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Determination of new antifouling agents in seacoasts in Korea by gas chromatography-mass spectrometry

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GC/MS를 이용한 한국연안의 새로운 방오제 분석

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Abstract: Antifouling agents including tributyltin (TBT) compound and its derivatives have been used for many years, but the usage of TBT in Korea was legally restricted in 2003, due to its significant environmental impact. Following this, many new alternative antifouling agents have been used. In this experiment, four major antifouling agents were selectively analyzed to study their release in seawater and tidal flats on the Korean Peninsula. These new antifouling agents were extracted from the seawater and tidal flats using a liquid-liquid extraction method and microwave extraction, respectively. The measured concentrations of Irgarol 1051, Sea-Nine 211, Dichlofluanid and Chlorothalonil ranged from N.D.-23.80 ng/L, N.D.-15.30 ng/L, N.D.-61.69 ng/L and N.D.-4.19 ng/L in the seawater samples and from N.D.-159.45 ng/g, N.D.-476.57 ng/g, N.D.-59.79 ng/g and N.D.-21.27 ng/g in the tidal flat samples, respectively. Interestingly, these new antifouling agents were not detected in any area in the tidal flats at Pusan, whereas a certain amount of them was found in the seawater.

요 약: 부틸주석화합물로(TBT)를 포함한 여러 방오제와 그 유도체 들은 지난 수년 동안 사용되어 왔다. 그러나 이중 특히 환경적으로 심각한 영향을 미치는 TBT의 사용이 한국에서 2003년에 법적으로 사용이 제한 되었다. 이 법적인 제한 이 후 TBT를 대신하여 새로운 방오제가 지금까지 사용 되어 왔다. 본 실험에서는 4가지의 주요한 새로운 방오제가 한국의 바닷물과 갯벌에 얼마큼 용출이 되어 있는지 분석을 하였다. 이 새로운 방오는 바닷물의 경우 액체-액체 방법에 의하여, 갯벌의 경우 마이크로웨이브 방법에 의하여 추출되었다. 바닷물의 경우 Irgarol 1051의 경우 N.D.-23.80 ng/L의 농도로 검출되었으며 Sea-Nine 211의 경우 N.D.-15.30 ng/L의 농도로 검출되었으며, Dichlofluanid 의 경우 N.D.-61.69 ng/L의 농도로 검출이 되었으며, Chlorothalonil의 경우 N.D.-4.19 ng/L의 범위로 검출이 되었다. 갯벌의 Irgarol 1051의 경우 N.D.-159.45 ng/g의 농도로 검출되었으며 Sea-Nine 211의 경우 N.D.-476.57 ng/g의 농도로 검출되었으며, Dichlofluanid 의 경우 N.D.-59.79 ng/g 의 농도로 검출이 되었으며, Chlorothalonil 의 경우 N.D.-21.27 ng/g 의 범위로 검출이 되었다.

Key words : irgarol 1051, chlorothalonil, dichlofluanid, sea-nine 211, seawater, tidal flat, korea

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1. Introduction

Tributyltin compound (TBT) has been used as an antifouling agent for many years in Korea. However, significant concerns were raised about the environmental damage it caused, as a result of which its usage was legally prohibited in 2003. After the official restriction of TBT, the concentrations of butyltin compounds have decreased along the coast, and the usage of new antifouling agents has led to the appearance of these compounds in coastal environments. Approximately eighteen new, tin-free antifouling agents are available on the market and the use of four them has been selectively examined worldwide. Irgarol 1051, which is used as a booster biocide in combination with copper-based marine antifouling agents, has been developed to replace the organotin-based ones. It is a triazine compound that functions by selectively blocking a key step in the electron transportation process in photosystem-II.[1] The new antifouling agent, Sea-Nine 211 biocide, was first introduced in 1996. The active ingredient of Sea-Nine 211 biocide was shown to affect a wide spectrum of bacteria, fungi and algae.[2] Chlorothalonil[3] and Dichlofluanid[4] are also widely used as fungicides in agriculture and are both toxic.

Contaminations resulting from antifouling agents are significant in large harbors in which large ships are anchored. This work is designed to determine the level of contamination resulting from these new antifouling agents in large harbors in Korea. The pollution arising from these new antifouling agents has adverse effects on the marine ecosystem. Contamination occurring particularly in tidal flats requires constant monitoring, because it directly influences marine organisms such as clams and oysters. Tidal flats are horizontal areas that are caused by tiding sand and clay in quiet wave areas. Korean tidal flats are considered to be one of the five major flats in the world. The other major flats are situated on the east coast of Canada, the east coast of America, the coast of the North sea and the Amazon basin. For a long time, tidal flats were considered to be useless land, but they have recently been found to play an important role in the purification and restoration of rivers and seawater, as well as in the control of floods, with the result that they are now considered to be worthy of ecological consideration and preservation. Tidal flats are a particularly important place for living shellfish such as oysters and *Ruditapes philippinarum* as well as shellfish that are gathered for use as food. The pollution of tidal flats is of considerable concern to people who eat these contaminated creatures. Contamination by antifouling agents is known to create a serious problem in the marine ecosystem. In particular, organotin compounds have been reported to induce imposex, which led the Korean government to ultimately prohibit their usage.[5]

Tidal flats can be classified based on the size of the particles as tidal sand flats (size : 200-700 μm, tidal mud flats (<200 μm) and tidal sandy flats. Because fine particles are more capable of adsorbing pollutants than sand size fractions, this study included the investigation of the effect of the particle size.[6] Liquid-liquid extraction (LLE)[7-9] and solid phase extraction (SPE)[10] are the major methods of extracting antifouling agents from seawater. Several methods have been applied to the extraction of antifouling agents from tidal flats, however microwave assisted extraction,[11,12] sonication[13] and Soxhlet extraction[14] have been especially used, since they reduce the working time. Usually GC-MS[6,10] and LC-MS[21,21] are used for their rapid and easy detection. This investigation is the first to attempt to determine the levels of these new antifouling agents in seawater and tidal flats on the Korean coast and the effect of the particle size of the tidal flats on the absorption of these antifouling agents.

2. Experiments and methods

2.1. Chemicals

Dichlofluanid (99%) and Chlorothalonil (98%) were purchased from SUPELCO. Irgarol 1051 was purchased from Ciba-Geigy (UK) and Sea Nine 211 was a generous donation from Rohm & Hass. 9-Bromoanthracene was purchased from Aldrich and used as an internal standard. HPLC grade toluene

was purchased from J.T Baker. These standard materials were used without further purification. Stock toluene solutions containing 1,000 ppm of each new antifouling agent were prepared in a 25 mL volumetric flask and stored at 4°C. The working solutions were prepared daily from this stock solution.

2.2. Samplings

Seawater samples were collected from ten different sites. A total of 49 samples were obtained from fishing harbors (13 sites), ports (33 sites), and bays (3 sites), as illustrated in *Table* 1 and *Fig.* 1. Samplings were carried out in the period of July 18th to 21st of 2006. One liter of subsurface water at a depth of 20 cm was collected in sterilized PE (Polyethylene) bottles. The samples were stored at -20°C.

The tidal flats in Korea are mainly found on the west and south coast. Therefore, the sampling sites

Table 1. Location of the sampling sites and the classification of seawater

Site		Latitude °N	Longitude °E	Date	Classification
Kanghwa	KAs1	37°38′ 21.46″	126°23′ 06.56″	2006.6.21	Fishery harbor
	KAs2	37°40′ 48.12″	126°23′ 55.33″	2006.6.21	Fishery harbor
	KAs3	37°42′ 00.51″	126°22′ 52.26″	2006.6.21	Fishery harbor
Inchon	Is1	37°27′ 12.03″	126°36′ 41.45″	2006.6.21	Port
	Is2	37°27′ 28.28″	126°36′ 04.15″	2006.6.21	Port
	Is3	37°30′ 11.09″	126°38′ 22.78″	2006.6.21	Port
Asan	As1	36°59′ 03.55″	126°49′ 23.02″	2006.6.21	Port
	As2	36°58′ 15.43″	126°49′ 57.59″	2006.6.21	Port
	As3	36°57′ 26.90″	126°50′ 36.49″	2006.6.21	Port
	As4	36°56′ 50.99″	126°48′ 17.83″	2006.6.21	Bay
	As5	36°58′ 58.28″	126°45′ 38.95″	2006.6.21	Bay
	As6	36°53′ 24.63″	126°49′ 35.12″	2006.6.21	Bay
Kunsan	KUs1	35°58′ 38.21″	126°36′ 05.16″	2006.6.20	Port
	KUs2	35°58′ 15.94″	126°37′ 06.73″	2006.6.20	Port
	KUs3	35°58′ 40.99″	126°37′ 30.95″	2006.6.20	Port
	KUs4	35°58′ 58.51″	126°40′ 35.26″	2006.6.20	Fishery harbor
	KUs5	35°59′ 30.33″	126°42′ 46.27″	2006.6.20	Fishery harbor
Yosu	Ys1	34°45′ 09.50″	127°45′ 10.35″	2006.6.20	Port
	Ys2	34°44′ 48.43″	127°44′ 57.63″	2006.6.20	Port
	Ys3	34°44′ 33.90″	127°45′ 25.36″	2006.6.20	Port
	Ys4	34°44′ 17.74″	127°44′ 12.09″	2006.6.20	Fishery harbor
	Ys5	34°43′ 46.63″	127°43′ 37.15″	2006.6.20	Fishery harbor
Masan	MAs1	35°10′ 30.39″	128°34′ 16.74″	2006.6.20	Port
	MAs2	35°11′ 35.39″	128°34′ 16.74″	2006.6.20	Port
	MAs3	35°12′ 37.91″	128°35′ 38.21″	2006.6.20	Port
	MAs4	35°12′ 10.05″	128°35′ 38.42″	2006.6.20	Port
	MAs5	35°10′ 47.21″	128°35′ 33.79″	2006.6.20	Port
Pusan	Ps1	35°04′ 02.31″	128°59′ 44.71″	2006.6.19	Port
	Ps2	35°04′ 57.75″	128°59′ 50.91″	2006.6.19	Port
	Ps3	35°04′ 19.94″	129°00′ 19.47″	2006.6.19	Port
	Ps4	35°04′ 52.80″	129°01′ 34.12″	2006.6.19	Port
	Ps5	35°05′ 38.06″	129°02′ 09.60″	2006.6.19	Port
	Ps6	35°07′ 22.14″	129°04′ 11.79″	2006.6.19	Port

Table 1. Continued

	Site	Latitude °N	Longitude °E	Date	Classification
Ulsan	Us1	35°31′40.80″	129°22′34.28″	2006.6.19	Port
	Us2	35°31′20.28″	129°22′33.70″	2006.6.19	Port
	Us3	35°30′47.59″	129°23′06.73″	2006.6.19	Port
	Us4	35°30′12.06″	129°23′15.66″	2006.6.19	Port
	Us5	35°30′10.13″	129°22′44.35″	2006.6.19	Port
	Us6	35°30′10.88″	129°22′01.91″	2006.6.19	Port
Tonghae	TOs1	37°29′40.17″	129°08′36.00″	2006.6.18	Port
	TOs2	37°29′29.81″	129°08′04.72″	2006.6.18	Port
	TOs3	37°29′27.85″	129°07′52.40″	2006.6.18	Port
	TOs4	37°29′40.04″	129°07′56.61″	2006.6.18	Port
	TOs5	37°30′08.33″	129°07′58.91″	2006.6.18	Port
Sokcho	SOs1	38°12′24.15″	128°35′54.07″	2006.6.18	Fishery harbor
	SOs2	38°12′21.76″	128°35′37.34″	2006.6.18	Fishery harbor
	SOs3	38°12′32.87″	128°35′39.31″	2006.6.18	Fishery harbor
	SOs4	38°12′38.26″	128°35′49.58″	2006.6.18	Fishery harbor
	SOs5	38°12′30.64″	128°36′08.34″	2006.6.18	Fishery harbor

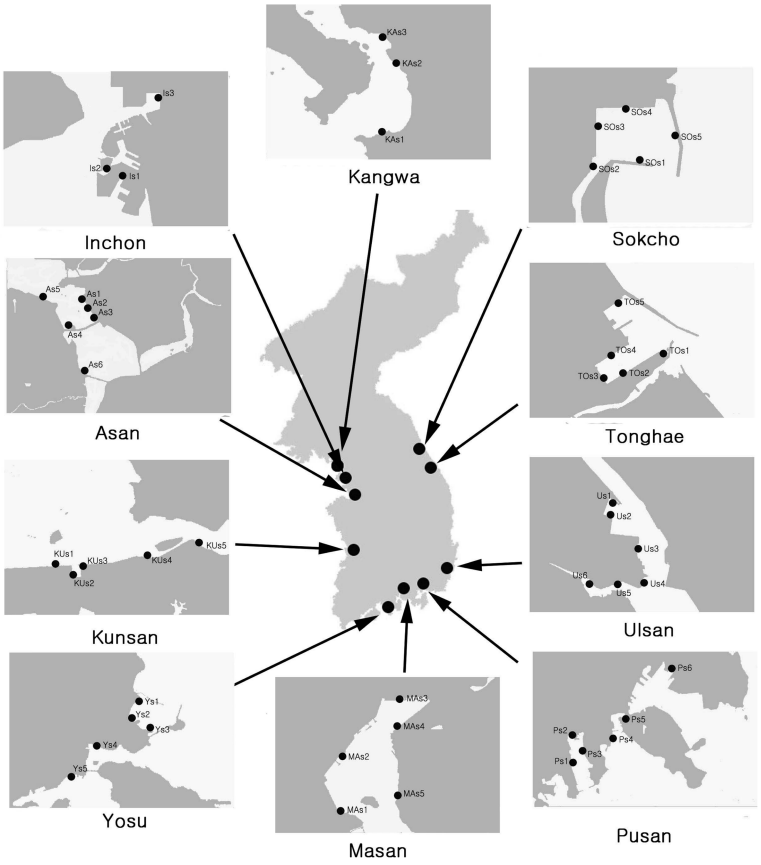

Fig. 1. Map of the sampling sites of seawater along the Korea coast (the 10 main harbors).

Table 2. Location of the sampling sites and classification of the tidal flats

	Site	Latitude °N	Longitude °E	Date	Classification
Incheon	It1	37°29′ 31.78″	126°34′ 48.50″	2006.8.19	Fishery harbor
	It2	37°29′ 43.15″	126°34′ 47.39″	2006.8.19	Fishery harbor
	It3	37°29′ 25.80″	126°34′ 55.16″	2006.8.19	Fishery harbor
	It4	37°29′ 23.16″	126°34′ 54.36″	2006.8.19	Small shipyard
	It5	37°29′ 21.42″	126°34′ 49.67″	2006.8.19	General tidal flat
Jebu Island	Jt1	37°10′ 40.56″	126°37′ 09.61″	2006.8.19	General tidal flat
	Jt2	37°10′ 42.50″	126°37′ 14.11″	2006.8.19	Fishery harbor
	Jt3	37°10′ 41.44″	126°37′ 18.43″	2006.8.19	General tidal flat
	Jt4	37°10′ 45.28″	126°37′ 23.89″	2006.8.19	General tidal flat
	Jt5	37°10′ 32.06″	126°37′ 31.73″	2006.8.19	General tidal flat
Taean	Tt1	36°42′ 01.12″	126°13′ 52.15″	2006.8.22	General tidal flat
	Tt2	36°41′ 42.75″	126°13′ 38.07″	2006.8.22	Fishery harbor
	Tt3	36°41′ 25.42″	126°12′ 32.42″	2006.8.22	Fishery harbor
Kunsan	KUt1	36°00′ 31.37″	126°40′ 33.05″	2006.8.20	General tidal flat
	KUt2	36°00′ 28.32″	126°41′ 15.10″	2006.8.20	General tidal flat
	KUt3	36°00′ 27.09″	126°41′ 44.67″	2006.8.20	Fishery harbor
	KUt4	36°00′ 19.01″	126°42′ 17.86″	2006.8.20	Small shipyard
	KUt5	35°59′ 21.96″	126°41′ 46.22″	2006.8.20	Fishery harbor
	KUt6	35°59′ 33.06″	126°42′ 40.49″	2006.8.20	Fishery harbor
Mokpo	MOt1	34°47′ 29.94″	126°25′ 18.21″	2006.8.21	General tidal flat
	MOt2	34°47′ 32.17″	126°24′ 49.85″	2006.8.21	General tidal flat
	MOt3	34°47′ 42.92″	126°25′ 58.55″	2006.8.21	General tidal flat
	MOt4	34°50′ 14.71″	126°23′ 34.96″	2006.8.21	General tidal flat
	MOt5	34°50′ 48.14″	126°24′ 29.72″	2006.8.21	General tidal flat
Suncheon	SUt1	34°52′ 13.71″	127°29′ 25.45″	2006.8.20	General tidal flat
	SUt2	34°52′ 29.60″	127°29′ 37.32″	2006.8.20	General tidal flat
	SUt3	34°52′ 27.68″	127°30′ 14.82″	2006.8.20	General tidal flat
	SUt4	34°52′ 50.90″	127°30′ 45.55″	2006.8.20	Fishery harbor
	SUt5	34°52′ 26.05″	127°31′ 02.39″	2006.8.20	General tidal flat
Gwangyang	GWt1	34°55′ 56.62″	127°41′ 59.02″	2006.8.20	General tidal flat
	GWt2	34°56′ 10.41″	127°42′ 11.58″	2006.8.20	General tidal flat
	GWt3	34°56′ 10.47″	127°42′ 37.11″	2006.8.20	General tidal flat
	GWt4	34°56′ 29.97″	127°42′ 50.60″	2006.8.20	General tidal flat
	GWt5	34°56′ 45.84″	127°43′ 04.73″	2006.8.20	General tidal flat
Pusan	Pt1	35°03′ 23.61″	128°57′ 23.49″	2006.8.21	General tidal flat
	Pt2	35°03′ 04.34″	128°57′ 31.32″	2006.8.21	General tidal flat
	Pt3	35°02′ 47.66″	128°57′ 55.58″	2006.8.21	General tidal flat
	Pt4	35°02′ 44.54″	128°58′ 12.28″	2006.8.21	Fishery harbor
	Pt5	35°02′ 58.05″	128°58′ 27.35″	2006.8.21	Fishery harbor

in the experiment were selected from the tidal flats of this region. The sampling sites are illustrated in *Table* 2 and *Fig.* 2. The sites of the tidal flats were classified into three types by considering the amount of new antifouling agents released. The first group consists of the tidal flats located in a harbor in which fishery ships are anchored, where can be directly affected by the new antifouling agents. The second group consists of those areas where a repair shipyard is located, viz. It4 in Incheon and KUt4 in Kunsan,

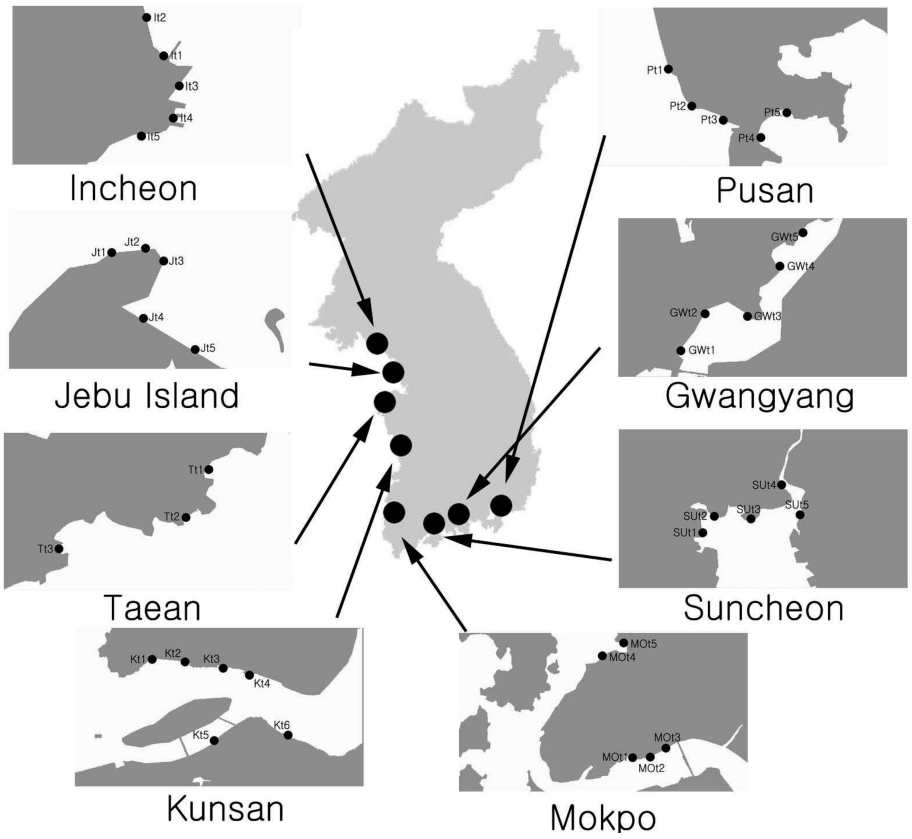

Fig. 2. Map of the sampling sites of the tidal flats along the Korean coast (the 8 main points).

Table 3. Particle sizes of the tidal flat samples

	It5	Jt1	Tt4	Kt3	MOt1	SUt4	GWt1	Pt2
>500 μm(%[a])	0.50	0.23	8.18	3.97	29.78	1.40	23.33	0.00
500-300 μm(%)	2.42	0.60	1.28	5.54	5.15	0.34	5.10	0.00
300-125 μm(%)	25.32	12.49	28.78	18.63	14.54	33.66	14.42	66.85
125-63 μm(%)	69.40	78.03	57.68	68.54	55.19	62.09	54.71	32.45
<63-32 μm(%)	2.37	8.66	4.07	3.32	2.46	2.52	2.44	0.70

a : wt %

and which may be highly contaminated by wastewater. The third group consists of general tidal flat areas where many people gather shellfish. 39 samples were obtained from fishing harbors (13 sites), small shipyards (2 sites), and general tidal flats (25 sites). The sites are shown in *Table* 2 and *Fig.* 2. The samplings were carried out in the period of August 19th to 22nd of 2006. The tidal flats can be divided into three groups based on their size, viz. tidal sand flats, tidal mud flats and tidal sandy flats. The sampling site for the particle size analysis is very important, because the particle size affects the absorption of contaminants. In this experiment, the particle size was classified into 5 groups according to previous reports regarding the analysis of sediment,[6,15] viz. >500 μm, 500-300 μm, 300-125 μm, 125-63 μm, and <63 μm and listed in *Table* 3. A representative point was selected out of the sampling

sites. In most sampling sites, the particle size ranged from 63 to 125 μm, whereas Pusan has a larger particle size of 125-300 μm.

2.3. Extraction

All samples of seawater were passed through a membrane filter (0.45 μm pore size, 90 mm, Advantec). The liquid-liquid extraction procedure was used to extract the four new antifouling agents from the seawater samples. One liter of seawater was transferred to a volumetric flask and 200 μL of the 1ppm internal standard (9-bromoanthracene) (was added?). 1 mL of toluene was added and the solution vigorously stirred at 2,000 rpm for one hour to extract the agents. The agitation was then stopped, the organic phase was transferred into a vial and 3 μL of each aliquot was injected into the GC-MS.

The samples collected in the tidal flats were dried at 80°C in an oven and then pulverized using a rubber hammer. 3 g of the tidal flat powder, 20 mL of concentrated acetic acid and 20 mL of deionized water were added to a Teflon tube and spiked with 50 μL of the internal standard solution containing 10 ppm of 9-bromoanthracene. The sample was inserted into a microwave oven operated at 1,200 W at 100°C for 15 minutes. The sample was cooled down to ambient temperature and centrifuged for 5 minutes at 4,000 rpm. The upper transparent phase of the solution was transferred to a 50 mL volumetric flask and 1 mL of toluene was then added. After stirring for 1 hour, the agitation was stopped, the organic phase was transferred to a vial and 3 μL of each aliquot was injected into the GC-MS.

2.4. Determination of particle size of tidal flats

The tidal flat samples were dried at 80°C in a drying oven and then pulverized using a rubber hammer. A sample of the resulting powder was placed in a sieve shaker and shaken for 20 min. The separated tidal flat component was weighed using a chemical balance.

2.5. Instrumental measurement

The same GC-MS conditions were used for analyzing both the seawater and tidal flat samples. A Hewlett Packard 5890 II GC equipped with an HP 5970 mass selective detector and an Ultra-2 column (crosslinked 5% phenyl-methylpolysiloxane with a length of 50 m, *i.d.* of 0.2 mm, and film thickness of 0.11 μm was used. After tuning the machine, the operating conditions were optimized as follows: injection volume, 3 μL; injector temperature, 300°C; and split time, 80 sec. The oven temperature was programmed to stay at 105°C for 1 min and then ramp up to 180°C at 25°C/min. After staying at this temperature for 1 min, the temperature was ramped up to 230°C at 5°C/min and kept at this temperature for 20 min. The transfer line temperature was set to 280°C. Helium was used as the carrier gas with a flow rate of 2 mL/min. The target and qualifier ions placed with the SIM descriptor are as follows: for Irgarol 1051, *m/z* 253, 238 182 and 111; for Sea-Nine 211, *m/z* 281, 246, 169 and 154; for Chlorothalonil, *m/z* 266, 229, 205 and 168; and for Dichlorofluanid *m/z* 332, 224, 167 and 154; for 9-Bromoanthracene *m/z* 256, 176, and 147. An electron

Table 4. Recovery and detection limits of the selected booster biocides from the spiked seawater and tidal flat samples

	Analytes	Mean recovery (%)	RSD% (n=3)	LOD
Seawater	Irgarol 1051	73.54	1.64	5.39 ng/L
	Sea-Nine 211	80.88	2.84	5.37 ng/L
	Dichlofluanid	93.83	2.34	1.89 ng/L
	Chlorothalonil	120.28	4.87	0.43 ng/L
Tidal flat	Irgarol 1051	102.85	4.06	10.38 ng/g
	Sea-Nine 211	92.53	4.46	11.86 ng/g
	Dichlofluanid	87.51	4.48	5.64 ng/g
	Chlorothalonil	95.13	5.16	11.16 ng/g

multiplier operating at approximately 200 volts was used as the detector. For the acquired particle size data, a sieve shaker manufactured by Jo-il science (model RO-TAP) was used to acquire the sieved material.

2.6. Recovery rates and detection limits

For the seawater recovery analysis, 100 ng/L of the new antifouling agent were added to a standard sample as well as to a real seawater sample containing the matrix. Replicate analyses of the spiked matrices (with n=3) confirmed that adequate precision with good recovery and reproducibility was achieved, and the results are shown in *Table* 4. The mean recoveries (RSDs) for Irgarol 1051, Sea-Nine211, Dichlofluanid and Chlorothalonil were 73.55±1.64%, 80.88±2.84%, 93.83±2.34, and 120.28±4.87, respectively. The detection limits for Irgarol 1051, Sea-Nine 211, Dichlofluanid and Chlorothalonil were 5.39, 5.37, 1.89 and 0.43 ng/L, respectively.

For the tidal flat recovery analysis, 1,000 ng/3 g of the new antifouling agents were added to a standard sample and a tidal flat sample containing the matrix. Replicate analyses of the spiked matrices (with n=3) revealed adequate precision with good recovery and repeatability. The RSDs for Irgarol 1051, Sea-Nine211, Dichlofluanid and Chlorothalonil were 102.85±4.06%, 92.53±4.46%, 87.51±4.48, and 95.13± 5.16, respectively. The detection limits for Irgarol 1051, Sea-Nine 211, Dichlofluanid and Chlorothalonil were 10.38, 11.86, 5.64 and 11.16 ng/g, respectively.

3. Results and Discussion

3.1. Distribution of new antifouling agents in seawater

The measured concentrations of the four new antifouling agents are summarized in *Table* 5. Interestingly, one of the new antifouling agents,

Table 5. New antifouling residues in seawater

Site		New antifouling agents					
		Chlorothalonil	Dichlofluanid	Sea-Nine 211	Irgarol 1051	Total	Classification
Kanghwa	KAs1	1.19	2.83	N.D.	N.D.	4.02	Fishery harbor
	KAs2	N.D.	N.D.	N.D.	N.D .	0	Fishery harbor
	KAs3	N.D.	5.11	N.D.	N.D.	5.11	Fishery harbor
Inchon	Is1	2.77	8.27	N.D.	N.D.	11.04	Port
	Is2	2.22	11.71	N.D.	N.D.	13.93	Port
	Is3	0.60	13.25	N.D.	12.48	26.33	Port
Asan	As1	N.D.	4.30	13.24	N.D.	17.54	Port
	As2	2.07	5.17	N.D.	N.D.	7.24	Port
	As3	0.68	9.76	N.D.	N.D.	10.44	Port
	As4	1.36	3.63	N.D.	N.D.	4.99	Bay
	As5	1.87	2.45	N.D.	N.D.	4.32	Bay
	As6	0.51	4.10	N.D.	N.D.	4.61	Bay
Kunsan	KUs1	0.83	13.03	N.D.	N.D.	13.86	Port
	KUs2	N.D.	5.34	N.D.	N.D.	5.34	Port
	KUs3	N.D.	29.24	N.D.	N.D.	29.24	Port
	KUs4	1.63	12.15	N.D.	N.D.	13.78	Fishery harbor
	KUs5	1.25	4.53	N.D.	N.D.	5.78	Fishery harbor
Yosu	Ys1	1.86	7.69	N.D.	N.D.	9.55	Fishery harbor
	Ys2	N.D.	12.15	N.D.	10.32	22.47	Fishery harbor
	Ys3	0.68	7.88	N.D.	N.D.	8.56	Fishery harbor
	Ys4	N.D.	2.97	15.30	N.D.	18.27	Fishery harbor
	Ys5	N.D.	N.D.	N.D.	N.D.	0	Fishery harbor

Table 5. Continued

Site		New antifouling agents					
		Chlorothalonil	Dichlofluanid	Sea-Nine 211	Irgarol 1051	Total	Classification
Masan	MAs1	1.02	N.D.	N.D.	N.D.	1.02	Port
	MAs2	N.D.	7.27	N.D.	N.D.	7.27	Port
	MAs3	N.D.	12.92	N.D.	N.D.	12.92	Port
	MAs4	1.42	27.31	N.D.	N.D.	28.73	Port
	MAs5	2.50	6.25	N.D.	N.D.	8.75	Port
Pusan	Ps1	N.D.	N.D.	N.D.	6.41	6.41	Port
	Ps2	N.D.	61.69	N.D.	N.D.	61.69	Port
	Ps3	4.19	2.71	N.D.	N.D.	6.9	Port
	Ps4	2.17	7.01	N.D.	11.54	20.72	Port
	Ps5	N.D.	N.D.	N.D.	N.D.	0	Port
	Ps6	N.D.	N.D.	N.D.	N.D.	0	Port
Ulsan	Us1	0.55	2.06	N.D.	N.D.	2.61	Port
	Us2	0.6	18.96	N.D.	N.D.	19.56	Port
	Us3	0.73	8.45	8.13	N.D.	17.31	Port
	Us4	2.55	2.12	N.D.	N.D.	4.67	Port
	Us5	N.D.	5.21	N.D.	N.D.	5.21	Port
	Us6	1.76	3.94	N.D.	N.D.	5.70	port
Tonghae	TOs1	N.D.	3.14	11.018	N.D.	14.16	Port
	TOs2	1.45	3.45	N.D.	N.D.	4.90	Port
	TOs3	N.D.	N.D.	6.00	23.80	29.80	Port
	TOs4	2.89	3.98	N.D.	N.D.	6.87	Port
	TOs5	N.D.	N.D.	N.D.	N.D.	0	Port
Sokcho	SOs1	N.D.	N.D.	N.D.	N.D.	0	Fishery harbor
	SOs2	N.D.	4.84	5.28	N.D.	10.12	Fishery harbor
	SOs3	1.29	5.36	N.D.	N.D.	6.65	Fishery harbor
	SOs4	1	2.98	N.D.	N.D.	3.98	Fishery harbor
	SOs5	N.D.	N.D.	N.D.	N.D.	N.D.	Fishery harbor

Dichlofluanid, was detected at all of the sampling sites of seawater in Korea. When, in the past, only butyltin compounds were allowed to be used, the total concentration of antifouling agents was limited to the summation of the TBT, DBT and MBT concentrations. However, the restriction of butyltin compounds has led to the development of various antifouling agents that are selectively used by ship owners. The total level of antifouling agent contamination can be determined by summing the concentrations of the eighteen different new antifouling agents. However, in the current study, for the sake of convenience, the total concentration of new antifouling agents is defined as the sum of the four major antifouling agents.

Kanghwa has three point harbors located at a narrow current of seawater and the total concentration new antifouling agents in the seawater was N.D. -5.11 ng/L, which was not higher than those in the other sites. Although Incheon has a large port, we were unable to collect samples there owing to the official access restrictions. Therefore, samplings were carried out at the surrounding, nearby harbors. Dichlofluanid was found in all areas at Incheon. The total concentration ranged from 11.04 to 26.33 ng/L, which is higher than those of the other sites in Korea. In the case of Asan having both ports for large ships and a fishery harbor, the total concentration of new antifouling agents in the ports of 7.25-17.54 ng/L was observed to be higher than that of 4.32-4.99 ng/

L in the bay. The Dichlofluanid concentration of the As3 site in Asan was higher than that measured anywhere else in the Asan area. The reason for this is thought to be that the As3 area is situated at the corner of the harbor where the water circulation is not smooth. In the case of Kunsan, the total concentration of agents at KUs1-3 having large ports ranged from 5.34 to 29.24 ng/L, and the concentrations at KUs4 and KUs5 having fishery harbors were 5.78 and 13.78 ng/L, respectively. Therefore, the total concentration at the port sites was higher than that at the fishery harbor sites in the Kunsan area. In Yosu, the Ys2 and Ys4 sites at which small fishing boats are anchored exhibited higher total concentrations, viz. 22.47 and 18.27 ng/L, respectively, than those of the other sites for the same reason as that described for As3 above. Since MAs3 and MAs4 in Masan harbor are located at the edge of the bay, the total concentrations in these areas were quite high, viz. 12.92 ng/L and 28.73 ng/L, respectively, as shown in *Table* 5. In Ps2 at Pusan, the concentration of Dichlofluanid of 61.69ng/L was higher than those of the other sites in Korea. Large ships such as those over 2,000 tons anchored and stayed at this site. High turbidity can be seen even with the naked eye. The highest concentration of Chlorothalonil of 4.19 ng/L was observed at Ps3 in the Pusan area. Although the harbors in Ulsan have large ships, these sites are placed at a specific location where the water circulation is smooth, with the result that relatively low total concentrations were observed compared to those at the other sites in Korea. In Tonghae, the observed Irgarol 1051 concentration of 23.80 ng/L at TOs_3 was higher than those in the other sites in Korea. In Sokcho, because sites SOs2-4 are located at the corner of the harbor, the total concentrations of the new antifouling agents were as high as 10.12 ng/L, 6.65 ng/L and 3.98 ng/L, respectively, as shown in *Fig.* 1 and *Table* 5. In particular, the Dichlorofluanid and Sea-Nine 211 concentrations at SOs_2 were 4.84n g/L and 5.28 ng/L, respectively.

Table 6. New antifouling residues in the tidal flat samples

	Site	New Antifouling agents					
		Chlorothalonil	Dichlofluanid	Sea-Nine 211	Irgarol 1051	Total	Classification
Incheon	It1	N.D.	13.46	N.D.	N.D.	13.60	Fishery harbor
	It2	N.D.	19.15	N.D.	N.D.	19.15	Fishery harbor
	It3	N.D.	N.D.	14.32	42.31	56.63	Fishery harbor
	It4	N.D.	20.00	75.37	24.92	120.29	Small shipyard
	It5	N.D.	N.D.	15.58	33.87	49.45	General tidal flat
Jebu Island	Jt1	N.D.	N.D.	14.79	71.47	86.26	General tidal flat
	Jt2	N.D.	N.D.	12.06	54.48	66.54	Fishery harbor
	Jt3	N.D.	N.D.	19.11	20.13	39.24	General tidal flat
	Jt4	N.D.	N.D.	31.50	159.45	190.95	General tidal flat
	Jt5	N.D.	17.19	N.D.	67.16	84.35	General tidal flat
Taean	Tt1	N.D.	19.29	157.78	25.89	202.96	General tidal flat
	Tt2	N.D.	59.79	N.D.	N.D.	59.79	Fishery harbor
	Tt3	N.D.	23.06	16.32	88.29	127.67	Fishery harbor
Kunsan	KUt1	N.D.	22.86	127.26	N.D.	150.12	General tidal flat
	KUt2	N.D.	37.11	476.57	114.88	628.56	General tidal flat
	KUt3	N.D.	19.67	25.42	14.72	59.81	Fishery harbor
	KUt4	13.09	26.77	36.62	N.D.	76.48	Small shipyard
	KUt5	N.D.	28.49	37.72	84.94	151.15	Fishery harbor
	KUt6	N.D.	N.D.	51.85	N.D.	51.85	Fishery harbor

Table 6. New antifouling residues in the tidal flat samples

Site		New Antifouling agents					
		Chlorothalonil	Dichlofluanid	Sea-Nine 211	Irgarol 1051	Total	Classification
Mokpo	MOt1	N.D.	N.D.	N.D.	66.00	66.00	General tidal flat
	MOt2	N.D.	10.76	13.77	43.90	68.43	General tidal flat
	MOt3	N.D.	9.64	13.97	26.03	49.64	General tidal flat
	MOt4	N.D.	7.52	67.28	N.D.	74.80	General tidal flat
	MOt5	N.D.	N.D.	N.D.	44.96	44.96	General tidal flat
Suncheon	SUt1	N.D.	13.89	20.04	39.58	73.51	General tidal flat
	SUt2	N.D.	9.62	31.94	27.71	69.27	General tidal flat
	SUt3	N.D.	10.77	N.D.	37.89	48.66	General tidal flat
	SUt4	N.D.	0.36	11.91	16.77	29.04	Fishery harbor
	SUt5	N.D.	10.39	N.D.	31.81	42.20	General tidal flat
Gwangyang	GWt1	N.D.	6.92	79.74	38.05	124.71	General tidal flat
	GWt2	N.D.	7.79	34.85	80.44	123.08	General tidal flat
	GWt3	N.D.	N.D.	20.15	85.44	105.59	General tidal flat
	GWt4	N.D.	N.D.	N.D.	114.34	114.34	General tidal flat
	GWt5	21.27	N.D.	N.D.	27.24	48.51	General tidal flat
Pusan	Pt1	N.D.	N.D.	N.D.	N.D.	0	General tidal flat
	Pt2	N.D.	N.D.	N.D.	N.D.	0	General tidal flat
	Pt3	N.D.	N.D.	N.D.	N.D.	0	General tidal flat
	Pt4	N.D.	N.D.	N.D.	N.D.	0	Fishery harbor
	Pt5	N.D.	N.D.	N.D.	N.D.	0	Fishery harbor

3.2. Distribution of the new antifouling agents in the tidal flats

The concentration of Dichlofluanid was dominant for most of the seawater sites. However, high concentrations of Sea-Nine 211 and Irgarol 1051 were observed in the tidal flats, as shown in *Table* 6. The reason why the concentration of Dichlofluanid is low in the tidal flats is that it has a short half-life of 8-86 hours when exposed to sunlight.[16]

The total concentration at It4 in Inchon located at a repair shipyard was measured to be 120.29 ng/g, which is higher than those in any of the other sites in Inchon. In Jt4 in Jebu Island, the Irgarol 1051 concentration was measured to be 159.45 ng/g, which is the highest concentration measured at any of the sites in Korea. At Tt2 in Taean, the concentration of Dichlofluanid was measured to be 59.79 ng/g, which is the highest concentration measured among all of the sites in Korea. The concentration of Sea-Nine 211 in Kut2 in Kunsan located near a port was 476.57 ng/g, which is the highest concentration among all of the sites in Korea. The total concentration (of Sea-Nine 211?) in Mokpo ranged from 44.96 to 74.80 ng/g. The tidal flats in Suncheon are open to the public and are used as a place for ecological area. Although only present in trace amounts, total concentrations ranging from 29.04 to 73.51 ng/g were observed. The concentration of Chlorothalonil in Gwangyang (GWt5) was 21.27 ng/g, which is the highest concentration measured at any of the sites in Korea. The total concentration of new antifouling agents in Gwangyang ranged from 48.51 to 124.71 ng/g. Since the particle sizes in Pusan are bigger than those of the other sites, all of the concentrations measured at these locations were below the detection limits. A detailed explanation for this is given in the following section.

3.3. Particle size and the accumulation of the new antifouling agents

Because fine particles have much more surface area than bigger size particles do, they are more

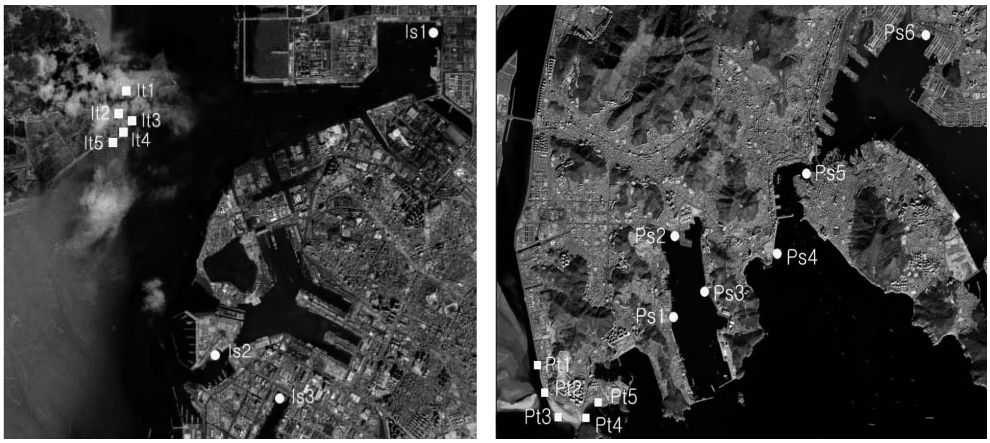

Fig. 3. Sampling sites of seawater(○) and tidal flats(□) at Incheon(left) and Pusan(right).

capable of adsorbing pollutants.[6] Inchon and Pusan are representative harbors in Korea, and the sampling sites of seawater and tidal flats for Inchon and Pusan are shown in *Figs.* 3(a) and 3(b), respectively. In the case of Inchon, the concentrations of Chlorothalonil and Dichlofluanid in seawater were measured to be in the range of 0.60 to 2.77 ng/L, and 8.27 to 13.25 ng/L, respectively, as shown in *Table* 7(a-1). Dichlofluanid, Sea-Nine 211 and Irgarol 1051 were also observed to be in the range of N.D. -20.00 ng/g, N.D.-75.37 ng/g, and N.D.-131.12 ng/g, respectively, as shown in *Table* 7 (a-2) in the tidal

Table 7. Measured concentrations and particle sizes of seawater and tidal flat sample sites

(a-1) Incheon seawater concentrations (ng/L)

Site	Chlorothalonil	Dichlofluanid	Sea-Nine 211	Irgarol 1051
Is1	2.77	8.27	N.D.	N.D.
Is2	2.22	11.71	N.D.	N.D.
Is3	0.60	13.25	N.D.	12.48

(b-1) Pusan seawater concentrations (ng/L)

Site	Chlorothalonil	Dichlofluanid	Sea-Nine 211	Irgarol 1051
Ps1	N.D.	N.D.	N.D.	6.41
Ps2	N.D.	61.69	N.D.	N.D.
Ps3	4.19	2.71	N.D.	N.D.
Ps4	2.17	7.01	N.D.	11.54
Ps5	N.D.	N.D.	N.D.	N.D.
Ps6	N.D.	N.D.	N.D.	N.D.

(a-2) Incheon tidal flat concentration (ng/g)

Site	Chlorothalonil	Dichlofluanid	Sea-Nine 211	Irgarol 1051
It1	N.D.	13.46	N.D.	N.D.
It2	N.D.	19.15	N.D.	131.12
It3	N.D.	N.D.	14.32	42.31
It4	N.D.	20.00	75.37	24.92
It5	N.D.	N.D.	15.58	33.87

(b-3) Pusan tidal flat concentration (ng/g)

Site	Chlorothalonil	Dichlofluanid	Sea-Nine 211	Irgarol 1051
Pt1	N.D.	N.D.	N.D.	N.D.
Pt2	N.D.	N.D.	N.D.	N.D.
Pt3	N.D.	N.D.	N.D.	N.D.
Pt4	N.D.	N.D.	N.D.	N.D.
Pt5	N.D.	N.D.	N.D.	N.D.

(a-3) Incheon tidal flat sieve size (% mass percent)

site	>500 μm	500-300 μm	300-125 μm	125-63 μm	<63-32 μm
It5	0.50	2.42	25.32	69.40	2.37

(b-3) Pusan tidal flat sieve size (%mass percent)

site	>500 μm	500-300 μm	300-125 μm	125-63 μm	<63-32 μm
Pt2	0.00	0.00	66.85	32.45	0.70

flats. In the seawater of Pusan harbor, Chlorothalonil and Dichlofluanid were detected to be in the range of N.D.-4.19 ng/L and N.D.-61.69 ng/L, respectively. However, the concentrations of these two new antifouling agents were below the detection limits in the tidal flats near Pusan port, as shown in *Tables* 7(b-1) and 7(b-2), respectively. This is attributed to the particle sizes of the tidal flats in Pusan. The particle size data obtained from the Pusan tidal flats showed that the major particle sizes ranging from 125-300 μm contained up to 66.85% of the new antifouling agents, as shown in 7(b-3), whereas the major particle sizes in Inchon ranging from 63-125 μm contained up to 69.40%, as shown in 7(a-3). Hence, the results demonstrate that the absorption of the new antifouling agents is dependent on the size of the particles since this affects their surface area.

3.4. Comparison between korea and other countries

The monitoring of these new antifouling agents has been carried out worldwide for many years. The results obtained for seawater are as follows. The concentrations of Irgarol 1051 in the North Sea and Baltic Sea are within 11-170 ng/L and 80-440 ng/L, respectively, as shown in *Table* 8, however a low concentration of N.D.-23.80 ng/L was observed in Korea. Dichlofluanid was observed to be present in the range of N.D.-61.69 ng/L at all sites in Korea, but high concentrations ranging from N.D.-214 ng/L were observed in Piraeus-Elefsina. The concentration of Chlorothalonil found in the Blackwater

Table 8. Comparison of concentrations of new antifouling agents in seawater

	Location	Date	Seawater(ng/L)	Reference
Irgarol 1051	North sea (Germany)	July – September 1999	11 – 170	19
	Baltic sea (Germany)	August 2005	80 – 440	14
	Blackwater Estuary (UK)	June 1999	N.D – 680	17
	This experiment	June 2006	N.D. – 23.80	
Dichlofluanid	Piraeus-Elefsina	October 1999-September 2000	N.D – 214	18
	This experiment	June 2006	N.D. – 61.69	
Chlorothalonil	Blackwater Estuary (UK)	June 1999	N.D – 810	17
	Thessaloniki		N.D – 68	19
	This experiment	June 2006	N.D. – 4.19	
Sea-Nine 211	Danish	January-August 1999	N.D – 283	20
	Catalonia	June 2000	N.D – 3000	21
	This experiment	June 2006	N.D. – 15.30	

Table 9. Comparison of concentrations of new antifouling agents in sediment

	Location	Date	Tidal flat (ng/g)	Reference
Irgarol 1051	North sea (Germany)	July – September 1999	N.D. – 15	19
	Baltic sea (Germany)	August 2005	2 – 80	14
	Blackwater Estuary (UK)	June 1999	N.D – 222.3	17
	This experiment	June 2006	N.D. – 159.45	
Dichlofluanid	Thessaloniki	October 1999 – September 2000	N.D. – 65	22
	This experiment	June 2006	N.D. – 59.79	
Chlorothalonil	Blackwater Essex (UK)	October 1998 – June 1998	16.0 – 34.3	20
	This experiment	June 2006	N.D. – 21.27	
Sea-Nine 211	Catalonia	June 2000	N.D. – 4	22
	Patra	August 2000	N.D. – N.D.	21
	This experiment	June 2006	N.D. – 476.57	

Estuary (UK) was N.D.-810 ng/L, whereas that in Korea was N.D.-4.19 ng/L. The concentration of Sea-Nine 211 was N.D.-283 ng/L in Denmark, whereas that in Korea was N.D.-15.30 ng/L. The results show that Korean seawaters are not as seriously contaminated as those in other countries.

The results obtained for the tidal flats are as follows. Because there are not many tidal flats in other countries to investigate, most studies have focused on the sediment containing soil at the bottom of the harbor. As shown in *Table* 9, the concentrations of Irgarol 1051 in the Blackwater Estuary sediment in England, North Sea sediment and Baltic sediment were within N.D. -222.3 ng/g, N.D. -15 ng/g and 2-80 ng/g, respectively, but that in Korea was within N.D.-159.45 ng/g. The concentration of Irgarol 1051 in Korea is higher than those in the other countries. The Dichlofluanid concentration ranged from N.D. to 65 ng/g and N.D. -59.79 ng/g in Thessaloniki and Korea, respectively. The concentration of Dichlofluanid in Korea was similar to those in the other countries. The concentration of Chlorothalonil ranged from 16.0 to 34.3 ng/g in the Blackwater Estuary sediment and was N.D.-21.27 ng/g in Korea, which is similar to those in the other countries. The concentration of Sea-Nine 211 was as low as 4 ng/g in the other countries, but that in Korea ranged from N.D.-476.57 ng/g.

4. Conclusion

Following the legal restriction imposed on the use of TBT in 2003, the characteristic distributions of antifouling agents near the Korean coast have changed. In this study, the new antifouling agents were successively extracted from seawater by using a liquid-liquid extraction method and from tidal flats by microwave extraction. We were able to detect the new antifouling agents in the coastal regions by using GC-MS. The Dichlorofluanid concentration in the seawater samples was observed to be much higher than those of the other new antifouling agents, while the Irgarol 1051 and Sea-Nine 211 concentrations in the tidal flat samples were observed to be very high. These results indicate that out of the 4 new antifouling agents, Dichlorofluanid was used to a significant extent in Korea, but was easily degraded and therefore detected at a very low concentration in the tidal flats. In comparison with other countries, the seawater in the Korean coastal regions is not seriously contaminated with these new antifouling agents. However, further work will be needed to monitor their levels, in order to improve the coastal environment in Korea.

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