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Determination of Organotin Compounds in Marine Sediments Using Graphite Furnace Atomic Absorption Spectrometry

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Organotin compounds, especially tributyltin, began to cause concern 10 years ago due to a high toxicity towards marine organisms. Several methods of analysing organotin compounds in various matrices have already been developed to determine organotin species simultaneously, but these are quite expensive as special equipment and specialized staff are needed. A simple screening method, which determines the organic tin compounds in the sediment, has therefore been developed and validated. The method can easily be implemented in laboratories accustomed to trace-element analyses; the sediment is extracted by a two-phase extraction and the organic extract is analysed using graphite furnace atomic absorption spectrometry (GF AA.) The screening method has been validated using high-pressure liquid chromatography-inductively coupled plasma mass spectrometry (HPLC–ICP MS).

Keywords: graphite furnace atomic absorption spectrometry; organotin determination; marine sediment analysis; tributylin

INTRODUCTION

Organotin compounds are widely used biocidal agents. They are effective as antifouling agents, e.g. to prevent growth of algae and molluscs on commercial and merchant ships and pleasure craft as well on nets in fish and shellfish farms. Some of the most common organotin compounds in antifouling paints are bis(tributyltin) oxide and tributyltin fluoride. Unfortunately they have severe biological effects on nontarget organisms at very low concentrations.[1] Gibbs *et al.*[2] showed results indicating that some sterile females of dogwhelk *Nucella lapillus* had been exposed to tributyltin levels as low as 1–2 ng Sn l^{-1}.

Tributyltin species introduced to natural waters will mainly be adsorbed onto particles including sediment. Tributyltin compounds have a relatively low mobility in the aquatic environment because of their low solubility in water and high affinity for sediment. Once adsorbed, tributyltin decreases mainly by degradation. The half-time for degradation of tributyltin in seawater at 20 °C is 3–8 days in light and 7–13 days in darkness.[3] It is known that the degradation rate of tributyltin in sediment is slower than in the water column, particularly under anaerobic conditions. In aerobic sediment the half-time has been measured to be between half to one year and about two years in anaerobic sediment.[3,4]

Enhanced concentrations of organotin in the marine environment are still a problem because antifoulants containing it are used on merchant ships, even though it has been banned for use on small ships in many countries. Due to the relatively slow decomposition rate, high concentrations of organotin are expected to be found in the future, especially in harbours and in the surroundings, where high concentrations have already been found. Dumping of dredged material from harbours to less polluted areas will result in increased numbers of contaminated areas in the future.

Many methods have been published for determination of organotins in environmental samples, including sediment. They are usually based on a two phase extraction in which the organotin compounds are extracted into an organic solvent followed by a separation step often including a derivatization procedure and then quantitative determination using techniques such as atomic absorption spectrometry, mass spectrometry, flame photometric detection and electron capture detection.[5–16] These methods are in general quite expensive as special equipment and specialized staff are needed. It is not always possible to estimate the accuracy and recovery of the methods based on the standard addition experiments

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often used for this purpose, but the introduction of certified standard sediment for organotin[14] can facilitate this process in the future.

Our aim was to develop a simple sensitive screening method for routine analysis of the total organic tin concentration in sediments, which can be implemented easily in laboratories accustomed to trace-element analyses.

The sediment is extracted by a two-phase extraction and the organic extract is analysed using graphite furnace atomic absorption spectrometry (GF AA). The screening method has been validated using high pressure liquid chromatography–inductively coupled plasma mass spectrometry (HPLC–ICP MS).

Organic Sn in the text means the amount of extractable tin and the concentrations given are calculated as Sn.

MATERIALS AND METHODS

Glassware

Solvent extraction was carried out in 50 ml glass centrifuge tubes with glass stoppers. All water used was purified in a Millipore quality demineralization system to 18 MΩ. The cleaning procedure for glassware was as follows: rinsing three times with water, soaking for 24 h in 1% EDTA solution, rinsing again three times, soaking for at least two hours in 0.7% HNO_3 solution, and finally rinsing three times with water and drying at room temperature.

Reagents

All reagents were of pro-analysis grade: methanol, iso-octane, potassium dichromate, ammonium dihydrogenphosphate and magnesium nitrate were all from Merck, Germany and palladium nitrate (10 mg Pd ml^{-1}) was from Techno Lab, Norway. The 9.5 M (30%) hydrochloric acid and 14.4 M (65%) nitric acid, both from Merck, Germany, were of suprapur grade. Organotin compounds used for standard solutions and spiking experiments were also of pro-analysis grade: tributyltin chloride and dibutyltin dichloride were both from Fluka, Switzerland, and monobutyltin trichloride was from Ventron, Germany.

Two freeze-dried certified reference sediments, PACS-1 and BCSS-1, from the National Research Council of Canada (CNRC) were used to optimize and validate the method. The certified values of the sediments are given in Table 1.

Table 1 Certified values for organotins and inorganic tin in standard sediments, PACS- 1 and BCSS-1 as Sn

	(mg Sn/kg dry matter)	
Tin species	PACS-1	BCSS-1
Tributyltin	1.27 ± 0.22	Not certified
Dibutyltin	1.16 ± 0.18	Not certified
Monobutyltin	0.28 ± 0.17	Not certified
Inorganic tin	41.1 ± 3.1	1.85 ± 0.20

Apparatus

The sediment samples were analysed using a Perkin–Elmer 5100 PC atomic absorption spectrometer equipped with a 600 HGA 600 Zeeman graphite furnace and a AS-60 autosampler. The instrumental condition and furnace programme are given in Tables 2 and 3 respectively. The screening method was validated using a Perkin–Elmer model Elan 5100 inductively coupled plasma mass spectrometer connected to a Perkin–Elmer model 250 biocompatible binary liquid chromatograph; the analysis parameters for HPLC and ICP MS are listed in Tables 4 and 5 respectively.

Analyses

The extraction method used in this work was based on the method by Siu[14] but some of the extraction parameters have been modified to improve the recovery. The influence of the parameters pretreatment, digestion procedure, amount of extraction solvent, time duration plus intensity of shaking and dilution of solution, were investigated. The certified standard sediment

Table 2 Parameters for the graphite furnace atomic absorption spectrometer

Wavelength	286.3 nm
Irradiation source	Tin electrodeless discharge lamp, 8 mA
Slit width	0.7
Signal	Atomic absorption with background correction
Signal processing	Peak area
Integration time	3 s
Sample volume	15 µl
Alternative volume	5 µl matrix modifier (potassium dichromate)
Inert gas	Argon
Rods	Pyrolytically coated with L'vov platform

Table 3 Temperature programme for the graphite furnace

Step	Temperature (°C)	Ramp time (s)	Hold time (s)	Flow (ml min^{-1})
1	90	3	10	300
2	140	5	40	300
3	500	15	30	300
4*	2100	0	3	30
5	2500	1	3	300

* Signal processing step.

PACS-1 was used in all the experiments unless otherwise stated.

RESULTS

Optimized analytical procedure

The final recommended sample extraction procedure, as the result of the optimization, was as follows. A 1 g portion of freeze-dried sediment was weighed accurately and transferred into a 50 ml centrifuge tube, then 2 ml methanol and 4 ml 9.5 M hydrochloric acid were added to dissolve the organotin compounds. The mixtures were mixed well using a whirl-mixer and the tubes were left overnight with a glass stopper at room temperature. Then 8 ml iso-octane was added and the stoppered tubes were shaken vigorously for 60 min on a mechanical shaker, treated for 30 min in an ultrasonic bath and centrifuged at 2000 rpm for 10 min. Using a pipette, 1 ml of the iso-octane phase was transferred into a 10 ml bottle and the volume was made up to 10 ml with 0.1 M HNO_3 in methanol. The rest of the iso-octane was removed, another 8 ml iso-octane was added and the extraction procedure was repeated as described above. The extracts were stored in the dark in a refrigerator at 5 °C until analysis and all the experiments had been done in duplicate. The samples could be stored at least overnight, as an analysis of the same extracts immediately after the extraction and after 24 h storage gave the same results.

Table 4 Parameters for liquid chromatography separation

Apparatus	Perkin–Elmer model 250 biocompatible binary LC pump with titan tubes and a valve injector (Rheodyne model 7161) with 100 µL loop
Column	10 µM cation exchange column, (Whatman, Partisil SCX, 250 mm × 4.6 mm)
Separation	Isocratic elution at pH 6
Flow rate	1 ml min^{-1}
Mobile phase	0.18 M diammonium citrate in methanol–water (60:40)

Table 5 Analysis parameters of the inductively coupled plasma mass spectrometer

Apparatus	Perkin–Elmer, model Elan 5100 ICP MS
RF power	1300 W
Nebulizer flow rate	0.73 l min^{-1}
Auxiliary flow rate	2.0 l min^{-1}
Plasma flow rate	12 l min^{-1}

Pretreatment

Three natural sediment samples, both wet and freeze-dried, were analysed and the results are given in Table 6. In the sediment sample from location 1 more organotin was extracted from the wet sediment sample than from the freeze-dried sample. This could have been due to inhomogeneity in the sediment sample, caused for example by scraps of antifouling paint used on the boats. Wet sediment samples could vary in water content and they had a tendency to clot; it was also easier to take a homogeneous subsample from a freeze-dried sediment sample. All sediments were freeze dried in the further experiments even though no firm conclusion regarding the influence of the pretreatment of the sediments on the recovery could be drawn on the basis of the few results.

Digestion procedure

The influence on the recovery of varying the ratios of methanol and hydrochloric acid to the amount of sediment was investigated. The highest recovery was achieved when digesting 1 g freeze-

Table 6 Comparison of the organotin content calculated as Sn in wet and freeze dried sediment samples from Skovshoved marina collected on 12 August 1991

	Wet sample (µg org.Sn/g dry matter)		Freeze-dried sample (µg org.Sn/g dry matter)	
Location	Average	SD[a]	Average	SD[a]
1	8.52	4.16	1.25	0.13
2	1.76	0.29	3.82	1.11
3	<d.l.[b]	<d.l.	0.45	0.42

Number of replicates, $n = 6$.
[a] SD, standard deviation. [b] d.l., detection limit.

Table 7 The influence of the extraction parameters on the recovery percent of organotin in PACS-1

Parameter	Test 1	Test 2	Test 3
Wt PACS-1 (gram)	2	1	1
Volume iso-octane	1 × 4 ml	2 × 4 ml	2 × 8 ml
Duration of ultrasound (min)	0	10	30
Recovery (%)	10	45	80

dried sediment with 4 ml hydrochloric acid and 2 ml methanol. Leaving sediment, hydrochloric acid and methanol overnight for 16–18 h resulted in the same recovery as treating the mixture with ultrasound for 1 h. A further increase in the time of ultrasound had no effect on the recovery. The overnight digestion procedure was chosen to save time on the day of extraction.

Using water instead of methanol resulted in a reduced recovery, presumably because of less efficient mixing with the extraction solvent and a change in the equilibrium of organotin between the sediment and the solvent due to the higher solubility of organotin in methanol than in water.

Extraction

The influence on the recovery of the parameters weight of sediment, volume of extraction solvent and duration of ultrasound treatment is shown in Table 7. The recovery increased from 10% using the method described by Siu *et al.*[14] to 45% by extracting 1 g of sediment twice with 4 ml iso-octane followed by 10 min of ultrasound after the mechanical shaking (see Table 7). The recovery was 80% when the volume of extraction solvent was doubled and the ultrasound time was increased to 30 min.

Two shaking techniques for carrying out the extraction were also compared. Vigorous shaking for 3 min on a whirl-mixer or 1 h in a mechanical shaker gave the same recovery. Mechanical shaking for 1 h was preferred, because of the ergonomic aspect.

Matrix modification

Various types of matrix modifiers had previously been used to determine organotin in various samples analysed with GF AA.[2, 10, 11, 13, 15] The efficiency of five matrix modifiers has been compared by analysing tributyltin standards in acidic methanol.

The matrix modifiers were:

M1: Palladiumnitrate (10 mg Pd ml^{-1})
M2: Ammonium dihydrogenphosphate/ magnesium nitrate (3.33 mg ml^{-1}: 0.33 mg ml^{-1}) in 0.04 M nitric acid
M3: 1.36 mM potassium dichromate (0.4 mg Salt ml^{-1}) in 0.29 M nitric acid
M4: 1.2 M nitric acid
M5: 4.8 M nitric acid

In all experiments, 5 µl of the matrix modifier was added to 15 µl of the sample solution. The analysed samples were standard solutions containing various concentrations of tributyltin (0, 5, 10, 50 and 100 µg Sn l^{-1}) in an acidic methanol solution (0.1 M HNO_3 in methanol). The standard deviation of the linear regression and the correlation coefficient are given in Table 8. The matrix modifier 4.8 M HNO_3 gave the greatest sensitivity, but the correlation coefficient was not satisfactory, whereas the use of potassium dichromate gave the lowest standard deviation and the highest correlation coefficient. A standard curve based on three replicates and using potassium dichromate as matrix modifier is shown in Fig. 1.

Standard curves—dilution of solvent

Standard solutions of the same concentrations of tributyltin and dibutyltin dissolved in iso-octane gave various atomic absorption responses using GF AAS. If tributyltin and dibutyltin standards were dissolved in 0.1 M HNO_3 methanol the sensitivity was the same, but it varied when the standards were dissolved in iso-octane (see Fig. 2). Tributyltin dissolved in 0.1 M HNO_3 methanol was therefore used for the preparation of calibration curves in spite of the lower sensitivity compared with iso-octane. It was therefore also necessary to dilute the iso-octane extracts of the sediment samples with acidic methanol solution.

Using only iso-octane as solvent for the organotin compounds might underestimate the concentration in the sample. This is illustrated in Table 9, where the results of four sediment extracts, of either pure iso-octane extracts or extracts diluted in acidic methanol, are analysed against tributyltin standards in the respective solvents. Analysing the pure iso-octane extracts gave concentrations 50% lower than if the same extract were diluted in acidic methanol.

Detection limit

To determine the detection limit, eight samples of certified standard sediment BCSS-1, which has no detectable organotin content, were analyzed. The

Table 8 Comparison of the influence of different matrix modifiers on the absorbance signal of standard solutions in regard to the correlation coefficient (r) and the percentage standard deviation [CV (%)] of the linear regression curve

Matrix modifier	Organotin content (µg org.Sn/l)	CV (%)	r
10 µg $Pd(NO_3)_2$/ml	0	86.8	0.9955
	5	21.7	
	10	0.0	
	50	7.1	
	100	7.8	
3.3 µg $NH_4H_2PO_4$/ml + 0.3 µ $Mg(NO_3)_2$/ml	0	43.3	0.9991
	5	10.8	
	10	9.1	
	50	1.2	
	100	1.4	
1.36 mM $K_2Cr_2O_7$ (0.4 mg salt/ml) in 0.29 M HNO_3	0	24.7	0.9993
	5	0.0	
	10	0.0	
	50	0.0	
	100	1.1	
1.2 M HNO_3	0	32.7	0.9979
	5	29.4	
	10	0.0	
	50	3.7	
	100	7.8	
4.8 M HNO_3	0	8.7	0.9965
	5	9.6	
	10	2.1	
	50	11.5	
	100	3.9	

detection limit, calculated as three times the standard deviation of the eight samples, was 0.050 mg organic Sn/kg dry matter.

Recovery

The recovery of different organotin compounds in pure liquid–liquid extractions was examined. The methanol/hydrochloric acid solution was spiked with either tributyl-, dibutyl- or monobutyl-tin, left overnight and extracted with 8 ml iso-octane using the procedure described previously. It is possible to extract 98% of the tributyltin, 62% of the dibutyltin and 28% of the monobutyltin; the results are listed in Table 10. The recovery of tributyltin spiked with 1 g BCSS-1 was also exa-

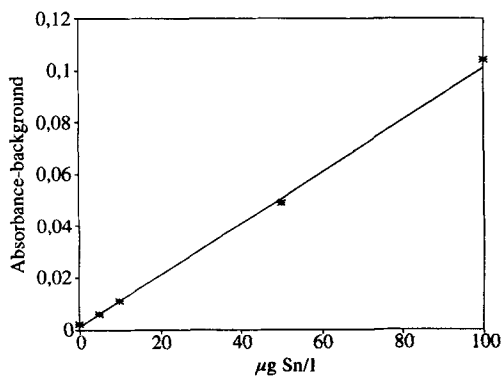

Figure 1 The influence of a potassium dichromate matrix modifier on the absorbance of standard solutions of tributyltin.

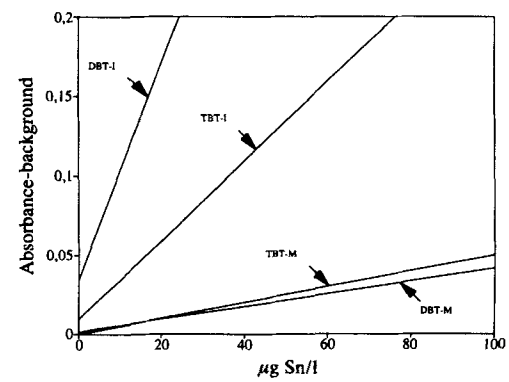

Figure 2 Tributyltin and dibutyltin standards in 0.5% nitric acid/methanol solution (TBT-M and DBT-M respectively) and in iso-octane (TBT-I and DBT-I respectively) using GF AAS

Table 9 Concentrations found in pure iso-octane extracts compared with extracts diluted with 0.1 M HNO_3 methanol (1:10)

Sample	Organotin content (μg org.Sn/g) Iso-octane extracts	Methanol-diluted extract
A	0.60	1.20
B	0.54	1.20
C	0.49	0.80
D	0.52	1.04

mined. The sediment was only spiked with this compound and the spiked sediment was left overnight before the extraction. The recovery of the sediment standard-addition experiments was in agreement with the results found for the liquid–liquid extractions.

PACS-1 was analysed to determine the recovery for the method. The results given in Table 11 show that it was possible to extract 69% of the total certified organotin content from PACS-1. Since the recovery of the liquid–liquid extractions showed that it was only possible to extract 98% tributyltin, 62% dibutyltin and 28% monobutyltin, reduced recovery of organotin from the sediment was expected. In Table 11 the calculated amount of extractable organotin from PACS-1 by the method described is given, using the results from the liquid/liquid extractions. As the calculated recovery is 92% it is reasonable to believe that all the organotins in PACS-1 were dissolved in the acidic methanol.

Only butyltin compounds were detected in the PACS extracts using the HPLC–ICP MS technique for validation. There have not to our knowledge been published any data which disagree with these findings. This is also in agreement with the fact that triphenyltin compounds, for example, were not used frequently in Canada in the

Table 10 Percentage recovery of organotin species extraction from liquid–liquid phase and spiked sediment, BCSS-1

Organotin species and range of amount added	Liquid phase R (%)[a]	s (%)	Spiked BCSS-1 R (%)	s (%)
Tributyltin 0.5–5 μg org.Sn	98 ($n=6$)	4	103 ($n=8$)	11
Dibutyltin 3 μg org.Sn	62 ($n=4$)	12	—	—
Monobutyltin 0.5–3 μg org.Sn	28 ($n=4$)	13	—	—

[a] R, average percentage recovery; n, no. of replicates.

Table 11 Recovery of organotin species extracted from ten replicates of PACS-1

Organotin species	Organotin content (μg org.Sn/g dry matter) Certified	Measured	Calculated[a]
Tributyltin	1.27	—	1.24
Dibutyltin	1.16	—	0.72
Monobutyltin	0.28		0.08
Σ Organotin	2.71	1.87	2.04
Recovery (%)		69	92

[a] Based on the results achieved from the liquid–liquid extractions given in Table 10.

period before the sampling of the PACS sediment.

Precision

The precision of the method was estimated to be 0.13 mg organic Sn/kg dry matter, calculated as the standard deviation of the difference between 12 duplicates of PACS-1 determined during an investigation of the organotin levels in Danish marinas.

Validation using HPLC–ICP MS

To validate the screening method using the GF AA analysis technique, sediment extracts were also analysed for their content of the organotin compounds tributyltin and dibutyltin using HPLC–ICP MS. By this technique it was possible to control which organotin species were present in the extract, as well as whether the screening method would overestimate the organotin content due to the presence of inorganic tin in the sediment. The analysis parameters for HPLC and ICP MS are listed in Tables 4 and 5 respectively. The HPLC parameters are those according to McLaren *et al.*[8] but the ICP MS parameters have been slightly modified with respect to gas flows and RF power. An example of a separation chromatogram for tributyltin and dibutyltin is shown in Fig. 3.

The results of four sediment extractions using the two technique are compared in Table 12. The results are in good agreement, which indicates that the main organotin compounds in the sediment were tributyltin and dibutyltin as well as that the screening method did not overestimate the organotin concentration due to the inorganic tin content of the sediment.

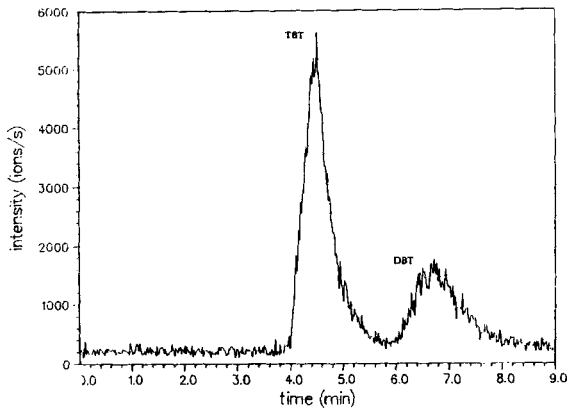

Figure 3 Chromatogram of separation of tributyltin and dibutyltin using liquid chromatography followed by inductive coupled plasma mass spectrometry (HPLC–ICP MS).

Table 12 Comparison of results achieved with GF AA and HPLC–ICP MS

	Organotin content (μg org.Sn/g dry matter)	
Sample	GF-AA	HPLC–ICP MS
Sediment A	2.64	2.08
Sediment B	1.68	1.76
Sediment C	1.36	1.68
Sediment D	1.44	1.28

Analysis of natural sediment samples

To test the method on natural sediment, samples from a marina near Copenhagen (Skovshoved marina) were collected at different dates during the summer of 1991 and analysed. The sediment samples were scraped off by a diver and transferred to plastic bags; 100 g of each sample was freeze-dried immediately after returning to the laboratory and finally sieved through a 2 mm sieve. The rest of the wet sediment was also sieved through a 2 mm sieve and stored in the dark in an acid-cleaned container at 5 °C. Table 13 gives the organotin concentration determined in the freeze dried sediments from Skovshoved marina.

Up to 4.30 mg organic Sn/kg dry matter was found in the organic extract, equal to 10.5 mg tributyltin ion/kg dry matter. Many hydrophobic compounds such as organotin compounds are adsorbed onto the organic fraction of the sediment. Ignition loss can be used as an estimate of the organic fraction. The concentration of organotin is therefore given as a function of ignition loss for locations 1 and 2 in the marina in Fig. 4. The Figure indicates there is a correlation between the ignition loss and the organotin content, as samples with high ignition loss also contain large amount of organotin.

Some of the wet sediment samples were analysed as well (Table 6). From the sample at location 1, larger amounts of tributyltin were extracted from wet sediment than from freeze dried sediment. This effect might due to the removal of porewater during freeze-drying, which may have resulted in the organotin compounds bonding more strongly to the sediment and leading to a lower recovery, or due to the inhomogeneity of the sediment. It is not possible from this study, however, to decide whether freeze-dried samples give lower concentrations than wet samples, because of the high variability and limited number of results. Quevauviller and Donard

Table 13 Organotin content, percentage of dry matter (DM) and ignition loss (LI) in natural sediment samples from Skovshoved marina collected during 1991

Location	Date	Organotin content (μg org.Sn/g DM)	SD	DM (%)	LI (mg/g DM)
1	22 May	1.60	1.32	50	56
	25 Jun.	1.92	1.26	50	58
	24 Jul.	4.30	2.60	54	57
	12 Aug.	1.25	0.13	47	54
2	22 May	0.12	0.14	70	28
	25 Jun.	1.13	0.09	56	46
	24 Jul.	1.13	0.20	53	59
	12 Aug.	3.82	1.11	40	96
3	22 May	0.44	0.20	20	153
	25 Jun.	0.51	0.10	18	207
	24 Jul.	0.36	0.06	22	189
	12 Aug.	0.45	0.42	48	49

No. of replicates $n = 6$.

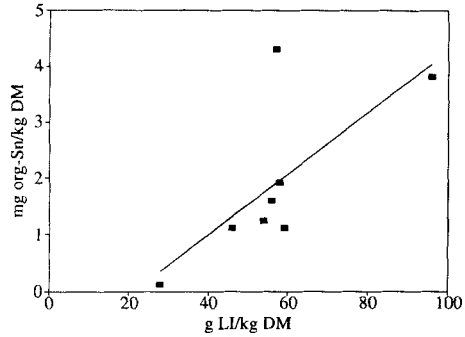

Figure 4 The organotin concentration as a function of ignition loss (LI) at locations 1 and 2.

found that both wet storage and freeze-drying are suitable for preserving tributyltin in sediments but, in general, the mono- and di-butyltin concentrations will change during storage regardless of the storage method.[12]

DISCUSSION

Organotin compounds have a high affinity for sediment, which can complicate the quantitative extraction of each individual compound during an analysis. The extractability probably varies in natural sediments with their character viz. their organic and water contents as well as the age of the sediments. It is possible with the screening method described to recover 69% of the organotin content in a certified standard sedi ment, PACS-1 (see Table 11). It is not possible to recover a greater amount of the total content with this method, because of the limitations of the liquid–liquid extractions, which extract only 98% of the tributyltin, 62% of the dibutyltin and 28% of the monobutyltin. Standard addition experiments show that it is possible to extract all of the added spike of tributyltin from the sediment. The tributyltin content in a natural sediment sample is therefore probably determined quantitatively, while the di- and mono-butyltin contents can be underestimated by up to 50% using this screening method. However, tributyltin is far more toxic to the marine ecosystem than di- and mono-butyltin. An underestimation of the concentration of these compounds is therefore regarded to be an acceptable drawback of a screening method. The detection limit for the method is 0.050 mg organic Sn/kg dry matter, calculated as three times the standard deviation of the organotin concentration of eight samples of sediment with very low organotin content. The precision of the method is 0.13 mg organic Sn/kg dry matter, calculated from duplicate analysis of PACS-1.

The results achieved with the GF AA technique have been validated using the HPLC/ICP MS technique and they gave comparable results. The organotin concentration in a sediment sample determined using the screening method was not overestimated due to the presence of inorganic tin. The main organotin compounds found in the extracts of the natural samples were tri- and di-butyltin.

CONCLUSION

The screening method that has been developed is simple and the equipment is readily available in most laboratories accustomed to trace-element analysis. The method is intended to be used for obtaining an overview of the concentration levels of organotin compounds in sediments, where the most polluted samples can be further investigated by more sophisticated methods.

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