APPLIED ORGANOMETALLIC CHEMISTRY, VOL. 8, 665–676 (1994)

Study of the Distribution of Methylmercury and Total Mercury in Grain Size Fractions of Freeze-Dried Estuarine Sediment Samples

A. M. Carro, E. Rubi, M. H. Bollain, R. A. Lorenzo and R. Cela*
Departamento de Quimica Analítica, Nutrici6n y Bromatología, Facultad de Quimica, Universidad de Santiago de Compostela, Avda de las Ciencias, 15706 Santiago de Compostela, Spain

A study of the total mercury and methylmercury distribution in esturine sediments is presented. Core sediments obtained in a polluted area were freeze-dried and sieved to obtain four fractions. Fraction 1, having grain size <0.30 mm; Fraction 2, having grain size <0.30 mm; Fraction 3, with grain size <1 mm and Fraction 4, having particle size >1 mm. In all these fractions total mercury (Cold Vapor AAS) and methylmercury (Gas Chromatography with ECD detection) have been analyzed. As expected high concentrations of total mercury and small concentrations of methylmercury were obtained in Fractions 1 and 2 but were not detected in Fractions 3 and 4, although the study of the data obtained suggests that significant amounts of geochemical mercury remain in these fractions. Attempts have been made to correlate data for both species when freeze dried sediments and the raw wet samples were analyzed.

Keywords: Capillary gas chromatography, mercury, methylmercury, core sediments, grain size, analysis

INTRODUCTION

Marine sediments on the coast near large industrial and urban areas are typically polluted by heavy metals, which may be present at amounts several times higher than their natural background level.[1] In the biosphere, mercury and its compounds undergo many interconversions that could be induced by chemical, photochemical or biochemical processes. In soils, sediments and aquatic systems, microbial pathways are of great significance. In this sense, the formation of methylmercury by microorganisms is probably the most important biological process.[2] A dynamic equilibrium exists between methylation and demethylation in aquatic systems and sediments. The greatest potential for mercury methylation has been observed in surface sediments that are in contact with the overlying water phase; it decreases in sediment cores with depth.[3]

Numerous authors have pointed out the importance of sediment characteristics as well as seasonal and other environmental conditions in mercury levels and mobility in polluted esturaries.[4] Also, much work has been carried out in order to standardize analytical operations in sediments[5] for a variety of heavy metals, including mercury. Most studies conclude that grain size is one of the most important factors in controlling the distribution of natural and anthropogenic trace metals in sediments.[6] In most estuarine and coastal sediments, fractionation of the particles accounts for increasing heavy-metal concentrations with decreasing grain size, because of their association with fine-grained clay minerals and organic matter. As a result, it is very difficult to make direct comparisons of sedimentary trace-metal concentrations without some prior normalization for the grain size effect. The mud fraction (<63 μm) is accepted as the most convenient for these purposes for several reasons:[7,8] one is that natural and anthropogenic heavy-metal inorganic and organic carriers are present as, and/or associated with, clay and silt size particles and that this fraction is mostly equivalent in size to the material carried in, and deposited from, suspension.

However, little information is available about the binding of methylmercury to sediments. It is known that clays,[9] humic acids[10] and sulfides[11] show high adsorption capacity for methylmercury. Some results have been obtained on frozen raw sediment samples,[12-14] but clearly freeze-dried samples are much more convenient to store, any sedimentary reference material has to be pre-

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* Author to whom correspondence should be addressed.

CCC 0268-2605/94/070665-12

Received 21 February 1994
Accepted 15 July 1994

Table 1 Grain size distribution of the sediment samples analyzed

Fraction	Grain diameter (mm)	Average weight (% of whole sample) Sediment	Mud
1	<0.060	1.2±0.5	31.5±8.1
2	<0.30	15.0±10.3	46.9±5.3
3	<1	44.3±10.8	0.15±0.05
4	>1	39.5±18.1	0.06±0.09

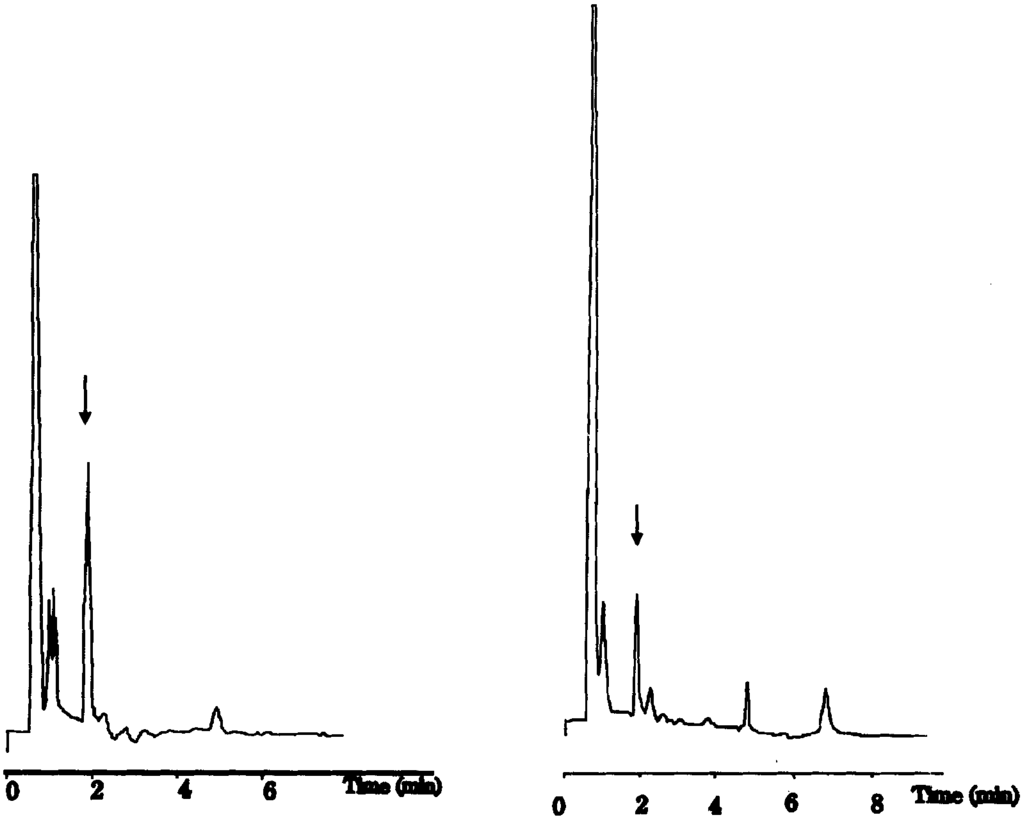
pared in this way and the decision has to be taken about the most convenient particle size. However, we have not been able to find references for studies on the distribution of methylmercury in grain fractions of freeze-dried sediments.

Here we present the results of a study on the distribution of total and methylmercury in several grain fractions of freeze-dried estuarine polluted sediments.

EXPERIMENTAL

Sampling

Sediment samples were taken in the estuary of Pontevedra, Spain, at 11 sampling locations near a chloralkali factory waste dump covering an area of about 0.3 km^2 with the intention of obtaining samples with different pollution levels. Comparative samples of sediments were taken from the opposite margin of the estuary, assuming that this area was not polluted by mercury discharges. Sediment samples were collected in cores 50 cm long and 5 cm in diameter, and immediately frozen. Five mud samples taken from the deep channel of the estuary were also analyzed. Once in the laboratory, they were divided in two parts. One of these was maintained frozen and then analyzed as wet raw sediment. The other was freeze-dried and then sieved in order to obtain the fractions shown in Table 1. Values in this table correspond to the average weight of each



(a) (b)

Figure 1 Chromatograms of (a) a standard solution of 20 ng ml^{-1} methylmercury and (b) a toluene extract from a sediment sample (<0.060 mm).

Table 2 Optimal CV AA conditions for the determination of total mercury

Reagent: 10% $SnCl_2$ in HCl	5 ml
Argon flow-rate	5–6 l min^{-1}
Reaction time	1 min
HCl concentration	1 M

fraction and the standard deviation of the 23 samples. These data were used to refer concentration values obtained in each fraction to the overall concentration in the sediment and compare the results with those obtained for the raw wet samples.

Geochemistry of the sediments at sampling locations

Geochemical data of the estuarine sediments in the sampling locations were available.[15] Clay and mud fractions of these sediments are basically micaceous–kaolinitic including variable quantities of 2:1 integrated (mica–vermiculite, chlorite–vermiculite and hydroxyaluminic vermiculites) and gibbsite.

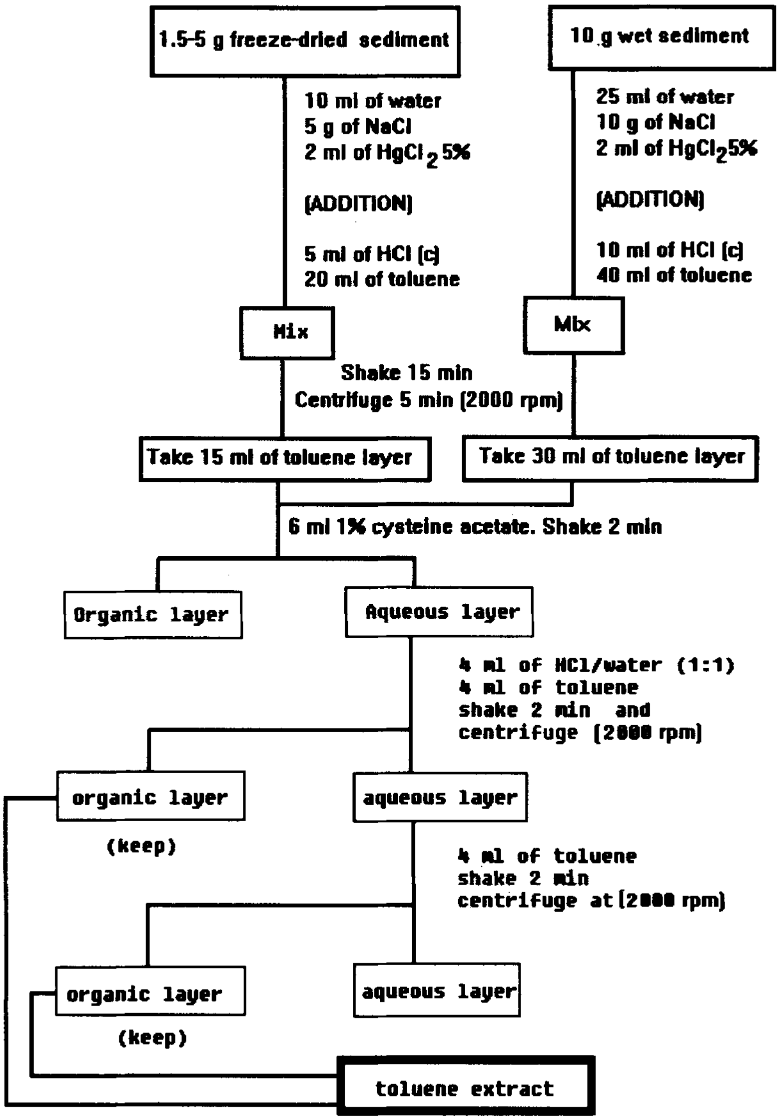


Figure 2 Extraction procedure.

Table 3 Summary of the results obtained for the analysis of sediment samples

Sample 1	Total Hg (μg g^{-1}) in Fraction 1 2	Total Hg (μg g^{-1}) in Fraction 2 3	Total Hg (μg g^{-1}) (dry samples) 4	Total Hg (μg g^{-1}) (water content corrected) 5	Total Hg (μg g^{-1}) (wet raw samples) 6	Methyl-mercury In Fraction 1 (μg g^{-1}) 7	Methyl-mercury in Fraction 2 (μg g^{-1}) 8	Methyl-mercury (dry samples) (ng g^{-1}) 9	Methyl-mercury (water content corrected) (ng g^{-1}) 10	Methyl-mercury (wet raw samples) (ng g^{-1}) 11
11	4.90	0.81	0.172	0.142	0.11	0.265	0.034	8.08	6.65	6.00
21	3.60	0.87	0.129	0.108	0.20	0.115	0.011	2.39	2.00	6.00
21	4.10	1.83	0.157	0.134	0.33	0.192	0.046	4.72	4.02	4.20
31	4.60	2.07	0.352	0.287	0.33	0.084	0.021	4.19	3.41	nd
32	3.70	0.60	0.344	0.291	0.20	0.086	0.008	5.25	4.46	4.20
32	2.60	0.96	0.246	0.193	0.45	0.075	0.020	5.28	4.16	3.20
41	5.10	1.23	0.242	0.194	0.31	0.067	0.011	2.53	2.03	7.00
23	2.70	0.87	0.096	0.081	0.14	0.100	nd	0.57	0.48	nd
33	4.35	1.33	0.980	0.782	0.59	0.150	0.021	16.10	12.86	nd
43	0.92	nd	0.008	0.005	0.17	0.055	nd	0.50	0.28	2.80
12	3.37	3.26	0.563	0.468	0.14	0.116	0.041	8.64	7.20	nd
12	1.02	0.43	0.060	0.050	0.20	0.040	nd	0.49	0.40	nd
28	3.42	1.11	0.079	0.064	0.41	0.085	nd	0.40	0.33	nd
53	nd[a]	nd	nd	nd	nd	0.075	nd	0.39	0.32	nd
70	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
10	nd	nd	nd	nd	nd	nd	0.012	4.49	1.24	2.80
20	nd	nd	nd	nd	nd	nd	nd	nd	nd	3.70
30	nd	nd	nd	nd	nd	nd	nd	nd	nd	2.70
10	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
L2BZ	3.09	1.99	1.825	0.574	0.78	0.040	0.027	19.49	6.13	6.40
L3AZ	3.12	3.01	2.525	0.476	0.68	0.039	0.032	23.33	4.40	7.30
L1BZ	3.53	3.19	2.511	0.567	0.70	0.028	0.025	15.55	3.51	6.20
LZ	1.96	1.41	1.276	0.322	0.59	0.029	0.025	17.06	4.31	7.60
L3BZ	2.12.	3.31	2.453	0.482	0.57	0.023	nd	8.30	1.63	5.40
L2AZ	2.65	1.59	1.294	0.469	0.49	nd	nd	nd	nd	7.00

[a] Abbreviation: nd, not determined.

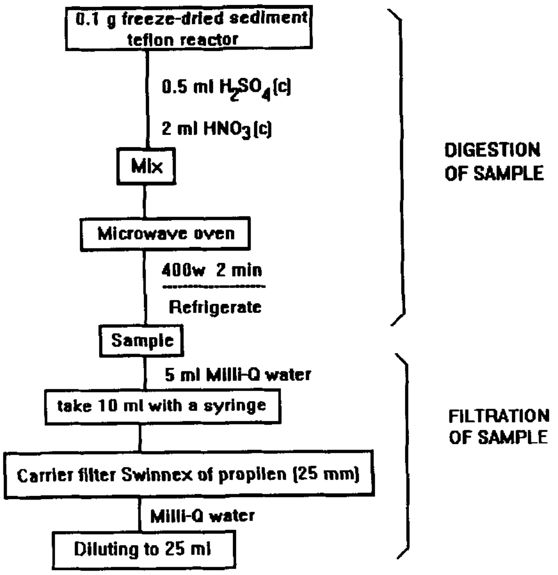


Figure 3 Digestion of filtration procedures.

Apparatus

Determination of methylmercury

The analyses of toluene extracts were carried out using an HP Model 5890A gas chromatograph, equipped with a nickel-63 electron capture detector. Data were acquired using an HP integrator Model 3396A. The chromatographic column used was an SGE (Australia) BP-1 (methylsilicone chemically bonded) fused silica 25 m in length and of 530 μm i.d. The column conditioning basically resembled the procedure proposed by O'Reilly,[16] except for the working temperature. According to our experiments,[17] if the treatment was carried out 25 °C above the temperature used in separation, the conditions were more efficient, the system became cleaner faster and the effect of the treatment lasted longer. Chromatographic conditions have been published elsewhere.[17] Chromatographic and other experimental details can be found in Ref. 18, in this issue.

All reagents used were of analytical-reagent grade and solvents were of spectroscopic or HPLC quality. The working solutions were prepared from a stock solution of 1 g l^{-1} mercury in

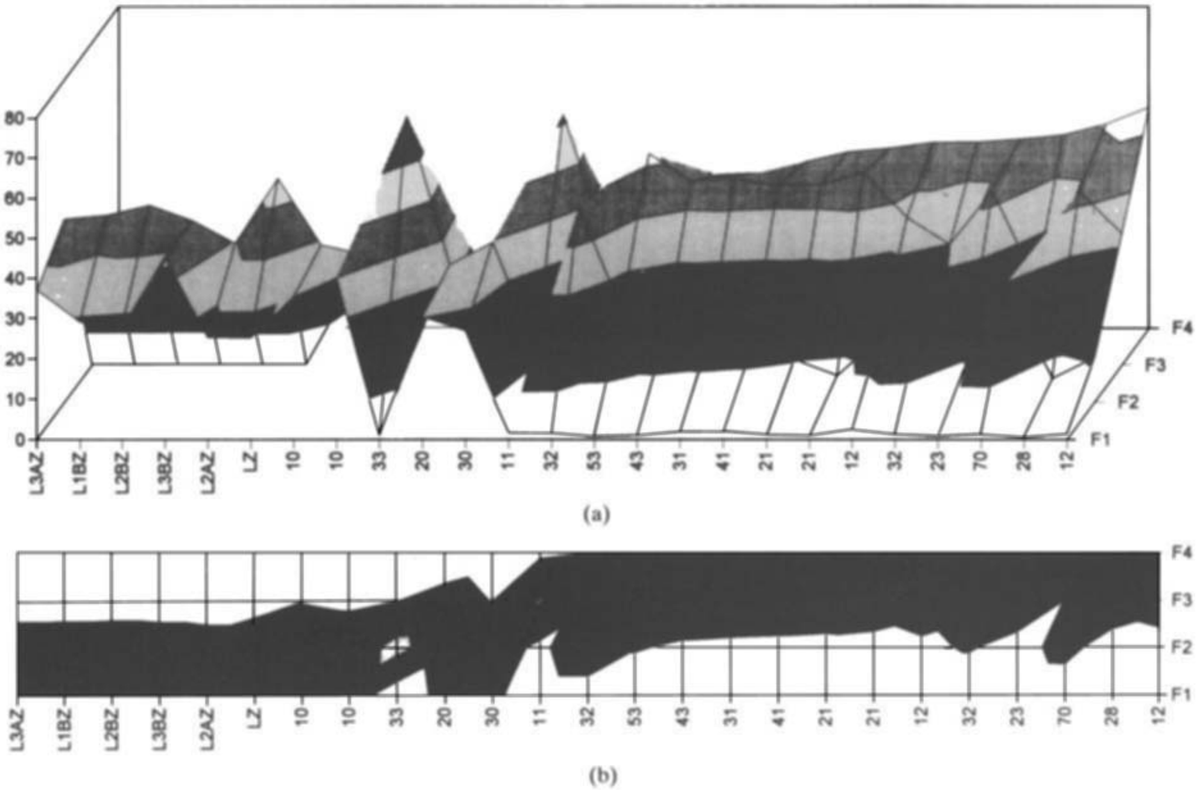
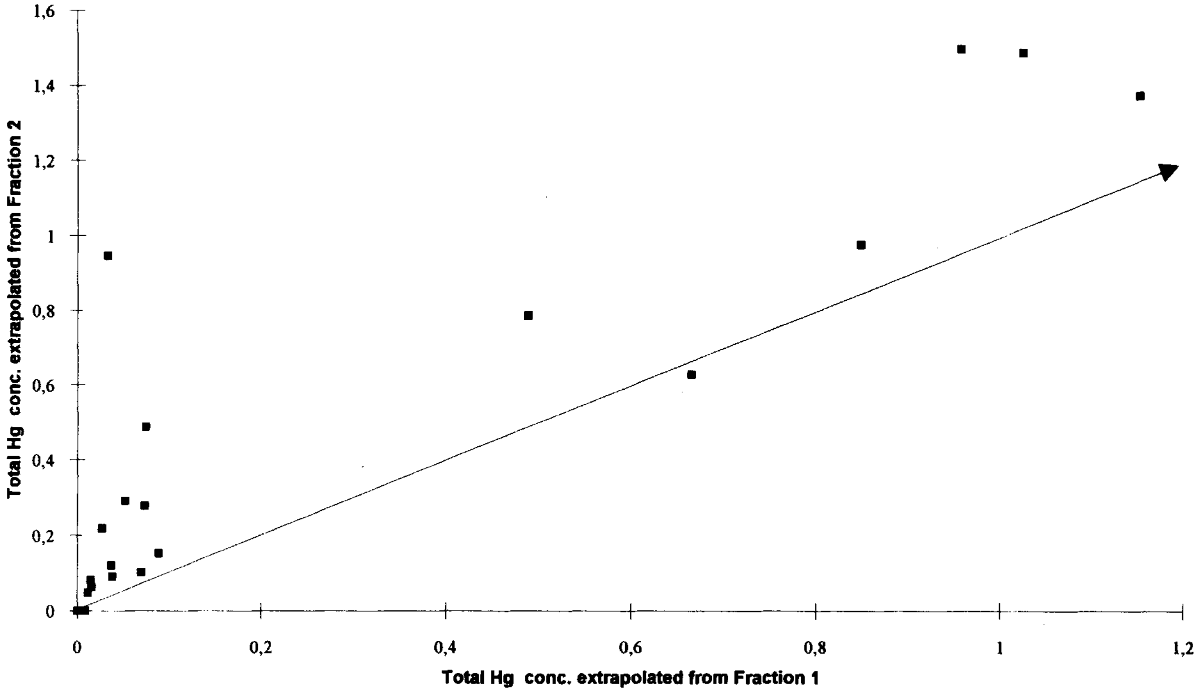
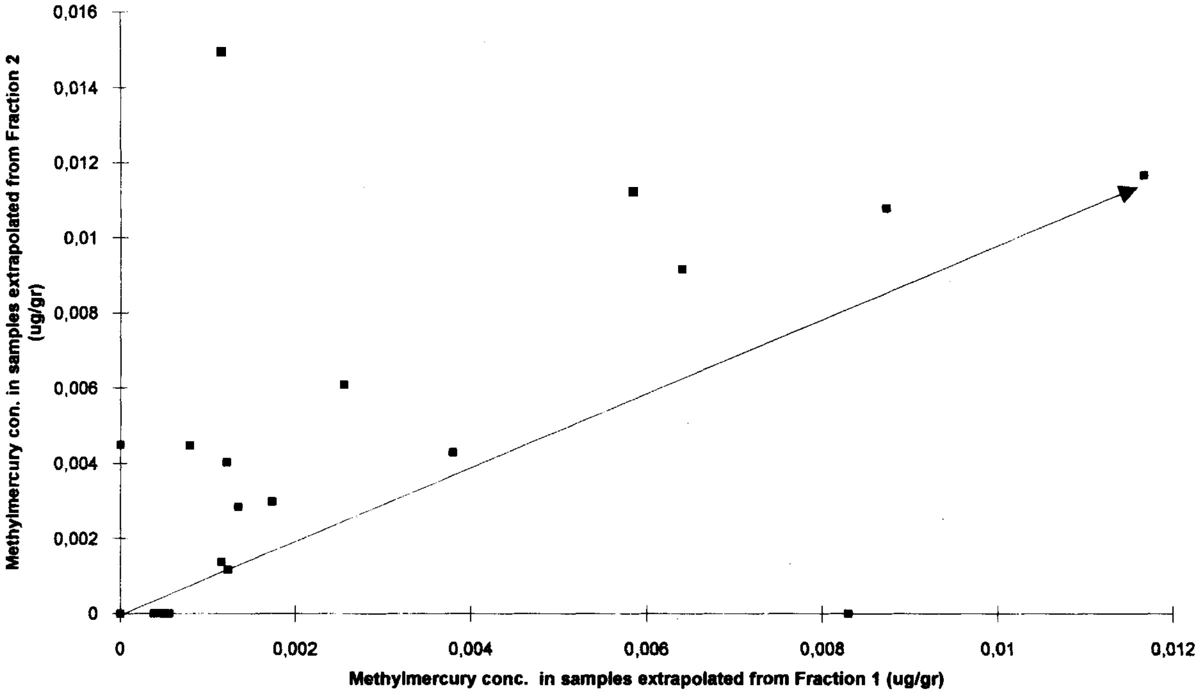


Figure 4 Proportion of each size fraction in the samples: (a) ; (b)

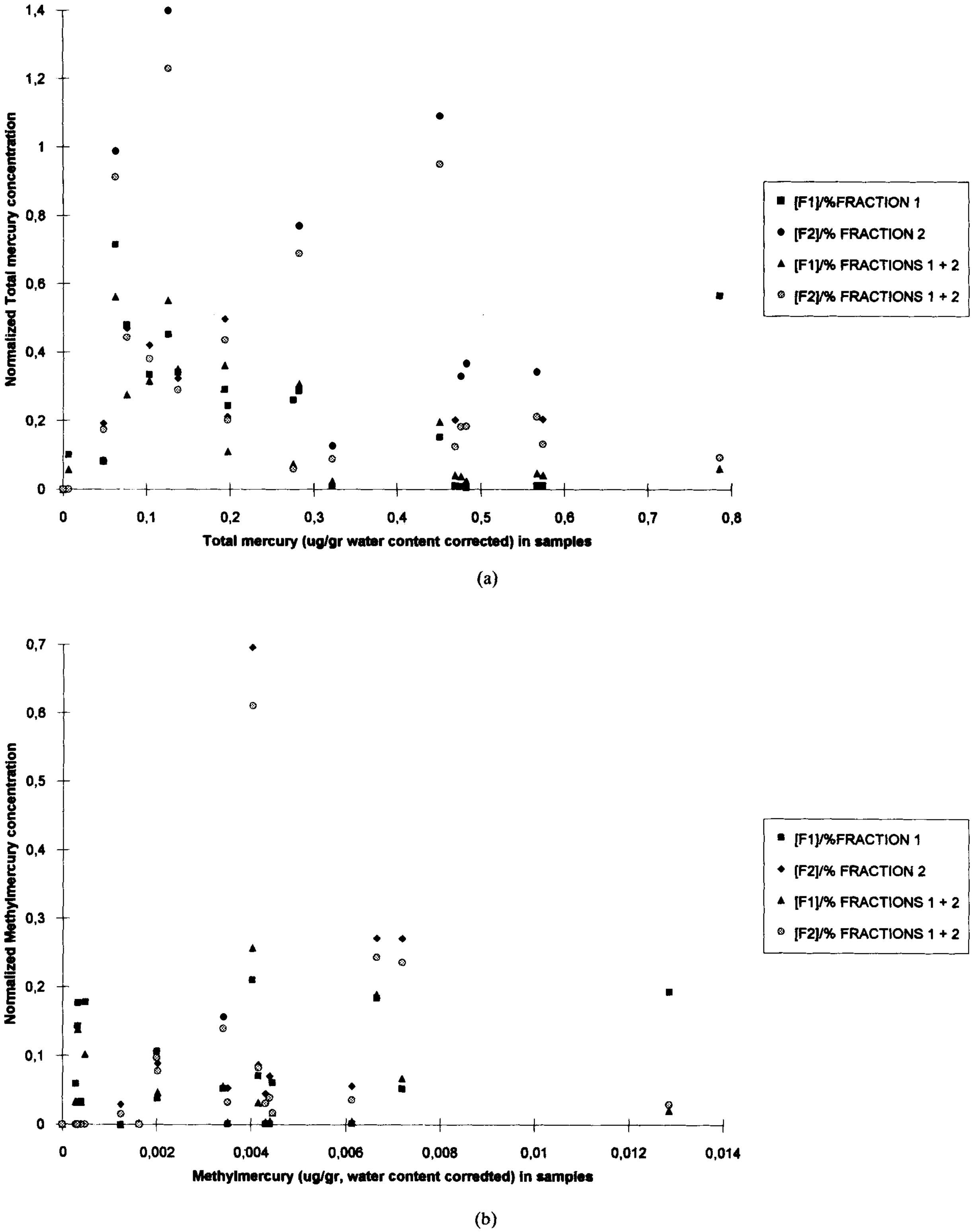


(a)



(b)

Figure 5 Comparisons of the concentrations extrapolated from Fractions 1 and 2: (a) total mercury; (b) methylmercury.



(a)

(b)

Figure 6 Correlation of normalized values with total values: (a) total mercury; (b) methylmercury.

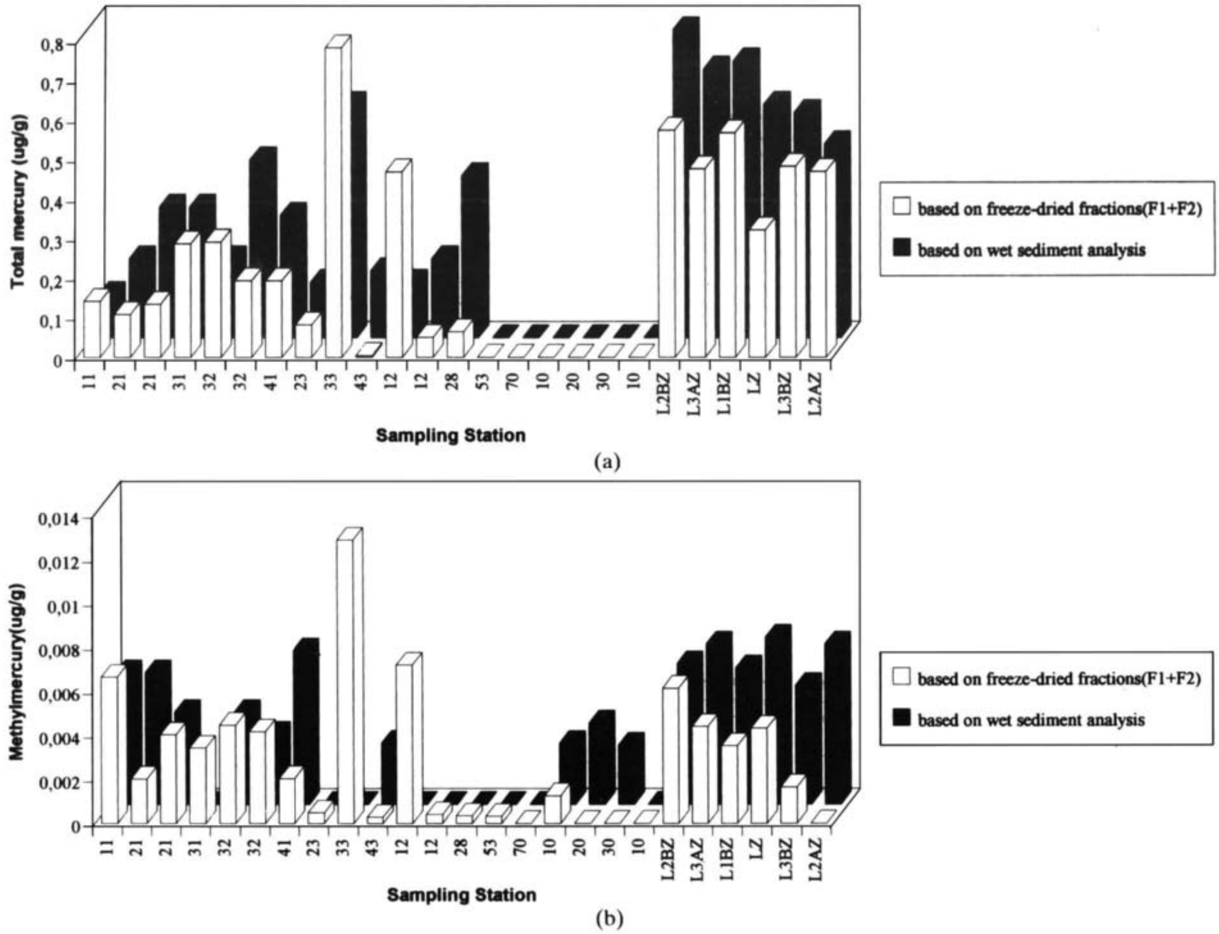


Figure 7 Comparison of analysers of wet raw sediments with those of freeze-dried samples: (a) total mercury; (b) methylmercury.

toluene (prepared from methylmercury chloride; Merck). These solutions were kept in the dark and refrigerated (4 °C) when not in use. The column-treatment solution used was 1% mercury(II) chloride (Merck) in toluene.

Calibration curves were prepared at four levels in the range of 10–40 ng ml^{-1} of methylmercury in toluene. Figure 1(a) shows the chromatogram of a standard solution of 20 ng ml^{-1} methylmercury and Fig. 1(b) shows the chromatogram of a toluene extract from a sediment sample (fraction < 0.060 mm). Under the conditions established, the recovery of methylmercury from the sediments was 67%. Considering sample preparation stages and the method used, the reproducibility of the procedure was acceptable (S.D. 0.016). Under optimum chromatographic conditions, the detection limit found was 5 ng ml^{-1} expressed as mercury.[19] The performance of the determination technique was verified with DORM-1 Certified Reference Material (from the National Research Council of Canada). This verification included the whole experimental procedure in biological samples. However, this verification was not possible for sediment analysis owing to the lack of Certified Reference materials for methylmercury.

Determination of total mercury

All measurements were made using a Perkin–Elmer model 5000 atomic absorption spectrophotometer, equipped with an electrodeless mercury discharge lamp (Perkin–Elmer) tuned at the 253.7 nm line under background correction. The spectrometer was attached to a cold-vapor automatic device (Thermo Jarrell Ash Corp.; model ILAVA). The system was fully automated. Table 2 shows the optimum working conditions for the cold-vapor device. All the reagents used were of analytical or Suprapur (acids)

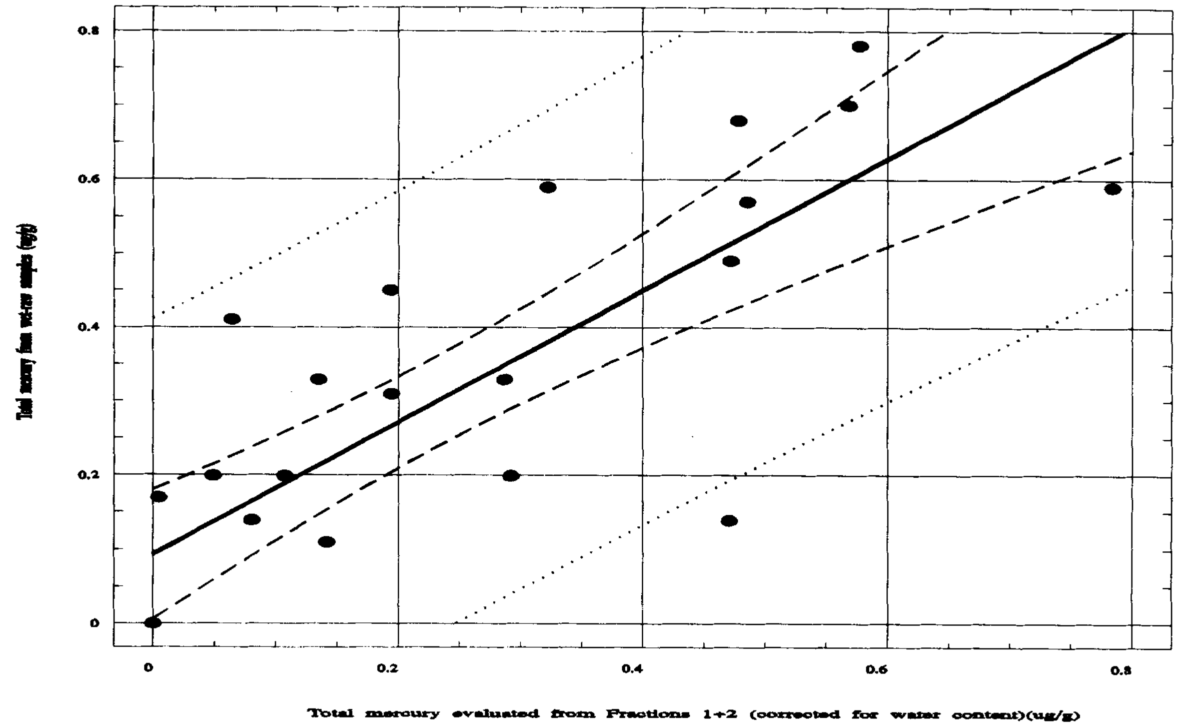


Figure 8 Correlation of wet raw data with extrapolated analyses for Fractions 1 and 2.

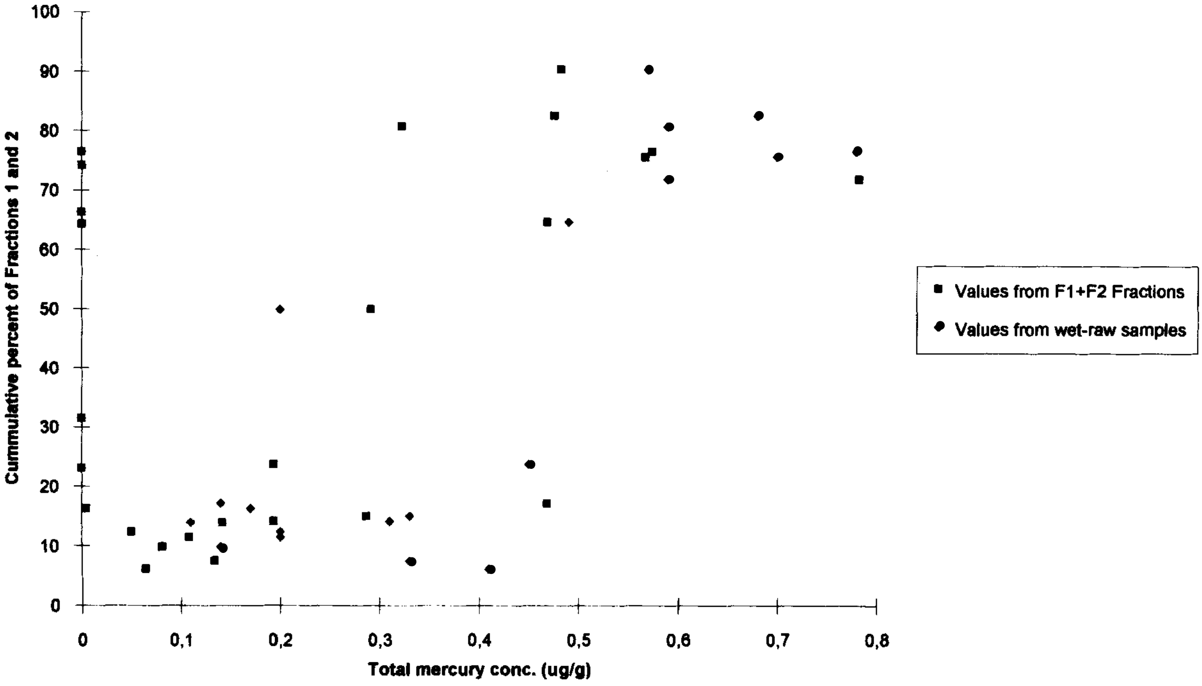


Figure 9 Correlation of total mercury with percentage of Fractions 1 and 2.

grade. Calibration curves were obtained daily by using standard solutions of mercury (5, 7.5, 10, 15 and 20 ng ml^{-1} levels) in 1 M hydrochloric acid. The validation of the experimental procedure was carried out by means of PACS-1 Certified Reference Material (from the National Research Council of Canada).

Procedure for the analysis of samples

Determination of methylmercury

Samples were prepared following the method proposed by Olson and Cooper.[20] Methylmercury was extracted with toluene from each fraction of the sediment. Figure 2 depicts schematically the procedure used.

Determination of total mercury

Samples were digested with a mixture of nitric and sulfuric acids in microwave pressure pumps (Parr; 45 ml). After cooling, the pumps were opened and the sample diluted with water and filtered through 5 μm pore-size Nylon membrane filters. Figure 3 shows the two phases of the procedure used.

RESULTS AND DISCUSSION

All the measurements were carried out at least in duplicate on the different fractions of the sieved sediments in order to study methylmercury and total mercury distributions. Results are expressed in all cases as mercury. Table 3 summarizes the results obtained. Figure 4 depicts the proportion of each size fraction in the samples. Data have been sorted in ascending order of percentage of Fraction 4. This means that sandy sediments appear on the right side of the graph. It can be seen that samples cover a wide range of particle size distribution, from those with a high proportion of very fine particles to those in Fraction 1 representing a negligible proportion. According to these data, it has to be expected that samples having higher proportions of Fraction 1 show higher concentrations of mercury and, perhaps, methylmercury. As we will see later, this assumption is probably true for total mercury but not for methylmercury.

In Table 3 it can be seen that in most cases mercury as well as methylmercury has been detected in Fractions 1 and 2. In no case did Fractions 3 or 4 give detectable amounts of mercury. For sediment samples where mercurial species were detected (samples from locations 11–28 in Table 3), noticeable differences appear between the concentrations found in Fractions 1 and 2. In fact, the concentration of mercury in Fraction 2 is on average about three times less than the concentration found in Fraction 1. In the case of methylmercury, the average ratio is about 1:7 (concn in F1 divided by concn in F2 fractions). However, in this type of sample, when the relative percentages of each fraction over the total sediment are considered (see Table 1) it can be concluded that Fraction 2 contains three times more mercury (and about the same absolute quantity of methylmercury) than Fraction 1. Therefore, it is necessary to decide whether the values obtained on analyzing either of the two fractions can be used as a reliable estimate of the total content of mercury and/or methylmercury in the raw sediment sample. Obviously, one can consider the mercury contained in Fraction 1 or in Fraction 2 as a representative value of the mercury content of the whole sediment if the Fraction 1 values were correlated with the Fraction 2 values and, thus, with the total mercury content.

Actual concentrations in Fractions 1 and 2 do not appear to be correlated ($r = 0.579$ for total mercury and $r = 0.572$ for methylmercury). Figure 5 shows a comparison of the concentrations in samples obtained by extrapolating the actual concentrations in both Fractions 1 and 2, considering the relative proportions in each sample. Figure 5(a) shows the results for total mercury while Fig. 5(b) corresponds to methylmercury. In each graph the arrow represents the ideal values if both fractions extrapolate to the sample concentration. It is evident from these graphs that sample concentrations evaluated from Fraction 2 lead to higher estimates than those obtained from Fraction 1. The conclusion is that none of these fractions gives a good estimate of the sample concentration. In fact, no apparent correlation was obtained, even using normalized values of the data obtained from Fraction 1 or 2. The graphs in Fig. 6 show some attempts to correlate normalized values with the total mercury content of the samples. Only dividing the actual concentration found in Fraction 1 by its percentage in the sediment sample gave apparently correlated points. However, the small number of samples available does not allow one to obtain any reliable conclusion.

Since no mercury was detected in Fractions 3 and 4 for any sample, the sum of the extrapolated values from Fractions 1 and 2 was considered the more realistic estimate of the overall pollution of the sediment samples. These values appear in columns 4 and 9 of Table 3, for total mercury and methylmercury respectively, referred to dry samples, and in columns 5 and 10 once they are corrected for the water content of each sample.

Furthermore, it is necessary to check if these estimates agree with the concentration values obtained in the analysis of wet raw sediment samples. The graphs in Fig. 7 depict the results of this comparison when the extrapolated values from Fractions 1 plus 2 were corrected for sample water content. With a few exceptions, higher results were obtained on analyzing wet raw sediments. These results could be explained by losses in the freeze-drying process or by assuming that small amounts of mercury (and methylmercury, of course) remain in Fractions 3 and 4 beyond the detection limits of the applied procedures. This second explanation is probably more realistic. This means that wet raw sediment data apparently produce better estimates of sediment pollution. However, because of the inherent heterogeneity of the samples, one cannot expect reproducible values when analyzing wet raw sediment samples. Moreover, this lack of reproducibility cannot be remedied, because two main factors can influence the results: first, the heterogeneity of the material and thus, sampling errors on taking portions for the analysis; second, the possible heterogeneous distribution of the pollutant in the sediment core.

In contrast, freeze-dried and sieved sediments are much more homogeneous materials, showing good variation coefficients when replicate analyses are carried out.

An attempt was made to correlate the values obtained by extrapolating the sample concentrations from Fractions 1 and 2 (corrected for sample water content) with the values obtained when wet raw sediments were analyzed. In the case of total mercury some correlation appeared, as shown in Fig. 8. However, in the case of methylmercury no correlation existed.

Finally, in Fig. 9 the correlation graph between the total mercury concentration in the samples vs the percentage of Fractions 1 and 2 is shown. Although the number of data does not allow a clear conclusion it is evident that a tendency appeared in the case of total mercury, whose concentration increases with increase of the percentage of small size particles in the samples. On the contrary no apparent pattern appeared in the case of methylmercury, considering either the extrapolated values from freeze-dried samples or the wet raw samples result.

Acknowledgements Financial support from the Xunta de Galicia (General Directorate for Universities and Scientific Policy), through Project XUGA20905B93, is gratefully acknowledged.

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