ± 05	–27.4 ± 05	35.2 ± 05	44.8 ± 00	7.6 ± 03
		16.2 ± 02	–21.2 ± 01	26.8 ± 05	43.2 ± 01	7.4 ± 02
	24	16.0 ± 05	03.1 ± 02	36.7 ± 05	50.0 ± 05	6.5 ± 03
		16.4 ± 00	14.2 ± 01	39.7 ± 05	53.7 ± 05	6.3 ± 02
4	48	16.1 ± 02	2.8 ± 05	32.6 ± 05	45.1 ± 05	7.1 ± 05
		16.3 ± 02	25.0 ± 05	39.6 ± 01	53.7 ± 05	6.6 ± 02
	72	15.0 ± 04	13.0 ± 05	32.7 ± 01	45.3 ± 05	6.9 ± 02
		14.6 ± 04	26.1 ± 02	39.3 ± 05	54.0 ± 05	6.6 ± 05
	96	16.7 ± 02	51.3 ± 02	33.1 ± 05	45.8 ± 05	5.8 ± 03
		16.0 ± 04	41.4 ± 03	37.8 ± 05	52.4 ± 01	6.3 ± 01
	0	15.8 ± 05	–27.1 ± 05	35.0 ± 05	51.2 ± 05	7.5 ± 03
		16.3 ± 02	–23.1 ± 05	37.1 ± 05	45.4 ± 01	7.5 ± 03
	24	16.0 ± 02	2.2 ± 05	37.3 ± 05	51.7 ± 05	6.5 ± 02
		16.2 ± 04	11.2 ± 01	39.6 ± 05	51.7 ± 05	6.4 ± 02
8	48	16.3 ± 02	0.8 ± 02	36.3 ± 05	49.7 ± 05	7.1 ± 01
		16.2 ± 05	23.1 ± 00	39.9 ± 05	53.9 ± 05	6.7 ± 05
	72	15.3 ± 05	13.5 ± 01	35.0 ± 05	48.3 ± 05	6.9 ± 01
		14.7 ± 02	23.4 ± 02	39.6 ± 01	54.2 ± 05	6.7 ± 02
	96	16.7 ± 03	50.2 ± 05	34.7 ± 05	47.6 ± 01	5.8 ± 05
		16.4 ± 04	39.9 ± 05	40.5 ± 05	54.7 ± 05	6.3 ± 05
	0	15.7 ± 02	–27.1 ± 05	35.5 ± 05	42.5 ± 00	7.5 ± 01
		16.3 ± 02	–22.8 ± 03	38.4 ± 05	51.9 ± 05	7.5 ± 01
	24	16.1 ± 05	0.6 ± 02	37.8 ± 05	51.6 ± 05	6.5 ± 02
		15.7 ± 00	8.8 ± 02	39.4 ± 05	53.6 ± 05	6.4 ± 01
12	48	16.3 ± 02	-0.6 ± 01	38.1 ± 05	52.0 ± 05	7.1 ± 03
		16.2 ± 04	13.0 ± 02	38.4 ± 05	52.2 ± 05	6.9 ± 01
	72	15.0 ± 05	13.9 ± 02	38.9 ± 05	52.6 ± 05	6.9 ± 05
		14.8 ± 02	20.9 ± 05	39.7 ± 05	53.7 ± 05	6.7 ± 03
	96	16.7 ± 05	47.0 ± 05	32.2 ± 05	45.2 ± 00	5.9 ± 04
		16.9 ± 02	38.1 ± 05	32.3 ± 05	44.6 ± 01	6.3 ± 01

Table 2. Physico-chemical parameters during the 2004 assay

Mean ± standard deviation (above, treatments with cadmium; below, control)

Depth [cm]	Hours	Temperature [C°]	Potential [mV]	Salinity [PSU]	Conductivity [mS cm^{-1}]	pH
0	0	15.7 ± 0.10	2.2 ± 0.10	43.5 ± 0.10	58.4 ± 1.80	7.4 ± 0.01
		15.0 ± 0.10	3.5 ± 0.10	44.1 ± 0.00	59.2 ± 0.90	7.3 ± 0.01
	24	16.5 ± 0.10	–28.3 ± 0.10	45.5 ± 0.00	60.5 ± 3.00	7.4 ± 0.00
		15.2 ± 0.00	–22.3 ± 0.20	46.2 ± 0.00	61.4 ± 0.00	7.3 ± 0.01
	48	16.6 ± 0.00	–37.3 ± 0.00	45.4 ± 0.00	60.5 ± 0.00	7.0 ± 0.00
		16.9 ± 0.00	–35.0 ± 0.10	46.8 ± 0.00	62.1 ± 0.00	6.8 ± 0.00
	72	17.7 ± 0.00	–5.4 ± 0.00	46.3 ± 0.00	61.4 ± 0.00	7.2 ± 0.00
		18.4 ± 0.00	4.7 ± 0.10	42.0 ± 0.00	54.4 ± 0.00	7.1 ± 0.01
	96	18.7 ± 0.30	–24.1 ± 0.00	46.3 ± 0.10	61.4 ± 0.10	7.1 ± 0.07
		19.1 ± 0.10	–20.2 ± 3.80	48.4 ± 0.10	63.8 ± 0.00	7.0 ± 0.06
4	0	15.0 ± 0.00	–7.3 ± 0.80	40.5 ± 0.10	55.4 ± 1.90	7.5 ± 0.01
		15.7 ± 0.10	2.1 ± 1.70	42.8 ± 0.10	57.6 ± 2.50	7.4 ± 0.01
	24	15.8 ± 0.10	–29.5 ± 0.10	40.2 ± 0.20	55.3 ± 0.00	7.4 ± 0.00
		15.5 ± 0.00	–24.5 ± 0.20	45.5 ± 0.00	60.7 ± 0.00	7.3 ± 0.01
	48	16.2 ± 0.00	–37.5 ± 0.00	44.3 ± 0.10	59.1 ± 0.00	7.1 ± 0.00
		16.6 ± 0.00	–35.7 ± 0.10	45.9 ± 0.00	61.0 ± 0.00	6.9 ± 0.00
	72	17.4 ± 0.00	–7.8 ± 0.00	46.1 ± 0.10	61.2 ± 0.00	7.2 ± 0.00
		17.8 ± 0.00	1.6 ± 0.00	45.7 ± 0.00	60.9 ± 0.00	7.2 ± 0.00
	96	18.9 ± 0.010	–25.3 ± 4.00	46.3 ± 0.10	60.8 ± 0.20	7.1 ± 0.06
		18.7 ± 0.10	–21.9 ± 4.30	47.2 ± 0.10	62.2 ± 0.10	7.0 ± 0.07
8	0	14.9 ± 0.00	7.7 ± 0.40	40.3 ± 0.10	52.7 ± 0.10	7.6 ± 0.11
		15.7 ± 0.00	7.4 ± 0.50	39.1 ± 0.10	52.4 ± 1.30	7.4 ± 0.01
	24	15.2 ± 0.20	–30.2 ± 0.00	44.3 ± 0.00	59.0 ± 0.10	7.4 ± 0.00
		14.9 ± 0.00	–26.1 ± 0.20	44.9 ± 0.10	60.2 ± 2.0	7.3 ± 0.00
	48	16.0 ± 0.00	–37.6 ± 0.00	40.1 ± 0.10	53.8 ± 0.00	7.1 ± 0.00
		16.3 ± 0.00	–35.0 ± 0.00	45.3 ± 0.00	60.3 ± 0.00	7.0 ± 0.00
	72	17.4 ± 0.00	–9.7 ± 0.00	45.9 ± 0.10	61.2 ± 0.00	7.2 ± 0.00
		17.7 ± 0.00	–0.7 ± 0.10	46.5 ± 0.00	61.1 ± 0.10	7.2 ± 0.00
	96	18.4 ± 0.10	–25.8 ± 4.10	45.6 ± 0.00	60.8 ± 0.00	7.1 ± 0.06
		18.3 ± 0.20	–22.8 ± 4.10	46.7 ± 0.00	61.6 ± 0.10	7.0 ± 0.07
12	0	14.9 ± 0.00	8.0 ± 1.40	40.5 ± 0.20	54.1 ± 0.00	7.9 ± 0.08
		15.6 ± 0.00	7.5 ± 0.60	38.6 ± 1.30	53.3 ± 2.60	7.5 ± 0.01
	24	14.8 ± 0.00	–30.5 ± 0.30	44.1 ± 0.00	59.1 ± 0.00	7.4 ± 0.00
		15.6 ± 0.00	–27.2 ± 0.10	41.8 ± 0.20	56.9 ± 2.00	7.4 ± 0.00
	48	16.4 ± 0.00	–37.7 ± 0.00	42.9 ± 0.10	57.9 ± 0.10	7.2 ± 0.00
		16.2 ± 0.10	–35.3 ± 0.00	44.9 ± 0.10	59.6 ± 0.00	7.0 ± 0.00
	72	17.6 ± 0.00	–11.7 ± 0.00	45.5 ± 0.00	60.5 ± 0.10	7.2 ± 0.00
		17.7 ± 0.00	–3.4 ± 0.10	45.9 ± 0.10	61.2 ± 0.00	7.2 ± 0.00
	96	17.8 ± 0.10	–26.5 ± 3.80	45.3 ± 0.10	60.0 ± 0.10	7.1 ± 0.06
		18.7 ± 0.10	–23.4 ± 4.00	46.7 ± 0.00	62.0 ± 0.00	7.0 ± 0.07

respect to salinity at 8 cm depth, and in February 2004, there was a significant difference with respect to the mean values of redox potential per depth between the control and the treatments with cadmium. The pH tended to decrease during the experimental period, whereas the reverse was the case for salinity. The redox potential displayed higher mean values at all depths in the treatments with cadmium than in the control. The pH of the microcosms varied between 6.32 and 7.67, the oxidation-reduction potential between −37.7 and 81.6 mV, the temperature between 14.8 and 20.5°C and the salinity between 32.2 and 48.4 PSU. The mean size grain was 730 μm in 2002 and 1025 μm in 2004.

The communities of protists. Figures 1 and 2 show the variations (percentages of mean values) in the density, number of species and biomass in the 2002 and 2004 assays. The components of the Variance Analysis indicated that density was the factor contributing most to the maximum of variance with respect to depth.

Effects of cadmium exposure. In contrast to the control, there was a decrease in the three above-mentioned parameters in the cadmium treatment after 24 h, and a recovery was observed after 72 h (Figs. 1a and 2a). As a result, variations over time and depth were more pronounced in the cadmium treatment than in the control. In 2004, the number of species increased slightly after 24 h; the subsequent increase was observed after 72 h (Fig. 2a).

Depth-related effects. There was a decrease in the three parameters from the surface to deeper levels, with some exceptions: the percentage of biomass in the treatments with cadmium was higher at 8 cm than at 4 cm, the percentage of biomass in the control was higher at 12 cm than at 8 cm in 2002 (Fig. 1b), and the number of species at 12 cm was slightly higher than that at the shallower depth in 2004 (Fig. 2b).

Time-related effects. The same was observed with respect to time, although the differences were less than the ones due to depth.

Density.

Depth- and time-related effects. The effects on protist density varied with depth. In the 2002 assay, at the surface and at 12 cm depth there was a sudden drop in density after 24 h, but at 4 and 8 cm similar effects were recorded after 72 h (Table 3). After 72 h, the community showed a recovery at 0, 4 and 8 cm, which was more remarkable at the surface. Multiple Comparison Analysis performed on the density data after 24 h showed that there was a significant difference between all the depths (F 3.77; $p \leq 0.05$). Likewise, after 72 h, when a recovery was observed, there was

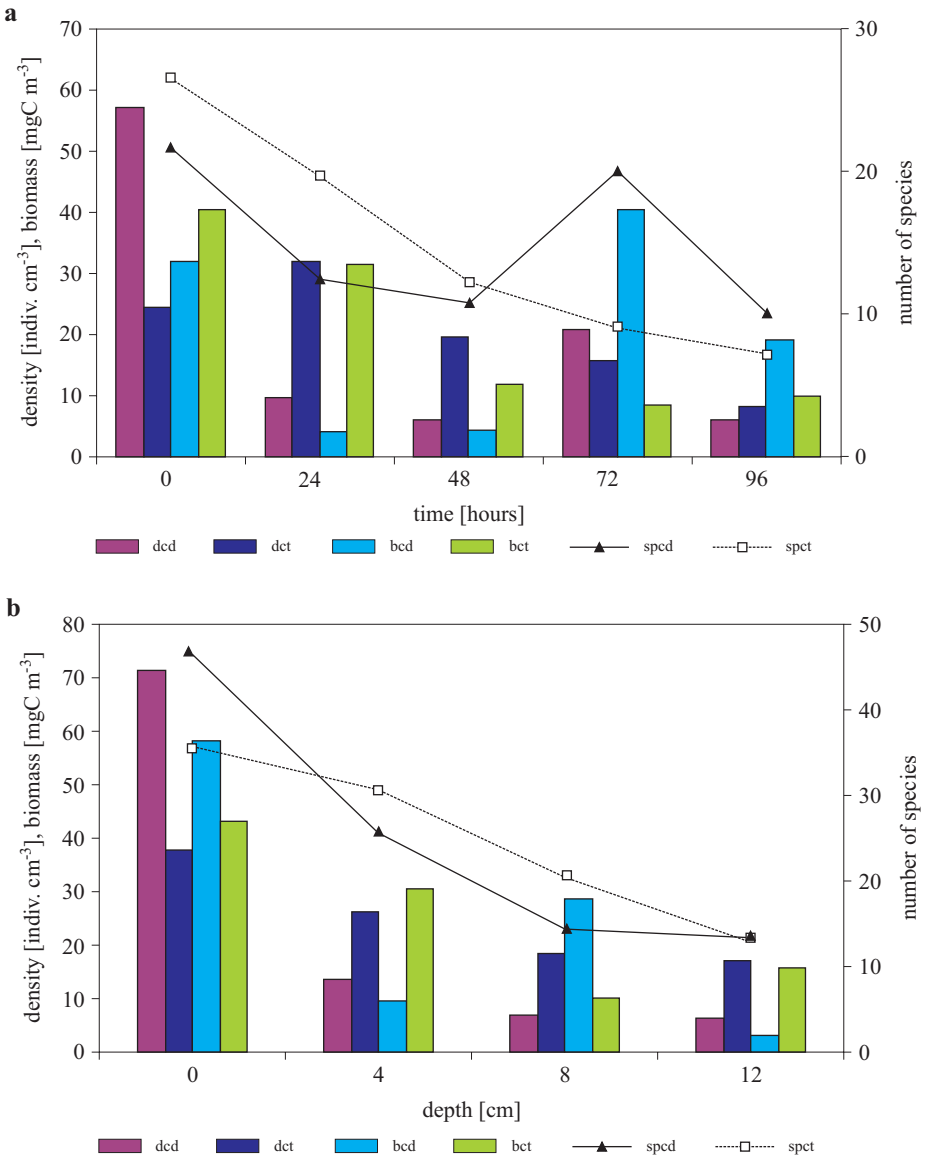

Fig. 1. Percentages of density, number of species and biomass of protists in mean values with respect to the stages of the assay (a), and the different depth levels (b) in 2002. (dcd, density in treatments with cadmium; dct, density in the control; spcd, number of species in treatments with cadmium; spct, number of species in the control; bcd, biomass in treatments with cadmium; bct, biomass in the control)

a significant difference between depths (F 4.87; $p \leq 0.05$). On the other hand, the variation during the assay was the greatest at 4 cm, where there was a significant difference with respect to the density of protists during all the

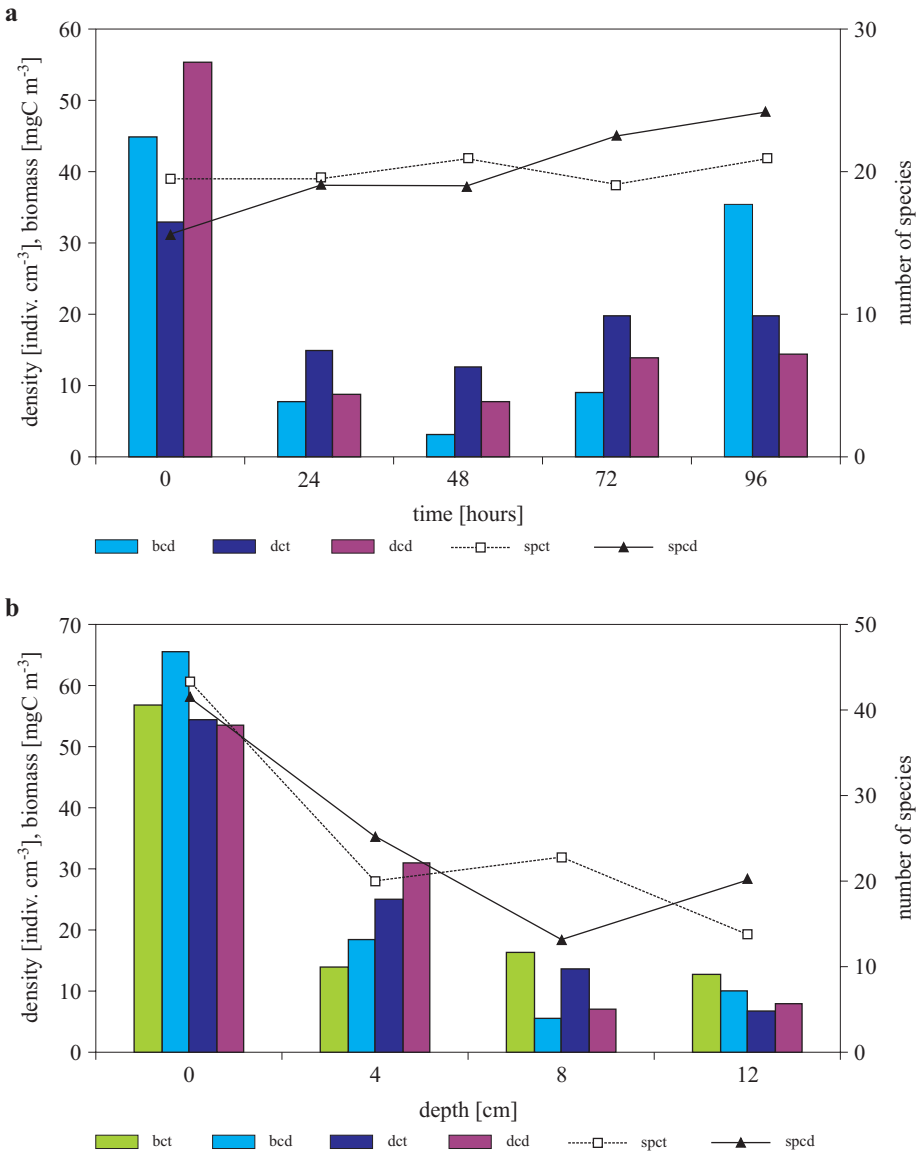

Fig. 2. Mean values of density, biomass and number of species at the stages of the assay (a), and the different depth levels (b) in 2004. (bct, biomass in the control; bcd, biomass in the treatments with cadmium; dct, density in the control; dcd, density in the treatments with cadmium; spct, number of species in the control; spcd, number of species in the treatments with cadmium)

stages of the treatment (F 3.36; $p \leq 0.05$). In the 2004 assay, there was an abrupt decrease in density between 0 and 24 h in the fractions with cadmium at all depths that was greater than in the control (Table 4). Anova/Manova

Table 3. Mean density values [indiv. cm^{-3}] of the protist groups during the 2002 assay

Mean ± standard deviation (above, treatments with cadmium; below, control)

Depth [cm]	Hours	Autotrophic flagellates	Heterotrophic flagellates	Dino-flagellates	Ciliates	Sarcodines	Diatoms
	0	45.220 ± 53	3.553 ± 50	1.224 ± 52	1.938 ± 50	7.174 ± 52	2.040 ± 50
		3.519 ± 53	306 ± 56	1.360 ± 51	612 ± 05	3.043 ± 565	578 ± 15
	24	289 ± 10	1.258 ± 50	1.428 ± 52	204 ± 57	00 ± 00	306 ± 56
		238 ± 05	1.717 ± 53	612 ± 05	952 ± 28	2.278 ± 15	00 ± 00
0	48	00 ± 00	867 ± 05	1.105 ± 56	629 ± 17	306 ± 05	255 ± 27
		00 ± 00	1.207 ± 56	00 ± 00	1.887 ± 05	1.071 ± 41	00 ± 00
	72	1.360 ± 50	5.338 ± 38	578 ± 05	4.488 ± 51	1.020 ± 53	2.159 ± 34
		00 ± 00	476 ± 05	00 ± 00	1.547 ± 28	578 ± 05	00 ± 00
	96	306 ± 05	884 ± 05	323 ± 05	1.377 ± 05	850 ± 32	731 ± 05
		00 ± 00	816 ±	00 ± 00	1.292 ± 09	204 ± 13	00 ± 00
	0	952 ± 54	2.006 ± 38	901 ± 15	00 ± 00	850 ± 50	00 ± 00
		00 ± 00	1.972 ± 56	00 ± 00	204 ± 05	170 ± 06	306 ± 52
	24	255 ± 05	1.955 ± 50	1.955 ± 51	306 ± 37	1.139 ± 50	612 ± 51
		1.377 ± 50	1.479 ± 50	357 ± 50	646 ± 50	2.295 ± 51	00 ± 00
4	48	00 ± 00	527 ± 50	255 ± 50	272 ± 50	204 ± 50	00 ± 00
		00 ± 00	408 ± 50	00 ± 00	238 ± 50	782 ± 50	816 ± 50
	72	00 ± 00	1.258 ± 54	816 ± 50	629 ± 50	901 ± 50	442 ± 50
		272 ± 34	1.377 ± 55	493 ± 50	1.462 ± 52	918 ± 50	00 ± 00
	96	00 ± 00	272 ± 50	00 ± 00	00 ± 00	00 ± 00	306 ± 50
		00 ± 00	204 ± 50	00 ± 00	714 ± 51	238 ± 50	272 ± 50
	0	952 ± 50	00 ± 00	00 ± 00	00 ± 00	00 ± 00	00 ± 00
		272 ± 50	714 ± 56	646 ± 52	00 ± 00	00 ± 00	306 ± 50
	24	00 ± 00	1.224 ± 55	595 ± 50	00 ± 00	00 ± 00	204 ± 50
		00 ± 00	1.428 ± 51	731 ± 50	918 ± 50	476 ± 50	1.054 ± 51
8	48	204 ± 50	544 ± 50	272 ± 50	00 ± 00	238 ± 50	00 ± 00
		357 ± 50	1.190 ± 51	289 ± 50	00 ± 00	714 ± 50	00 ± 00
	72	00 ± 00	1.088 ± 50	918 ± 54	476 ± 50	238 ± 50	2.380 ± 51
		476 ± 50	1.207 ± 50	306 ± 50	289 ± 50	00 ± 00	00 ± 00
	96	00 ± 00	289 ± 50	238 ± 50	00 ± 00	00 ± 00	459 ± 50
		00 ± 00	00 ± 00	00 ± 00	408 ±50	204 ±49	00 ±00
	0	272 ± 50	1.377 ± 53	1.190 ± 50	272 ± 50	00 ± 00	00 ± 00
		00 ± 00	306 ± 50	850 ± 50	00 ± 00	00 ± 00	544 ± 50
	24	00 ± 00	00 ± 00	00 ± 00	00 ± 00	204 ± 50	00 ± 00
		00 ± 00	323 ± 50	1.734 ± 56	00 ± 00	00 ± 00	1.921 ± 55
12	48	289 ± 50	731 ± 50	442 ± 50	00 ± 00	00 ± 00	204 ± 50
		00 ± 00	289 ± 50	3.298 ± 51	00 ± 00	00 ± 00	00 ± 00
	72	238 ± 50	476 ± 50	442 ± 50	00 ± 00	204 ± 50	00 ± 00
		00 ± 00	00 ± 00	765 ± 50	00 ± 00	00 ± 00	00 ± 00
	96	00 ± 00	748 ± 50	00 ± 00	00 ± 00	00 ± 00	646 ± 50
		00 ± 00	476 ± 50	00 ± 00	204 ± 44	00 ± 00	306 ± 50

Table 4. Mean density values [indiv. cm^{-3}] of the protist groups during the 2004 assay

Mean ± standard deviation (above, treatments with cadmium; below, control)

Depth [cm]	Hours	Autotrophic flagellates	Heterotrophic flagellates	Dino-flagellates	Ciliates	Sarcodines	Diatoms
0	0	27693±0.10	1105±0.27	4998±0.20	0±0.00	1326±0.20	340±0.25
		3757±0.20	10166±0.20	2822±0.25	833±0.20	833±0.10	2397±0.20
	24	2040±0.20	968±0.25	918±0.25	306±0.10	0±0.00	680±0.20
		0±0.00	2788±0.20	1258±0.20	272±0.20	1530±0.20	4301±0.25
	48	0±0.00	3672±0.10	442±0.15	442±0.25	0±0.20	1496±0.15
		1479±0.20	3179±0.20	493±0.20	2074±0.20	0±0.00	2737±0.20
	72	918±0.10	4777±0.27	1462±0.10	0±0.10	306±0.20	612±0.15
		2006±0.20	12920±0.20	493±0.12	1955±0.20	1972±0.15	2601±0.20
	96	1343±0.25	2805±0.20	1377±0.15	1836±0.10	323±0.18	1734±0.20
		697±0.20	11169±0.10	4590±0.20	629±0.25	646±0.12	3621±0.13
4	0	5270±0.25	17272±0.20	0±0.00	0±0.00	1632±0.15	0±0.00
		4896±0.20	13600±0.10	0±0.00	1530±0.10	1309±0.20	952±0.25
	24	0±0.00	1326±0.25	0±0.00	663±0.19	272±0.10	612±0.17
		663±0.10	2057±0.20	510±0.16	1581±0.20	0±0.20	0±0.00
	48	0±0.00	527±0.25	187±0.11	1173±0.20	0±0.00	119±0.10
		0±0.10	1156±0.20	527±0.10	612±0.21	0±0.00	1462±0.24
	72	867±0.15	1530±0.25	646±0.27	442±0.10	136±0.22	782±0.15
		0±0.00	2210±0.10	0±0.00	323±0.12	306±0.15	272.00
	96	0±0.00	1462±0.20	493±0.13	1343±0.20	0±0.00	561±0.10
		833±0.18	2346±0.10	0±0.00	697±0.20	731±0.20	289±0.16
8	0	0±0.00	1632±0.19	442±0.17	663±0.23	0±0.00	0±0.10
		323±0.20	1326±0.20	1054±0.20	714±0.20	1258±0.20	680±0.24
	24	0±0.10	884±0.15	323±0.20	204±0.24	0±0.00	0±0.00
		0±0.00	4318±0.20	289±0.10	952±0.20	544±0.25	629±0.20
	48	0±0.00	102±0.20	0±0.00	0±0.20	0±0.00	0±0.00
		0±0.00	1037±0.10	0±0.00	1394±0.10	408±0.17	0±0.00
	72	0±0.10	1445±0.18	0±0.00	493±0.25	153±0.10	374±0.21
		0±0.00	1768±0.20	0±0.10	204±0.11	1513±0.13	748±0.20
	96	0±0.00	765±0.12	0±0.00	969±0.27	0±0.10	0±0.00
		0±0.10	1326±0.20	323±0.10	306±0.20	0±0.00	238±0.25
12	0	0±0.00	2703±0.10	323±0.16	816±0.10	0±0.00	0±0.00
		0±0.00	1428±0.20	510±0.25	306±0.19	0±0.00	238±0.25
	24	0±0.00	527±0.20	119±0.21	459±0.22	119±0.23	0±0.00
		0±0.10	374±0.17	272±0.10	544±0.20	272±0.25	0±0.00
	48	0±0.00	510±0.25	272±0.10	340±0.15	0±0.00	0±0.00
		0±0.10	1377±0.16	901±0.20	680±0.15	0±0.10	0±0.00
	72	0±0.00	1139±0.10	272±0.25	119±0.17	0±0.00	0±0.00
		0±0.00	646±0.25	272±0.11	357±0.10	0±0.00	0±0.00
	96	0±0.00	561±0.20	527±0.25	425±0.10	544±0.18	0±0.10
		0±0.10	918±0.13	272±0.20	833±0.25	306±0.19	0±0.00

performed using protist densities during the treatment showed that the main effect was due to depth, followed by time. The effect of depth was greater in the fractions with cadmium, but less in the control.

Number of species.

In the 2002 assay, the total number of species was lower in the control (176) than in the treatments with cadmium (187). Hierarchical Conglomerate Analysis performed using the number of species showed that the dissimilarities between the depths corresponding to the control were noticeably lower that those corresponding to the cadmium treatments (Fig. 3).

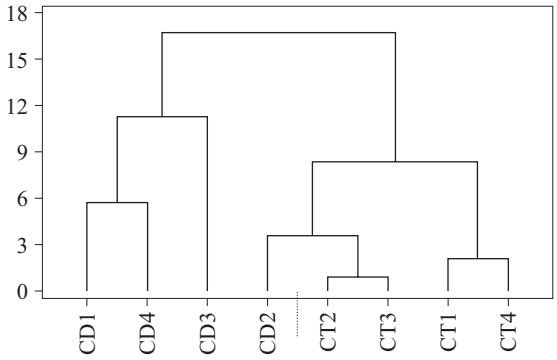

Fig. 3. Dendrogram of the Hierarchical Cluster Analysis obtained using the number of species during the treatment in 2002. The y-axis represents the metric distance (Manhattan distance). CT, control; CD, fractions with cadmium

In the 2004 assay, after 24 h there was a decrease in the number of species at all depths except 8 cm, where the drop was registered after 48 h. The diminutions were more pronounced than in the control. (Fig. 4).

Depth-related effects. In the 2002 assay, the highest number of species was recorded at the surface (87, 46.52%), and this number decreased with depth, so that the percentages at 8 and 12 cm were conspicuously low (14.44 and 13.37% respectively). The greatest variations occurred at 4 cm (F 2.96; $p \leq 0.05$), thus coinciding with the results relating to density (Multiple Comparison). In the 2004 assay, both in the control and in the fractions with cadmium, depth was also a significant factor influencing the number of species (F 19.24; $p \leq 0.05$).

Time-related effects. At the surface and at 12 cm depth there was a decrease in the number of species after 24 h, while at 4 cm a drop was observed after

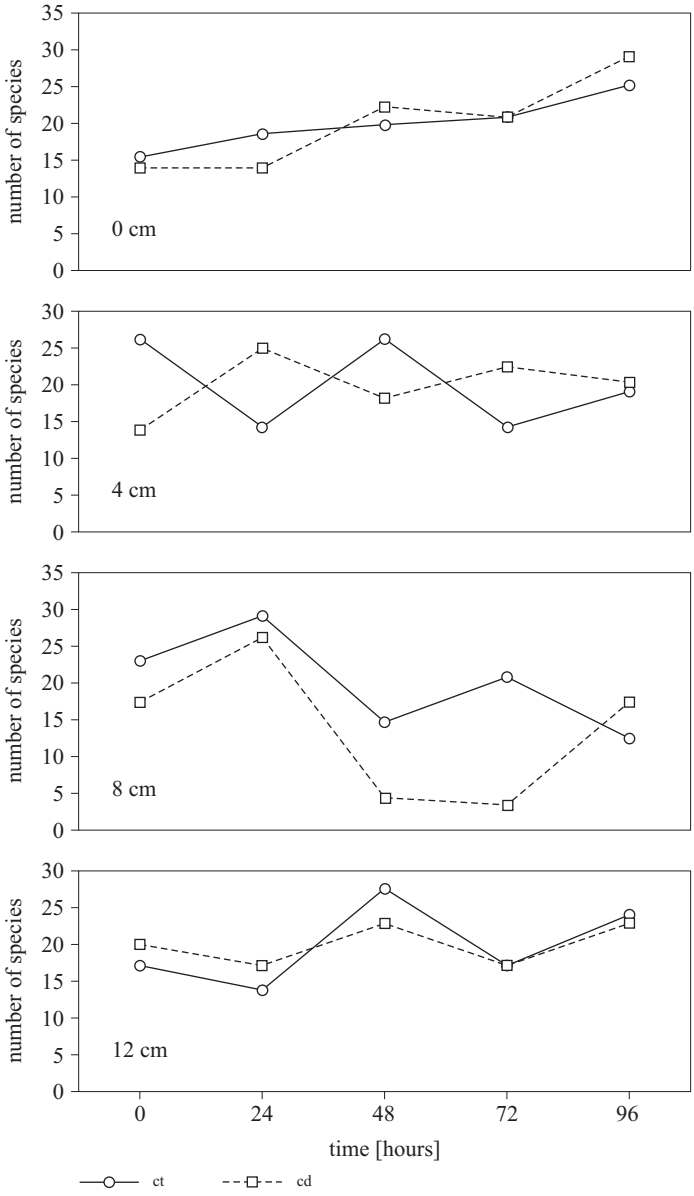

Fig. 4. Percentages of the mean number of species at the different depths during the 2004 assay (ct, control; cd, treatments with cadmium)

48 h, and at 8 cm there was no reduction. A recovery in the number of species was observed after 72 h at 0 and 4 cm, and after 48 h at 12 cm.

Variation in the protist groups. In the 2002 assay, the drop in the number of species at the surface after 24 h was due mainly to diatoms, whereas

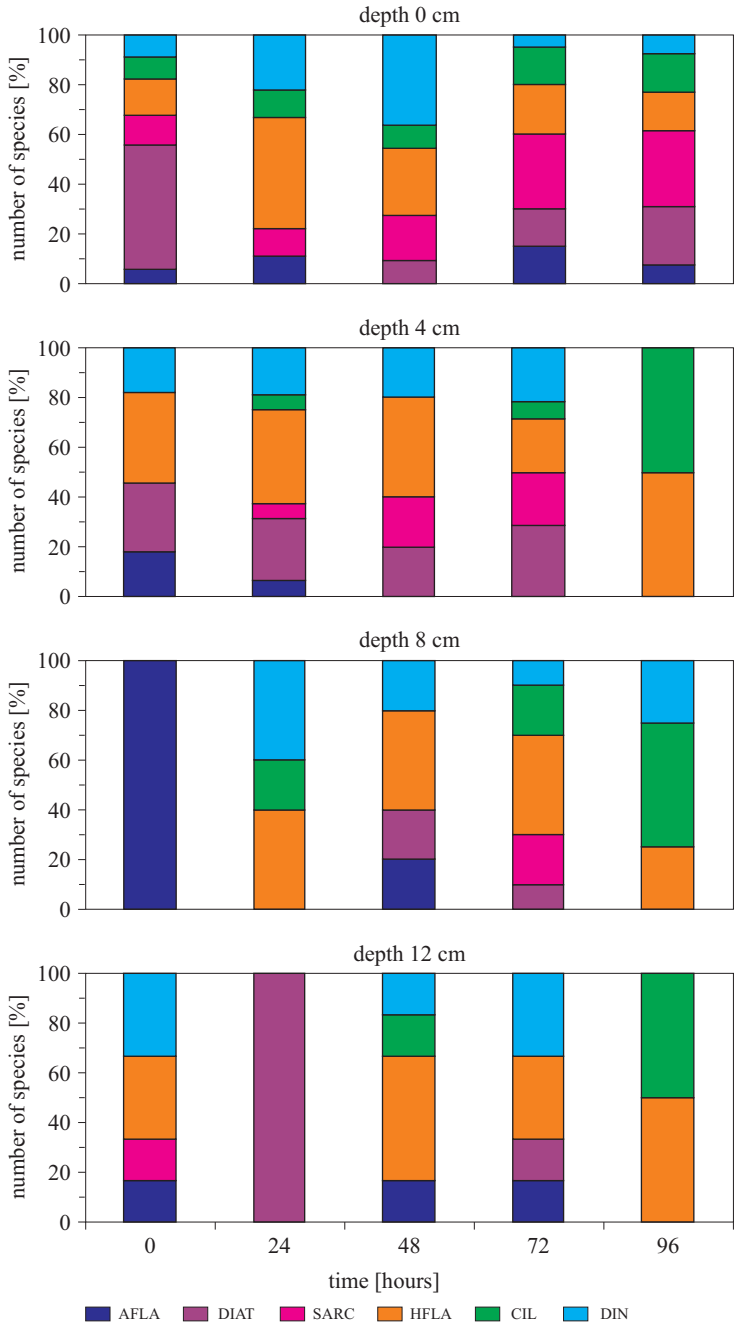

Fig. 5. Percentages of the number of species from the different protist groups (AFLA, autotrophic flagellates; DIAT, diatoms; SARC, sarcodines; HFLA, heterotrophic flagellates; CIL, ciliates; DIN, dinoflagellates) during the treatment with cadmium at the different depths in the 2002 assay

at 12 cm this decrease was due to all groups except diatoms. The drop in the number of species at 4 cm after 48 h was due to all groups except sarcodines. The recovery in the number of species at the surface and at 4 cm after 72 h was due to all groups except dinoflagellates at the surface, and autotrophic flagellates at 4 cm. The recovery observed after 48 h at 12 cm was due to all groups except sarcodines and diatoms (Fig. 5). Multiple Comparison showed that after 24 h there was a significant difference between all the depth levels (F 3.74; $p \leq 0.05$), and that the same thing occurred after 72 h (F 3.13; $p \leq 0.05$). These results coincided with those found with respect to the density. In the 2004 assay the groups contributing most to the variance were different in the control and in the fractions with cadmium. The only exception was recorded at 0 cm depth, in which heterotrophic flagellates were the principal group. In the control, heterotrophic flagellates, autotrophic flagellates and dinoflagellates were the groups contributing most to the variance at 4, 8 and 12 cm depth, respectively. In the treatments with cadmium, at the same depths the groups contributing most were diatoms, dinoflagellates and ciliates.

Biomass. The effects on biomass were similar to those observed with regard to the density and number of species.

Principal Component Analysis of the protist biomass in the 2002 experiment showed that cadmium treatment and control samples belonged to two distinct groups (Fig. 6).

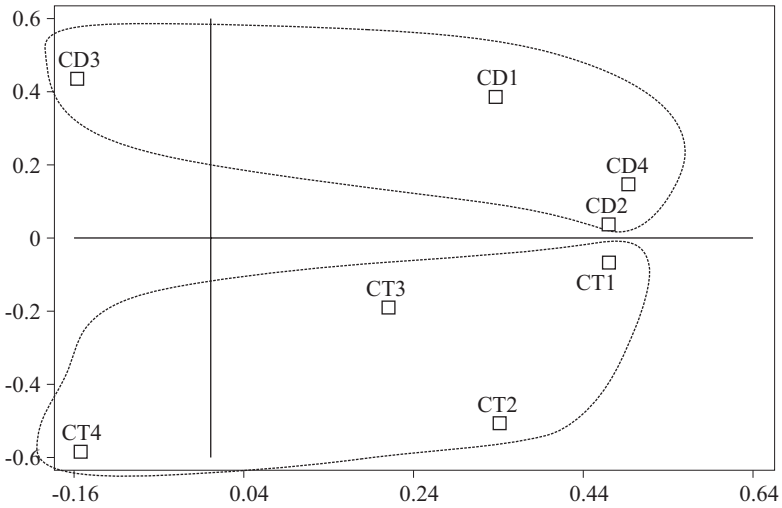

Fig. 6. Principal Component Analysis performed with the biomass values during the 2002 treatment; x-axis, first principal component; y-axis, second principal component; CT, control; CD, fractions with cadmium

Depth- and time-related effects. In the 2002 assay, at the surface and at 12 cm, a biomass diminution was observed after 24 h, while at 4 cm and, to a lesser extent, at 8 cm, this effect was observed after 48 h (Table 5). A recovery of biomass was recorded after 48 h at the surface and at 12 cm, and after 72 h at 4 cm and 8 cm. After 24 h, at the surface all the protist groups decreased noticeably in biomass, and the same occurred at 12 cm with the exception of diatoms. At 4 cm, all groups dropped in biomass with the exception of sarcodines after 48 h. At 8 cm, after 48 h, there was a slight diminution due mainly to heterotrophic flagellates. The recovery of biomass at the surface was due to diatoms, sarcodines and dinoflagellates, whereas at 12 cm, it was due to all groups except diatoms. The recovery of biomass at 4 and 8 cm was due to all groups except autotrophic flagellates.

In the 2004 assay (Table 6), there was a noticeable diminution of the biomass after 24 h, which continued until 48 h at the depths of 0, 4 and 8 cm. At 12 cm a slight recovery was recorded after 48 h, which lasted until 72 h, at which time a recovery was observed at the other depths. In the control, biomass at 4 and 8 cm decreased, though to a lesser extent than in the treatment with cadmium, while in the surface and 12 cm there was a rise in biomass during the first phases of the assay. In the control, a significant difference was observed between the total protist biomass at the different depths (F 5.88; $p \leq 0.05$).

Component Variance Analysis showed that the surface level of the sediment presented the maximum contribution in biomass with respect to the variation in time and depth, both in the control and in the cadmium treatments.

Dominant protist groups.

2002 assay. Effects due to cadmium exposure. In the treatments with cadmium in the 2002 assay, heterotrophic flagellates were the dominant group (31.09–60.50% of the protist density). They were dominant in 12 (60%) of the 20 fractions of the assay (4 depths + 5 stages). The second group were ciliates (46.55–52.94% of the protist density), which were dominant in 3 (15%) of the 20 fractions of the assay. Autotrophic flagellates (73.95–100%) were dominant in two (15%), as were dinoflagellates (34.95–36.10%) (15%). Sarcodines were the last group (30.80%), dominant in one fraction (5%) (Table 7). In the control in the 2002 assay, there were two dominant groups: heterotrophic flagellates and sarcodines, which were dominant in 6 fractions (30%). The third group were dinoflagellates (15%). The fourth group were diatoms (10%), and the last group were autotrophic flagellates, dominant in one fraction (5%) (Table 7). In comparison with the cadmium treatments, sarcodines were dominant in the control,

Table 5. Mean biomass values [mgC m^{-3}] of the protist groups during the 2002 assay

Mean ± standard deviation (above, treatments with cadmium; below, control)

Depth [cm]	Hours	Autotrophic flagellates	Heterotrophic flagellates	Dino-flagellates	Sarcodines	Diatoms	Ciliates
	0	4.198±57	535±05	1.272±05	18.923±52	31.605±50	4.131±51
		1.609±55	11.475±52	2.245±54	4.550±50	8.265±51	2.842±50
	24	104±56	127±44	258±27	1.792±56	00±00	911±49
		90±10	83±05	326±45	2.889±10	3.876±52	00±00
0	48	00±00	219±11	1.825±52	3.803±57	297±05	46±05
		00±00	351±29	00±00	15.490±55	2.726±16	00±00
	72	685±05	503±05	109±05	20.283±50	1.012±51	3.401±57
		00±00	170±44	00±00	1.702±05	413±05	00±00
	96	58±05	67±05	140±10	37.757±34	1.505±56	3.053±28
		00±00	103±	2.524±	2.620±	146±	00±00
	0	448±51	3.658±10	00±00	00±00	4.457±50	00±00
		00±00	1.521±50	1.103±50	12.534±52	771±50	99±05
	24	578±50	672±05	00±00	196±50	1.738±50	117±52
		195±53	870±52	289±50	7.854±53	5.427±52	00±00
4	48	00±00	45±05	177±50	1.065±50	892±51	00±00
		00±00	08±02	1.814±52	4.478±56	1.497±51	4.553±51
	72	00±00	218±50	00±00	1.869±53	1.341±53	49±05
		26±05	44±05	47±05	1.401±50	597±50	00±00
	96	00±00	63±05	00±00	00±00	00±00	74±05
		00±00	103±33	00±00	468±50	542±55	463±50
	0	227±50	00±00	00±00	00±00	00±00	00±00
		1.279±54	308±50	1.103±50	00±00	00±00	1.102±50
	24	00±00	1.223±50	504±50	00±00	00±00	45±03
		00±00	415±50	765±50	1.434±56	558±50	1.052±50
8	48	88±05	27±05	118±50	00±00	585±51	00±00
		39±03	312±50	70±05	00±00	952±50	00±00
	72	00±00	110±50	273±50	63.089±53	839±51	145±50
		103±10	111±50	347±50	2.996±51	00±00	00±00
	96	00±00	164±50	88±05	00±00	00±00	697±52
		00±00	00±00	00±00	1.287±51	420±50	00±00
	0	235±50	123±50	46±05	3.656±53	00±00	00±00
		00±00	661±50	305±50	00±00	00±00	176±50
	24	00±00	00±00	00±00	00±00	308±50	00±00
		00±00	47±05	560±50	00±00	00±00	13.085±53
12	48	47±10	282±34	167±50	00±00	00±00	310±50
		00±00	20±05	1.371±50	00±00	00±00	00±00
	72	116±45	50±05	46±10	00±00	308±50	150±28
		00±00	00±00	305±50	00±00	00±00	00±00
	96	00±00	26±05	00±00	00±00	00±00	1.788±50
		00±00	74±05	00±00	788±50	00±00	5.350±51

Table 6. Mean biomass values [mgC m^{-3}] of the protist groups during the 2004 assay

Mean ± standard deviation (above, treatments with cadmium; below control)

Depth [cm]	Hours	Autotrophic flagellates	Heterotrophic flagellates	Dinoflagellates	Ciliates	Sarcodines	Diatoms
0	0	2034.90 ± 0.10	1035.90 ± 0.20	48194.28 ± 0.27	0.00 ± 0.10	5370.30 ± 0.12	202.63
		524.79 ± 0.20	837.45 ± 0.10	149.94 ± 0.20	1437.05 ± 0.25	218.66 ± 0.20	2183.48 ± 0.10
	24	215.42 ± 0.20	799.43 ± 0.13	5026.05 ± 0.17	565.49 ± 0.25	0.00 ± 0.00	1058.22 ± 0.24
		0.00 ± 0.10	205.11 ± 0.25	2632.84 ± 0.20	1948.09 ± 0.24	1998.65 ± 0.20	3619.06 ± 0.25
	48	0.00 ± 0.20	227.66 ± 0.10	352.52 ± 0.15	470.18 ± 0.25	0.00 ± 0.00	1213.87 ± 0.20
		378.75 ± 0.25	1287.27 ± 0.20	73.95 ± 0.20	14602.85 ± 0.10	0.00 ± 0.00	4431.42 ± 0.20
	72	270.96 ± 0.20	362.29 ± 0.15	4176.17 ± 0.10	539.77 ± 0.18	154.91 ± 0.25	1575.59 ± 0.25
		310.59 ± 0.25	489.50 ± 0.20	1155.47 ± 0.27	2976.66 ± 0.20	3495.03 ± 0.13	2173.39 ± 0.20
	96	1429.37 ± 0.10	168.10 ± 0.15	10286.21 ± 0.19	9372.99 ± 0.20	3100.80 ± 0.20	1867.86 ± 0.16
		702.58 ± 0.20	915.46 ± 0.25	19498.24 ± 0.20	4755.24 ± 0.15	96.90 ± 0.10	3310.56 ± 0.25
4	0	296.44 ± 0.12	2574.19 ± 0.27	0.00 ± 0.00	0.00 ± 0.00	2798.88 ± 0.20	0.00 ± 0.00
		802.47 ± 0.10	255.00 ± 0.20	0.00 ± 0.10	2346.76 ± 0.18	1417.01 ± 0.10	1787.80 ± 0.11
	24	0.00 ± 0.00	357.19 ± 0.10	0.00 ± 0.00	898.75 ± 0.25	137.70 ± 0.17	368.65 ± 0.20
		335.64 ± 0.25	514.46 ± 0.10	637.50 ± 0.20	620.67 ± 0.10	0.00 ± 0.00	0.00 ± 0.00
	48	0.00 ± 0.00	50.96 ± 0.13	116.98 ± 0.15	843.61 ± 0.19	0.00 ± 0.00	63.97 ± 0.25
		0.00 ± 0.00	30.43 ± 0.25	1481.41 ± 0.27	632.24 ± 0.15	0.00 ± 0.00	861.28 ± 0.11
	72	103.90 ± 0.20	26.71 ± 0.16	166.72 ± 0.21	530.40 ± 0.23	1156.68 ± 0.22	1050.68 ± 0.27
		0.00 ± 0.00	108.00 ± 0.12	0.00 ± 0.00	840.28 ± 0.20	45.90 ± 0.21	137.09 ± 0.24
	96	0.00 ± 0.00	403.68 ± 0.25	8150.68 ± 0.17	7759.77 ± 0.10	0.00 ± 0.00	491.18 ± 0.29
		31.24 ± 0.10	691.31 ± 0.18	0.00 ± 0.00	3557.25 ± 0.10	1228.08 ± 0.16	467.31 ± 0.25
8	0	0.00 ± 0.00	30.60 ± 0.24	530.40 ± 0.15	1884.74 ± 0.23	0.00 ± 0.00	0.00 ± 0.00
		387.60 ± 0.11	109.08 ± 0.10	3790.52 ± 0.20	2592.71 ± 0.25	575.77 ± 0.10	99.96 ± 0.15

Table 6. (*continued*)

Depth [cm]	Hours	Autotrophic flagellates	Heterotrophic flagellates	Dinoflagellates	Ciliates	Sarcodines	Diatoms
8	24	0.00 ± 0.10	241.36 ± 0.19	1007.96 ± 0.25	103.28 ± 0.22	0.00 ± 0.00	0.00 ± 0.00
		0.00 ± 0.00	796.79 ± 0.15	221.95 ± 0.10	481.95 ± 0.13	1275.00 ± 0.21	357.60 ± 0.10
	48	0.00 ± 0.00	15.30 ± 0.25	0.00 ± 0.00	0.00 ± 0.20	0.00 ± 0.00	0.00 ± 0.00
		0.00 ± 0.00	339.17 ± 0.10	0.00 ± 0.00	1242.22 ± 0.10	61.20 ± 0.25	0.00 ± 0.00
	72	0.00 ± 0.00	172.14 ± 0.12	0.00 ± 0.00	1191.49 ± 0.25	80.33 ± 0.19	274.89 ± 0.16
		0.00 ± 0.10	74.80 ± 0.15	0.00 ± 0.00	1071.00 ± 0.20	2360.06 ± 0.10	611.06 ± 0.15
	96	0.00 ± 0.00	117.24 ± 0.20	0.00 ± 0.00	3167.10 ± 0.20	0.00 ± 0.10	0.00 ± 0.00
		0.00 ± 0.00	829.10 ± 0.20	4086.14 ± 0.25	500.55 ± 0.18	0.00 ± 0.00	257.00 ± 0.17
12	0	0.00 ± 0.00	351.98 ± 0.15	2765.00 ± 0.20	604.19 ± 0.13	0.00 ± 0.00	0.00 ± 0.00
		0.00 ± 0.00	361.82 ± 0.25	521.42 ± 0.27	528.77 ± 0.10	0.00 ± 0.00	101.21 ± 0.21
	24	0.00 ± 0.00	36.66 ± 0.20	80.97 ± 0.10	714.97 ± 0.25	153.06 ± 0.17	0.00 ± 0.00
		0.00 ± 0.10	7.01 ± 0.10	1101.60 ± 0.12	1000.97 ± 0.20	1101.60 ± 0.10	0.00 ± 0.10
	48	0.00 ± 0.00	19.39 ± 0.18	722.16 ± 0.25	557.49 ± 0.15	0.00 ± 0.00	0.00 ± 0.00
		0.00 ± 0.10	109.83 ± 0.10	2310.65 ± 0.20	816.00 ± 0.25	0.00 ± 0.00	0.00 ± 0.00
	72	0.00 ± 0.00	268.16 ± 0.16	1468.80 ± 0.20	131.01 ± 0.15	0.00 ± 0.00	0.00 ± 0.00
		0.00 ± 0.00	77.11 ± 0.25	53.73 ± 0.10	1210.01 ± 0.10	0.00 ± 0.00	0.00 ± 0.00
	96	0.00 ± 0.00	30.79 ± 0.10	4163.06 ± 0.20	1600.88 ± 0.10	1815.60 ± 0.27	0.00 ± 0.00
		0.00 ± 0.00	271.55 ± 0.20	2115.07 ± 0.20	5131.88 ± 0.10	514.08 ± 0.15	0.00 ± 0.00

Table 7. Dominant protist groups (density) during the 2002 assay

CD cm	0 h	24 h	48 h	72 h	96 h
0	AFLA, DIAT (73.95), 11.73	HFLA, DIN (36.10, 40.98)	DIN, HFLA (34.95, 27.42)	HFLA, SARC (35.72, 30.03)	SARC, HFLA, DIAT, CIL (30.80, 19.77, 19.01, 16.34)
4	HFLA, AFLA, DIN, DIAT (42.60, 20.22, 19.13, 18.05)	HFLA, DIN, DIAT (31.42, 31.42, 18.31)	HFLA, SARC, DIN, DIAT (41.89, 21.62, 20.27, 16.22)	HFLA, DIAT, HDIN (31.09, 22.27 20.17)	CIL, HFLA (52.94, 47.06)
8	AFLA (100.00)	HFLA, DIN (60.50, 29.41)	HFLA, DIN, DIAT (43.24, 21.62, 18.92)	CIL, HFLA (46.66, 21.33)	CIL, HFLA, DIN (46.55, 29.31, 24.13)
12	HFLA, DIN (44.26, 38.25)	DIAT (100.00)	HFLA, DIN (43.88, 26.53)	HFLA, DIN AFLA, DIAT (35.00, 32.50, 17.50, 15.00)	HFLA, CIL (53.66, 46.34)
CT cm	0 h	24 h	48 h	72 h	96 h
0	AFLA, DIAT (37.36, 32.31)	DIAT, HFLA (39.30, 29.62)	SARC, HFLA DIAT (45.31, 28.98, 25.71)	SARC, DIAT, HFLA (59.48, 22.22, 18.30)	SARC, HFLA (55.88, 35.29)
4	HFLA (74.36)	DIAT, HFLA, AFLA (37.29, 24.03, 22.38)	CIL, DIAT, HFLA (36.36, 34.85, 18.18)	SARC, HFLA DIAT (32.33, 30.45, 20.30)	SARC, CIL DIAT, HFLA (50.00, 19.04, 16.67, 14.29)
8	HFLA, DIN (36.84, 33.33)	HFLA, CIL DIN (31.00, 22.88, 15.87)	HFLA, DIAT (46.67, 28.00)	HFLA, AFLA (52.99, 20.90)	SARC, DIAT (66.67, 33.33)
12	DIN, CIL HFLA (50.00, 32.00, 18.00)	CIL, DIN (48.29, 43.59)	DIN (91.94)	DIN (100.00)	HFLA, CIL, SARC (48.28, 31.03, 20.69)

whereas they were infrequent in the former. Also, ciliates were not dominant in the control, but were second highest in the treatments with cadmium.

2004 assay. Figures 7 and 8 show the percentages of each protist group in the mean values of biomass in the control and cadmium treatments respectively. In both microcosms heterotrophic flagellates were dominant (34–54%, mean 41.6% of the whole protist groups), except at the beginning of the assay, when autotrophic flagellates were the dominant group. Heterotrophic flagellates were followed in dominance by different protist groups according to the phase of the assay: after 48 and 96 h ciliates were dominant,

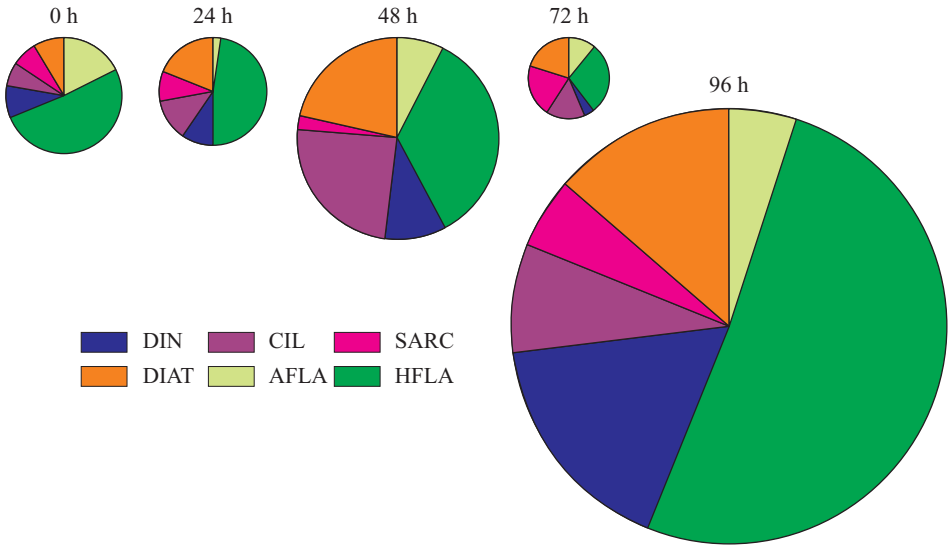

Fig. 7. Control dominant protist groups during the 2004 assay: HFLA, heterotrophic flagellates; AFLA, autotrophic flagellates; DIN, dinoflagellates; DIAT, diatoms; CIL, ciliates; SARC, sarcodines. The diameters are proportional to the biomass

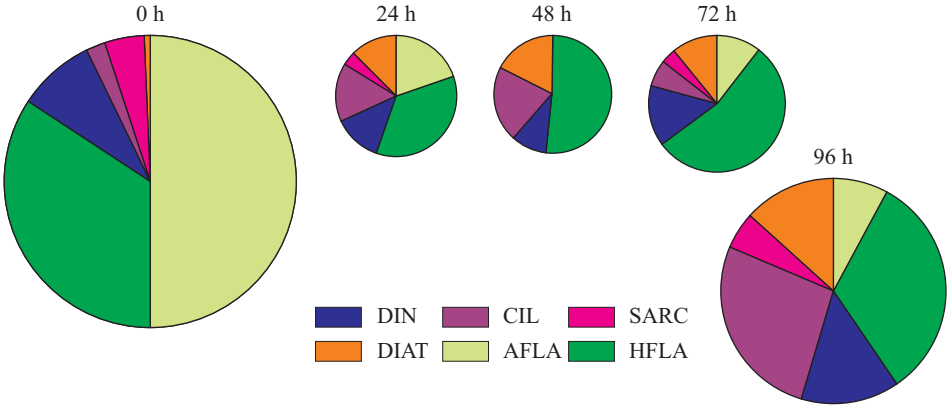

Fig. 8. Treatments with cadmium. Dominant protist groups during the 2004 assay: HFLA, heterotrophic flagellates; AFLA, autotrophic flagellates; DIN, dinoflagellates; DIAT, diatoms; CIL, ciliates; SARC, sarcodines. The diameters are proportional to the biomass

but after 72 h it was the turn of the dinoflagellates. In percentages of mean density, the most important groups after the heterotrophic flagellates were the autotrophic flagellates (17.8%), followed by the ciliates (14.4%), the dinoflagellates (12%), and the diatoms (10.8%). The protists with the

lowest percentage were the sarcodines (3.4%). The dominant group in the control during the experiment were the heterotrophic flagellates (28–52%, mean 42.2%, of all the groups of protozoa). In percentages of mean density, the dominant groups after the heterotrophic flagellates were the diatoms (16.6%), followed by the ciliates (13.6%). Other groups presented similar values (autotrophic flagellates, 9%; dinoflagellates, 9.8%; sarcodines, 8.8%).

It is important to note that in the control all protist groups were present during the assay, whereas in the treatments with cadmium, autotrophic flagellates and sarcodines were not found after 48 h. Figure 9 shows the mean density percentages of the protist groups at the different depths during the 2004 assay. In the control the dominant group at all depths were the heterotrophic flagellates (45–55%, mean 48.25% of the total protist groups). Various groups followed the heterotrophic flagellates in dominance: diatoms at the surface, autotrophic flagellates at 4 cm, sarcodines and ciliates at 8 cm, and ciliates at 12 cm.

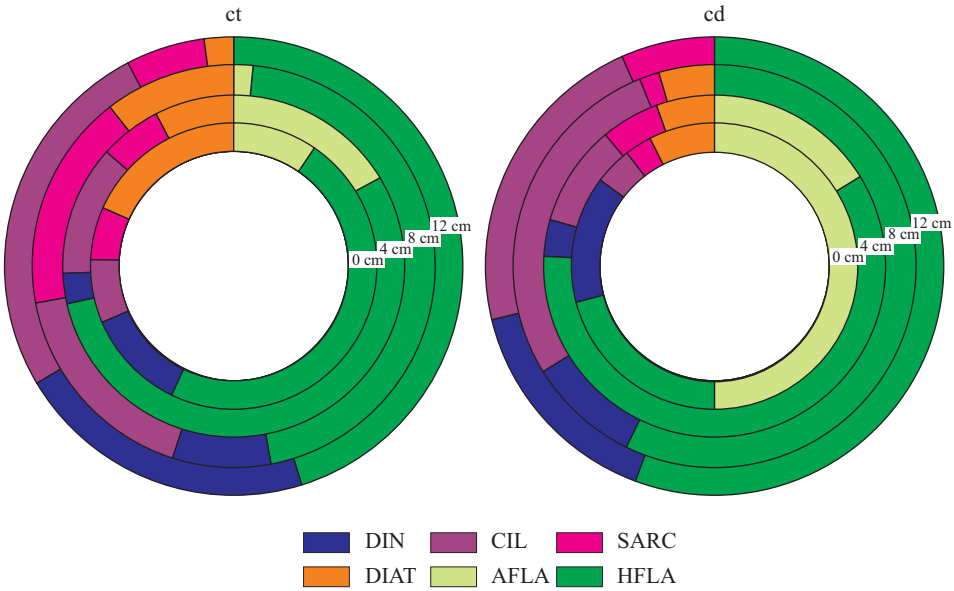

Fig. 9. Dominant protist groups density (HFLA, heterotrophic flagellates; AFLA, autotrophic flagellates; DIN, dinoflagellates; DIAT, diatoms; CIL, ciliates; SARC, sarcodines) at different depths in the sediment both in the control (ct) and the treatments with cadmium (cd) in the 2004 assay

Bioaccumulation.

Figure 10 shows the percentages of cadmium bioaccumulated in the protists during the 2004 assay. The values ranged from 0 to 167.84 μg Cd g^{-1} d.w. At all depths the proportion of cadmium bioaccumulated increased

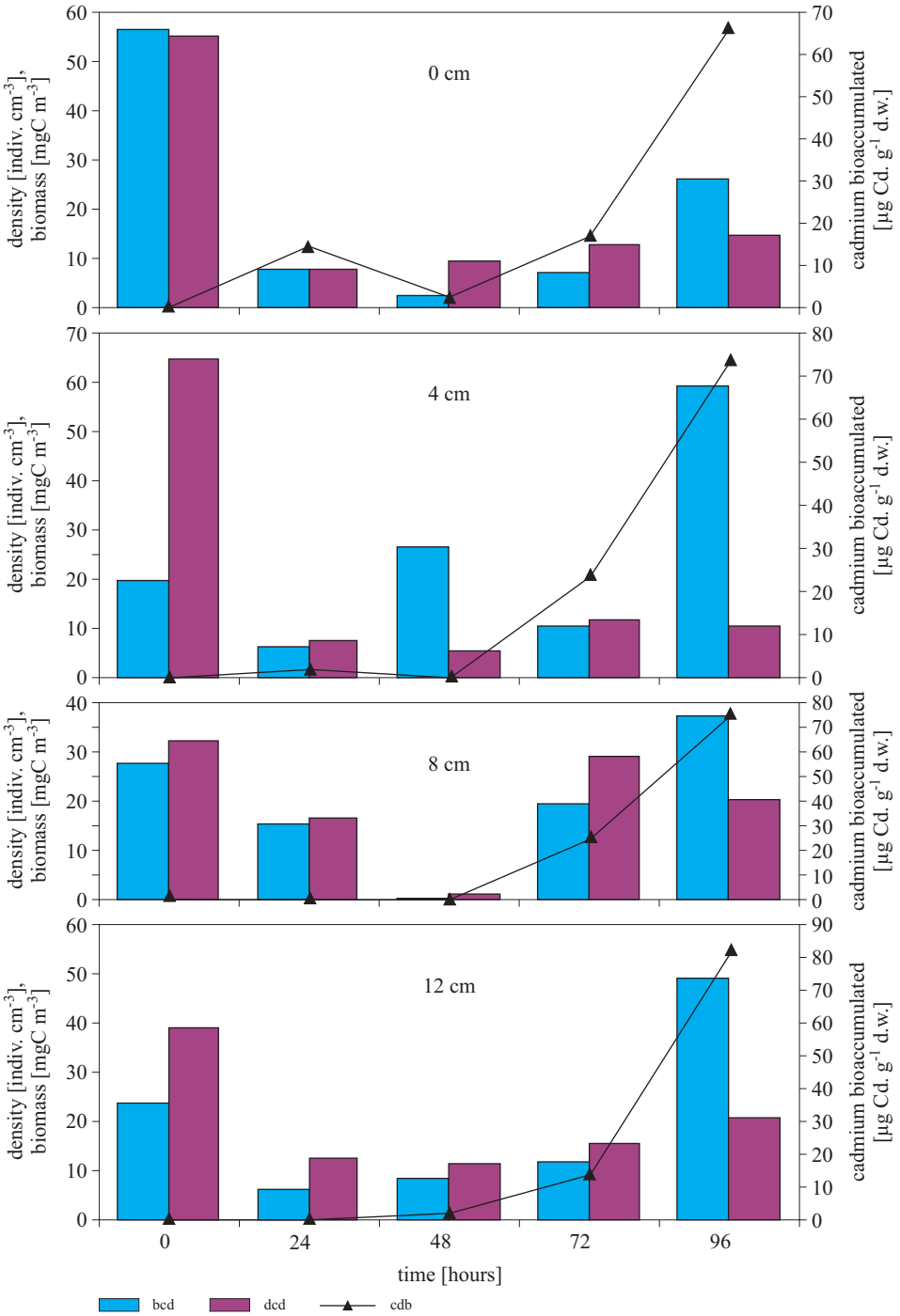

Fig. 10. Mean biomass (bcd), density (dcd) and cadmium bioaccumulated (cdb) (μg g^{-1} d.w.) in protozoa at different depths during the 2004 assay

towards the end of the experiment. In the initial phases of the treatment the proportion of cadmium bioaccumulated decreased with depth (14.52% at the surface, 1.76% at 4 cm, after 24 h). At 8 and 12 cm depths bioaccumulation was clearly in evidence after 72 h (24.55% and 14.16%, at 8 and 12 cm depth, respectively). After 96 h bioaccumulation was at a maximum, ranging between 66.22% at the surface and 81.79% at 12 cm. It is important to indicate that there was a significant correlation between the cadmium bioaccumulated and the biomass of protozoans at all depths (0.91–0.99; $p \leq 0.05$).

4. Discussion

In most of the documented effects of cadmium on marine communities of protists (Fernandez-Leborans & Novillo 1994, Fernandez-Leborans & Olalla-Herrero 1999), the communities used were epibenthic. In these previous studies the effects on the biomass and density of protists were observed in surficial sediments after 24 h.

Although the concentration of cadmium in seawater is low, it can become concentrated in sediments in polluted areas. In the present work, the initial concentration in water was chosen to be very high (1000 ppm), since cadmium was added only once. Moreover, this concentration allowed for the loss of cadmium through adsorption to the glass walls of tanks.

Metal toxicity is dependent on various environmental factors: temperature, salinity, pH, concentration of free metal ions, metal complexation by inorganic and organic ligands, etc. (Rai et al. 1981). According to Larsen (1989), minor fluctuations of the pH do not affect the speciation of cadmium. In the microcosms of the present study, pH varied between 6.32 and 7.67, and there were no significant pH differences between the control and the treatment with Cd. Cadmium uptake decreases with increasing salinity. Generally, it is accepted that only free Cd^{2+} can be accumulated in organisms, whereas there is no uptake by organisms of cadmium chlorocomplexes in seawater. This explains why cadmium is considered to be more toxic in freshwater, where its bioaccumulation is higher than in seawater (Stratford 1985).

Dissolved cadmium concentrations are extremely low in open ocean surface waters (≤ 1 ng dm^{-3}) and increase with depth to a maximum at the level of the nutrient maximum (about 900 to 1000 m), where total cadmium concentrations can reach 145 ng dm^{-3}. Concentrations then decline slightly at greater depths (Pohl et al. 1993, De Baar et al. 1994, Yeats et al. 1995).

Effects of cadmium in the assay. In the present study, after 24 h there was an important decrease in density, number of species and biomass. These

effects of heavy metals on communities of protists are due to the effects of the metals at a cellular level. It is known that toxic metal ions are able to cross membranes either by non-bilayer metal-induced structures or by non-specific multivalent ion carriers, causing membrane depolarisation and cytoplasmic acidification (Cumming & Gregory 1990). Heavy metals can also alter membrane function through displacement of calcium present as a structural component of membrane phospholipids (Green et al. 1980). In fact, membrane injury is one important effect of metal ions that may lead to the disruption of cellular functions. Protist mortality may then lead to oxidation of labile organic matter and a corresponding decrease in dissolved oxygen. These chemical changes could reduce the toxicity of cadmium, especially in the final phases of the assay, in which the amount of organic matter is high and the cadmium can form complexes. Redox potential has an indirect effect on the forms of cadmium in sediments. In oxic sediments, cadmium is associated primarily with the carbonate plus Fe/Mn oxide fractions; meanwhile, in hypoxic or anoxic sediments, most of the cadmium is associated with the carbonate and sulphide/insoluble humic substance phases. The cadmium species in oxidised sediment layers are more exchangeable and bioavailable than those in the anoxic layers (Dive et al. 1982, Houba & Remacle 1982, Nilsson 1989, Fernandez-Leborans & Novillo 1995). Tables 1 and 2 showed that the redox potential rose during the assay and it was higher at the surface than at depth 12 cm. In summary, cadmium species were less exchangeable and bioavailable at the end of the assays and at the surface than at the beginning of the assays and at 12 cm depth.

The recovery observed after 72 h (Figs. 1 and 2) in the density, number of species and biomass could be due to mechanisms of metal tolerance, including cellular adaptations such as exudation of chelating compounds and active efflux of metal ions by primary ATPase pumps (Cumming & Gregory 1990). Adaptations at the sub-cellular level have also been described; they mostly involve the induction of protective proteins, such as metallothioneins, phytochelatins, heat shock-proteins and antioxidant enzymes (Hassan & Scandalios 1990, Steffens 1990, Vierling 1990 Robinson et al. 1994). In the assay the variations in depth-averaged values with time were smaller than the variations in time-averaged values with depth. This may be due to the composition in functional groups of the communities present at the different depths.

With regard to the groups of protists, in a previous study (Fernandez-Leborans & Olalla-Herrero 1999) photoautotrophs decreased noticeably in biomass and density after 48 h. In the present study, the most remarkable reduction at the surface was also observed after 48 h. The density of ciliates at the surface decreased after 24 h and 48 h – a similar observation had

been made in the previous survey – and the same occurs with respect to the flagellates as a whole (heterotrophic and autotrophic flagellates).

Of the three dominant classes of coastal phytoplankton – diatoms, dinoflagellates and cyanobacteria – diatoms are the least sensitive to acute metal toxicity (Brand et al. 1986). Thus, the toxic effects of trace metals reported by Reinfelder et al. (2000) may be more pronounced in non-diatom phytoplankton species and could affect the phytoplankton species composition in natural waters. This observation is in line with the results of the present study, although in this case the communities belong to the benthic environment: the number of species and biomass at 12 cm depth decreased for all protist groups except diatoms. In biomass, diatoms were also the second most dominant group after sarcodines in 6 fractions with cadmium (39.46–100%; 30%), and also in the control (dominant in 3 fractions; 15%). This fact may indicate that the effects in the water column were reflected in the sediment. The flagellate *Olisthodiscus luteus* displayed a decrease in cellular volume, growth rate and levels of photosynthetic pigments as effects of cadmium exposure (Fernandez-Leborans & Novillo 1995). These facts could be compared with the results of the effects of cadmium on diatoms and autotrophic flagellates. In the experiment, after 24 h at the surface all the protist groups decreased noticeably in biomass, and the same occurred at 12 cm, except for diatoms, which increased.

Fernandez-Leborans & Novillo (1994) observed that after a 96 h exposure to a concentration of 0.5 mg dm^{-3} cadmium there was a reduction in biomass, and bacterivorous ciliates were replaced by small flagellates. Higher concentrations affected abundance and biomass after 48 hours. A change in the community structure through the loss of Cd-sensitive species and the appearance of Cd-resistant species occurred. The structure of the community was simplified and its diversity reduced. The bacterivore-detritivore and photoautotroph groups, the main components in the control, were noticeably reduced in the presence of cadmium. The metal content of protozoans increased during the experiment, indicating a certain bioassimilative role of these organisms (Fernandez-Leborans & Novillo 1994). In the present study there was a decrease in the number of species at 12 cm after 24 h, while at 4 cm, this drop was observed after 48 h; at 8 cm there was no reduction. The drop in the number of species at the surface was due mainly to diatoms, whereas at 12 cm this decrease was due to all groups except diatoms. The highest number of species was recorded at the surface and decreased with the depth, being noticeably low at 8 and 12 cm.

In a previous study carried out on protist communities inhabiting the sediment surface (Fernandez-Leborans & Olalla 1999), the protozoa

accumulated between 16.29 and 87.83 μg Cd g^{-1} d.w. in the same period of treatment. In the 2004 assay, the amount of cadmium bioaccumulated was higher (36.79–167.84 μg Cd g^{-1} d.w). There are several possible reasons for this difference. Taking into consideration the abiotic parameters, the pH was noticeably lower in the 2004 assay (7.09–7.48; mean 7.26; previous study 7.83–8.05, mean 7.88). Skowronski et al. (1991) reported that the toxicity of cadmium was highest at pH 6–7, whereas at higher pH hydrolysis of environmental components and the formation of complexes with hydroxyl ions caused a significant decrease in the concentration of available cadmium as measured electrochemically. The lower pH values in the present study may be responsible for the greater bioaccumulation. It must be borne in mind, however, that if the chemistry of cadmium in marine systems is controlled by the formation of chloro-complexes, salinity, rather than pH is the main environmental variable influencing the species of cadmium. Additionally, differences in the net electrical changes of the various chloro-complexes might have affected the tendency of cadmium to adsorb onto sediment particles. Two other parameters could be related to the bioaccumulation: the redox-potential and the sediment grain-size. In the previous study (Fernandez-Leborans & Olalla 1999), the mean grain-size was 0.2207 mm smaller than that in the 2004 assay (1.025 mm). This difference may partly explain why bioaccumulation was lower in the previous study. In another survey, it was observed that the fraction with the lowest grain-size showed the highest concentrations (Danis et al. 2004).

5. Conclusions

Cadmium caused an important decrease, after 24 h, in the density, number of species and biomass of the protist communities. In general, there was a drop in these three parameters as a function of depth.

The effect of depth was greater than time in the fractions with cadmium. The surface level of the sediment showed the maximum contribution with respect to the variation in time and depth.

Cadmium bioaccumulation increased towards the end of the assay. Between the initial and final phases of the assay, the proportion of cadmium bioaccumulated rose with depth.

The recovery of communities after 72 h may be due to metal tolerance mechanisms. Diatoms seem to be the group least sensitive to cadmium toxicity. The effect of cadmium determines a change in the community structure with the appearance of Cd-resistant species. The structure of the community was simplified.

Salinity is the main environmental variable influencing the species of cadmium; the sediment grain-size is also important.

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