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Microfluorometrical study of bioaccumulation of benzo(a)pyrene and marker xenobiotics in the bivalve *Donax trunculus* from clean and polluted sites along the Mediterranean shore of Israel

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ABSTRACT: This study focused on bioaccumulation of benzo(a)pyrene [B(a)P] from a polluted environment and 2 xenobiotic markers under experimental conditions in various organs of the marine bivalve *Donax trunculus*. The cationic dye acridine orange (AO) and anionic dye fluorescein (FLU) were used as markers. Test molluscs were collected from 3 sites along the Israeli Mediterranean shore: a relatively clean site, an oil-polluted site and a site polluted by chemical effluents from a polyvinylchloride (PVC) factory. B(a)P was determined in tissue samples using benzene extraction and solid-surface luminescent analysis. Bivalves from the oil-polluted site contained 55.6 ng B(a)P g^{-1} wet wt. B(a)P concentration varied among body compartments: hepatopancreas > total soft tissues > kidney > shell > mantle edge = gills. In experiments, living bivalves from these 3 sites were kept in seawater with 10 µM AO or 100 µM FLU for 24 to 96 h. Accumulation and distribution of AO and FLU in the molluscs was determined by vital contact microfluorometry and from ethanolic extraction of the markers. Following exposure to the markers, *D. trunculus* from the clean site showed AO concentrations as follows: mantle edge > kidney > hepatopancreas > gills > central part of the mantle. For FLU the order was: kidney > gills > mantle edge > hepatopancreas > central part of the mantle. *D. trunculus* from polluted sites demonstrated a decrease in AO concentration and an increase in FLU concentration, especially in specimens from the site polluted by the PVC factory. Experimental thermal stress produced a drastic decrease in AO bioaccumulation. The observed bioaccumulation of B(a)P, AO and FLU reflects the environmental quality and can thus be used for monitoring.

KEY WORDS: Bivalve mollusc *Donax trunculus* · Bioaccumulation of xenobiotics · Defense mechanisms against xenobiotics

INTRODUCTION

Molluscs are numerically and energetically important in numerous benthic communities. Many are filter-feeding and long-lived, and they are in constant contact with various xenobiotics dissolved in water or associated with bottom sediments or suspended material. Some molluscs accumulate considerable quantities of potentially toxic anthropogenic or natural xenobiotics with no noticeable toxic effect (Dindal & Wurzinger 1971, Neff et al. 1976, Roberts 1976, Eriksson et al. 1986, 1989, Paasivara et al. 1992). Others, upon exposure to anthropogenic pollutants, exhibit various physiological, histopathological, bio- or histochemical alterations (Couch et al. 1979, Lowe 1988, Moore 1988, Triebskorn et al. 1991, Yawetz et al. 1992) and mass mortality (Okland 1992, Fishelson et al. unpubl.).

Along the Mediterranean shore of Israel, the bivalve *Donax trunculus* occurs in population densities of up to 2 kg biomass m^{-2} (Fishelson et al. unpubl.). Following a local long-term oil spill along the Mediterranean shore, mass mortality of the bivalve *Mastra corallina* occurred, the shells of which covered the shore, along with lower mortality of *Venus* spp., *Angulus planatus* and *Ensis* spp. The latter 3 species dwell in deep water and dig deeper into the sand than *D. trunculus*. However the shallow-water *D. trunculus* population was apparently unaffected, as only a few died. Atomic ab-

sorption analysis of the soft tissues of *D. trunculus* from this oil-polluted site showed a marked increase in the content of some trace metals, especially chrome, lead and copper, that were probably derived from oil (Fishelson et al. unpubl.).

The main biologically active, cytotoxic, mutagenic, carcinogenic components of oil are polycyclic aromatic hydrocarbons (PAHs), which are often accumulated by molluscs (Neff et al. 1976, Couch et al. 1979, Kauss & Hamdy 1991). Accumulation and distribution of heavy metals in various molluscs, including *Donax trunculus*, have been studied and are well established (Hornung & Oren 1981, George 1984, Romeo & Gnassia-Barelli 1988, Jenner et al. 1991); however, the accumulation and biological activity of PAHs derived from oil have not been studied in exposed *D. trunculus*, whose apparent resistance to oil spills has not been investigated. Enzymes for detoxification of xenobiotics have been studied, but in many hosts, especially invertebrates, other protective systems may be important in antichemical defense (Harborne 1982, Bresler et al. 1985, 1990, Triebskorn et al. 1991). Therefore, the present study attempted to explore the sensitivity of a new methodological approach for detection of physiological, biochemical or cytomorphological mechanisms of antixenobiotic defense. For this purpose methods were adapted and developed encompassing vital light microscopy, luminescent microscopy, microfluorometry and special fluorescent probes.

The availability of modern fluorescent methods and probes facilitates the detailed study of cellular organisation, function and metabolism (Goldberg 1989, Taylor & Wang 1989, Bresler et al. 1990, Darzynkiewicz & Kapuscinski 1990). The present study investigated accumulation and distribution of benzo(a)pyrene [B(a)P] and xenobiotic markers in *Donax trunculus* from both relatively clean and polluted sites along the Mediterranean shoreline of Israel, and the reactions of these molluscs to oil and other pollutants.

MATERIAL AND METHODS

Test molluscs and experimental design. In this study approximately 450 specimens of adult *Donax trunculus* with a mean length of 24.4 ± 0.5 mm (± 95% confidence limit) were used, some of which (n = 165) were collected from a relatively clean site along the Israeli Mediterranean shore (Akko), and others from sites polluted by local long-term oil spills (n = 165, Kiryat Yam) or by chemical effluents from a PVC factory (n = 135, Frutarom). These 3 stations have been used and characterized by Yawetz et al. (1992) and Fishelson et al. (unpubl.). All collections were performed from July to October. Samples of live *D. trunculus* were quickly transported to the laboratory in plastic containers with ambient seawater. The specimens were kept there for a short time (2 to 10 d) in aerated aquaria with seawater at 25 °C without substrate and without artificial feeding. For comparison, 23 live specimens of the bivalve *Mactra corallina* — a species which is very sensitive to given pollutions — were taken from the same clean site where *D. trunculus* was sampled.

Test chemicals. Xenobiotic markers in the tissues of molluscs were directly quantified with contact fluorescent microscopy and microfluorometry. Two fluorescent xenobiotic markers were used: acridine orange (AO; N,N,N',N'-tetramethyl-3,6-acridine diamine; pK_b = 10.4) was selected as a cationic lysosomotropic compound (Wittekind 1973, Swanson 1989, Murphy 1990) and fluorescein (FLU; 3',6'-dihydroxy spiro[isobenzofuran-1(3M)9'-xanthen]-3-one, disodium salt; pK_a = 7.0) was selected as an anionic analogue of numerous natural and anthropogenic weak organic acids which are constantly present in the environment (Zanker & Peter 1958, Perdue & Gjessing 1989) and as a marker for determining the viability of plasma membranes (DuBois et al. 1980, Bowen 1981, Pieters et al. 1989).

In preliminary tests, the optimal concentrations of AO and FLU for microfluorometry and vital microscopic investigations were found to be 10 and 100 µM l^{-1} respectively. Subsequently, sufficient AO or FLU was added to seawater to maintain the predetermined exposure level of AO at 10 µM and FLU at 100 µM. In additional experiments to study possible run-out of accumulated marker, the tested molluscs were transferred for 24 h into clean seawater for depuration after 96 h incubation with AO or FLU.

Thermal stress produces marked alterations in cell structures and metabolism in molluscs (Moore 1976). Therefore, the effect of high temperature (24 h incubation at 35 °C) on AO accumulation by *Donax trunculus* from the clean site was also studied.

Analytical procedures. After incubation with AO or FLU, and after depuration, the valves of the molluscs were opened by cutting the adductor muscles. Intensity of fluorescence was determined by vital contact microfluorometry on the natural surface of: mantle edge along the pallial line; central part of mantle; gills; renocardial area (kidney); hepatopancreas (digestive diverticula); periostracum; and extrapallial surface of the nacreous shell layer. Autofluorescence intensity was also measured. For each specimen, 10 separate measurements were taken from both periostracum and shell, and 20 measurements from each of the other tissues. For each separate experiment (site of collection, type of marker, time of incubation) 15 specimens of *Donax trunculus* were used.

The technique of vital microfluorometry was described in detail by Bresler et al. (1975, 1979, 1985,

1990) and Bresler & Nikiforov (1977). A contact objective of 25 × 0.75 was used for microfluorometry of *Donax trunculus* with a rectangular photometric diaphragm that had an aperture of 75 × 75 µm in the plane of the object. The electric current from the photomultiplier tube measured the intensity of fluorescence at 530 nm in arbitrary units (a.u.). Fluorescence of accumulated marker was calculated as the difference between the experimentally measured fluorescence and autofluorescence.

For cuvette fluorometry, after incubation with markers and microfluorometry, whole soft bodies of specimens or samples of the mantle edge along the pallial line, central part of the mantle, renocardial area, and hepatopancreas were rapidly dissected, weighed and transferred into 30 % ethanol. Fluorochrome concentration in these extracts was determined by microcuvette fluorometry, as described earlier (Sernetz & Thaer 1973, Bresler & Nikiforov 1977, Bresler et al. 1975, 1990). In each determination 12 bivalves were used for whole soft body samples and 12 for tissue samples.

Tissue samples for determination of B(a)P equivalents were prepared from *Donax trunculus* from all collection sites as described above and extracted with benzene. For each determination a total of 15 specimens were used for both whole soft body and shell samples, and 12 to 15 were used for samples of various tissues. Samples of the sediment present permanently on the bottom of containers holding collected molluscs were also investigated. Nine samples of such sediment from each collection site were weighed, dried and extracted with benzene. B(a)P in benzene extracts was determined by room-temperature solid-surface fluorescence, at wavelength pair excitation/emission 380/430 nm (Hurtubise 1981, Hurtubise et al. 1989). B(a)P equivalent includes B(a)P, pyrene and fluoranthene (Krahn et al. 1987).

For microfluorometry and fluorometry results, means and 95 % confidence limits were calculated. When necessary the correlation coefficient (r) and regression equation were calculated.

RESULTS

During incubation from 24 to 96 h with AO or FLU at 25 °C no mortality of *Donax trunculus* was observed and no marked alteration occurred in their behavior (opening of valves, syphonal activity, movement). During 24 h incubation at 35 °C (experimental thermal stress) 15 of the 25 incubated *D. trunculus* from the clean site died while others drew in their syphons and partially closed their valves.

The maximal amount of B(a)P equivalents was found in soft body and shells of *Donax trunculus* from the oil-polluted site; bivalves from the PVC factory site contained approximately half as much B(a)P, and those from the relatively clean site had only traces in their soft bodies (Table 1). In specimens from polluted sites the B(a)P distribution in various tissues in decreasing order was: hepatopancreas ≫ total soft body > kidney > shell > mantle edge = gills (Table 1). B(a)P concentration was highest in sediments from the oil-polluted site, and lowest in the sediment from the relatively clean site (Table 1).

Microfluorometrical data demonstrated that the distribution of AO in the tissues of *Donax trunculus* from both the relatively clean and polluted sites was very irregular (Table 2): the maximal fluorescence intensity of AO was observed in numerous large (50 ± 9 µm) bodies along the pallial line of the mantle (Fig. 1); the lowest activity was observed on the shell surface. In the central part of the mantle, gills, hepatopancreas and kidney, AO was detected as green diffuse fluorescence of the cytoplasm and orange or red fluorescence of lysosomes and related structures; in the kidney AO was also present in the lumen of tubules.

A steady-state level of AO accumulation in the tissues of *Donax trunculus* from the clean site was observed within 48 h of incubation (Fig. 2).

After depuration, a statistically significant decrease of AO content in the mantle edge and hepatopancreas

Table 1. Concentration of benzo(a)pyrene equivalents [B(a)P] in the tissues of *Donax trunculus* (ng g^{-1} wet weight) from a relatively clean site and polluted sites along the Mediterranean shore of Israel (mean ± 95 % confidence limit, n = number of individuals tested). All differences between samples from clean and polluted sites are significant at the 95 % level

Sample	Relatively clean		Oil polluted		PVC-factory-polluted	
	B(a)P	n	B(a)P	n	B(a)P	n
Sediment	131.0 ± 15.6	9	1390.0 ± 34.2	9	744.6 ± 53.3	9
Whole mollusc	4.3 ± 0.7	15	55.6 ± 13.8	15	24.7 ± 7.7	15
Whole soft body	5.0 ± 0.5	15	80.0 ± 6.3	15	40.4 ± 11.6	15
Shell	0.0	15	36.0 ± 4.4	15	8.7 ± 1.2	15
Hepatopancreas	8.9 ± 1.4	12	138.4 ± 5.8	15	74.7 ± 10.3	15
Renocardial area	3.7 ± 0.8	12	55.7 ± 3.4	15	23.3 ± 4.7	15
Mantle	0.0	12	23.0 ± 1.9	15	7.5 ± 1.7	15
Gills	0.0	12	22.8 ± 1.3	15	6.5 ± 1.6	15

Table 2. Fluorescence intensity (arbitrary units) of acridine orange in body parts of *Donax trunculus* from the clean and polluted sites along the Mediterranean coast of Israel (mean ± 95% confidence limit, n = 300 measurements from 15 specimens)

Origin of molluscs	Incubation (h)	Fluorescence intensity						
		Mantle edge	Mantle center	Gills	Renocardial area	Hepato-pancreas	Periostracum	Shell surface
Clean site	24	395.4 ± 28.1	22.0 ± 0.8	63.4 ± 2.2	98.4 ± 3.3	54.7 ± 1.7	31.6 ± 1.0	0.5 ± 0.4
	96	551.0 ± 44.8	22.8 ± 0.8	84.0 ± 2.9	193.9 ± 10.5	109.8 ± 3.3	32.4 ± 2.2	0.9 ± 0.4
	96/24[a]	404.5 ± 29.6	16.3 ± 0.6	63.3 ± 1.0	182.5 ± 5.5	84.4 ± 1.9	26.7 ± 1.5	0.5 ± 0.4
Oil-polluted site	24	335.8 ± 11.0*	18.4 ± 0.7*	55.3 ± 1.5*	94.3 ± 2.9	46.5 ± 1.2*	32.0 ± 1.8	1.6 ± 0.4*
	96	479.0 ± 42.7	20.9 ± 1.1	67.3 ± 1.1*	188.3 ± 4.7	71.8 ± 1.9*	32.3 ± 2.1	0.5 ± 0.3
	96/24[a]	408.0 ± 30.9	16.1 ± 0.6	44.4 ± 1.4*	181.8 ± 8.1	72.1 ± 1.3*	27.2 ± 1.7	0.4 ± 0.3
PVC-factory-polluted site	24	272.3 ± 22.7*	13.6 ± 3.2*	40.8 ± 5.6*	75.1 ± 13.6*	40.1 ± 13.8*	34.9 ± 5.9	0.5 ± 0.2
	96	362.4 ± 5.8*	17.9 ± 0.9*	56.0 ± 1.5*	143.2 ± 2.9*	58.1 ± 1.5*	31.1 ± 2.4	0.5 ± 1.2
	96/24[a]	351.4 ± 19.1*	15.8 ± 2.1	54.8 ± 3.4*	139.0 ± 9.6*	55.5 ± 5.1*	32.0 ± 5.6	0.2 ± 0.1
Clean site; experimental thermal shock applied	24	69.7 ± 3.6*	3.4 ± 0.8*	4.2 ± 1.0*	4.2 ± 1.7*	1.4 ± 1.1*	31.4 ± 2.9	0.4 ± 1.2

*Significantly different from control (the same tissue of *D. trunculus* from the clean site), $p < 0.05$
[a]96 h incubation with marker; 24 h incubation in clean sea water (depuration)

of *Donax trunculus* from the clean site was observed, whereas in *D. trunculus* from polluted sites the AO content in these tissues was unchanged (Table 2). AO accumulation in the tissues of *D. trunculus* decreased drastically after 24 h incubation at 35 °C (Tables 2 & 3, experimental thermal stress).

The distribution of FLU in various tissues of *Donax trunculus* from both clean and polluted sites also showed a very irregular pattern (Tables 4 & 5): the highest fluorescence intensity was detected in renocardial tissues, and the lowest in the central part of the mantle. After depuration, a significant decrease in fluorescence intensity in all soft tissues, except the renocardial area, was observed (Table 4).

Quantitative determination of AO or FLU concentration after 96 h incubation in various tissues of bivalves by ethanolic extraction showed that in *Donax trunculus* from the clean site, the mantle edge along the pallial line contained about 3000 µg AO g^{-1} wet tissue weight (ww), the renocardial area (kidney) about 1000 µg g^{-1} ww, and total soft tissues about 400 µg g^{-1} ww (Table 3). All tissues of *D. trunculus* from the clean site contained only small amounts of FLU after 96 h incubation: about 2.6 µg g^{-1} ww in the renocardial area and about 0.364 µg g^{-1} ww in total soft tissues (Table 5). After depuration total soft tissues contained only 0.18 µg g^{-1} ww (data not shown in Table 5). There is a strong correlation ($r = 0.9$) between the data on AO or FLU determined by ethanolic extraction and by microfluorometry.

Comparison of data from Tables 2 to 5 shows significant differences between *Donax trunculus* sampled from relatively clean and polluted sites. First, AO accumulation in the mantle edge, gills and hepatopancreas of molluscs from polluted, especially the PVC-factory-polluted, sites was less than in the same tissues of *D. trunculus* from the relatively clean site. Second, FLU accumulation in the mantle, gills and hepatopancreas of *D. trunculus* from polluted, especially the PVC-factory-polluted, sites was greater than in the same tissues of specimens from the relatively clean site. After 96 h incubation, the renocardial area of *D. trunculus* from the oil-polluted site contained the highest concentration of FLU; a lower concentration was observed in molluscs from the clean site, and the lowest was found in specimens from the PVC-factory-polluted site (Tables 4 & 5). Third, during depuration, run-out of FLU from mantle, gills and hepatopancreas of *D. trunculus* from polluted, especially the PVC-factory-polluted, sites was weaker than from the same tissues of molluscs from the clean site. However, after depuration, no run-out of FLU or AO from the renocardial area of *D. trunculus* from both clean and polluted sites was observed.

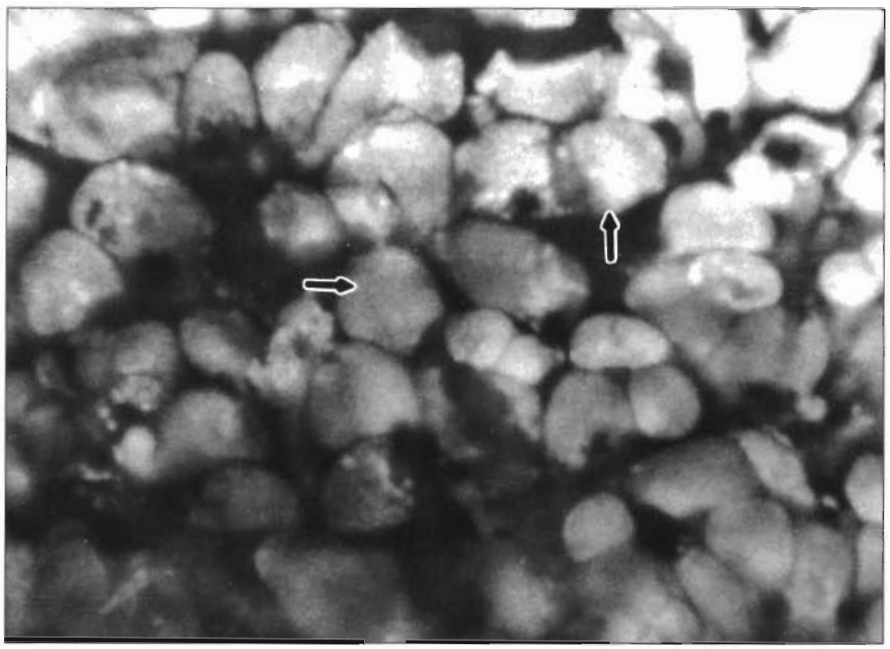

Fig. 1. *Donax trunculus* incubated with 10 µM acridine orange for 24 h showing numerous intensely fluorescent large bodies (arrows) in the mantle edge along pallial line. Vital contact fluorescent microscopy; objective 25 × 0.75, ocular ×5, photomagnification ×4

DISCUSSION

Information on PAH accumulation from oil-polluted sites as well as on PAH distribution in these molluscs is very limited. Our data show that some typical PAHs [B(a)P equivalents] were accumulated by *Donax trunculus* from the oil-polluted site. Their distribution in the tissues of *D. trunculus* was very irregular, showing concentrations as follows: hepatopancreas > total soft body > kidney > shell > mantle edge = gills. Neff & Anderson (1975) described a similar distribution of B(a)P in the clam *Rangia cuneata*, and Couch et al. (1979) demonstrated a similar uptake pattern in oysters using ^{14}C-labeled B(a)P. These results show that PAH accumulation in the various molluscs studied is tissue-specific. In this study the soft body of *D. trunculus* from the oil-polluted site had from 65 to 98 ng B(a)P g^{-1} ww. Kauss & Hamdy (1991) showed that in whole soft bodies of mussels from highly polluted sediments the maximal concentration of B(a)P equivalent was up to 1220 ng g^{-1} dry weight. Such concentrations may be cytotoxic and would be important markers to unmask any signs of cytopathological and cytogenetic alterations produced by accumulated PAHs.

Table 3. Acridine orange (AO) content (µg g^{-1} wet wt) in various tissues of *Donax trunculus* from the clean site, oil-polluted site and site polluted by effluents of the polyvinylchloride (PVC) factory, and in the same tissues of *Mactra corallina* from the clean site of the Mediterranean coast of Israel. AO content was determined by ethanolic extraction after 96 h incubation and microcuvette fluorometry (mean ± 95% confidence limit)

Species and sampling origin	Mantle edge	Mantle center	Gills	Kidney	Hepato-pancreas	Total soft tissues
D. trunculus from clean site	2987.0 ± 150.0	147.5 ± 37.0	450.1 ± 57.7	985.0 ± 80.8	590.0 ± 53.4	385 ± 36.1
D. trunculus from oil-polluted site	2487.5 ± 300.3*	101.2 ± 17.6	391.2 ± 48.3	923.7 ± 111.4	491.2 ± 112.2	311.0 ± 46.3
D. trunculus from PVC-factory-polluted site	2271.2 ± 385.4*	78.7 ± 21.9*	330.0 ± 82.4	771.2 ± 126.8*	445.0 ± 87.5*	280.7 ± 35.8*
D. trunculus from clean site; acute thermal shock applied	507.5 ± 49.2*	22.2 ± 3.5*	31.5 ± 4.9*	41.0 ± 6.5*	14.7 ± 3.5*	46.5 ± 2.0*
M. corallina from clean site	383.7 ± 48.3*	206.2 ± 58.6*	612.5 ± 87.2*	1420.0 ± 78.6*	812.0 ± 52.6*	491.7 ± 25.1*

*Statistically significant ($p < 0.05$) from control (the same tissue of *D. trunculus* from the clean site)

Table 4. Fluorescence intensity (arbitrary units) of fluorescein in tissues of *Donax trunculus* from the clean or polluted sites along the Mediterranean coast of Israel (mean ± 95% confidence limit, n = 300 measurements from 15 specimens)

Origin of molluscs	Incubation (h)	Fluorescence intensity						
		Mantle edge	Mantle center	Gills	Renocardial area	Hepato-pancreas	Periostracum	Shell surface
Clean site	24	6.9 ± 1.0	0.4 ± 0.4	13.7 ± 0.9	41.7 ± 1.6	2.3 ± 0.6	37.5 ± 1.4	0.8 ± 0.5
	96	30.1 ± 2.7	5.7 ± 0.4	43.4 ± 1.9	144.5 ± 9.7	7.5 ± 0.6	134.0 ± 3.5	8.9 ± 0.7
	96/24[a]	3.2 ± 1.2	0.0 ± 0.4	0.0 ± 0.3	120.2 ± 5.0	0.0 ± 0.5	25.2 ± 1.6	0.7 ± 0.6
Oil-polluted site	24	17.2 ± 0.9*	1.0 ± 0.5	21.9 ± 1.3*	65.7 ± 1.6*	1.8 ± 0.3	78.2 ± 2.2*	1.8 ± 0.5
	96	56.0 ± 6.9*	6.8 ± 0.4*	64.2 ± 2.2*	218.8 ± 11.9*	27.9 ± 0.9*	168.2 ± 3.9*	18.0 ± 1.2*
	96/24[a]	26.2 ± 2.0*	1.5 ± 0.6*	3.8 ± 0.7*	232.3 ± 7.4*	6.7 ± 0.7*	50.3 ± 3.7*	2.3 ± 0.6*
PVC-factory-polluted site	24	27.4 ± 4.7*	17.5 ± 4.2*	31.2 ± 5.9*	24.3 ± 6.5*	11.7 ± 3.3*	46.7 ± 5.6	1.6 ± 0.5
	96	84.9 ± 3.2*	49.3 ± 1.5*	65.9 ± 1.6*	71.2 ± 2.2*	31.0 ± 1.1*	164.1 ± 7.6*	2.3 ± 1.5*
	96/24[a]	76.3 ± 6.9*	26.2 ± 5.8*	26.7 ± 6.6*	67.3 ± 6.4*	24.6 ± 9.2*	77.6 ± 8.5*	1.6 ± 0.4*

*Significantly different from control (the same tissue of *D. trunculus* from the clean site), $p < 0.05$
[a]96 h incubation with marker; 24 h incubation in clean sea water (depuration)

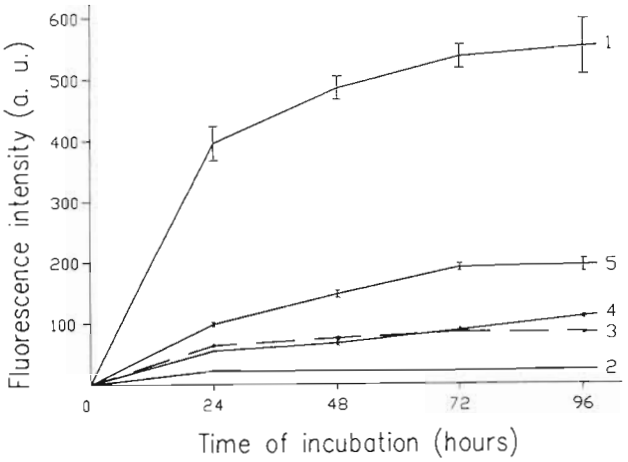

Fig. 2. *Donax trunculus*. Accumulation of acridine orange in various tissues of molluscs from the clean site along the Israeli Mediterranean shore as a function of incubation time. Concentration of acridine orange in seawater is 10 µM. (1) Mantle edge, (2) central part of the mantle, (3) gills, (4) hepatopancreas, (5) renocardial area. Vertical bars indicate 95% confidence limit

The relationship between the concentration of contaminants in the organism or its tissues and that in the surrounding water or sediment is known as the bioconcentration factor (BCF). Recently, attention has been focused on correlations of BCF with various physicochemical properties of xenobiotics (Kauss & Hamdy 1991, Niimi & Lee 1992). However, the influence of various biological factors on BCF requires more study.

BCF for B(a)P in various tissues of *Donax trunculus* from the oil-polluted site was very low: for the whole soft body it was 0.173 (wet weight basis). This is interesting, as PAHs are very lipophilic. In short-term experiments on oysters with ^{14}C-labeled B(a)P, BCF was very high (Couch et al. 1979). However, many authors found very low (usually < 1) BCF for mussel/sediment PAHs under field conditions (Roesijadi et al. 1978, Heit et al. 1980, Elder & Dressler 1988, Kauss & Hamdy 1991). Due to the low water solubility of PAHs, during long-term exposure they interact with dissolved organic carbon and are absorbed by fine suspended matter and bioaccumulated by plankton (Kauss & Hamdy 1991). Therefore, under field conditions, PAHs and other lipophilic xenobiotics penetrate into molluscs mainly in the particulate-associated form via the digestive canal, especially the hepatopancreas, and only a small fraction extens in dissolved form via the gills (Brodtman 1970, Dindal & Wurzinger 1971, Kauss & Hamdy 1991).

B(a)P accumulation in the hepatopancreas of *Donax trunculus* from polluted sites also indicates that the rate of B(a)P detoxification in the hepatopancreas is slower than the rate of its uptake and bioaccumulation.

Table 5. Fluorescein (FLU) content (µg g^{-1} wet wt) in various tissues of *Donax trunculus* from the clean site, oil-polluted site and site polluted by effluents of the polyvinylchloride (PVC) factory, and in the same tissues of *Mactra corallina* from the clean site of the Mediterranean coast of Israel. FLU content was determined by ethanolic extraction after 96 h incubation and micro-cuvette fluorometry (mean ± 95% confidence limit)

Species and sampling origin	Mantle edge	Mantle center	Gills	Kidney	Hepato-pancreas	Total soft tissues
D. trunculus from clean site	0.57 ± 0.06	0.06 ± 0.006	0.70 ± 0.08	2.60 ± 0.29	0.10 ± 0.035	0.364 ± 0.033
D. trunculus from oil-polluted site	1.16 ± 0.05*	0.10 ± 0.033*	0.94 ± 0.16*	3.75 ± 0.62*	0.32 ± 0.132*	0.455 ± 0.047*
D. trunculus from PVC-factory-polluted site	1.62 ± 0.37*	1.30 ± 0.25*	1.39 ± 0.48*	1.66 ± 0.35*	0.46 ± 0.15*	0.595 ± 0.099*
M. corallina from clean site	1.49 ± 0.77*	1.05 ± 0.40*	1.37 ± 0.60*	1.87 ± 0.37*	0.11 ± 0.03	0.471 ± 0.020*

*Statistically significant ($p < 0.05$) from control (the same tissue of *D. trunculus* from the clean site)

Yawetz et al. (1992) showed that the significant increase in cytochrome P450 content in *D. trunculus* from the same oil-polluted site was accompanied by a drastic decrease in 7-ethoxyresorufin O-deethylase catalytic activity. Consequently, PAH distribution and BCF values in molluscs from polluted sites are tissue-specific and possibly dependent upon specific uptake pathways.

Bioaccumulation and distribution of AO and FLU were very different in the various tissues of molluscs. For AO, BCF (wet weight basis) was high considering its octanol/water partition coefficient: in *Donax trunculus* from the relatively clean site BCF was 1118.7 in the mantle edge along the pallial line, 368.9 in the kidney, and 55.2 in the central part of the mantle. In contrast, for FLU, BCFs were very low, decreasing from 0.08 in the kidney to 0.0018 in the central part of the mantle. *Mactra corallina* from the same clean site had different BCF values; for AO, BCF was 531.8 in the kidney, 143.7 in the mantle edge, and 77.2 in the central part of the mantle, and for FLU it was 0.14 in the kidney and 0.03 in the central part of the mantle. Consequently, the data for *D. trunculus* and *M. corallina* suggest that BCF values for AO and FLU are species-specific.

A non-ionized form of AO penetrates through plasma membranes; in the cells AO binds with some proteins, especially mucoproteins, and nucleic acids, and is also accumulated by cell compartments with low pH, such as lysosomes and related structures (Wittekind 1973, Swanson 1989, Darzynkiewicz & Kapuscinski 1990, Murphy 1990). As intralysosomal pH and, accordingly, lysosomal accumulation of cationic chemicals depend on cellular energetic metabolism, each cell injury will induce a proportional decrease in the accumulation of lysosomotropic markers (Swanson 1989, Dierickx & Van de Vyver 1991, Saito et al. 1991). Therefore, the level of AO accumulation in the cell is determined by its specific structure and function, i.e. by production and massive intracellular accumulation of the mucoproteins, or by the presence of numerous lysosomes. Our data show that in *Donax trunculus* the main part of the accumulated AO is bound by large bodies distributed along the pallial line. Thus, in this tissue, marker binds with some intracellular mucoproteins, and such binding is inhibited significantly by pollution and especially by pollution from the PVC factory. Xenobiotic markers probably compete with environmental pollutants for binding sites within the intracellular mucoproteins. Binding of xenobiotics with structures which contain mucoproteins is a known defense mechanism against environmental pollutants in flatworms and molluscs (Dindal & Wurzinger 1971, Triebskorn et al. 1991). This defense mechanism is well developed in *D. trunculus*, but it is undeveloped in *Mactra corallina*.

In other tissues of *Donax trunculus* AO is accumulated mostly by lysosomes and related structures, and such accumulation is also inhibited by environmental pollutants. Saito et al. (1991) and Dierickx & Van de Vyver (1991) showed that accumulation of another lysosomotropic marker, neutral red, was inhibited significantly by various anthropogenic xenobiotics in experiments *in vitro*. Lysosomal accumulation of xenobiotics is believed to defend the cells against some pollutants (George 1984, Jenner et al. 1991). Using AO as a marker shows that this defense mechanism is well developed in both *D. trunculus* and *Mactra corallina*.

Our data show that B(a)P and especially AO and FLU were concentrated by the molluscan kidney. Harrison (1962) postulated the presence of active excretory processes for anionic xenobiotics in the kidney of the abalone *Haliotis rufiens*. The kidney of other molluscs

can concentrate organic anions such as paraaminohippurate and phenol red (Harrison & Martin 1965, Martin et al. 1965, Martin & Harrison 1966). In vertebrates the renal proximal tubules, liver and choroid plexus, and Malpighian tubules, contain 2 transport systems: one for active elimination of organic acids and one for active elimination of organic bases (Weiner 1973, Bresler et al. 1975, 1979, 1985, 1990, Maddrell 1980, Moller & Sheikh 1982). Similar defense systems may exist in the tubules of the molluscan kidney, and in *D. trunculus* these systems were probably inhibited by pollutants from the PVC factory.

Deposition of B(a)P and marker xenobiotics was also found in the periostracum and shell of *Donax trunculus*. Bias & Karbe (1985) found a loosely bound fraction of cadmium in the periostracum of the freshwater mussel *Dreissena polymorpha*. The mechanisms whereby such xenobiotics are deposited into the shell have not been studied.

A sharp decrease in AO accumulation in all soft tissues of *Donax trunculus* after experimental thermal stress demonstrated that all the above-mentioned mechanisms of AO accumulation were dependent on cellular metabolism and nativity of cell structures.

It is axiomatic that the plasma membrane of intact living cells is inpermeable to water-soluble organic anions, including FLU. Therefore, determination of cell permeability to anionic dyes is widely used to detect cell injury or changes in cell viability (DuBois et al. 1980, Bowen 1981, Pieters et al. 1989). Increase in FLU accumulation in the mantle, gills and hepatopancreas of *Donax trunculus* from all polluted sites reflects an increase in plasma membrane permeability, i.e. the presence of cell injury produced by environmental pollutants.

We draw the following conclusions from our study:

(1) Bioaccumulation and BCF values for B(a)P, AO and FLU are compound-dependent, tissue-specific and possibly species-specific.

(2) Under field conditions BCF values for B(a)P are very low, and B(a)P in the tissues of *Donax trunculus* from polluted sites is accumulated mainly in the hepatopancreas, kidney and shell.

(3) Under experimental conditions the level of AO and FLU accumulation in the tissues of *Donax trunculus* depends on the site of collection, i.e. environmental conditions, and probably reflects the action of pollutants on cell viability.

(4) The kidney of *Donax trunculus* and *Mactra corallina* may contain at least 2 separate transport systems to eliminate anionic and cationic xenobiotics.

(5) *Donax trunculus* are able to deposit most of the accumulated AO into large bodies in the mantle edge along the pallial line, as well as B(a)P, AO and FLU into their shells.

This system of ecotoxicological analysis should be incorporated into studies of population stability examining numerical changes in community structures and determining intrinsic markers for organismal health.

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