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Use of sand-living microalgal communities (epipsammon) in ecotoxicological testing

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ABSTRACT: A new ecotoxicological test system employing sand-living microalgal communities is presented. Equipment and procedures were developed for sampling and transportation of epipsammon to the laboratory where they can be kept and prepared for subsequent toxicity testing. The proposed test system make use of small-volume (60 µl) subsamples in the measurement of metabolic activities in short-term toxicity tests. The replicability is therefore very good and the variance between subsamples was shown to be low. After several hours of storage between sampling and analysis, epipsammon showed only minor changes in metabolic activities and no significant changes in sensitivity to the investigated toxicant tri-*n*-butyltin (TBT). The use of a community that has been established on a natural, but still homogenous, substratum has several advantages. Similar to other attached communities, but in contrast to phytoplankton, epipsammon represents a certain site and can thus have a known pollution history. The preparation and monitoring of sampling equipment for colonization of periphyton on artificial substrata is avoided. In addition, since epipsammon has been colonizing the sand for a long time, the community is also likely to be more natural than the opportunistic assemblages that are the early colonizers of artificial substrata. Epipsammon from the investigated locality also showed a fairly low spatial variability in most employed structural and functional parameters which indicated that the test system is robust and the actual sampling site not too critical. However, the estimated toxicity of TBT was shown to be lower to epipsammon than to previously investigated phytoplankton and periphyton. From indirect evidence, we suggest that this difference is not explained by different bioavailability of the toxicant in the short-term test systems but rather to the adaption of algae to natural environmental conditions in the sediments.

KEY WORDS: Microalgae · Epipsammon · Microphytobenthos · Sediment Community level · Multispecies test system · Ecotoxicology · Tolerance Tributyltin · TBT

INTRODUCTION

Epipsammon communities consist of more or less immobile microalgae living attached to sand grains. These attached life-forms dominate the benthic microflora in such sediments where the sand is regularly moved by water currents or wave exposure (Round 1965, 1971, Moss & Round 1967, Amspoker 1977, Admiraal 1984, Sundbäck 1984, de Jonge 1985). Epipsammon shows a high diversity particularly of diatom species but cyanobacteria also contribute significantly to the total biomass (Meadows & Anderson 1968, Sundbäck 1983, Sundbäck et al. 1990, Asmus & Bauerfeind 1994). The occasional burial of epipsammon within the sand for longer periods of time puts certain constraints on their physiology; important adaptional aspects are the requirements to withstand elevated concentrations of sulfides and the maintenance of a functioning photosynthetic apparatus even after long periods in darkness (du Preez & Bate 1992). Epipsammon have been reported to contribute significantly to the primary production of shallow sandy habitats, and to be a significant source of energy for secondary production (Asmus & Asmus 1985, Daehnick et al. 1992).

Aquatic algae are nowadays an integrated part of the hazard assessment of chemicals and have long

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been used in biological monitoring programs (Round 1991, Thursby et al. 1993, McCormick & Cairns 1994, Lewis 1995a, b). However, the extrapolation of test results from single-species toxicity tests to natural communities may be questioned since algal sensitivity to chemicals is highly species dependent (e.g. Blanck 1984, Blanck et al. 1984) and furthermore since most of these tests are performed under ecologically unrealistic conditions (Blanck et al. 1978, National Research Council 1981, Kimball & Levin 1985, Landner et al. 1989, Cairns et al. 1992). These doubts have led to the development of more or less complex multispecies test systems, using natural or artificial assemblages of algal species in the laboratory or in the field (see reviews by Cairns & Cherry 1993, Lewis 1995b). Both phytoplankton (Lewis & Hamm 1986, Kusk & Nyholm 1991) and periphyton (Blanck 1985) communities have been used in short-term toxicity tests, but the epipsammon seems so far to have been overlooked.

The ideal community to be used for testing purposes should contain a representative set of algal species, exhibit a low variability between replicates, and be easy to sample and handle. Preferably the community should also maintain its original metabolic activity and sensitivity to toxicants during transport and storage prior to the testing.

In this paper, we introduce the epipsammon as a new tool in community-level ecotoxicological testing. We report a set of procedures to collect, handle and characterize epipsammon community subsamples. Temporal and spatial variability in functional and structural properties of the communities is described as well as the variability in the estimated toxicity of the antifouling agent tri-*n*-butyltin (TBT).

MATERIALS AND METHODS

Sampling and handling procedure. The subtidal zone of a sandy shore, in the Gullmar fjord on the west coast of Sweden (58° 16' N, 11° 28' E), was sampled for epipsammon communities during the period April 22 to August 12, 1992. All sediment samples were taken at approximately 0.5 m water depth.

Two different ways of collecting sand from the sampling site were employed. A detritus sledge (Ockelmann 1964), with the chain removed in order to keep the sediment undisturbed in front of the sledge, was towed over the bottom by hand for a distance of approximately 10 m and the collected sample was transferred to a polyethylene box. The knife edge of the sledge was adjusted to get an approximate depth of the sediment sample of approximately 2 cm. The box was filled with water from the station to prevent rapid temperature changes during transport and covered with a lid to protect samples from high light intensities. The samples were kept in dim light in the laboratory with sea water from the fjord flowing through the box to keep the temperature stable and close to the temperature at the sampling site. Sediment cores were taken by hand using Perspex cylinders (Fig 1), with a diameter of 11 cm and height of 30 cm, by pressing cylinders into the sediment and closing the end with a Perspex disc and a plastic stopper before lifting the core out of the sediment. The cores were immediately placed in a transport box filled with sea water from the sampling site and covered with a lid. At the laboratory, cores were kept shaded and with a slow flow of water through the box. Cores were divided into subsamples representing different depth ranges. A core-dividing unit was put on top of the cylinder (Fig 1). Sediment in the core was pushed upwards until a chosen depth range was obtained, above the edge of the Perspex cylinder, and the subsample was moved sideways from

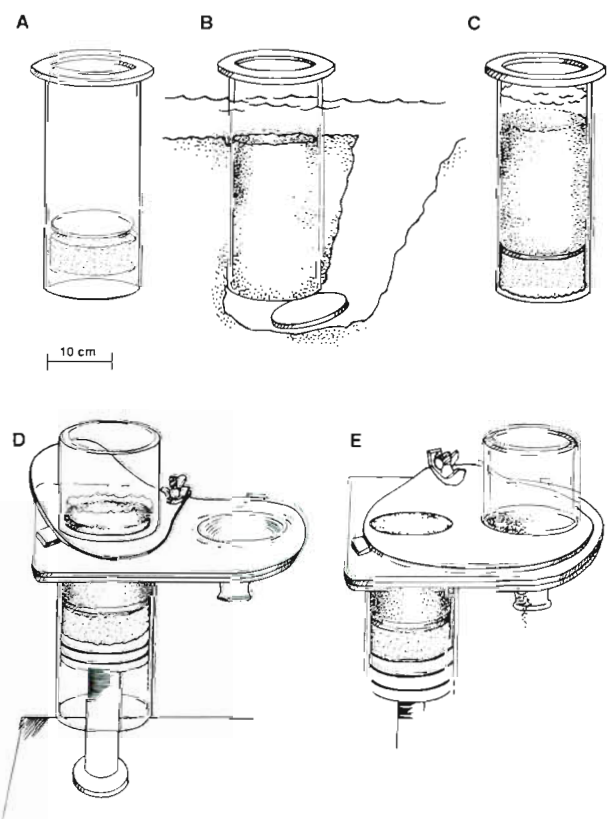

Fig. 1. Epipsammon sampling device: (A) Perspex cylinder, (B) sediment sample and Perspex disc, and (C) plastic stopper. At the laboratory, the Perspex cylinder was assembled with the core-dividing unit put on its top (D) used to produce subsamples with desired depth ranges (D, E). See 'Materials and methods' for a more detailed outline of the sampling procedure

the cylinder to a funnel from where the sediment was washed into a glass beaker using filtered (Whatman GF/F) sea water.

The >500 µm grain size fraction consisted mainly of broken shells and was removed by sieving the sediment through a 500 µm nylon mesh mounted on a wide Perspex cylinder. Sieving was achieved by gently lifting the sieve up and down in a polyethylene bowl filled with filtered sea water. By this procedure strong physical action on the sand was minimized, thus preventing high losses of attached algae to the water. The same water was used when the silt-clay fraction (grain size <63 µm) was removed using a 63 µm nylon mesh. The loss of microalgae during sieving was estimated by measuring the photosynthetic activity in the water used in the sieving procedure diluted to 1 l with filtered sea water. The sand was stored in glass beakers with filtered sea water after sieving and kept at approximately the same temperature as at the sampling site and in dim light until further analysis.

A sand-distributing device was used to make small volume subsamples of the sand for different analytical procedures. The device (Fig. 2) consisted of 2 Perspex plates, 3 and 6 mm thick, mounted on top of each other. Plates had 50 holes each (diameter of 5 mm), symmetrically distributed in 5 rows and 10 columns. The sand distributor was placed above a rack containing 50 glass scintillation vials (20 ml) centred beneath the holes. The lower plate was moved sideways in order to close the holes in the upper plate. The holes in the upper plate were then filled with sand using an excess of filtered sea water and a polyethylene spatula to even out the sand surfaces in the holes to the level of the Perspex plate. After distributing sand into the holes, the lower plate was slid back to open the passage from the upper plate to the scintillation vials. The subsamples were washed into the vials using 1.0 ml of filtered sea water. The procedure took about 10 min and no sign of samples being dried during this exercise could be noticed. By this procedure, 50 subsamples of 60 µl sand each could be achieved in a very short period of time.

Photosynthesis measurement. Photosynthesis was measured using a ^{14}C technique in a similar way to that described for periphyton communities (e.g. Blanck & Wängberg 1988, Molander et al. 1992, Dahl & Blanck 1996). In addition to the filtered sea water already present in the vials (1 ml) another 1 ml was added and the vials were placed in a thermostated waterbath at the ambient temperature of the water at the sampling station. Four to five replicates were used in all photosynthesis experiments and 2 additional samples were incubated with 100 µl of formaldehyde (37%) added from the start in order to estimate the abiotic carbon

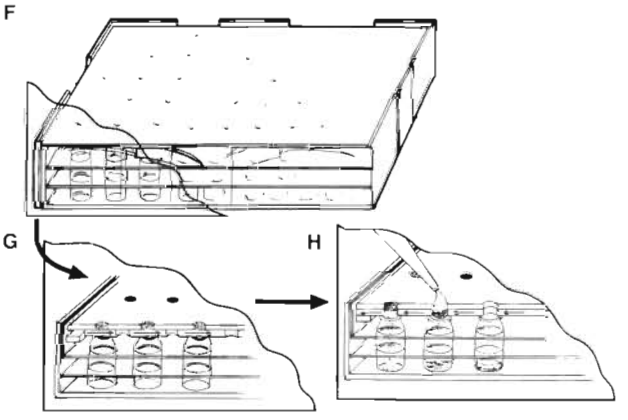

Fig. 2. Sand distributing device (F) used to generate small-volume subsamples of the sediment. Sand was evenly distributed into the holes (G) before being washed into the scintillation vials (H). See 'Materials and methods' for a more detailed outline of the procedure

dioxide fixation. The light from fluorescent tubes (Osram Lumilux Daylight L18W/11) was filtered through 20 cm water and regulated to give a photon flux density of 125 µmol photons m^{-2} s^{-1}. Samples were gently shaken during the incubation time. After 30 min incubation in light, 50 µl of a ^{14}C-labelled bicarbonate solution was added to the samples. The radiolabelled bicarbonate solution was prepared by a 50-fold dilution of a stock solution (Amersham CFA2, 2 mCi ml^{-1}, 55 mCi $mmol^{-1}$) with filtered sea water (approximately 1.9 mM total inorganic carbon) giving a final activity of 2 µCi (74 kBq) per 50 µl aliquot. After 15 min, 100 µl of formaldehyde was added to terminate carbon fixation activity. The volume was adjusted to 5 ml by adding 3 ml of water, purified by reversed osmosis, and the remaining inorganic carbon was driven off by acidifying the samples with 0.1 ml of concentrated hydrochloric acid followed by air bubbling for 10 to 15 min. A 7 ml volume of a scintillation cocktail (Ready Gel™, Beckman Inc., Fullerton, CA, USA) was added and the samples were thoroughly mixed. A liquid scintillation counter (LS 3801, Beckman Inc.) was used to determine the amount of incorporated ^{14}C. The activities, as disintegrations per minute (dpm), were calculated from the counts per minute (cpm) data, using an external standard technique and the correction factors for the sample quench characteristics and machine efficiency.

Tolerance quantification. Epipsammon photosynthetic activity was measured in a concentration series of tri-*n*-butyltin (TBT, 97% purity, Fluka AG, Neu-Ulm, Germany). Four to five replicates were used in each measurement. Test solutions were prepared by diluting stock solution of TBT in acetone with filtered sea water The acetone concentration in test solutions were 10 µl l^{-1} in the photosynthesis experiments. From the

concentration-effect curves generated, the epipsammon TBT tolerance could be estimated, i.e. as the effective concentrations inhibiting 20% (EC20) or 50% (EC50) of the photosynthetic activity. EC values were quantified by log-linear interpolation giving the average photosynthetic activity in TBT-treated samples as a percentage of the average activity of the controls which was set to 100%.

Chlorophyll and phaeophytin analysis. Five 60 µl sand portions were pooled and the water removed by decanting the vials gently onto a paper tissue. 1 ml of 95% acetone was added and the samples were stored overnight at –20°C. At analysis, 1 ml of 90% acetone was added and the samples were sonicated in an ice-water filled water bath (Bransonic 220, Misonix, Farmingdale, NY, USA) for 10 min. Samples were centrifuged to remove debris and sand particles and analysed for chlorophyll *a* and phaeophytin according to Lorenzen (1967) using the equation of Jeffrey & Humphrey (1975). Three to five replicates were analysed in each determination.

Abundances of algal species. In order to estimate the taxonomic diversity at the sampling site and to get some information on patchiness, 3 samples (0 to 1 cm, 63 to 500 µm fraction) were taken in August in a transect parallel to the shoreline and analysed. At first, epifluorescence microscopy was used on fresh samples to identify the cyanobacteria and the larger diatom species. Further analysis was performed on diatoms, removed from sand grains with nitric acid and embedded in Naphrax, in a phase contrast microscope. A semi-quantitative analysis of species abundances was performed. Bray-Curtis dissimilarity indices (Bray & Curtis 1957), as described by Sheehan (1984) and Clarke (1993), between samples were calculated based on the ranked abundances of species.

Organic content and grain size analysis. The organic content in the sand was estimated as the loss on ignition. Approximately 100 ml of sand was freeze-dried upon sampling and stored until analysis. 5 g of sand (n = 3) was dried at 100°C overnight before being burned at 470°C for 6 h (Wassman 1991).

For the grain size analysis, freeze-dried samples were mixed in a 150 ml 10% (v/v) hydrogen peroxide (puriss) solution and the sediment was allowed to settle in an E-flask overnight. The same procedure was repeated 3 times with water, purified by reversed osmosis, but without hydrogen peroxide. Samples were dried and the grain size distributions were determined in the ranges of >500, 500–250, 250–125, 125–63 and <63 µm. Cumulative percentage dry weight of each fraction was transformed to a probability scale before calculating sorting coefficient (graphic standard deviation method) and median grain size according to Gray (1981).

RESULTS

Activity and inhibition of photosynthesis

Photosynthetic activity could readily be detected using small epipsammon subsamples, i.e. 60 µl aliquots of sieved sand. Using surface sediments from the 0 to 2 cm depth range of the sediment, 10 to 40000 dpm (range, n = 42) of radiolabelled carbon was incorporated during the 15 min incubation period. The measurements were made with a fairly high precision (Fig. 3), the average coefficient of variation being 14 ± 6% (SD). Only a minor fraction of algae was lost during sieving (range 4 to 10%, n = 4) as estimated by the photosynthetic activity in water collected from the sieving procedure.

Inhibition of epipsammon photosynthesis after short-term exposure (i.e. 45 min) to TBT was concentration dependent (Fig. 4) and TBT toxicity to epipsammon could be quantified by log-linear interpolation. The average EC50 for inhibition of photosynthesis for 5 consecutive tests during 1 d was 140 ± 28 nM TBT (mean ± (SD) (Fig. 4).

Influence of storage

Epipsammon was stored in dim light and at a temperature close to field conditions before the experi-

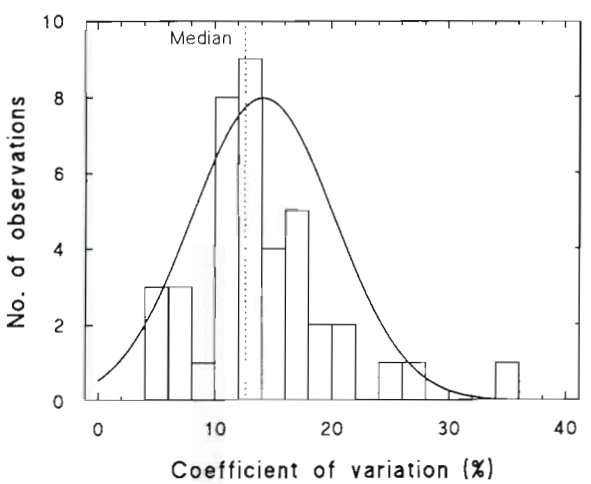

Fig. 3. Frequency distribution of coefficients of variation for measurements of epipsammon photosynthetic activity. Values originate from 40 measurements during a day employing 5 replicates in each measurement. Samples originate from approximately 0 to 2 cm depth and were collected with an Ockelmann detritus sledge. The 63 to 500 µm fraction was employed in the experiments. The frequency distribution was not found to be significantly different from normal (chi-squared test, p = 0 273, df = 3). Normal distribution, fitted to the original values, together with the median value, is indicated

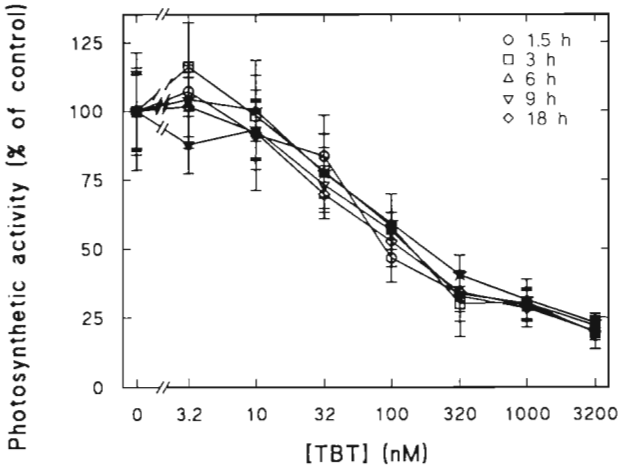

Fig. 4. TBT inhibition of photosynthesis after different times of storage of the epipsammon samples. Epipsammon was exposed to TBT only during the short-term experiments that followed upon storage. Error bars indicate standard deviations. Origin of sediment as in Fig. 3

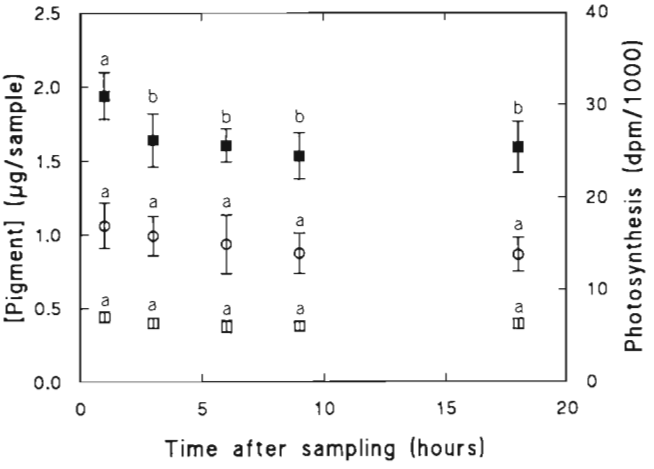

Fig. 5. Influence of storage time on epipsammon photosynthetic activity (○), chlorophyll *a* (□) and phaeophytin (■) content. Error bars indicate standard deviations. Different letters above symbols indicate significant difference at $p < 0.05$ (Tukey HSD). Origin of sediment as in Fig. 3

ments. No significant effect of storage time between 1.5 and 18 h could be detected on epipsammon photosynthetic activity (ANOVA, $p = 0.22$) or chlorophyll *a* content (ANOVA, $p = 0.053$) (Fig. 5). However, the phaeophytin content decreased (ANOVA, $p = 0.0047$) between 1.5 and 3 h of storage [Tukey Honest Significant Difference (HSD), $p < 0.05$] and then remained stable.

TBT toxicity to epipsammon photosynthesis, estimated as the EC20 or EC50, was not significantly dependent on the time between sampling and measurement, as judged by the linear regressions ($p > 0.10$), although some weak time dependence was implied (Fig. 6).

Toxicity modifying factors

The bioavailability of toxicants to epipsammon algae may be different than to phytoplankton and periphyton due to environmental conditions in the sandy sediment. We investigated indirectly whether organic and inorganic sites in the samples were competing with the algal cells for TBT. If so, any modification of test conditions that affected the available amount of TBT or the amount of competing sites would affect the estimated TBT toxicity. Incubation times, test medium volumes and organic content of the sand were assayed for such influence.

We found no significant effects of different incubation times or of different organic matter contents on the estimated toxicity of TBT ($p = 0.14$ and 0.054 respectively) (Fig. 7). The estimated TBT toxicity was found to be significantly different between different test medium volumes ($p = 0.0021$) (Fig. 7) although the response was variable and inconsistent. We were able to detect significant differences between the 2 sampling sites ($p = 0.016$, 0.013 and 0.072 respectively) in their response to the toxicity modifying manipulations (Fig. 7).

Influence of depth

Because of the occasional burial and redistribution of sand at epipsammon sampling sites, it is important to

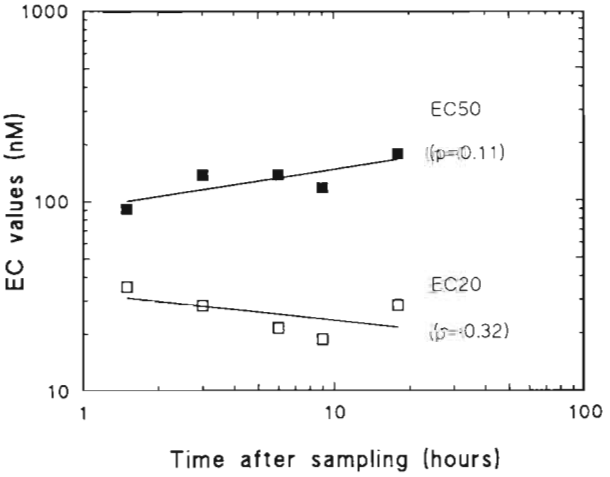

Fig. 6. Influence of storage time on EC20 and EC50 values of TBT inhibition of epipsammon photosynthesis. Linear regressions are fitted to the EC values. Origin of sediment as in Fig. 3

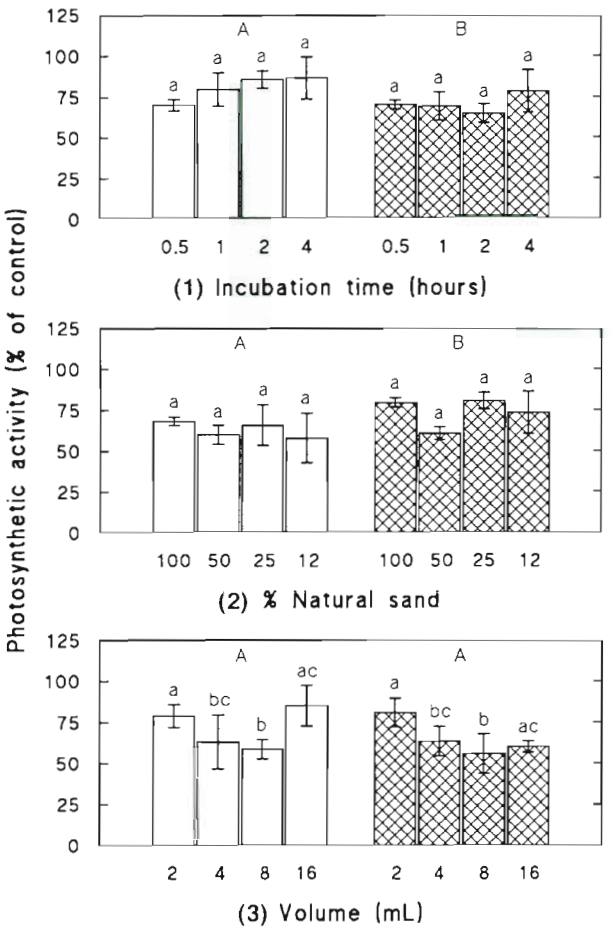

Fig. 7 Influence of incubation time, organic content and test volumes on the toxicity of TBT to epipsammon photosynthesis. Three sediment cores were collected and pooled together, from one exposed (open bars) and one sheltered (hatched bars) part of the locality respectively. The 63 to 500 µm grain size fraction from 0 to 1 cm depth was employed. Replicate subsamples (n = 3 to 5), with or without 100 nM TBT added, were (1) pre-incubated for different times, (2) diluted with sand free of organic content or (3) incubated in different volumes of test solutions. Photosynthetic activities are expressed as relative controls. Error bars indicate standard deviations. Effects of treatments and locality on the estimated toxicity of TBT were tested by a 2-way ANOVA (Statistica Software, StatSoft, Tulsa, OK, USA) on arcsin $(x)^{1/2}$ transformed data. Significant differences ($p < 0.05$) between treatments are indicated by different lowercase letters and between localities by different uppercase letters

understand the influence of depth on the ecotoxicological properties of epipsammon samples. These are likely to covary with the biotic and abiotic properties of the sediment.

Significant effects of depth were detected for all biotic parameters (ANOVA, $p < 0.0001$). The potential photosynthetic activity already decreased significantly at depths below 1 cm (Tukey HSD, $p < 0.05$; Fig. 8) compared to surface levels whereas chlorophyll *a* and

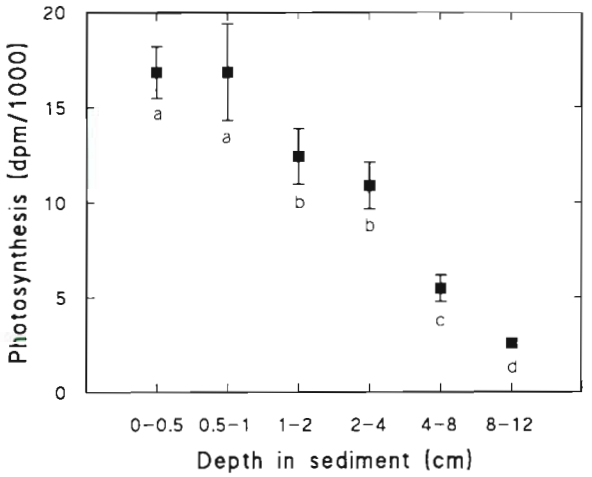

Fig. 8. Potential photosynthetic activity of epipsammon from different depths in the sediment. Two sediment cores were collected and samples representing the same depth range were pooled together. The 63 to 500 µm fraction was used. Error bars indicate standard deviation (n = 5). Symbols followed by different letters indicate significant difference at $p < 0.05$ (Tukey HSD)

phaeophytin content decreased at depths below 4 cm (Fig. 9). Also the content of organic matter in the sediment, estimated as the fraction of dry sediment lost on ignition, decreased significantly (ANOVA, $p = 0.013$) at depths below 4 cm (Tukey HSD, $p < 0.05$; Fig. 10).

TBT toxicity to epipsammon photosynthesis decreased linearly with depth (Fig. 11). Epipsammon from a depth of 8 to 12 cm were 5 times more tolerant than epipsammon from the surface. Sorting coefficients and median grain sizes did not show any depen-

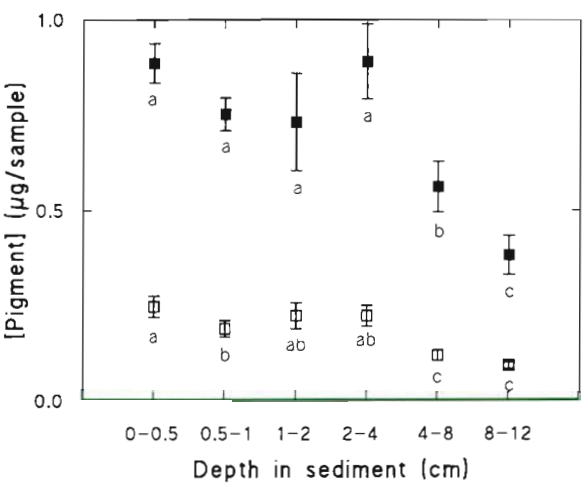

Fig. 9. Chlorophyll *a* (□) and phaeophytin (■) content in epipsammon from different depths in the sediment. Error bars indicate standard deviation (n = 5). Symbols followed by different letters indicate significant difference at $p < 0.05$ (Tukey HSD). Sediment was collected and handled as described in Fig. 8

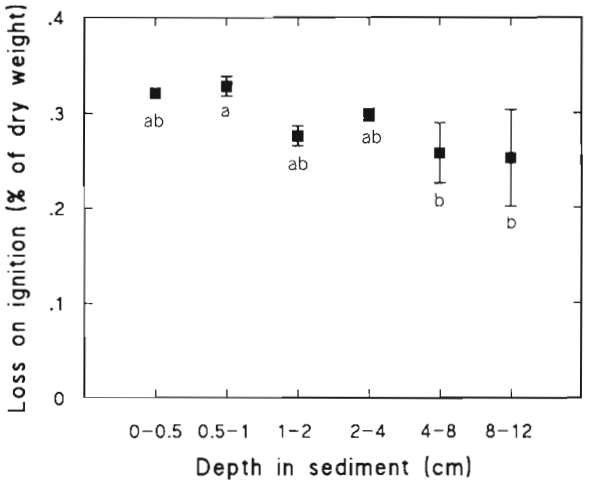

Fig. 10. Content of organic matter, estimated as the loss on ignition, in epipsammon from different depths in the sediment. Error bars indicate standard deviation (n = 3). Symbols followed by different letters indicate significant difference at $p < 0.05$ (Tukey HSD). Sediment was collected and handled as described in Fig. 8

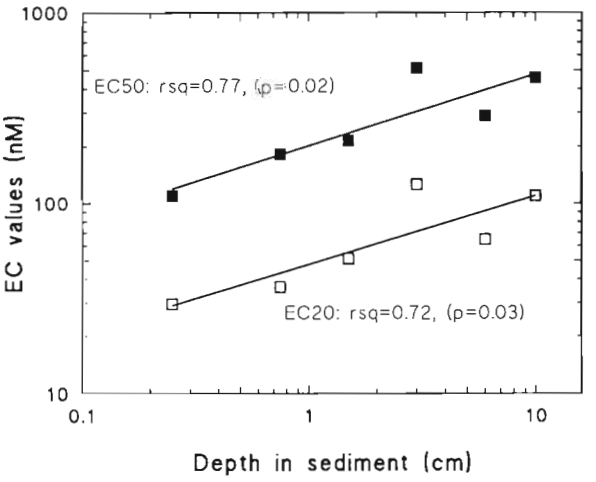

Fig. 11 EC20 (□) and EC50 (■) for TBT inhibition of photosynthetic activity in epipsammon collected from different depths in the sediment. Values are plotted against the average depth of each epipsammon sample. Linear regressions were fitted to the EC values. Sediment was collected and handled as described in Fig. 8

dence upon depth in the linear regression analysis (data not shown). All samples taken were classified as moderately well sorted according to the range of sorting coefficients (0.61 to 0.69). Median grain sizes were found in the range of 0.20 to 0.23 mm. Sediment from the 8 to 12 cm depth was rich in sulfide, judging from its smell, and was also darker than at the surface.

Spatial and temporal variability in the estimated TBT toxicity to epipsammon

TBT toxicity to epipsammon photosynthesis, as related to different biotic and abiotic sediment properties at the time and place of sampling, was investigated twice (in April and August 1992). Sediment cores were taken from the same beach but in different clusters or along transects from sheltered to more wind-exposed sites.

The spatial variances in biotic and abiotic sediment parameters in August (n = 10) are listed in Table 1. Only the phaeophytin content was highly variable with a coefficient of variation (CV) of 70%. Other parameters varied between 7 and 31%. The assimilation ratio, photosynthetic activity per unit of chlorophyll *a*, had a lower variance than either of photosynthetic activity or chlorophyll *a* alone since they were intercorrelated (linear regression, $p = 0.006$, $R^2 = 0.64$).

The estimated toxicity of TBT to epipsammon was highly variable (Table 2). The CV for EC20 and EC50 values was 71 and 84% respectively. Part of the variance may be attributed to the sampling occasion, because the estimated toxicity of TBT was significantly higher in April than in August (ANOVA, $p = 0.011$ and 0.004 respectively for EC20 and EC50).

Organic matter and phaeophytin content in the samples turned out to be the only significant variables in a stepwise multiple regression analysis using the EC50 for inhibition of photosynthesis as independent variable (Table 3, Fig. 12A). Variables like sorting coefficient, median grain size, chlorophyll *a* content and photosynthetic activity could all be excluded with only a minor decrease of the coefficient of determination

Table 1 Spatial and temporal variability in different sediment parameters. Sediment cores (n = 10) were taken 20 m apart in a transect parallel to the shoreline during 2 consecutive days. Sediment from 0 to 1 cm depth (the 63 to 500 µm fraction) was utilized in the characterisation of sediment. Coefficients of variation (CV) along with minimum (Min) and maximum (Max) values are given

	CV (%)	Min	Max
Sorting coefficient (ϕ)	7	0.53	0.66
Median grain size (mm)	12	0.14	0.22
Assimilation ratio (dpm h^{-1} µg chl a^{-1})	19	0.42×10^6	0.70×10^6
Organic content (µg g^{-1} dry wt)	23	0.26	0.59
Chlorophyll *a* content (µg subsample^{-1})	29	0.09	0.27
Photosynthesis (dpm h^{-1} subsample $^{-1}$)	31	0.05×10^6	0.15×10^6
Phaeophytin content (µg subsample^{-1})	70	0.01	0.88

Table 2. Spatial and temporal variability in the estimated toxicity of TBT to epipsammon photosynthetic activity. Three of the 5 April samples were taken 2 to 3 m apart whilst the other 2 were located approximately 20 m apart from each other and from the first cluster of 3. August samples were taken as outlined in Table 1. Sediment from 0 to 1 cm depth (the 63 to 500 µm fraction) was utilized in the measurements of TBT toxicity to epipsammon

	Average	SD	CV (%)	Min	Max	Factor[a] (10^x)
All (n = 15)						
EC20 (nM)	140	97	71	17	310	1.25
EC50 (nM)	1100	950	84	100	2900	1.46
April (n = 5)						
EC20 (nM)	45	33	73	17	98	0.75
EC50 (nM)	210	150	70	100	470	0.67
August (n = 10)						
EC20 (nM)	180	84	46	71	310	0.64
EC50 (nM)	1600	830	53	510	2900	0.75

[a]The difference in sensitivity between the most sensitive (Min) and tolerant (Max) sample is given in orders of magnitude (x)

(multiple R^2). The final regression was significant (ANOVA, $p < 0.001$) and the 2 variables together explained 89% of the variance in EC50 values (Table 3). The regression coefficients for organic and phaeophytin content, 0.78 and -0.62 respectively, indicated a positive correlation between organic content and EC50, whereas phaeophytin content showed a negative correlation. A similar result was obtained using the EC20 value as dependent variable (Table 3, Fig. 12B).

Abundances of algal species

A total of 73 microalgal species were observed in the 3 samples taken in August. A major part of the species (62) were attached small benthic diatoms in the size range of 5 to 20 µm. Also 8 different species of cyanobacteria were observed. Four different diatoms species from the *Achnanthes* and *Navicula* genera along with the species *Cocconeis peltoides* and *Nitzschia aurariae* dominated the diatom assemblage as did *Dermocarpa* cf., *Merismopedia* sp. and *Microcrocis* sp. in the group of cyanobacteria. Altogether, 29 species occurred as more than single specimens. The similarity among the 3 observations made along the sampled transect was relatively high according to the Bray-Curtis dissimilarity indices of 0.31 to 0.34.

Table 3. Stepwise multiple regression summaries using EC50 or EC20 for the inhibition of epipsammon photosynthetic activity as dependent variables. Variables were standardised by setting means to zero and standard deviations to unity in order to give all parameters equal influence on the outcome of the analysis. Chlorophyll *a*, photosynthetic activity, sorting coefficient and median grain size variables were all excluded as non-significant ($p > 0.05$) in the stepwise procedure

Dependent variable	Independent variables	Regression coefficient	p	R^2
EC50			<0.001	0.89
	Organic content	0.78	<0.001	
	Phaeophytin	-0.62	<0.001	
EC20			<0.001	0.78
	Organic content	0.79	<0.001	
	Phaeophytin	-0.48	0.004	

DISCUSSION

Equipment and procedures have been developed for sampling and transportation of epipsammon to the laboratory where they can be kept and prepared for subsequent toxicity testing (Figs. 1 & 2). The proposed multi-species test system makes use of small-volume subsamples which is convenient when measuring short-term effects on metabolic activities such as photosynthesis. The replicability is therefore very good and the variance between subsamples

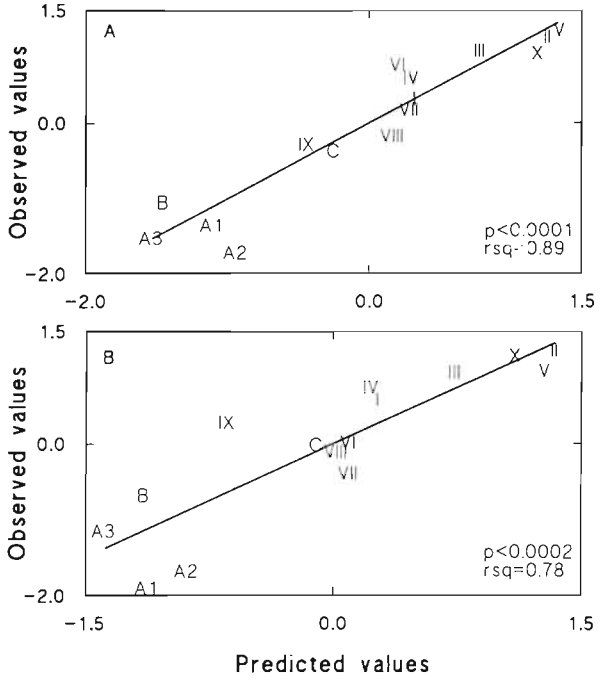

Fig. 12. Predicted versus observed (A) EC50 and (B) EC20 values for the inhibition by TBT of epipsammon photosynthetic activity using the multiple linear regressions presented in Table 3

acceptable (Fig. 3). The main advantage of the epipsammon test system is however that a natural community established on a natural, but still homogeneous, substratum can be used. This eliminates the tedious preparation of sampling equipment that has to stay *in situ* for several weeks, to allow the colonization of periphyton onto artificial substrata. The epipsammon community which has been established on the sand for a long time is also likely to be more natural than the opportunistic assemblages that are the early colonizers of artificial substrata. Furthermore, the epipsammon community has the advantage that it can tolerate several hours of storage with only minor changes of its metabolic activity (Fig. 5) and without significant changes in sensitivity (Figs. 4 & 6), at least to the studied toxicant TBT. Similar to samples of other attached communities but in contrast to phytoplankton, epipsammon represents a certain site and can thus have a known pollution history.

The spatial variability in structural and functional properties of the epipsammon at the investigated sampling site was fairly low (Table 1). Since the samples were deliberately taken in a transect along which wind and wave exposure differed markedly, we would have expected this variability to be higher. The low variability found implies that the epipsammon test system is robust and the actual sampling site not too critical.

However, the spatial and temporal variability in the estimated TBT toxicity to epipsammon was considerably higher (Table 2). When this variability was further investigated, we found a correlation to the content of phaeophytin and organic matter in the sediment samples (Fig. 12, Table 3). Since an 8-fold decrease in organic content of the sand samples did not affect short-term TBT toxicity to epipsammon significantly (Fig. 7), the influence of organic matter must be indirect or active only on a longer time scale than hours. The variability within each sampling occasion (0.6 to 0.7 orders of magnitude) was similar to findings of temporal and spatial variability in the sensitivity of periphyton and phytoplankton sensitivity to chemicals (Table 2; Blanck 1985, Kusk & Nyholm 1991, Blanck & Dahl 1996). Variability in the estimated toxicity of chemicals is thus not an exclusive property of the epipsammon test system.

There was a significant decrease in the estimated TBT toxicity with depth (Fig. 11) along with other parameters reflecting epipsammon structure or function (Figs. 8 & 9). This fact and the observation that active photosynthesis is present only in the top millimetres of the sediment surface (Revsbech & Jørgensen 1983) lead to our strong recommendation to use only the surface epipsammon for testing purposes.

TBT toxicity to epipsammon photosynthesis was approximately 4 to 9 times lower than indicated in earlier studies of periphyton (Molander et al. 1992, Blanck & Dahl 1996, Dahl & Blanck 1996) and phytoplankton (authors' unpubl. results) collected at low ambient TBT concentrations. Abiotic and biotic differences between the algal communities, or more specifically between the test systems, may affect the availability of toxicants during the short-term exposure. Uptake of the toxicant may be slower in epipsammon, or the accumulation of TBT into an inactive part of the system might modify the internal concentrations of TBT at its site of action in the algae. If so, (1) a longer exposure time, (2) a larger volume of test medium and thus a higher relative amount of the toxicant or (3) a decreased content of living and dead organic matter in the test would increase the estimated toxicity of the compound. However, none of these parameters could be shown to significantly increase toxicity of TBT towards epipsammon (Fig. 7), at least not in a consistent manner. It thus seems that the epipsammon or its environment possess other properties that make epipsammon photosynthesis less susceptible to TBT on a short-term basis.

It has been known for some time that organotins such as TBT act on the ATPase activity in chloroplasts and mitochondria by uncoupling or energy transfer inhibition (Kahn 1968, Selwyn et al. 1970, Watling & Selwyn 1970, Gould 1976, Millner & Evans 1980, Antonenko 1990). Other findings that dimercapto compounds effectively reverse the effects of TBT (Byington 1971, Singh & Bragg 1979, Gray et al. 1986) and that TBT, in bacteria, only affected sulfhydryl-containing enzymes such as ATPase (Tseng & Cooney 1995) support the view of sulfhydryl groups as a possible primary site of TBT action.

Sulfide has been suggested to bind to or react with disulfide bridges, a reaction not only suggested as a possible mode of action but also as a tolerance mechanism against sulfide toxicity (cf. review by Bagarinao 1992). Algae collected in or occasionally exposed to sulfide rich environments should, by their mere presence, be expected to possess properties that make them less sensitive to such stress. It can be argued that epipsammon, due to their occasional burial in the sediment, experience elevated sulfide concentrations more often than do phytoplankton and periphyton. High sulfide tolerance has also been demonstrated for diatom species isolated from organically enriched surface sediments (Admiraal & Peletier 1979, 1980). There is a possibility that adaptions to sulfide make the community less vulnerable not only to subsequent sulfide exposure, but also to TBT exposure due to the similar mode of action. This would not only explain the difference in sensitivity found at different depths but also support the correlations found between organic content and TBT sensitivity over spatial and temporal gradients and the overall difference found between different algal communities.

The use of epipsammon communities for toxicity testing appears to combine some of the advantages pertinent to either phytoplankton or periphyton toxicity tests. Epipsammon offers a diverse natural community that can be assayed with high precision, that is easy to sample and store for several hours, and that is attached and therefore representative of the conditions on the sampling site. This property is essential for the future application of epipsammon in PICT studies (Blanck et al. 1988). We report here the use of the epipsammon test system in the marine environment. However, the technique has recently been applied in both lotic and lentic fresh water environments. Actually, it may be utilized in any environment where sorted sand with microbial activity can be obtained.

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