Impact of Heavy Metals and PCBs on Marine Picoplankton

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Received 12 September 2005; revised 21 March 2006; accepted 29 April 2006

ABSTRACT: Synergistic/antagonistic effects of multiple contaminants in marine environments are almost completely unexplored. In the present study, we investigated the effects of heavy metals (Zn and Pb) and PCBs on picoplankton abundance, biomass, cell size distribution, and bacterial C production. Natural picoplankton assemblages were exposed to heavy metals (Zn or Pb), organic contaminants (PCBs, Aroclor 1260), and to a mixture of different contaminants. The results of the present study indicate that Zn addition stimulated heterotrophic growth, whereas Pb has a negative impact on heterotrophic picoplankton, particularly significant in the first 24 h. Heavy metals had no effects on the autotrophic component. The addition of Aroclor 1260 had a significant impact on abundance, biomass, and cell size of autotrophic and heterotrophic picoplankton, and reduced significantly bacterial secondary production. Three weeks after PCB treatment, heterotrophic bacteria displayed a clear resilience, both in terms of abundance and biomass, reaching values comparable to those of the controls, but not in terms of bacterial C production. Our results indicate that picoplankton can be sensitive indicators of impact determined by heavy metals and PCBs in coastal marine systems. © 2006 Wiley Periodicals, Inc. Environ Toxicol 21: 541–551, 2006.

Keywords: picoplankton; Pb; Zn; heavy metals; PCBs; bacterial C production

INTRODUCTION

Pollutants generally accumulate in marine coastal areas as a consequence of anthropogenic inputs via industrial run-off, river outflows, and domestic sewage. In the last decades, heavy metal concentrations have increased significantly, reaching highest levels (up to several micrograms per liter) in densely populated regions, such as the North-Western Mediterranean Sea (Danovaro, 2003). Most heavy metals may create complexes with organic particles, so that their accumulation in benthic organisms is typically coupled with organic matter content (Dell'Anno et al., 2003).

In pristine water systems, total zinc concentrations range usually from 0.002 to 0.1 μg L^{-1}, but in contaminated areas, such as in estuarine waters, concentrations up to 15.0 μg L^{-1} can be found (Van den Berg et al., 1987). Available literature on Zn impact on marine invertebrates suggests that concentrations ranging from 0.097 to 11.3 mg L^{-1} are highly toxic for mysids and shrimps, respectively (Negilski et al., 1981; Martin et al., 1989).

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The authors declare that they have no conflict of interest, and no interest in the commercial exploitation of the methods or results of the present research.

Contract grant sponsors: CNR, Rome.

Contract grant sponsor: ADIOS; INTERPOL.

Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/tox.20215

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Pb concentrations in seawater samples typically range from 0.01 to 27.0 $\mu g\ L^{-1}$ (Sadiq, 1992), displaying highest values in proximity of urban and industrialized areas. Pb levels are also high in estuarine and coastal areas, and studies carried out in the Tyrrhenian and Adriatic Sea suggest that their concentrations typically decrease toward the open sea (Fabiano et al., 1994; Dell'Anno et al., 2003). Studies carried out *in vitro* and *in vivo* reported that Pb concentrations of 0.2 $\mu g\ L^{-1}$ adversely affected aquatic biota (Sadiq, 1992; Jackson et al., 2005), but since Pb can occur in a wide range of forms, its impact on ecosystem components can change considerably. For instance, Pb(II), being the most stable ionic species, tends to bioaccumulate in most aquatic organisms (Nussey et al., 2000).

Sediments are the natural collectors of pollutants. Here, persistent contaminants are stored and partially buried, but can be potentially resuspended, thus re-entering the pelagic food web (Charles et al., 2005; Pusceddu et al., 2005). In impacted coastal areas, such as the Mar Piccolo of Taranto (Ionian Sea, Central Mediterranean), Zn and Pb are the most abundant heavy metals (with average concentrations of 264 ± 40 and 134 ± 29 $\mu g\ g^{-1}$, for Zn and Pb, respectively) (Pastore et al., 2002), reaching values 4–5 times higher than those typical of Mediterranean sediments (<70 $\mu g\ g^{-1}$ for Zn and 25 $\mu g\ g^{-1}$ for Pb) (EEA/UNEP, 1999; ICES, 2000).

In the 1970s, in the Mediterranean, polychlorinated biphenyls (PCBs) have been largely utilized for industrial purposes, but at that time their toxicity and impact on the environment were unknown (Colombo et al., 1990). Owing to their high stability and persistence, PCB levels in Mediterranean coastal areas are still high: in the Northern Adriatic Sea, for instance, PCB concentrations can reach 80 ng g^{-1} in the estuarine sediments of the Po Delta (Caricchia et al., 1993), whereas in the sediments of Sado Estuary (Portugal), PCB concentrations are <4.9 ng g^{-1} (Gil and Vale, 2001). PCBs easily bioaccumulate in fish, reaching concentrations at orders of magnitude higher than levels safe for human health (Olsson and Bignert, 1997). Studies conducted on the impact of Aroclor 1254 on phytoplankton have demonstrated a strong inhibitory effect on their growth rates, even at very low concentrations of 0.1 $\mu g\ L^{-1}$ (Biggs et al., 1978; IPCS/INCHEM, 1992).

Although the impact of PCBs and heavy metals have been, individually, investigated on a variety of marine organisms (Pridmore et al., 1992), the effects of a mixture of contaminants on picoplankton assemblages is completely unknown.

Picoplankton, which includes also the ultraplankton (*sensu*, Li, 1995), comprises three major groups: (i) cyanobacteria (*Prochlorococcus* and *Synechococcus*), (ii) picoeukariotic algae, which are primary producers with specific photosynthetic pigments, and (iii) heterotrophic microorganisms (Zubkov et al., 2000), which are the main decomposers and mineralizers of organic matter in marine ecosystems.

In the present study, we investigated the impact of different contaminants (Zn, Pb, and PCBs) and their potential synergistic effects on the abundance, biomass, and cell size of picoplankton as well as bacterial C production by means of microcosm experiments.

MATERIALS AND METHODS

Study Area and Experimental Design

Seawater samples were collected at Bosco Marziotta in the Northern Ionian Sea (40°28′50″ N, 17°02′46″ E) in March 2001 at a depth of 0.5 m below the sea surface, using 5-L sterile Niskin bottles, previously washed with 0.1 N HCl. Water temperature was of 15.4°C, salinity 38.19‰, and dissolved oxygen reached 93.7% of saturation.

Seawater samples were collected from oligotrophic coastal waters. Such condition was evident from the analysis of inorganic nutrient concentrations, as well as phytoplankton biomass (as chlorophyll *a* content). Nitrogen concentrations were 1.83 $\mu g\ L^{-1}$ for NH_4^+—N, 0.25 $\mu g\ L^{-1}$ for NO_2^-—N, 0.57 $\mu g\ L^{-1}$ for NO_3^-—N. Phosphate concentration was 0.25 $\mu g\ L^{-1}$, and silicate concentration was 0.91 $\mu g\ L^{-1}$. Finally chlorophyll *a* content was 0.38 $\mu g\ L^{-1}$.

In coastal seawater of the Northern Ionian Sea, Zn and Pb concentrations were 0.001 and 0.002 $\mu g\ L^{-1}$, respectively, whereas heavy metal content in the sediment was 0.087 and 0.005 $\mu g\ g^{-1}$ DW, respectively, for Zn and Pb (Buccolieri et al., 2004). Furthermore, in the mussels (*Mytilus galloprovincialis*) collected in the investigated areas, both Zn and Pb were present in concentrations of 5.15 and 1.19 mg kg^{-1}, respectively, assumed to be safe for human consumption (Storelli et al., 2000). In the pristine waters collected, PCB concentrations were below detection limits (APAT, 2003).

In the laboratory, the seawater samples were immediately filtered through 200-μm pore-size filters and then through 2-μm pore-size filters, to remove larger suspended particles (such as nanoflagellates, phytoplankton, and larger organisms). In addition, the removal of protozoa and microalgae was checked by epifluorescence microscopy (Danovaro et al., 1998). The response of picoplankton to the addition of different pollutants was tested by using replicate microcosm experiments. All microcosms were performed by using beakers of 2000 mL capacity filled with filtered seawater, and subjected to gentle stirring on a magnetic stirrer.

Stock solutions of heavy metals were prepared by dissolving the appropriate metal salts ($ZnCl_2$ and $PbCl_2$) in distilled water in 1000 mL glass volumetric flasks, which had previously been washed in 10% nitric acid and well rinsed with distilled water. They were then stored in darkness at 4°C until use. All contaminants were added after serial dilutions of the stock solution with natural seawater, previously filtered onto 0.2-μm pore filters.

The PCBs (Aroclor 1260, ULTRA Scientific, USA) used for the experiments had a purity of 98%. Aroclor 1260 was first dissolved into filtered seawater (dissolved in 100 μL of acetone) and placed in microcosms, to obtain a final concentration of 1 μg L^{-1}. All glassware was pretreated overnight with seawater, and then normalized with each respective contaminant in order to avoid any loss due to adsorption. The final concentrations utilized in the present study fall within the range of Zn, Pb, and Aroclor values observed in coastal and polluted Mediterranean areas (UNEP, 1996).

Five experimental sets were prepared, each set included three independent replicate microcosms subjected to the same treatments. In the first experimental set of three microcosms, we added $ZnCl_2$ with a final concentration of 100 μg L^{-1}; in the second set of three microcosms, we added $PbCl_2$ to a final concentrations of 100 μg L^{-1}; in the third set of three microcosms, we added Aroclor 1260 (1.0 μg L^{-1} final concentration). In the fourth set of three microcosms, we added Zn^{2+}, Pb^{2+}, and Aroclor 1260 at the same concentrations reported earlier. Finally, the fifth experimental set of three microcosms contained only natural seawater, without any treatment and served as controls.

All microcosms were incubated for 21 days (504 h) at the *in situ* seawater temperature and light intensity of 80 μE m^{-2} s^{-1} (Kana and Glibert, 1987) and a period of Light/Dark 12:12 h. The duration of the experiment was defined according with previous studies conducted on polyethylene microcosms (Danovaro and Corinaldesi, 2003). At each sampling time from each microcosm, a volume of 15 mL was withdrawn. Sampling intervals were defined in order to follow microbial response to contaminant addition. In particular, samples were taken at T_0 and 3, 12, 24, 48, 72, 96, 120, 144, 168, 336, and 504 h after starting the experiment.

The concentrations of heavy metals and PCBs at the end of the experiment were not measured, as we assumed that the amounts supplied were not susceptible of significant changes due to microbial incorporation. Such an assumption is reasonable as, for instance, heavy metal concentrations are at least 10 times higher than total picoplankton biomass.

Picoplankton Counts and Cell Size

For estimating picoplankton abundance, samples were preserved with buffered formaldehyde at a final concentration of 2% and kept in the dark at 4°C until analysis. Counts were performed using a Zeiss Standard Axioplan microscope equipped with a halogen lamp (Hg 100) light. For picophytoplankton analysis, triplicate slides were prepared from each experimental treatment at each time, by filtering 10 mL of seawater onto 0.2-μm black Millipore membranes (Maugeri et al., 1990). Blue light excitation with a BP 485/20 excitation filter, a FT 510 chromatic beam splitter, and a LP 520 barrier filter were used. For heterotrophic picoplankton, triplicate slides were prepared from each sample by filtering 1 mL of seawater onto a 0.2 μm Millipore filter, using 4,6-diamidino-2 phenyl-indole as fluorochrome (Porter and Feig, 1980). A G 365 excitation filter, a FT 395 chromatic beam splitter, and a LP 420 barrier filter were used. At least 20 microscopic fields were counted for each preparation, at $\times$1000 magnification.

Cell size of both autotrophic and heterotrophic picoplankton was estimated by epifluorescence microscopy, using microphotographs. Each cell size was determined after projection on a screen, and at least 60 cells per filter were measured manually. For biovolume estimation of the autotrophs, two morphotypes were considered: spheroid and ellipsoid, and the following formula was applied: $V = \pi/6\ W^2L$, where L and W are the cell length and width (Sieracki et al., 1989). Picoplankton cells were subdivided into three size classes: small size, medium size, and large size ($<$0.33, 0.34–0.64, and $>$0.65 μm^3 for picophytoplankton, and $<$0.065, 0.065–0.320, and 0.320–0.780 μm^3 for heterotrophic bacteria, respectively) (Palumbo et al., 1984; Danovaro et al., 1998). The accuracy of the estimates of cell sizes based on epifluorescence microscopy is debated. We selected the present protocol on the light of the availability of previous studies (Lee and Fuhrman, 1987; Fuhrman et al., 1989; Cho and Azam, 1990; Ducklow and Carlson, 1992; Kirchman et al., 1993; Moriarty et al., 1997), in which picoplankton biovolume was estimated visually by image analysis (based on epifluorescence microscopy) and intercalibrated with an ocular micrometer. Picoplankton biovolume was then converted into biomass, assuming a carbon content of 220 fg C μm^{-3} for picophytoplankton (Sondergaard et al., 1991) and 310 fg C μm^{-3} for heterotrophic bacteria (Fry, 1988).

Bacterial C Production

Bacterial carbon production was determined by [^{3}H] leucine incorporation (Smith and Azam, 1992). Triplicate subsamples and two blanks (1.7 mL) were added with ^{3}H-leucine (final concentration 20 nM) and incubated for 1 h in the dark. Incubation was stopped with 100% trichloroacetic acid (TCA, final concentration 5%) and stored at 4°C. Pellets were washed with 5% TCA and 80% ethanol, and supplemented with 1 mL liquid scintillation cocktail (Ultima Gold MV; Packard). The incorporated radioactivity was measured by determining the counts per minute with a liquid scintillation counter (Packard Tri-Carb 300).

Statistical Analyses

In order to test whether the different treatments had a significant impact on picoplankton variables, One-Way ANalysis Of VAriance (ANOVA) was utilized, using sampling times as a repeated measure. Since we collected consecutive samples from all microcosms, the significance thresholds were kept more conservatively at Bonferroni

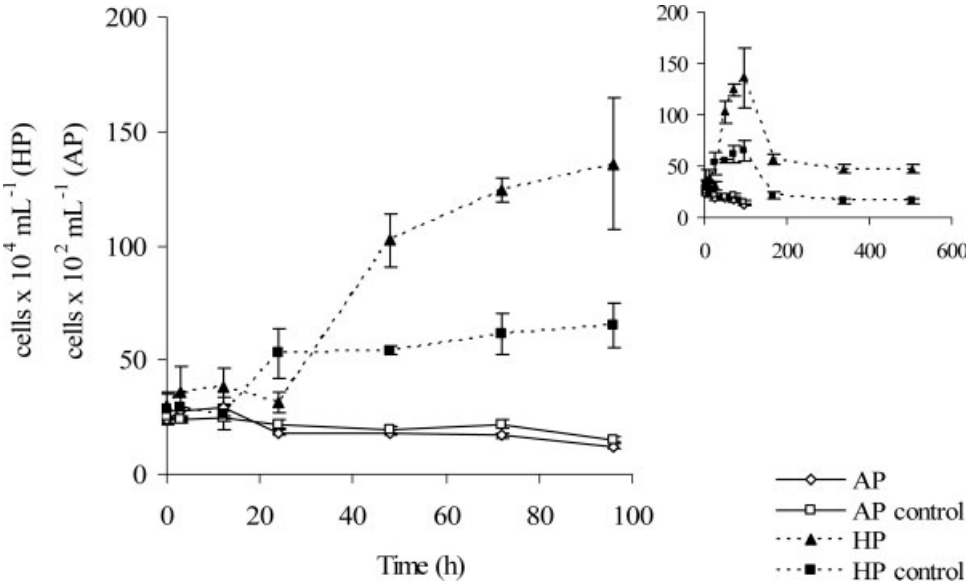

Fig. 1. Picoplankton abundance in microcosms treated with Zn and in the controls. Reported are values relative to the first 96 h (large graph) and the rest of the experiment up to day 21 (smaller graph). Mean values ± SDs are reported. AP, autotrophic picoplankton; HP, heterotrophic picoplankton; $n = 24$ for AP and 36 for HP.

corrected levels. Differences among treatments and controls were investigated after testing of variance homogeneity (Levene test). When differences were statistically significant, a *posthoc* Turkey's test was performed.

RESULTS

The abundance of autotrophic and heterotrophic picoplankton displayed clear differences depending on the treatment. When compared with the control, Zn addition resulted in a slight, though not significant decrease of autotrophic picoplankton fraction (abundance reduced by 25% after 72 h). In treated microcosms, the abundance of the autotrophic component collapsed after 96 h. This pattern was consistent throughout all treatments, controls included. By contrast, a stimulating effect of Zn was observed on the heterotrophic picoplankton growth, whose abundance increased significantly already after 48 h (when compared with the control; ANOVA, $P < 0.001$, up to 200% after 96 h; Fig. 1).

The addition of Pb had no significant impact on the picophytoplankton abundance, but determined a significant decrease of the heterotrophic picoplankton abundance after 24 h (ANOVA, $P < 0.05$, Fig. 2), with a 50% reduction. In the subsequent samplings, no significant differences were observed comparing treatments and controls.

The addition of Aroclor 1260 determined a significant decrease in picophytoplankton abundance, particularly evident after 24–48 h (ANOVA, $P < 0.001$, Fig. 3). Aroclor 1260 had an even stronger impact on the heterotrophic component after 24 h (ANOVA, $P < 0.001$, Fig. 3). After the initial decrease of the abundance of heterotrophic picoplankton, no significant differences were observed comparing treatments and controls, until the end of the experiments.

When all pollutants were simultaneously added, picophytoplankton abundance was significantly reduced after 72 h (by 80%; ANOVA, $P < 0.001$, Fig. 4). Moreover, after 24 h of incubation, heterotrophic picoplankton abundance was significantly reduced when compared with the control (by ~50%; Fig. 4).

Results reported earlier were confirmed by the analysis of bacterial C production, which remained rather constant in the control, but varied consistently with changes in cell abundance (Fig. 5). In fact, a slight increase of bacterial production was observed 48 h after the start of the Zn treatment. Moreover, a decrease after Pb addition, and a significant drop of bacterial production were observed after Aroclor 1260 addition (ANOVA, $P < 0.01$). Finally, also the addition of the mixture of contaminants determined a significant decrease (ANOVA, $P < 0.01$), though less relevant than after the addition of Aroclor alone.

Picoplankton response to the addition of different contaminants was also investigated in terms of biomass and cell size distribution (i.e., repartition between small, medium and large size cells). Table I reports the results of the ANOVA analysis conducted on picoplankton biomass and cell size distribution after different treatments with respect to the control. Zinc treatment did not significantly change total picophytoplankton biomass and size class distribution, when compared with control values [Fig. 6(a)], but significantly increased the biomass of heterotrophic picoplankton

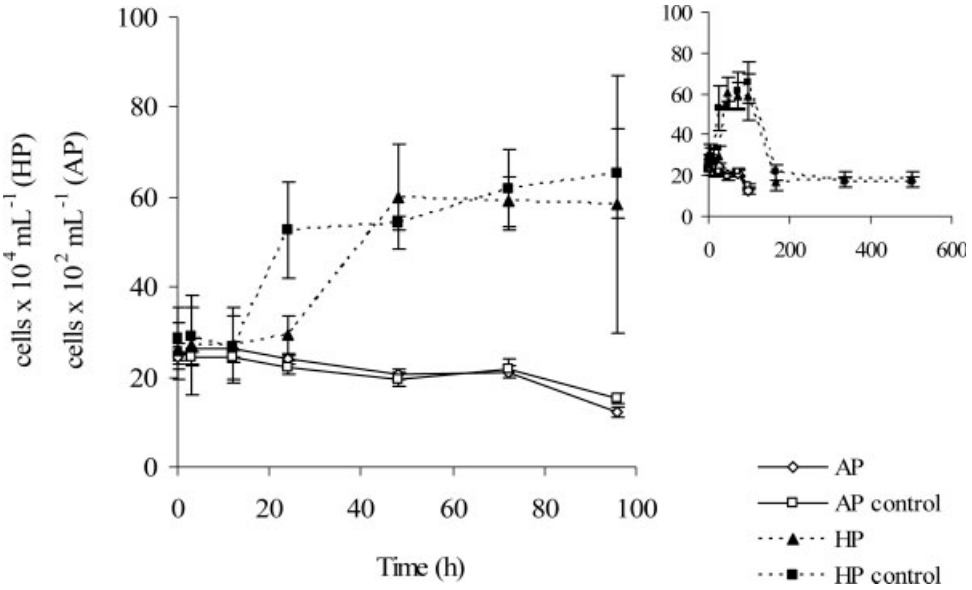

Fig. 2. Picoplankton abundance in microcosms treated with Pb and in the controls. Reported are values relative to the first 96 h (large graph) and the rest of the experiment up to day 21 (smaller graph). Mean values ± SDs are reported. AP, autotrophic picoplankton; HP, heterotrophic picoplankton; $n = 24$ for AP and 36 for HP.

(47.70 ± 0.07 *versus* 31.50 ± 0.05 μg C L^{-1}) [Fig. 7(a)], also inducing a shift toward the smaller size classes. Pb addition had a significant impact on total picophytoplankton biomass and size class distribution, determining a significant increase of average size [Table I, Fig. 6(b)]. The impact of Pb treatment on heterotrophic picoplankton biomass was clearly detectable within the first 24 h, when the total biomass was reduced by ~50% with respect to the control (24.08 ± 0.05 *versus* 45.50 ± 0.1 μg C L^{-1}, respectively). Then bacterial biomass remained rather constant till the end of the experiment (days 21; Table I). These results were reflected by cell size distribution.

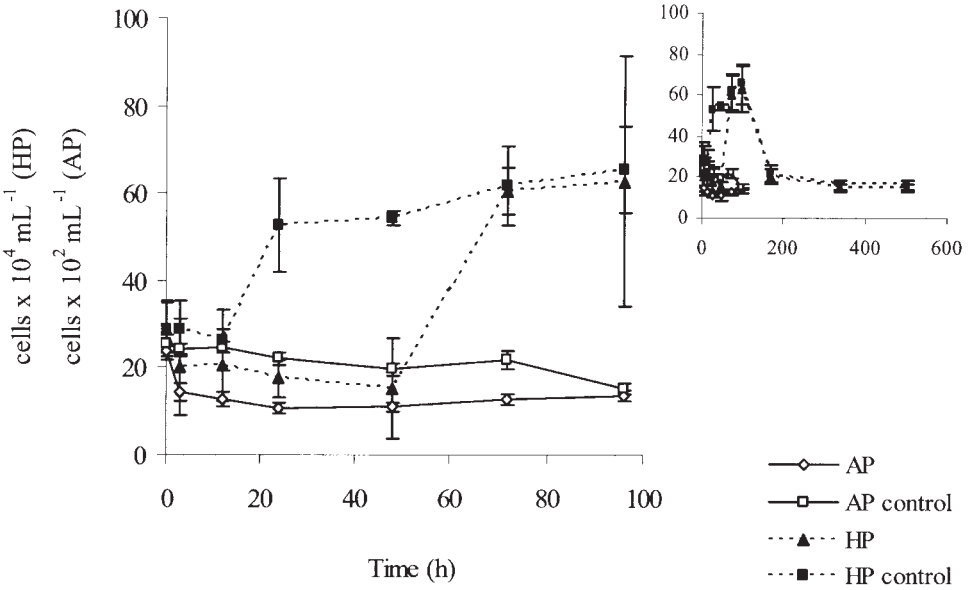

Fig. 3. Picoplankton abundance in microcosms treated with Aroclor 1260 and in the controls. Reported are values relative to the first 96 h (large graph) and the rest of the experiment up to day 21 (smaller graph). Mean values ± SDs are reported. AP, autotrophic picoplankton; HP, heterotrophic picoplankton; $n = 24$ for AP and 36 for HP.

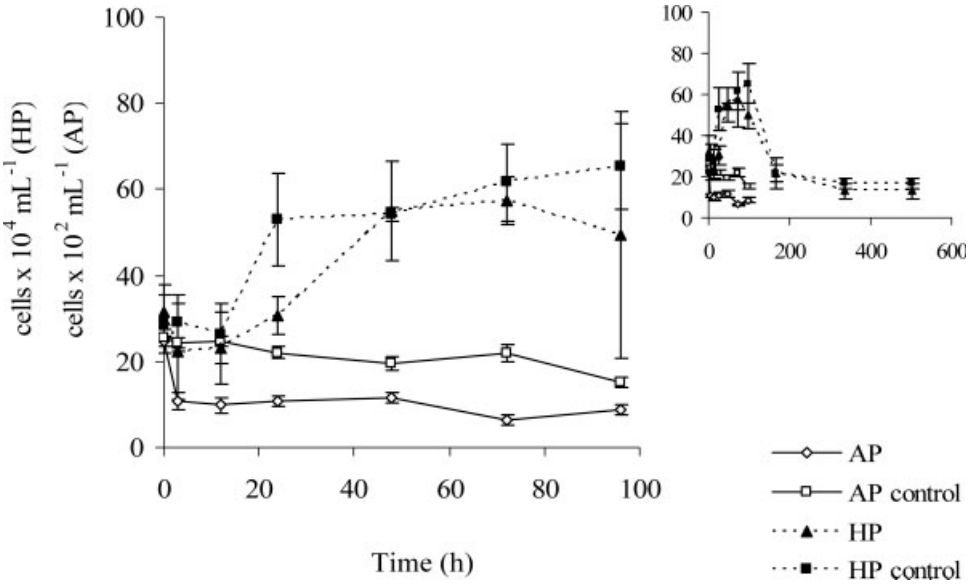

Fig. 4. Picoplankton abundance in microcosms treated with Zn, Pb, and Aroclor 1260 and in the controls. Reported are values relative to the first 96 h (large graph) and the rest of the experiment up to day 21 (smaller graph). Mean values ± SDs are reported. AP, autotrophic picoplankton; HP, heterotrophic picoplankton; $n = 24$ for AP and 36 for HP.

Total picoplankton (i.e., both autotrophic and heterotrophic) biomass was significantly lower in microcosms treated with Aroclor 1260 [Figs. 6(a) and 7(a)], whereas the effects on cell size distribution were less evident [Figs. 6(b) and 7(b); Table I]. When all contaminants were added simultaneously, the biomass of the autotrophic component decreased significantly (ANOVA, $P < 0.001$), along with a reduction of the relevance of large size classes (Table I). Finally, the biomass and cell size of heterotrophic bacterioplankton did not change significantly compared with treatments and controls.

DISCUSSION

Information on picoplankton response to different kinds of pollutants in the marine environment is almost completely lacking. As far as heavy metals are concerned, several studies have been carried out on their impact on organisms (Jak et al., 1996; Nayar et al., 2003; Morelli and Scarano, 2004), but little is known yet on their effects on the microbial food webs (Goulder et al., 1980). Previous investigations indeed focused on the effects of heavy metals on soil microorganisms (Babich and Stotzky, 1985; Baath, 1989; Giller et al., 1998; Kelly et al., 2003). Such studies, along with others carried out in soils contaminated by heavy metals, demonstrated that high concentrations of these contaminants can result in a decrease of microbial activity and cell size (Chander and Brookes, 1991; Konopka et al., 1999), with important implication for organic matter cycling (Chander and Brookes, 1991; Saeki et al., 2002; Rajapaksha et al., 2004).

Although pioneer laboratory experiments have shown the inhibitory effects of heavy metals on the activity of marine bacteria (Sunda and Gillespie, 1979; Zavenhuizen et al., 1979), information on the marine environment is still extremely limited. Experimental findings have been confirmed by field studies conducted on estuarine waters and sediments, where high concentrations of Cd, Pb, and Cu have been demonstrated to affect bacterial distribution (Fabiano et al., 1994) and to cause local inhibition of benthic bacterial activity (Goulder et al., 1980). However,

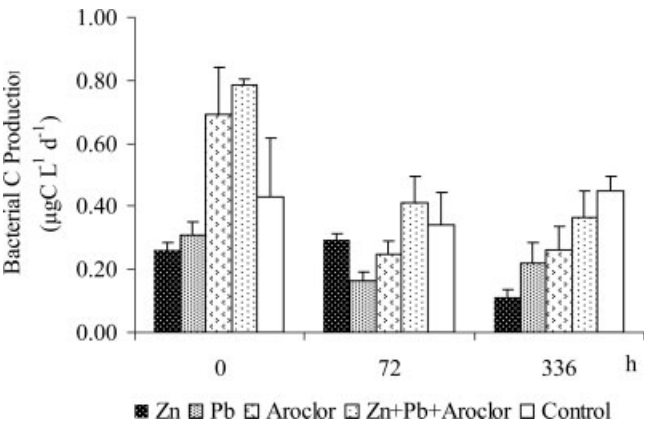

Fig. 5. Temporal changes in bacterial C production in microcosms treated with Zn, Pb, Aroclor 1260, and the combination of the three contaminants.

TABLE I. ANOVA of the picoplankton biomass and cell size in treated microcosms with respect to control values

	Zn	Pb	Aroclor	Zn + Pb + Aroclor
TAPB				
F	1.565	6.282	11.203	36.480
P	0.213	**<0.05**	**<0.005**	**$<10^{-4}$**
SAP				
F	0.387	1.057	0.964	0.281
P	0.548	0.321	0.343	0.601
MAP				
F	0.607	3.101	5.992	19.701
P	0.439	0.083	**<0.05**	**$<10^{-4}$**
LAP				
F	1.233	9.733	11.673	23.685
P	0.270	**<0.005**	**<0.001**	**$<10^{-4}$**
THPB				
F	7.461	0.259	6.491	0.725
P	**<0.01**	0.612	**<0.05**	0.395
SHP				
F	17.291	0.173	0.051	0.284
P	**<0.001**	0.679	0.822	0.596
MHP				
F	11.129	0.043	11.384	1.120
P	**<0.01**	0.836	**<0.005**	0.294
LHP				
F	0.189	0.686	1.517	0.350
P	0.665	0.410	0.222	0.556

Significative values are reported in bold. $N = 24$ (autotrophic picoplankton) and $N = 36$ (heterotrophic picoplankton). TAPB, total autotrophic picoplankton biomass; SAP, small size autotrophic picoplankton; MAP, medium size autotrophic picoplankton; LAP, large size autotrophic picoplankton; THPB, total heterotrophic picoplankton biomass; SHP, small size heterotrophic picoplankton; MHP, medium size heterotrophic picoplankton; LHP, large size heterotrophic picoplankton; F, Snedecor variable; P, significativity.

so far no studies proved the effect of heavy metals on marine picoplankton abundance, biomass, cell size, and on bacterial C production. Our results indicate that after 48 h, Zn increased the abundance and biomass of the heterotrophic bacterioplankton. Being involved in different metabolic activities, Zn is an essential element for several marine organisms (Bettger and O'Dell, 1981). Zinc availability is also crucial for the heterotrophic growth and contributes to the catalysis of essential metabolic reactions and in the transfer of genetic information (i.e., transcription and replication; Vallee and Auld, 1990). Therefore, a stimulating effect of this metal on picoplankton could be expected. As observed in previous studies, it is possible that heterotrophic picoplankton displayed one or more metabolic processes requiring Zn, and as such the addition of 100 μg Zn L^{-1} resulted to be beneficial in the short term (Macaskie et al., 1987; Macaskie and Dean, 1990; Gadd, 1992; Diels et al., 1993). At the same time, Zn had no effect on the abundance and biomass of the autotrophic component, which nonetheless disappeared completely in all systems (controls included) after 96 h. These results suggest that picophytoplankton was much more sensitive to experimental manipulation (including nutrient changes, light conditions, etc.; Alabiso et al., 2001), and other studies are needed to clarify the potential of this component in environmental toxicology studies.

Bacteria are known to be sensitive to Cd and Pb contaminations, and studies on benthic bacteria have proved that the toxicity of these heavy metals can be 10–100 times higher than that of other metals (Romero et al., 1999). Our study confirmed the negative impact of Pb on abundance, biomass, and production of heterotrophic picoplankton, even though significant changes were observed only at the beginning of the experiments. The abundance of autotrophic picoplankton was unaffected by the exposure to Pb, but a reduction of the average biomass was observed. Such a phenomenon could depend on the effect of Pb on cyanobacteria

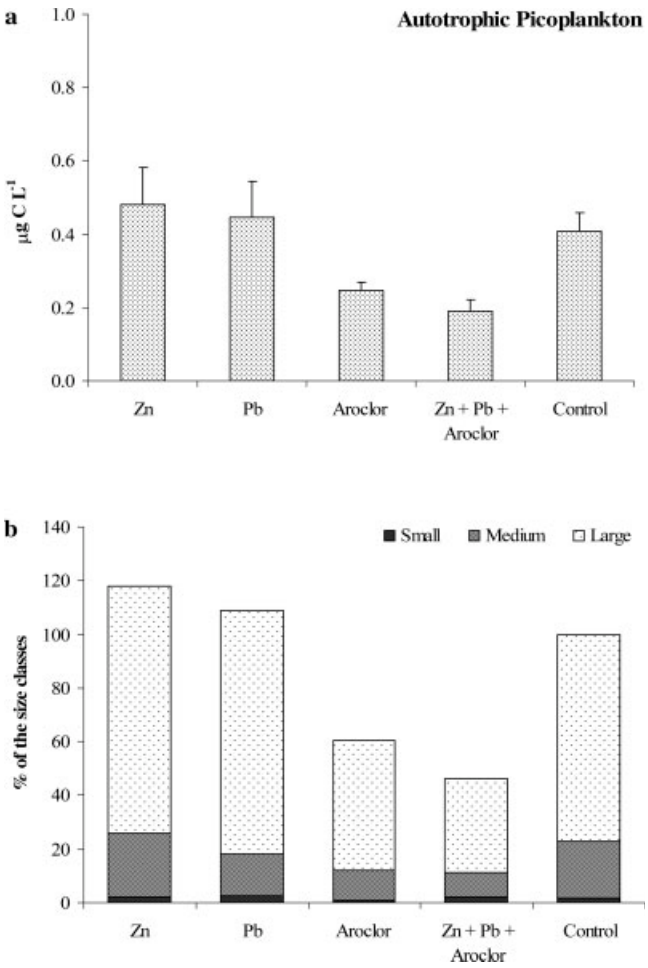

Fig. 6. Total autotrophic picoplankton biomass (a) and percentage contribution of the three size classes (small, medium, and large) (b). Data reported are relative to the first 96 h of experiment.

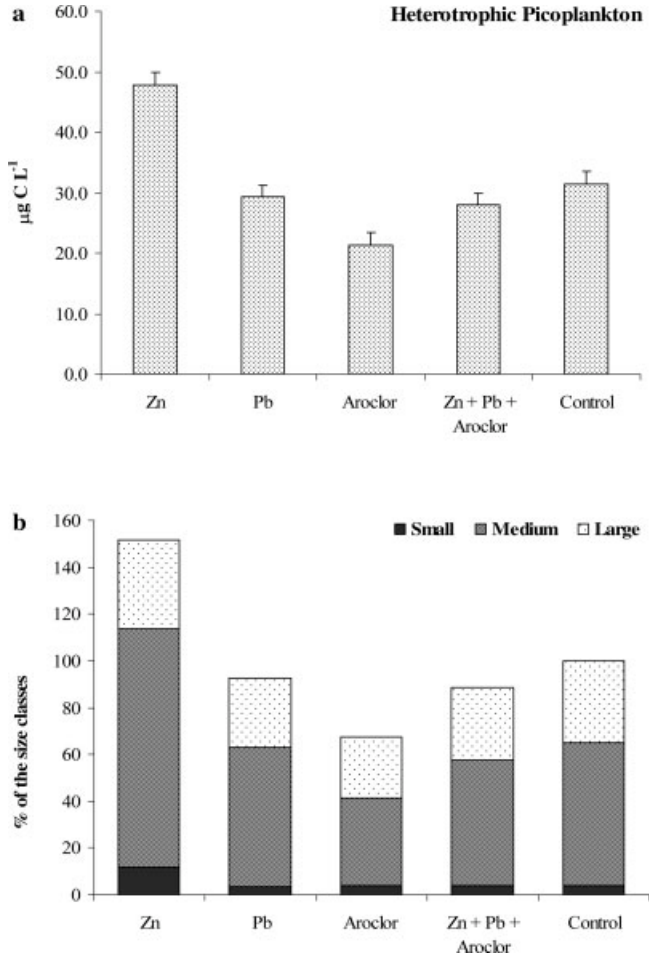

Fig. 7. Total heterotrophic picoplankton biomass (a) and percentage contribution of the three size classes (small, medium and large) (b). Data reported are relative to the entire duration of the experiment (21 days).

(the largest fraction of picophytoplankton), which have been reported to uptake heavy metals (Gardea-Torresday et al., 1998).

The different response of autotrophic and heterotrophic components to Pb exposure could be related to Pb binding to DOM (Yoona et al., 1999), which could determine an increase of the bioavailability of this metal to heterotrophic bacteria that utilize DOM (Cho and Azam, 1990). Since previous studies reported that cyanobacteria, such as *Spirulina platensis*, can accumulate Hg and Pb in contaminated sites (Bender et al., 1994), an alternative explanation could be that Pb sequestration within the cell is coupled with the synthesis of metallothioneins that would considerably reduce the metal toxicity (Ybarra and Webb, 1999).

Aroclor 1260 had an impact particularly evident on the heterotrophic component, determining a reduction of bacterial abundance (up to 80%) within 24 h. The relationship between bacterial production and cell size has been repeatedly investigated (Björnsen et al., 1989; Bird and Kalff, 1993). Recently, Servais et al. (1999) and Bernard et al. (2000) have shown that certain size classes have higher growth rates than others, thus, contributing to a larger fraction of bacterial C production. In our study, the impact on bacterial C production was reflected by a shift of the microbial assemblage, with a significant decrease of the medium sized cells. These results indicate that Aroclor 1260 has strong effects on the bacterial size distribution. After the first days, heterotrophic bacteria displayed a clear resilience both in terms of abundance and biomass, reaching values similar to those of the control at the end of the experiment. Several marine bacteria are able to produce resting stages, extracellular binding mechanisms, transformation of toxicants into less toxic compounds, and may use PCBs as carbon source (Ford, 2000). Some genera, such as *Pseudomonas* and *Cytophaga,* are involved in PCB degradation (Stolp, 1988). We can hypothesize that in our study the addition of the PCBs had an immediate impact on sensitive microorganisms, leaving a competitive advantage for more tolerant species/strains. As a consequence, in the absence of the competitors suppressed by the Aroclor, their abundance increased, reaching the observed values. Therefore, the picoplankton recovery after Aroclor 1260 addition would be associated with a shift of the composition of heterotrophic microbial assemblages. Frostegård et al. (1996) investigated changes following Zn addition in the microbial community (through phospholipids analysis and fatty acid patterns) structure in two different soils. They found that during the incubation of polluted soils in laboratory conditions, gradual changes over time occurred in the microbial community structure soil, when compared with unpolluted soils. Also, taxonomic investigations carried out in a small stream of West Yorkshire, characterized by a high concentration of chlorophenols, nitrophenols, and phenoxyalkanoic acids, demonstrated that the composition of bacterial communities in the polluted stream (dominated by *Pseudomonas*) was different from that of unpolluted streams (mainly characterized by other Gram-negative bacteria; Milner and Goulder, 1986). Further studies, possibly based on new developed fingerprinting techniques (e.g., ARISA, T-RFLP, DGGE), are needed to clarify this hypothesis in future (Muyzer and Smalla, 1998; Gremion et al., 2004; Danovaro et al., 2006). If this will be confirmed, novel strategies aimed at identifying microbial consortia for remediating (or at least mitigating) intensive and extensive pollution caused by PCBs will be needed.

Although synergistic/antagonistic interactions between multiple metals in marine environments are almost completely unexplored, there is evidence that different heavy metals can have a different impact on microbial components (Bruland et al., 1991). In fact, it is known that heavy metals without metabolic function, such as cadmium, lead, and mercury, are toxic for marine organisms even at low levels, while other metals, such as copper, iron, and zinc, are essential in trace amounts (Silver and Wauderhaug,

1992). Several prokaryotes show specific resistance determinants, tolerating a wide range in concentration of these elements by a variety of mechanisms. Microorganisms in fact have coexisted with metals since early history. This is reflected in the wide range of divalent or transition metals at the active centers of many enzymes (Vallas and de Lorenzo, 2002). This is the first study exploring bacterioplankton and picophytoplankton response to multiple contaminant exposure in the marine environment. Our data pointed out that Aroclor 1260 in addition with Zn and Pb had a toxic effect on the picophytoplankton abundance, biomass, and bacterial C production. However, when PCB addition was coupled with the addition of heavy metals the impact on the heterotrophic component was less rapid and negative than when PCB was alone. This result suggests a possible antagonistic effect between essential metals (in our case likely Zn) and PCBs (such as Aroclor 1260).

Picoplankton response to zinc, lead, and Aroclor 1260 addition was always, when detected, extremely rapid (i.e., within 3–72 h), suggesting that this class of microorganisms can be a sensitive indicator of stressed environmental conditions in coastal marine systems. Some toxicity tests (such as the Microtox) based on prokaryotes, such as the *Vibrio fischeri*, have the advantage of being rapid (El-Alawi et al., 2002; Phyu et al., 2005).

Results presented here indicate, for the first time, that the whole picoplankton assemblage can be utilized in experimental laboratory conditions to identify the presence of stressing agents of anthropogenic origin, such as heavy metals and PCBs, with the advantage of being rapid, sensitive, and relatively easy to manage, using small volumes of samples. Other studies are needed to have a more complete view of the responses of picoplankton to Zn, Pb, and PCB addition, to identify the occurrence of specific resistant strains, which might also be indicators of contamination. Research along these lines is in progress, but we recommend that in the future more attention will be given to picoplankton assemblages, a key component in the functioning of marine ecosystems.

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