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Antifouling paint booster biocide contamination in Greek marine sediments

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Abstract

Organic booster biocides were recently introduced as alternatives to organotin compounds in antifouling products, after restrictions imposed on the use of tributyltin in 1987. In this study, the concentrations of three biocides commonly used as antifoulants, Irgarol 1051 (2-methylthio-4-tertiary-butylamino-6-cyclopropylamino-*s*-triazine), dichlofluanid (*N*-dichlorofluoromethylthio-*N*′,*N*′-dimethyl-*N*-phenyl sulphamide) and chlorothalonil (2,4,5,6-tetrachloro isophthalonitrile) were determined in sediments from ports and marinas of Greece. Piraeus (Central port, Mikrolimano and Pasalimani marinas), Thessaloniki (Central port and marina), Patras (Central port and marina), Elefsina, Igoumenitsa, Aktio and Chalkida marinas were chosen as representative study sites for comparison with previous monitoring surveys of biocides in coastal sediments from other European countries.

Samples were collected at the end of one boating season (October 1999), as well before and during the 2000 boating season. All the compounds monitored were detected at most of sites and seasonal dependence of biocide concentrations were found, with maxima during the period June–September, while the winter period (December–February) lower values were encountered. The concentrations levels ranged from 3 to 690 ng/g dw (dry weight). Highest levels of the biocides were found in marinas (690, 195 and 165 ng/g dw, for Irgarol, dichlofluanid and chlorothalonil respectively) while in ports lower concentrations were observed. Antifouling paints are implicated as the likely sources of biocides since agricultural applications possibly contributed for chlorothalonil and dichlofluanid inputs in a few sampling sites.

Keywords: Antifouling biocides; Irgarol 1051; Chlorothalonil; Dichlofluanid; Marine sediments; Greece

1. Introduction

Pilot survey studies of antifouling agents affecting the marine environmental contamination has, to date, been focused on tributyltin (TBT), triphenyltin (TPT) and other copper-based biocides (Clark et al., 1988). Leaching of organotins from boats and ships coated with antifouling paints, resulted in potential toxic concentrations in the water column, sediments and tissues of marine organisms, during the 1970s and 1980s. Concentrations were especially high in areas of high shipping or boating activity and very serious biological impacts were inevitable in the most contaminated areas (Alzieu et al., 1986; Alzieu, 1991; Fent, 1996).

As a consequence, legislation in many countries banned the application of TBT-based paints to small vessels (<25 m) (European Community, 1989) and the International Maritime Organization has recently announced a proposed ban on the use of organotin antifouling paints on ships by 2003 (IMO, 1998; Christen, 1999; Julian, 1999). This ban will lead to an increase in alternative coating products containing organic biocides such as Irgarol 1051, dichlofluanid, chlorothalonil, Sea

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Nine 211, zinc pyrithione, that will replace TBT as antifouling compounds. The fate of TBT compounds in marine sediments have been extensively studied whilst very little work has been performed to assess the accumulation of organic booster biocides. Although the physico-chemical properties of biocides differ significantly and some are rapidly degraded, they will also accumulate in marine sediments if introduced as paint particles (Thomas et al., 2000).

For the more persistent compounds, increased biocide use and an associated increase in water column concentrations will lead to a build up in sediments due to sorption processes. The binding of biocides to sediment particles retards their migration and increases their persistence, causing ecological as well as ecotoxicological damage in the aquatic environment (Evans et al., 2000).

Irgarol 1051 is effective mostly against freshwater and seawater algae, and less against animal organisms. It belongs to the *s*-triazine group of compounds and its biochemical mode of action upon algae growth is via inhibition of the photosynthetic electron transport in chloroplasts (Hall et al., 1999). Chlorothalonil and dichlofluanid are widely used in agriculture as fungicides and they are both toxic (Heil et al., 1991; Caux et al., 1996).

Since these alternatives to TBT are also toxic, their contamination in the marine environment has been a topic of increasing importance the last years. Several studies have been carried out concerning their occurrence in coastal waters (Tolosa et al., 1996; Ferrer et al., 1997; Scarlett et al., 1997; Steen et al., 1997; Ferrer and Barceló, 1999; Liu et al., 1999; Thomas et al., 2001) but only limited data (mainly focused on Irgarol occurrence) were published on their presence in sediments (Gough et al., 1994; Tóth et al., 1996; Biselli et al., 2000; Boxall et al., 2000; Thomas et al., 2000; Voulvoulis et al., 2000).

This study was performed within the scope of the European research project “Assessment of the Antifouling Agents in Coastal Environment” (ACE), that focused on the fate, occurrence and distribution of biocides in marine water and sediments. The results presented in the present study is a part of this project and includes the monitoring and the temporal distribution of biocides in coastal sediments of Greece. To our knowledge, this represents the first survey on the monitoring of such biocides in Greek coastal sediments.

The purposes of this extended survey were: (1) to determine the concentration levels of the selected biocides (Irgarol, dichlofluanid and chlorothalonil) in coastal sediment samples from several Greek ports and marinas with high levels of commercial, leisure and yachting activity, (2) to study the seasonal variations of the biocides for a period of one year (October 1999 to September 2000).

2. Experimental

2.1. Description of the ports and marinas

Three of the busiest commercial ports and eight of the most popular marinas with high yachting activity around Greece have been selected in this study in order to investigate the environmental occurrence of antifouling paint biocides in sediments. The ports were at the cities of Piraeus, Thessaloniki and Patras. The main marinas selected were from Piraeus (Mikrolimano and Pasalimani), and from the cities of Patras, Chalkida, Thessaloniki (Kalamaria), Aktio, Elefsina and Igoumenitsa. Fig. 1 shows the location of the sampling stations.

Piraeus is Greece’s largest port located in the Southwestern Aegean Sea (Saronikos Gulf). It can be divided into three sections of port activity: the passenger port, the commercial port and the handling of bulk cargo ships. Ship traffic is very heavy both inside the harbor and outside the entrance area day and night. There are two main marinas adjacent to the Central Port, the Mikrolimano and the Pasalimani marinas that can accommodate more than 800 vessels. The port of Thessaloniki is located in the Northwest Aegean Sea on the northern shore of the Gulf of Thessaloniki (Thermaicos Gulf). The port of Thessaloniki, the natural gate of the Balkans to the sea is rapidly being developed to accommodate international cruise liners and local ferries as an outlet to the islands of the Aegean. The marina of Thessaloniki is situated in the region of Kalamaria beside the city. It can accommodate approximately 250 boats from 5 to 30 m long. About 50–60 boats moor in the marina throughout the year. Patra is located in the Northwest of Peloponnese region (Korintiakos Gulf) and its port is a major transportation center, linking Greece with Italy and the Peloponnese with the Ionian Islands. There is a closed marina near the port accommodating at about 100 vessels throughout the year. Chalkida is the capital of Evia, the second largest island of Greece, located in the Northern part of the island. Chalkidas’ marina can handle less than 60 vessels without direct connection to the sea, that implies a low ability of renewing its water. Aktio marina is located in the entrance of Amvrakikos Gulf in the middle of the Ionian Sea. It is built in a private land of 20.000 m^2 and its modern well-designed construction is capable of offering yacht repair and maintenance for more than 300 boats ashore.

Greeces’ second biggest developed shipyard is in Elefsina located in 30 km north of Athens. The Elefsina shipyard concentrates on building ships for the navy and repairing an average of 160 ships a year, mostly for the merchant marine. There is a small marina close to the ship-repairing zone which handles at about 40–50 boats throughout the year including a shipyard at the same

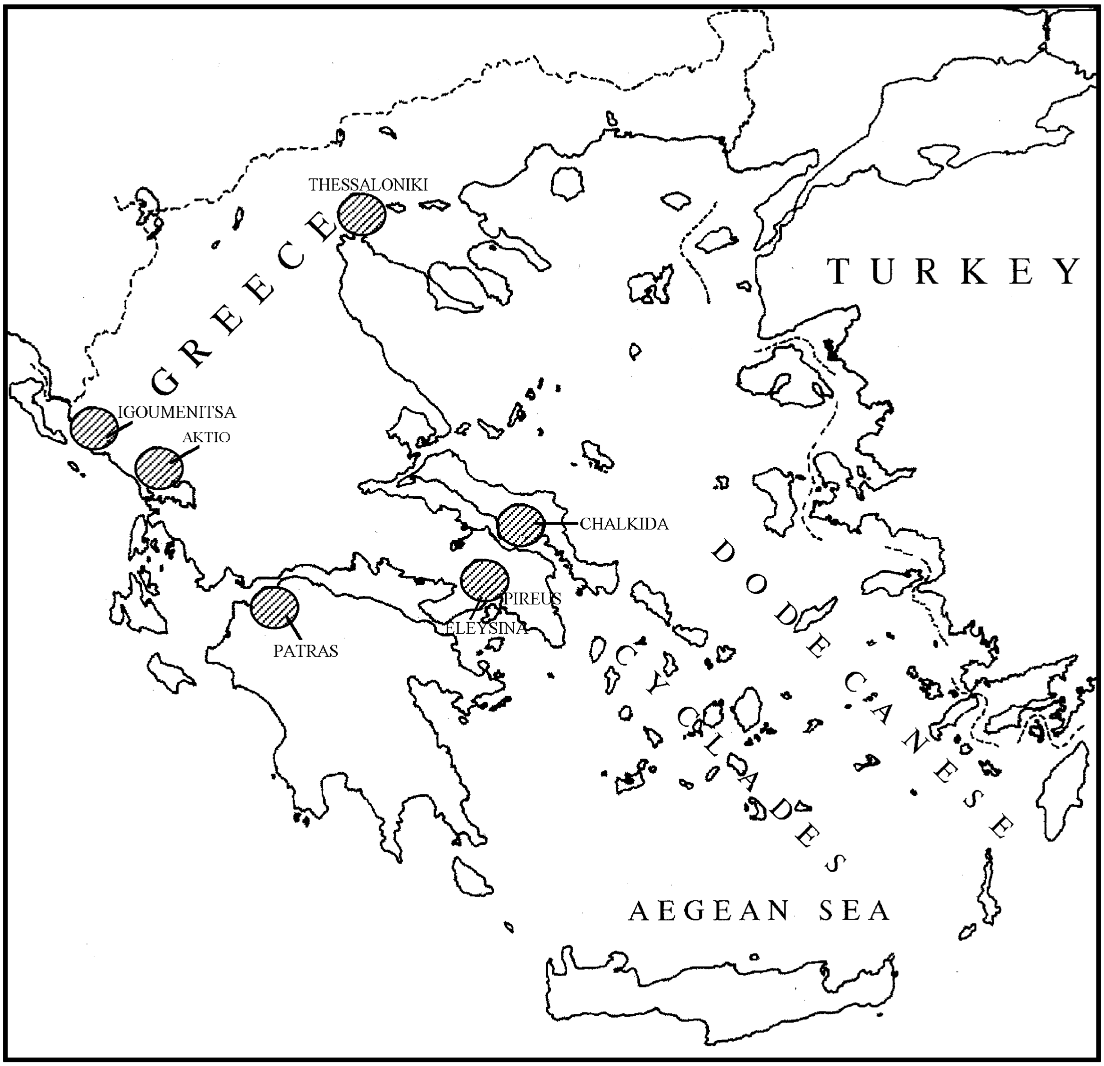

Fig. 1. Map of Greece—locations of the selected ports and marinas.

locality. Igoumenitsa is the capital city of Thesprotia, located in the Western part of Greece. Igoumenitsa is an important center of communication, due to the fact that the port is the most Northern one of Western Greece and connects Greece with Italy and from there with the rest of Europe. There is an enclosed marina near the port that accommodates less than 100 leisure craft throughout the year. The characteristic data of the ports and marinas are summarised in Table 1.

2.2. Sampling

Fine grained surface sediments (0–10 cm) were collected with a grab sampler, sieved through a 2 mm-mesh strainer immediately after the collection and stored in pre-cleaned 250 ml glass jars in a dark room, at −30 °C, until analysis (1–3 days). The samples were taken from three different points of each port and marina in order to be more representative for the biocide concentration level.

Monitoring of biocide concentrations started in October 1999 and was performed monthly until September 2000 (one year) in order to identify variations of biocide levels due to boating activity. By sampling in October the aim was to determine the biocide levels after the boating season. By sampling in November–April before the next boat season, any contamination found will be mainly due to persistence of biocide residues from the previous season. Finally, the May–September period represents the contamination during the yachting season.

Table 1
Characteristic data of the ports and marinas included in this study

Station	Coordinates	Number of vessels (max. capacity)	Water exchange[a]	Proximity to agricultural land	(%) O.C.[b]
Ports					
Piraeus	37°56′3″N23°37′3″W	–	++	none	14.0
Patras	38°15′6″N21°44′W	–	++	none	11.6
Thessaloniki	40°38′N22°56′1″W	–	++	none	12.9
Marinas					
Aktio	38°57′06″N20°45′55′W	300	++	30 km	5.0
Chalkida	38°27′9″N23°35′4″W	60	−	none	9.2
Elefsina	38°02′3″N23°33′1″W	50	+	none	13.8
Igoumenitsa	39°30′1″N20°15′7″W	100	++	none	5.6
Patra	38°15′6″N21°44′W	100	+	none	11.0
Piraeus (m1)[c]	37°56′3″N23°37′3″W	450	+	none	13.5
Piraeus (m2)[d]	37°56′3″N23°37′3″W	400	+	none	14.0
Thessaloniki (m)	40°38′N22°56′1″W	250	+	none	10.8

[a] ++ high, + medium, − low.
[b] Organic carbon content.
[c] m1 is Mikrolimano marina.
[d] m2 is Pasalimani marina.

2.3. Chemicals

Biocide standards, Irgarol 1051 (2-methylthio-4-tertiary-butylamino-6-cyclopropylamino-*s*-triazine), chlorothalonil (2,4,5,6-tetrachloro isophthalonitrile), and dichlofluanid (*N*-dichlorofluoromethylthio-*N*′,*N*′-dimethyl-*N*-phenyl sulphamide), were purchased from Riedel-de Häen (Germany). Pesticide grade *n*-hexane, methanol, acetone and dichloromethane were purchased from Pestiscan (Labscan Ltd., Dublin, Ireland). Sodium sulphate and ammonium acetate (pro-analysis) were from Merck (Darmstand, Germany). Florisil was from Fluka (Buchs, Switzerland). Paper filters of 70 mm diameter (type 40) were supplied from Whatman (England).

2.4. Analysis

The analytical method for the determination of biocides was based on the previously developed method by Hela and Albanis (1998) for the determination of priority pesticides in sediments. This method was slightly modified as follows: wet homogenised sediment samples (∼10 g) were mixed with 10 g of Na_2SO_4 (pre-purified with acetone and dried at 100 °C), 5 ml of ammonium acetate 0.2 N and 50 ml of distilled water. The mixture was extracted with 50 ml of acetone, shaken for 30 min in a mechanical flask-shaker, sonicated for 30 min and then filtered using a buchner filter. This process was then repeated twice. The filter was washed with 30 ml of acetone. The organic phases were combined and were subjected to successive liquid/liquid extraction by adding 50, 25 and 25 ml of dichloromethane. The extracts were dried through anhydrous sodium sulphate (5 g) and concentrated to 5 ml using a rotary evaporator and with a gentle stream of nitrogen to a final volume of 0.5 ml.

2.4.1. Clean up

All samples were purified by Florisil column chromatography and eluted with solvents of increasing polarity. The chromatographic glass columns (150 mm × 10 mm i.d.) were packed with 4 g of Florisil (60–100 mesh) pre-purified with hexane, activated at 300 °C and deactivated with 2% H_2O. The adsorbent was topped with 2 cm of anhydrous sodium sulphate. A sample of 0.5 ml was introduced to the column and eluted first with 50 ml of acetone/*n*-hexane (1 + 99 v/v) mixture followed by 50 ml of acetone/*n*-hexane (50 + 50 v/v) mixture. The eluents were combined and concentrated to 5 ml using a rotary evaporator and with a gentle stream of nitrogen to 1 ml. Then they were ready for chromatographic analysis.

2.5. Gas chromatographic conditions

The chromatographic analysis of chlorothalonil and dichlofluanid was performed with a Shimadzu 14B capillary gas chromatograph, equipped with a ^{63}Ni electron capture detector (ECD), at 300 °C. Analytes were separated with a DB-1 column (J&W Scientific, Folsom, CA, USA) 30 m of length, 0.32 mm i.d., coated with dimethylpolysiloxane of a film thickness of 0.25 μm. The temperature program and the analysis was: 55 °C (2 min) to 210 °C (15 min) at 5 °C/min and then to 270 °C at 10 °C/min. Helium was used as carrier at a flow of 1.5 ml/min and nitrogen was used as make-up gas at a flow of 35 ml/min. The injector temperature was set to 220 °C. The splitless mode was used for injection with the valve opened after 60 s. Injection volumes were 1.5 µl.

The analysis of Irgarol as well as the confirmatory analysis of chlorothalonil and dichlofluanid was performed on a Shimadzu 14A capillary gas chromatograph equipped with a flame thermionic detector (FTD). Analytes were separated on a DB-5 column (J&W Scientific, Folsom, CA, USA) of 30 m length and 0.32 mm i.d., coated with 5% phenyl 95%-methylpolysiloxane with a film thickness of 0.25 µm. The temperature program for the analysis was the same as for ECD. Helium was used as carrier (1.5 ml/min) and make up gases (40 ml/min), respectively. The detector gases were hydrogen and air at flows of 4 and 120 ml/min, respectively. The detector temperature was set to 250 °C and the injector temperature to 220 °C. An alkali metallic salt (Rb_2SO_4) bonded to a 0.2 mm spiral of platinum wire generated the ions. The splitless mode was used with the valve opened after 60 s. Injection volumes were 1.5 µl.

Confirmation of compounds was performed by GC–MS using a QP 5000 Shimadzu instrument equipped with a DB-5-MS capillary column (J&W Scientific) of 30 m length and 0.25 i.d., coated with 5% phenyl 95%-methylpolysiloxane using the following chromatographic conditions: injector temperature 220 °C, oven temperature program 55 °C ramped at 5 °C/min to 200 °C followed by another ramp of 1 °C/min to 210 °C, held for 2 min and finally to 270 °C at 20 °C/min (held for 3 min). Helium was used as carrier gas at a flow of 1 ml/min. The temperatures of the ion source and the interface were set at 240 and 290 °C respectively. The splitless mode was used for injection with the valve opened after 30 s. Three µl injections were made.

The mass spectrometer was operated in the electron impact (70 eV), selected ion monitoring (SIM) mode at 1.75 kV. In this way it was possible to establish the best conditions with respect to sensitivity and selectivity. Selection of the ions was restricted to specific retention time windows according to the following segments: 25.0–29.0 min at m/z 266 (100) and m/z 264 (77) for chlorothalonil, 29.0–34.0 min at m/z 123 (100) and m/z 167 (31) for dichlofluanid and finally 34.0–47.0 min at m/z 253 (56) and m/z 182 (100) for Irgarol 1051. The values in parentheses give the relative abundance (%).

2.6. Quantification and quality control

The found concentrations of Irgarol 1051, dichlofluanid and chlorothalonil are reported as the mean of duplicate determinations. Quantification was carried out by external standards with concentrations similar to those actually encountered in sediment samples. This quantification technique was preferred in view of the special characteristics of the FTD, in particular, with regard to the well-known temporal variations of its response (Bester and Huhnerfuss, 1993). Furthermore, it should be noted that additional problems with matrix effects might increase the variability. To control these variations a series of standards were injected between the samples. Analysis of blanks was performed for each batch of samples to exclude potential laboratory contamination. A typical gas chromatogram of a sediment sample from Elefsina marina is shown in Fig. 2. The GC–FTD and GC–ECD analysis of the selected

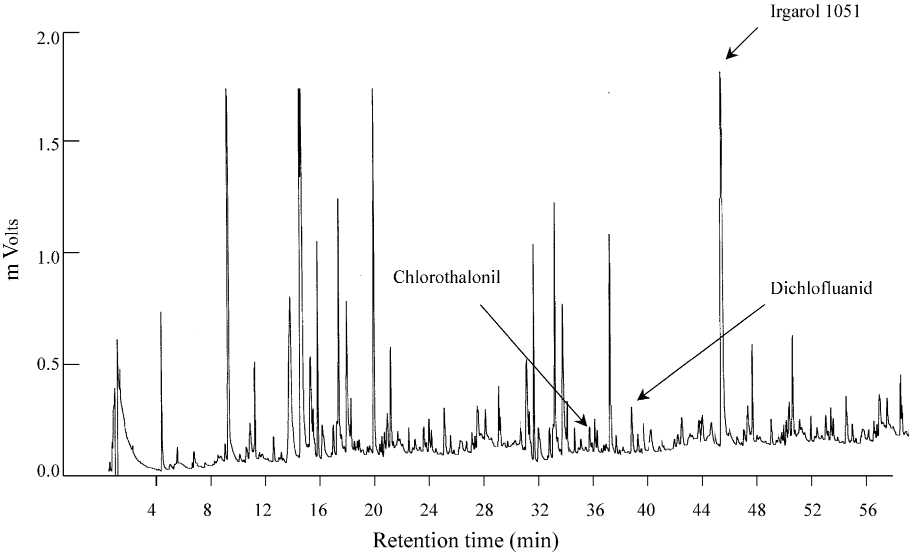

Fig. 2. GC–FTD chromatogram of sediment sample extract from Elefsina marina (June 2000).

Table 2
Recoveries, limits of determination (LOD) and RSD using GC–FTD, GC–ECD and GC–MS (SIM) techniques

Biocide	Recovery (%)	LOD[a]–RSD[b]					
		GC–MS (SIM)		GC–ECD		GC–FTD	
		(ng/g dw)	(%)	(ng/g dw)	(%)	(ng/g dw)	(%)
Chlorothalonil	92 ± 6	2.5	9	1.5	6	–	–
Dichlofluanid	87 ± 8	3.0	11	2.5	9	–	–
Irgarol 1051	82 ± 12	0.7	8	–	–	0.4	8

[a] Limit of determination of the whole analytical procedure under the chromatographic conditions selected (signal-to-noise ratio = 10), corrected for recovery.

[b] RSD is derived from replicate analysis of matrix ($n = 5$).

antifouling compounds provided good gas chromatographic resolution with low detection limits in the range of ng/g.

The verification of all compounds was accomplished by GC–MS using SIM. Full scan GC–MS confirmation (100–450 amu) was also performed on selected samples. The presence of chlorothalonil and dichlofluanid was also verified by a second GC-column (DB-5) coupled to the FTD. Quantification was only considered acceptable, if the respective peaks were present at the correct retention times on both columns.

Although the analytical method extracts wet sediment the results are normalised on dry sediment weight (dw) for comparison with other studies.

2.7. Recovery rates and limits of determination

A series of experiments using spiked sediment samples with known concentrations of biocide standard solutions was carried out to determine the recovery of the analytical method. The same range as of the analysed samples was covered. Replicate analysis of spiked sediments (with $n = 5$) revealed a sufficient recovery of 82–92% with a precision of 6–12%. The variation of the recovery rate of Irgarol 1051 of about 12% is due to the complex analytical method and the influence of the organic matrix of the sediments. The limit of determination varied between 0.4 and 3 ng/g dw. Relative standard deviations (RSD) of less than 11% were generally achieved (Table 2).

3. Results and discussion

3.1. Differences between sampling regions

The levels of biocides in coastal sediments during the period October 1999 to September 2000 are summarised in Table 3 and Fig. 3(a)–(c). The extent of contamination varied widely and considerable levels were determined at most of the ports and marinas under investigation. Because of this variation in biocide concentrations, a statistical approach was not possible to calculate significant differences between locations and months (time) versus the more frequently detected biocides. It is evident that the variation between the concentrations in sediment and the adjacent water column depends on the water flow and on specific characteristics of the station, e.g., the volume of the port/marina, the start of boating season and the number of vessels. Furthermore the sediment contamination is greatly influenced by the daily flooding of each harbour (Biselli et al., 2000; Thomas et al., 2001). However, higher levels of biocides were detected during and after the boating season compared to those before.

In addition, highest concentrations of most studied biocides are shown to be associated with yacht marinas, which primarily house pleasure craft. Ports, which generally have a lower density of pleasure craft and more and larger commercial vessels are shown to have lower concentrations of biocides (Table 3). These observations can easily be explained taking into account the above mentioned characteristics of the stations.

3.1.1. Irgarol 1051

Little is known about the aquatic partitioning of Irgarol 1051, although its water solubility of 7 mg/l (Ciba Geigy, 1988) implies a stronger tendency to be adsorbed to particles and sediments in comparison to other triazines that exhibit a higher water solubility (i.e. atrazine 33 mg/l, prometryne, 40 mg/l). In addition, the relatively high octanol/water partition coefficient ($\log K_{ow} = 3.95$) of Irgarol 1051 suggests an enrichment of the antifouling compound in the sediment.

Irgarol 1051 is not considered to be readily degraded in seawater with a half-life of about 100 days (Ciba Geigy, 1995; Scarlett et al., 1997). However biodegradation, photodegradation and chemical hydrolysis were found to occurred (Liu et al., 1999; Okamura et al., 1999; Köenig et al., 1999). In sediments, it has been shown that degradation is slow even under aerobic conditions with half-lives of 100–200 days for marine and freshwater sediment respectively, and considerably slower for anaerobic conditions (Tóth et al., 1996).

Table 3
Summary of October 1999–September 2000 yachting season monitoring data in sediments from Greek ports and marinas

Location	Range of biocide concentration (Mean[a])								
	Irgarol 1051			Dichlofluanid			Chlorothalonil		
	RSD			RSD			RSD		
	(ng/g dw)	(%)	n.d.[b]($N = 8$)[c]	(ng/g dw)	(%)	n.d. ($N = 8$)	(ng/g dw)	(%)	n.d. ($N = 8$)
Ports									
Piraeus	12–19 (9)	12	3	16–90 (32)	9	1	9–32 (13)	13	3
Patra	8–11 (6)	10	2	10–18 (10)	12	1	18–26 (14)	12	3
Thessaloniki	3–11 (5)	12	3	8–18 (9)	15	2	12–56 (31)	13	1
Marinas									
Aktio	11–58 (25)	7	–	30–195 (76)	9	–	13–32 (13)	10	3
Chalkida	22–88 (40)	8	2	18–75 (44)	12	–	18–48 (19)	9	3
Elefsina	86–690 (233)	13	–	27–65 (41)	13	–	10–126 (41)	10	1
Igoumenitsa	20–74 (31)	6	2	14–47 (21)	9	2	8–48 (15)	8	3
Patra (m)	14–37 (21)	10	2	15–55 (25)	12	1	12–65 (20)	9	3
Piraeus (m1)	15–43 (34)	9	–	25–60 (41)	12	–	14–48 (22)	12	2
Piraeus (m2)	10–22 (12)	11	1	12–58 (34)	13	–	11–27 (11)	14	3
Thessaloniki (m)	75–350 (170)	9	–	9–65 (31)	12	1	20–165 (73)	8	–

[a] Mean concentration.
[b] No detected.
[c] Number of samplings.

Irgarol 1051 was detected in 72% of the sediment samples analysed in the survey. The concentration levels ranged between 3 and 690 ng/g dw with a mean concentration of 53 ng/g dw. The concentration levels in the central ports (Patras, Thessaloniki and Piraeus) ranged between detectable and values below 19 ng/g dw. This can be explained by the high water exchange rates based on the direct connection to the sea and, therefore, the greater effect of the tidal cycles. Most of the contaminated surface water is presumably removed from the port prior to the transport of the pollutants to the sediment. Moreover, other studies suggested also that environmental pollution by antifouling biocides arose largely from pleasure craft rather than commercial shipping which occurred mostly in the central ports (Pidgeon, 1993; Readman et al., 1993).

On the other hand, higher levels of Irgarol 1051 were detected in marinas due to the high boating activity and the poor tidal water exchange, as they constitute more enclosed environments than ports. The low water renewing time increases the water column concentration and causes an associated increase in the binding of biocide on the sediment particles.

Elefsina, although being the smallest marina, accomodating about 50 vessels, demonstrated the highest concentration of Irgarol 1051 (686 ng/g dw in June) and generally is one of the most contaminated marinas in the survey. Obviously, the neighbourhood of the shipyard is clearly being reflected by the extremely high Irgarol 1051 value, so that a direct contamination with Irgarol 1051 occurred in addition to the normal leaching rate from the ships, due to the ship painting. Lower but considerable levels of Irgarol 1051 (22–88 ng/g dw) were detected in marina of Chalkida, which reflects also a small marina of about 60 vessels with a low water renewing time. Similar concentrations of Irgarol 1051 were also determined between the other marinas of Aktio, Igoumenitsa, Patra, Piraeus (Mikrolimano) and Piraeus (Pasalimani) as a result of the combination of their higher yachting capacity (can accommodate 100–450 vessels) causing higher leaching rates, and the medium or high water exchange. In the more open tidal marinas this runoff is quickly diluted and flushed away by the tide. The levels of Irgarol 1051 in the marina of Thessaloniki (Kalamaria) do not follow the same trend. High concentrations (75–350 ng/g dw) were detected because the majority of vessels were removed from the water and treated on scrubbing piles or on beaches at the same locality. As a result direct contamination with Irgarol 1051 due to the ship painting is probable.

The found concentrations of Irgarol 1051 are not high enough to have acute toxic effects to sediment biota. Chronic effects at lower concentrations are unknown and difficult to determine (Scarlett et al., 1997; Voulvoulis et al., 2000). To our knowledge, this is the first report on the presence of Irgarol 1051 in sediment samples of Greece. The concentrations found in the Greek environment are comparable to other studies concenring the environmental contamination of aquatic systems by Irgarol 1051 (see Table 5). The highest values were found in UK estuaries by Boxall et al. (2000) (<10–1011 ng/g dw), Voulvoulis et al. (2000) (3.3–222 ng/g dw)

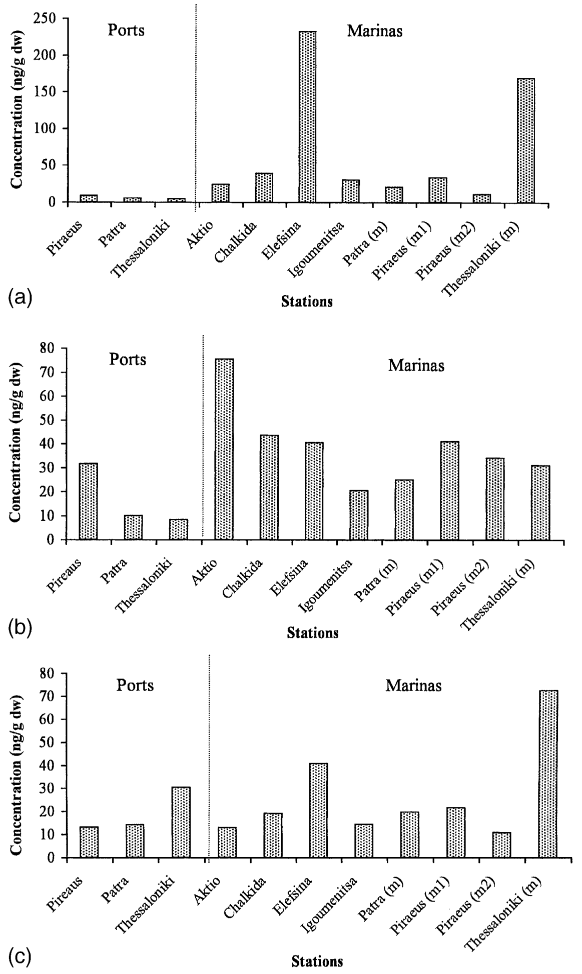

Fig. 3. Mean concentration levels (ng/g dw) of (a) Irgarol 1051, (b) Dichlofluanid and (c) Chlorothalonil in sediment samples from selected Greek ports and marinas.

Table 4
Average concentrations of the antifouling biocides for three sampling periods

Location	Mean[a] of biocide concentration (ng/g dw)		
	October–January	June–September	February–May
Irgarol			
Ports			
Piraeus	10	14	n.d.
Patra	5	7	4
Thessaloniki	n.d.	9	n.d.
Marinas			
Aktio	21	36	6
Chalkida	11	64	17
Elefsina	165	338	91
Igoumenitsa	11	51	10
Patra (m)	11	33	7
Piraeus (m1)	20	43	29
Piraeus (m2)	5	15	12
Thessaloniki (m)	99	249	82
Dichlofluanid			
Ports			
Piraeus	12	49	17
Patra	7	14	7
Thessaloniki	7	13	n.d.
Marinas			
Aktio	52	102	47
Chalkida	23	62	27
Elefsina	35	44	39
Igoumenitsa	8	33	8
Patra (m)	8	39	14
Piraeus (m1)	29	48	40
Piraeus (m2)	18	50	20
Thessaloniki (m)	4	50	20
Chlorothalonil			
Ports			
Piraeus	7	22	n.d.
Patra	9	24	n.d.
Thessaloniki	7	49	17
Marinas			
Aktio	7	22	n.d.
Chalkida	9	33	n.d.
Elefsina	5	73	12
Igoumenitsa	4	26	n.d.
Patra (m)	12	33	n.d.
Piraeus (m1)	7	34	11
Piraeus (m2)	6	18	n.d.
Thessaloniki (m)	25	120	26

and Gough et al. (1994) (<10–132 ng/g dw). Similarly high levels were noted in UK coastal sediments (<1–110 ng/g dw) by Thomas et al. (2000), and in North and Baltic Sea (3–220 ng/g dw) by Biselli et al. (2000). Generally lower values were detected in Lake Geneva (2.5–8 ng/g dw) by Tóth et al. (1996) and in Korlslund (<10 ng/g dw) by Köenig et al. (2000).

3.1.2. Dichlofluanid

The very low water solubility of dichlofluanid and the high octanol/water partition coefficient (2 mg/l at 20 °C and 3.7 respectively) suggest that this biocide probably becomes associated to particulate matter and has the potential to accumulate in sediment. Limited data are known about its environmental fate and effects although it was used extensively on various crops. Dichlofluanid has been sfound to degrade in seawater under natural sunlight as well as in darkness indicating that both photodegradation and hydrolysis of the compound are considerable (Sakkas et al., 2001).

Considerable concentrations of the biocide were observed in sediments. Levels were higher than for the other biocides indicating a tendency to partition to sediments.

Dichlofluanid was found in 86% of the samples and the concentration levels ranged between <8 and 195 ng/g dw with a mean value of 33 ng/g dw. The highest concentration was detected in the Aktio marina despite the high water exchange capacity of this marina. The transport of dichlofluanid residues from agricultural applications to the marine environment is possible since many crops growing areas are situated close to the marina. Chalkida marina had also high concentrations of dichlofluanid due to the low water renewing time as stated already for Irgarol 1051. In the marina of Elefsina similar concentrations were determined showing that its location close to the shipyard is an essential factor for the contamination. The concentrations in the rest of the marinas varied considerably and could not be related to boating activity or water exchange capacity. As far as ports are concerned, the concentration levels ranged from <8 to 90 ng/g dw showing the influence of the high water exchange capacity and the lower density of pleasure crafts.

There are almost no published data available about the presence of dichlofluanid in the marine environment. Concentration levels found in this survey were similar to the levels published by Voulvoulis et al. (2000) for coastal sites, in the Blackwater Estuary, Essex, UK.

3.1.3. Chlorothalonil

Chlorothalonil has been found to degrade after four weeks in natural seawater and even faster in water supplemented by cultured marine bacteria indicating that biodegradation of the compound can be considerable (Callow and Willingham, 1996). Davies (1988) has demonstrated that degradation also occur when the biocide is present in low concentrations. However, chlorothalonil was found at most stations indicating a persistence in sediments. A number of studies have shown that the biocide is more persistant in soils with a half-life of 1–2 months (Davies, 1988; Caux et al., 1996).

Chlorothalonil was found in 78% of the samples analysed. Measured concentrations ranged from <8 to 165 ng/g dw with a mean concentration of 25 ng/g dw. Similar concentration levels were also reported for marine sediments by Voulvoulis et al. (2000). The concentration levels followed the same pattern as for Irgarol 1051 and dichlofluanid. The highest concentration was detected in the marina of Thessaloniki. Transport of chlorothalonil residues from agricultural areas is not likely due to long distances to the marina. On the contrary, in the case of Aktio marina the contribution of agricultural runoff is possible. The concentration levels detected in the survey pose a major risk to biota in the marine environment and especially for sediment living organisms considering the high toxicity of chlorothalonil (Caux et al., 1996).

3.2. Seasonal dependence of biocide concentrations

Monitoring of biocide concentrations was performed monthly from October 1999 to September 2000 in order to gain insight into the seasonal dependence of the biocide concentrations in the sediment. The average concentrations of the sampling periods (October 1999–January 2000, February–May 2000 and June–September 2000) are summarised in Table 4 for Irgarol 1051, dichlofluanid and chlorothalonil, respectively.

The concentrations were higher in June–September period (after paintings use and during the yachting season) with levels peaking in early summer (Fig. 4). A decline of concentration was observed during the winter period, though measurable amounts of biocides remain in the sediment during that time.

This trend is similar to past TBT contamination profiles as well as to previous reported biocide seasonal variations observed in other European coastal sediments (Gough et al., 1994; Biselli et al., 2000; Thomas et al., 2001) suggesting that the use of biocides as constituents in antifouling paints is a major source of these compounds to the Greek marine environment.

However a contribution in the detected concentrations by agricultural runoff cannot be excluded in the case of chlorothalonil and dichlofluanid respectively. As they are applied in spring, runoff is likely to occur up to a month later (in May–June, depending on rainfall),

Table 5
Ranges of Irgarol 1051 in sediment samples, as reported in the literature

Region	Sediment (ng/g)	Reference
UK/Hamble Estuary	<10–132	Gough et al., 1994
UK/Black Water Estuary, Essex	3.3–222	Voulvoulis et al., 2000
UK/Hamble and Orwell Estuaries	<10–1011	Boxall et al., 2000
UK marinas	<1–110	Thomas et al., 2000
CH/Lake Geneva	2.5–8	Tóth et al., 1996
GER/North and Baltic Sea	3–220	Biselli et al., 2000
SWE/Korlslund, Stockholm	<10	Köenig et al., 2000

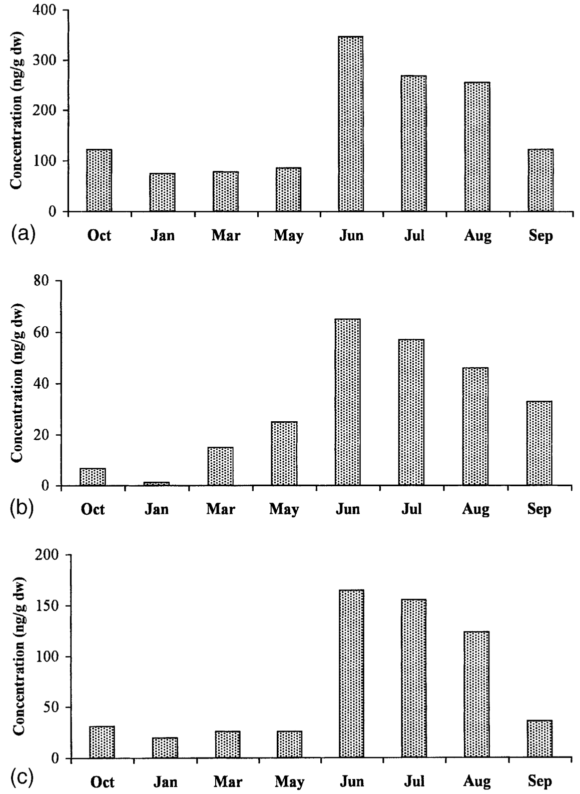

Fig. 4. Monthly time resolution of (a) Irgarol 1051, (b) Dichlofluanid and (c) Chlorothalonil in sediment samples from Thessaloniki marina (Kalamaria).

when there is a pulse of input into the aquatic environment (Flecher et al., 1994). On the contrary, this is not expected for Irgarol 1051, since this compound is not used on crops, as resulting by input data.

The sorption behaviour of the biocides is dictated by a number of physico-chemical parameters in the aquatic environment. Factorial experiment followed with the application of a Mackay fugacity model (Voulvoulis et al., 2002) demonstrated that there is evidence of the influence of total organic carbon on the sorptive behaviour of the biocides, however, further experiments are needed to investigate its relationship to the binding mechanism of antifouling biocides in the natural environment. Sorption appeared to be enhanced by increased suspended matter whilst salinity does not seem to play a significant role. The influence of pH is not clear.

The complete ban on the use of TBT in antifouling paints on all ships planned for the year 2003 will probably result in an increase in the occurrence of these biocides in the marine environment and thus, the environmental risk posed by this increase should be well established. A greater understanding of the long-term fate of these compounds in the environment would facilitate a more comprehensive risk assessment.

4. Conclusions

A survey over a period of one year has been conducted in order to evaluate the present state of accumulation of booster biocides (Irgarol 1051, dichlofluanid and chlorothalonil) in surface sediments from ports and marinas with high boating density in Greece. It was demonstrated that their recent use in antifouling paints on boats could lead to a significant presence in the coastal environment. Sources of these biocides have been identified mainly as antifouling paints but also agricultural inputs were possible in some cases.

Biocides were found in considerable concentrations (up to 690 ng/g dw in the case of Irgarol 1051) in marinas with high boating activity and lower water exchange rates. On the contrary, lower levels were found in ports due to stronger tidal cycles. An investigation of the seasonal dependence of biocide concentration levels showed that higher values were encountered, during the boating season (June–September 2000), while minima were encountered during the winter period. Such a trend reflects the seasonal application of antifouling paints indicating that the main sources of these biocides is their use in antifouling paints. Most of the biocide concentrations found in sediments are not high enough to reach the acute toxicity levels for marine biota but an ecotoxic long-term effect on marine organisms cannot be excluded.

It is suggested that further work need to be conducted to assess the fate of these biocides, in order to ensure that the likely increasing use of organic TBT substitutes does not result a harmful accumulation of these compounds or degradation products in marinas and ports.

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