

Occurrence of methylated tin and dimethyl mercury compounds in a mangrove core from Sepetiba Bay, Brazil

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Analyses of organotin and organic mercury compounds have been performed in a sediment core from Sepetiba Bay, Brazil, in order to investigate possible methylation pathways in a mangrove environment. The results have revealed that the physico-chemical conditions existing in this type of environment (high organic inputs, anaerobic conditions, microbial activity, etc.) account for high methyltin concentrations (mono-, di- and trimethyltin) in the sediments, which are dependent upon the total load of metal released (e.g. from anthropogenic sources). Furthermore, the presence of dimethylmercury and not monomethylmercury in the samples demonstrated a new pathway of transformation of mercury in the environment: this compound, thought to be unstable in sediment, is assumed to be stabilized by a conjunction of factors, such as high sulphide levels, anoxic conditions and constant inputs of methane into the medium.

Keywords: Mangrove, methylation, organotins, dimethylmercury, anoxic conditions, methyltins

INTRODUCTION

The natural occurrence of methylation processes for metals such as tin and mercury in the environment has been the subject of numerous controversies in the past few years¹ and the mechanisms of the chemical transformations of the different species are still not well understood.² Methylation processes were first demonstrated, for example in the case of mercury, after enrichment of sedi-

ments with micro-organisms, which led to the assumption that these pathways are controlled biologically.³ This hypothesis has been confirmed for other elements such as tin.¹ Biological methylation may also be controlled by the presence of methyl donor molecules such as methylcobalamin¹ or other methylating agents, e.g. *S*-adenosylmethionine.⁴ However, methylation via physico-chemical routes has also been suspected to occur in the environment due to naturally occurring methylating agents such as methyl iodide⁵ and the degradation products of humic and fulvic acids.⁵ It is now generally accepted that such phenomena may occur in various environmental compartments (water, sediment, biological tissues). Factors affecting extent and rates of methylation in sediment include total inputs of metals,⁶ the organic content,⁷ pH,⁸ redox potential,⁹ temperature,¹⁰ the nature of the micro-organisms present and the sulphide levels.¹¹

Some environments are assumed to favour such pathways, e.g. areas with high organic inputs and anaerobic conditions, such as mangroves. However, methylation processes in such media have never been demonstrated so far. Therefore we have investigated the occurrence of methylated species of tin and mercury in a sediment core collected in a mangrove area from Sepetiba Bay, Brazil, in order to confirm that methylation processes may be an important pathway of metal transformation in organic-rich environments.

MATERIALS AND METHODS

Sampling and sample treatment

A sediment core was collected in a mangrove area in the Bay of Sepetiba located on the south-east coast of Brasil 60 km from the city of Rio de

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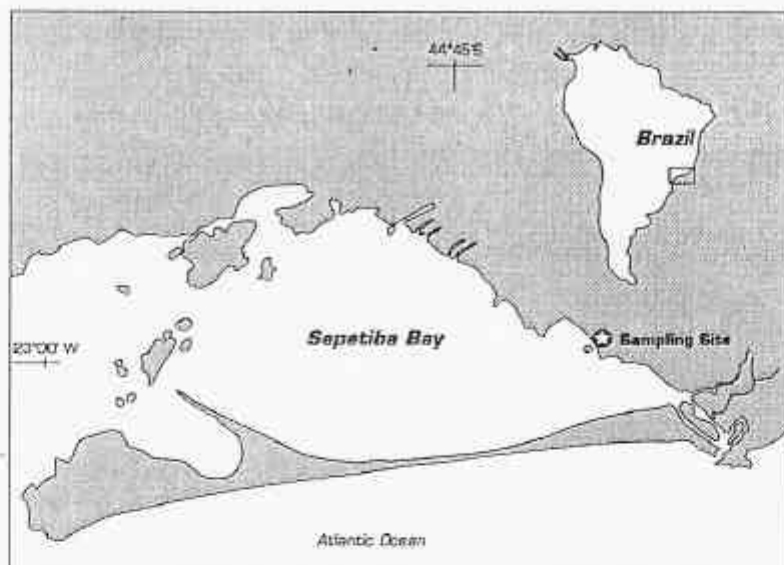


Figure 1 Sample location.

Janeiro (Fig. 1). The study focused on a single site rather than on a wide area as the aim was mainly to investigate the possible occurrence of methylation processes and not to perform a survey of metal concentrations. Special care was taken to perform sampling in an unperturbed zone, i.e. areas with trees such as *Avicenia schaueriana* (with underground roots) were avoided. The sampling location was in a zone of *Rhizophora mangle* of which the roots are aerial. The core was recovered with a gravity corer with PVC jackets and separated in 5 cm slices. Only the central part was sampled to avoid possible contamination from PVC. The samples were immediately frozen and later freeze-dried. This storage procedure was found suitable to preserve the organotin contents, i.e. no degradation or methylation phenomena were found in sediment samples stored in these conditions over four months.¹²

In the case of methylated mercury species, recent experiences have shown that freezing followed by freeze-drying is also suitable to preserve the sample integrity for methyl-mercury in fish-tissues.¹³

Sample pre-treatment and extraction

Trace-metal determinations (zinc, copper, manganese, lead and iron) were performed in order to assess anthropogenic metallic inputs in the area of sampling. The extraction was with 0.1 mol litre⁻¹ hydrochloric acid (1–3 g of sediment sample in 100 cm³ acid, agitated during 20 h); this method is currently used to determine the bioavailable frac-

tion of trace metals.¹⁴ The extraction of organic forms of metals (tin and mercury) was performed at room temperature using analytical-grade pure acetic acid (1 g in 20 cm³) by stirring overnight and agitating ultrasonically during 30 min.

Analyses

Determinations of trace metals were by flame atomic absorption spectrometry (copper, iron, manganese and zinc) using a Perkin-Elmer 420 instrument. Lead contents were determined on dry sediments using X-ray fluorescence with a Phillips PW 1400/1510 apparatus.¹⁵

Organotin and mercury compounds were determined by derivatization with NaBH₄ (5%), cryogenic trapping in a chromatographic column (packed with 60/80 mesh Chromosorb G-NAW coated with 3% SP 2100) and detection in an electrothermally heated quartz furnace by AA (Perkin-Elmer 5000) using an EDL source. To improve the rate of atomization, oxygen and hydrogen are introduced in the quartz cell with respective flows of 20 and 200 cm³ min⁻¹. Details of the analytical procedure are described elsewhere.¹⁶ This technique has been successfully employed by other laboratories both for tin¹⁷ and mercury¹⁸ compounds.

Calibration was by standard addition of the different compounds of interest (mono-, di- and tri-methyltin; mono- and di-methylmercury, and diethylmercury). All the samples were analysed in duplicate.

The particulate organic carbon (POC) content was determined using the Strickland and Parsons method modified by Eteheber.¹⁹ The content of humic acids was determined by spectrophotometry.²⁰ Finally, the sulphur content was determined by X-ray fluorescence.

RESULTS

Geochemistry of the core

The POC and sulphur contents detected were in the range 18–39 mg kg⁻¹ and 6.5–11.2 mg kg⁻¹ respectively (Table 1; Fig. 2a). Table 1 and Figs 2(b) and 2(c) show that anthropogenic inputs of metals gradually increased from the bottom to the top of the core; this was likely to originate from increasing industrial activities in the area of collection.²¹ A good correlation was found between zinc, copper, iron, lead and inorganic tin (TRIT): their coefficients of correlation, *r*, ranged from 0.7 to 0.9 (Table 2).

It was unfortunately not possible to obtain data on the sedimentation rate.

Distribution of the methylated tin species

The distribution of tin species is shown in Fig. 3. The content of total recoverable inorganic tin (TRIT) displayed the same pattern of increase

that was observed for other trace metals, but stabilized afterwards. Mono-, di- and trimethyltin (MMT, DTM and TMT respectively) concentrations appeared quite fluctuating over the core but an overall increasing gradient from the bottom to the top of the core may however be observed. The concentrations of TRIT are generally well correlated with those of DMT and TMT (*r* 0.9 and 0.7, respectively), whereas a lower coefficient was found for TRIT and MMT (Table 3).

No particular relationship could be evidenced between the contents of methylated tin species and POC and sulphur concentrations.

Determination of dimethylmercury

In the procedure used, it was shown that (CH₃)₂Hg⁺ may be determined as CH₃HgH after hydride generation at pH 3.5 in an acetic acid medium (absolute detection limit of 700 pg as mercury, (Fig. 4). It has been observed that methylated forms of tin remained stable for several months in freeze-dried samples and that no new organotin species could be detected.¹² However, both stability of methylated Hg species and absence of methylation of Hg upon storage has not been demonstrated so far. We may only assume that (CH₃)₂Hg has been formed in the medium, was stabilized by freezing and remained stable until the extraction step. (CH₃)₂Hg does

Table 1 Concentrations of trace metals, POC, sulphur and humic acids in the sediment core

Depth (cm)	Zn ^a	Cu ^a	Mn ^a	Fe ^a	Pb ^b	POC ^c	S ^d	HA ^e
0–1	596	5.2	2515	3753	34	35.4	6.48	3.6
1–3	582	3.6	9763	4012	64	33.9	8.56	4.5
3–5	642	5.7	1951	3781	66	31.5	7.81	8.3
5–8	628	4.9	1697	3475	63	28.0	7.76	4.7
8–12	637	2.8	1168	3794	60	31.0	9.13	4.9
12–15	623	3.0	1911	3333	58	33.1	8.39	2.6
15–20	506	3.0	1410	2936	54	36.0	9.01	2.8
20–30	89	0.2	478	2167	22	17.7	9.60	2.0
30–40	47	nd ^f	222	2137	26	37.4	11.15	2.3
40–50	27	nd	125	2025	22	33.7	8.82	— ^g

^a Zn, Cu, Mn and Fe: 0.1 mol litre⁻¹ HCl extraction. The concentrations are expressed in µg g⁻¹.

^b Pb: total content (XRF) expressed in µg g⁻¹.

^c POC: Particulate organic carbon expressed in mg g⁻¹.

^d S: total content (XRF) expressed in mg g⁻¹.

^e HA: Humic acid (total content) expressed in mg g⁻¹.

^f nd: Not detected.

^g —: Not analysed.

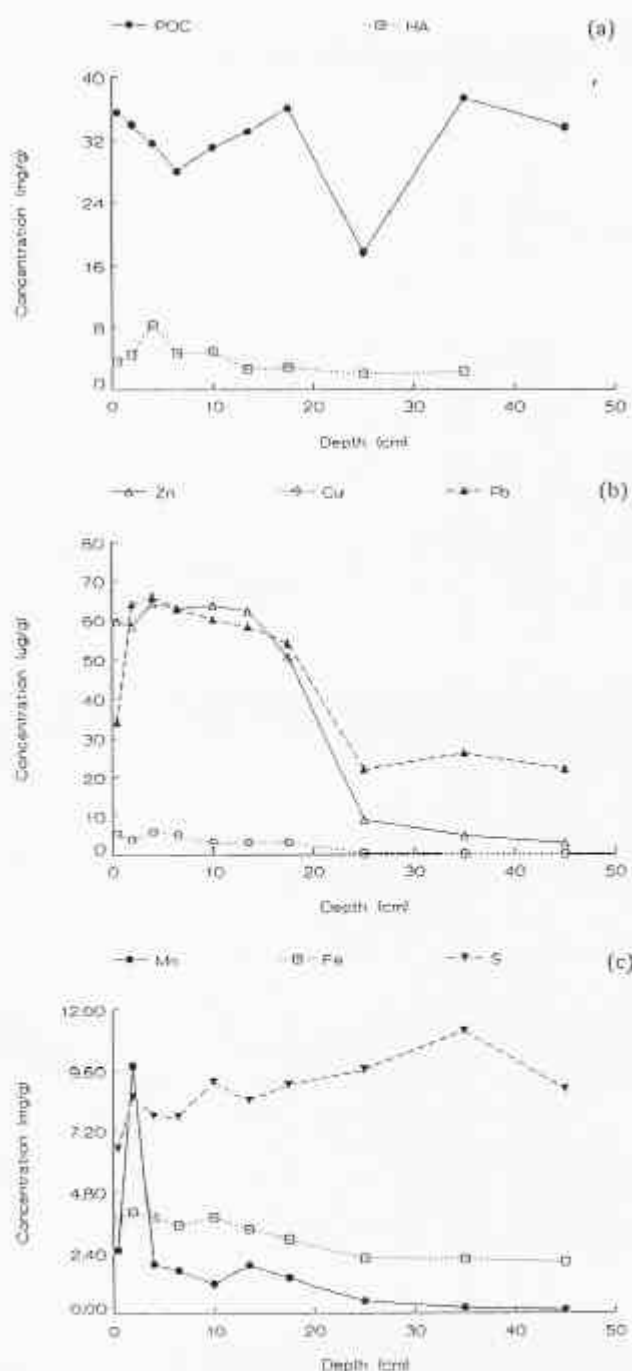


Figure 2 Variation of concentrations along the core: (a) POC and HA (humic acids); (b) copper, lead and zinc; (c) iron, manganese and sulphur.

not react with NaBH_4 and it was assumed that the high production of hydrogen in the reaction flask may favour a rapid sweeping of the volatile mercury compounds to the cryogenic trap without any reduction of these species. The retention times demonstrate this.

In order to verify that transmethylation processes did not occur during the derivatization step, sediment leachates were spiked with $(\text{CH}_3)\text{Hg}^+$ and derivatized. It was demonstrated that no additional amount of $(\text{CH}_3)_2\text{Hg}$ could be detected (Fig. 4). The identification of the compounds was based on their retention times. The detector is of course element-specific.

Stability of dimethylmercury in sediment

The stability of methylated mercury compounds for several months has already been demonstrated to be achieved in fish tissues.¹³ In the case of sediment samples rich in organic matter, it was assumed that the presence of high amounts of humic acids in the medium favoured the stability of $(\text{CH}_3)_2\text{Hg}$.

It was assumed that stable methyl-mercury hydride species could be formed after NaBH_4 derivatization which was confirmed recently by Filipelli *et al.*¹⁸ who showed that this reaction yields unexpectedly stable methyl-mercury hydride with a half-life of ca. two hours.

DISCUSSION

The results demonstrate the occurrence of methylation processes of tin and mercury in mangrove sediments. The concentrations detected for mono-, di- and tri-methyltin are of the same order of magnitude for concentrations already presented in the literature of which some data are presented in Table 4.²²⁻²⁵ Thus, it is confirmed that the methylation of tin is a widely occurring phenomenon which may be enhanced in organic-rich environments.

Mangroves display typical physico-chemical conditions of which the main characteristics are a high organic carbon and sulphur contents and anoxic conditions. Micro-organisms (e.g. methylcobalamin-utilizing bacteria) could account for the high methyltin concentrations found, of which the gradient is closely dependent upon anthropogenic inputs of metals; this observation is confirmed by the good correlation found between methyltin compounds and inorganic tin (Table 2). The maximum rate of methylation processes was found to occur in oxidizing anaerobic conditions,²⁶ which is likely to be the case in mangrove environments.

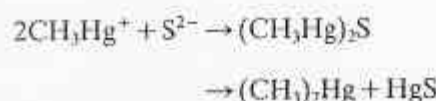
Table 2 Correlation coefficients, *r*, between the different elements and compounds investigated

	Zn	Cu	Mn	Fe	Pb	POC	S	HA	TRIT	MMT	DMT	TMT	(CH ₃) ₂ Hg
Zn	1												
Cu	0.9	1											
Mn	0.4	0.4	1										
Fe	0.9	0.9	0.6	1									
Pb	0.9	0.7	0.5	0.8	1								
POC	0.2	0.2	0.2	0.2	0.2	1							
S	-0.7	-0.8	-0.3	-0.7	-0.4	-0.4	1						
HA	0.6	0.8	0.3	0.7	0.7	0.1	-0.4	1					
TRIT	0.9	0.8	0.3	0.8	0.9	0.2	-0.5	0.5	1				
MMT	0.6	0.8	0.1	0.6	0.3	-0.1	-0.8	0.7	0.4	1			
DMT	0.9	0.9	0.4	0.9	0.7	0.1	-0.7	0.5	0.9	0.6	1		
TMT	0.7	0.7	0.5	0.7	0.8	-0.1	-0.5	0.4	0.7	0.4	0.8	1	
(CH ₃) ₂ Hg	0.6	0.8	0.3	0.6	0.5	-0.4	-0.8	0.7	0.5	0.8	0.6	0.6	1

The presence of dimethylmercury gives evidence for a new possible pathway for the transformation of mercury in the environment. Although this compound was known to be unstable in sediment, its formation and its stabilization could be explained by anaerobic conditions and the high amounts of humic acids which were found to be well correlated with the dimethylmercury content (Table 2). (CH₃)₂Hg⁺ is generally the main product formed in the presence of high concentrations of inorganic Hg(II) ions.¹ However, (CH₃)₂Hg is produced in alkaline anoxic sediments;²⁷ these physico-chemical conditions correspond to those observed in the environment studied.^{28, 29} The (CH₃)₂Hg compound may diffuse out of the sediment and through the water layer to the atmosphere but it may also be stabilized, depending upon the ligand binding.

Different pathways have already been discussed to explain the formation of (CH₃)₂Hg: this

compound could be formed from a further methylation of (CH₃)Hg⁺ in the presence of sulphide ions or hydrogen sulphide by a dismutation process:¹



This phenomenon could be favoured by the presence of sulphate-reducing bacteria which are the principal methylating agents of mercury in anoxic estuarine sediments.¹¹ The formation of (CH₃)₂Hg could also occur through transmethylation processes, e.g. with trimethyltin, with which a correlation coefficient of 0.6 was found (Table 2). This

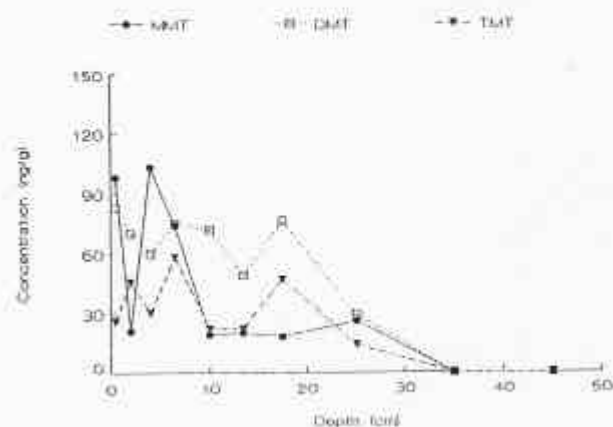
Table 3 Concentrations of methylated tin and mercury compounds along the sediment core

Depth (cm)	TRIT ^a	MMT ^a	DMT ^a	TMT ^a	(CH ₃) ₂ Hg ^b
0-1	164	98	83	26	211
1-3	171	21	70	46	212
3-5	213	103	60	30	278
5-8	153	74	75	58	233
8-12	211	19	72	22	164
12-15	203	20	49	22	187
15-20	239	18	76	47	144
20-30	46	26	29	14	195
30-40	nd	nd	nd	nd	43
40-50	nd	nd	nd	nd	150

^aTRIT: Total recoverable inorganic tin. MMT, DMT, TMT: respectively, mono-, di- and tri-methyltin. The concentrations are expressed in ng g⁻¹ (as Hg).

^b(CH₃)₂Hg: Dimethylmercury, in ng g⁻¹ (as mercury).

^cnd: Not detected.

**Figure 3** Variation of methylated tin compound concentrations along the sediment core.

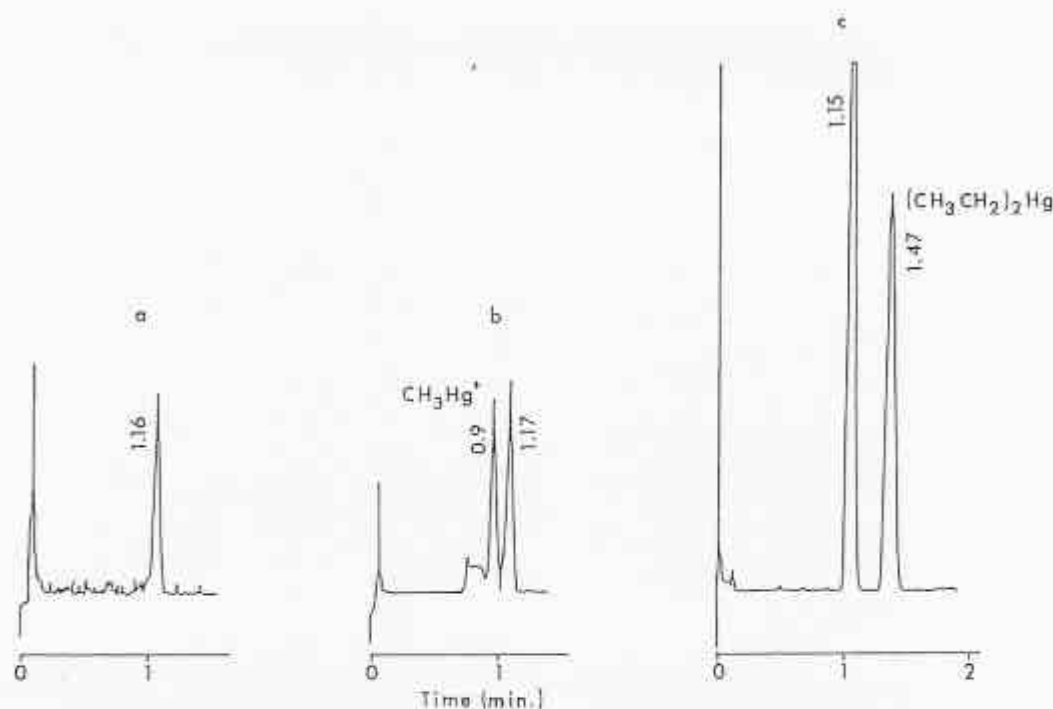


Figure 4 Identification of $(\text{CH}_3)_2\text{Hg}$. (a) $(\text{CH}_3)_2\text{Hg}$ in the sediment leachate; (b) replicate of the sediment leachate spiked with $(\text{CH}_3)_2\text{Hg}^+$; (c) standards of $(\text{CH}_3)_2\text{Hg}$ and $(\text{C}_2\text{H}_5)_2\text{Hg}$ analysed under the same conditions.

pathway is in agreement with the detection of $(\text{CH}_3)_2\text{Hg}^+$ and $(\text{CH}_3)_2\text{Hg}$ in an organic- and sulphur-rich sediment³⁰ containing high amounts of trimethyltin.²⁵

$(\text{CH}_3)_2\text{Hg}$ has been recently found in low-oxygen environments (subthermocline waters) and the authors suggested that anoxic conditions may conduct to the formation and stabilization of alkylmercury species.³¹ Finally, a recent paper suggested that dimethylmercury was actually the original product of mercury methylation and that methylmercury was only a degradation product.³²

It is important to emphasize that *no traces of monomethylmercury* were detected in the sediment core (monomethylmercury spikes were detectable but the method used was not suitable for inorganic Hg). This observation leads to two possible explanations for the presence of $(\text{CH}_3)_2\text{Hg}$ in the mangrove sediment: (1) the total amount of CH_3Hg has been methylated to form $(\text{CH}_3)_2\text{Hg}$; or (2) $(\text{CH}_3)_2\text{Hg}$ was originally present in the sediment and no demethylation processes occurred. The first hypothesis is unlikely since the methylation of CH_3Hg would not be likely to

Table 4 Concentrations of methyltin compounds in some coastal environments (ng g^{-1} , as tin)

Sample	Location	MMT	DMT	TMT	Ref.
Water	Great Bay	65	37	9	20
	Carthage	252	75	96	21
Algae	Great Bay	52	47	1	22
Mussel Reef	Sado Est.	nd-7	nd-10	nd-23	23
	Providencia	15-129	13-223	nd-21	21
	San Andrés	39-127	30-117	5-40	21
	San Andrés	2427	499	100	21
Sediment	Great Bay	nd-80	nd-49	nd	20
	Sado Est.	nd-21	nd-160	nd-120	23

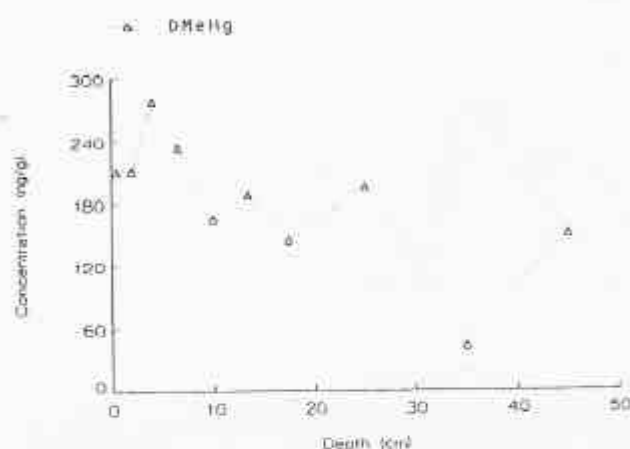


Figure 5 Variation of $(\text{CH}_3)_2\text{Hg}$ along the sediment core.

occur with a rate of 100%. However, the second hypothesis is in agreement with the results of Wood *et al.*,¹² assuming that this compound is the original methylation product and is not formed by a further methylation of CH_3Hg .

All the preceding remarks highlight the fact that a series of factors, such as anaerobic conditions, high sulphide and methyltin contents, are met in typical systems such as mangroves which could explain the formation of dimethylmercury. These conditions may also allow the stabilization of this compound: e.g. by possible binding with, for example, humic acids.

Further investigations should be carried out to understand better this new pathway of transformation of mercury in anaerobic environments such as mangroves. Laboratory experiments and field applications would be of paramount importance to assess the chemical reactions, extent of stabilization and volatilization, and possible toxic impacts of methylated tin and mercury compounds.

CONCLUSIONS

This study demonstrates the occurrence of methylation processes of tin and mercury in organic-rich sediments. Mangroves display particular physico-chemical conditions which account for the high methyltin concentrations found in a sediment core. Furthermore, new possible pathways are demonstrated by the presence of dimethylmercury, which could be the main methylation product of mercury in mangrove sediments and would not be due to a further methylation of

CH_3Hg as previously demonstrated in other sediment environments. This compound would be stabilized in the presence of high contents of humic acids, sulphur and anaerobic conditions. These processes illustrate the importance of speciation to understand the geochemical pathways of metals in the environment.

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