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Re-evaluation of distillation and comparison with HNO₃ leaching/solvent extraction for isolation of methylmercury compounds from sediment/soil samples

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Distillation was re-evaluated for the formation of artifacts arising from increasing naturally occurring mercury(II) concentrations, as opposed to previous identification of artifacts by spiking standard mercury(II) into samples. Naturally occurring mercury(II) concentrations lower than $2\,\mu g\,g^{-1}$ were found not to affect methylmercury (MeHg) results. However, when the natural concentrations of mercury(II) were greater than $2\,\mu g\,g^{-1}$, in contrast to standard mercury(II) spiked in samples, the MeHg concentrations measured were found to decrease (not increase) with increasing naturally occurring mercury(II) concentrations. This indicated that standard mercury(II) spiked in samples behaved differently from naturally occurring mercury(II) in the formation of MeHg artifacts during distillation. As a result, spiking standard mercury(II) into samples to identify the formation of MeHg artifacts is not adequate. It is difficult to explain why high naturally occurring mercury(II) suppresses MeHg measurements during distillation. In comparison with HNO₃ leaching/solvent extraction (and other existing techniques), distillation was found to generate results comparable for samples containing less than $2\,\mu g\,g^{-1}$ mercury(II). The HNO₃ leaching/solvent extraction showed significant advantages over other procedures, as this technique generated the highest recoveries with good precision for all samples analyzed, and the results were found to be independent of mercury(II) concentrations for both naturally occurring and spiked standard mercury(II). Thus, except for samples from high mercury-contaminated fields, distillation is still a good choice. Both the positive bias (possibly caused by artifact formation of MeHg) and the negative bias (due to incomplete leaching, back-adsorption, and/or decomposition of MeHg) were investigated. Geologically, physically, and chemically different samples were used for the investigation. Copyright © 2004 John Wiley & Sons, Ltd.

KEYWORDS: methylmercury; sediment/soil; distillation; HNO₃ leaching; artifacts

INTRODUCTION

Numerous recent studies have concluded that much, if not all, of the mercury that is bio-accumulated through the food chain is as methylmercury (MeHg).[1] As a result, accurate determination of MeHg in various matrices is important. Compared with other matrices, especially with biological samples,[2] analyzing MeHg accurately in sediment/soil samples seems to be more difficult.

Owing to its low detection limit and high efficiency for isolation of MeHg from complex matrices, the combination of distillation[3–6] and aqueous-phase ethylation, room temperature pre-collection, gas chromatography (GC) separation, and cold-vapor atomic fluorescence spectrum (CVAFS) detection[7,8] has been the most commonly used method for analysis of water and sediment/soil samples for MeHg. However, since the recent identification of

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formation of MeHg artifacts during distillation[9,10] (Liang and Horvat, unpublished results), the technique is no longer considered optimal for use for isolation of MeHg from sediment/soil samples. As a result of this, several procedures using acid or alkaline leaching/CH_2Cl_2 extraction have been employed to replace the distillation process for isolating MeHg from sediment/soils. These leaching procedures, including $KOH/CH_3OH/75\,^{\circ}C$,[11] $H_2SO_4/CuSO_4/KBr/20\,^{\circ}C$,[10] $HCl/20\,^{\circ}C$,[3] and $HNO_3/CuSO_4/20\,^{\circ}C$,[12] were critically evaluated and compared with distillation in this work.

The formation of artifacts has been identified by analyzing samples spiked with high concentrations of mercury(II) standard. In the presence of high-concentration mercury(II) standards, a positive bias in MeHg results was indeed generated during distillation, and these positive biases were considered to be artifacts[9,10] (Liang and Horvat, unpublished results). However, standard mercury(II) and naturally occurring mercury(II) need not necessarily behave in the same way with regard to formation of artifacts. Hence, this study investigates whether or not naturally occurring mercury(II) acts in the same way as mercury(II) standard spiked in samples.

The discovery of the formation of MeHg artifacts during distillation drew a significant amount of attention with regard to whether or not the procedures used for isolation of MeHg from matrices would make artifacts. Thus workers became aware of the need to assess whether their data included artifacts or not prior to using them for environmental assessments. Reference sediment/soils certified using methods involving distillation, such as IAEA356, were re-examined, and it seems that only results lower than previous certified values are considered to be reasonable.[9,10] Great efforts have since been made to minimize the formation of artifacts and to reduce the effects of data having artifacts on assessments of the environment. Unfortunately, the negative bias due to incomplete leaching was overlooked. In this work, both positive and negative biases are investigated.

HCl is commonly used both for preservation of water samples for MeHg analysis[13] and for leaching MeHg from soil and biological samples,[3] as it was considered not to decompose MeHg. Since oxygen-containing acids such as HNO_3 are possibly considered to decompose MeHg (except low concentrations of H_2SO_4[3]) few of them are used in preserving water samples to be analyzed for MeHg or in leaching MeHg from sediment/soil samples. Owing to its good capability of leaching metals from tied sites, HNO_3 is used extensively for decontamination of containers to be used for collection of samples for trace metals analysis. For the same reason, HNO_3 was a suitable choice for digestion of samples for analysis of total recoverable metals. If MeHg is stable to HNO_3 at concentrations and conditions that ensure the complete leaching of MeHg, then the acid clearly should also be the best choice for leaching MeHg from sediment/soils. Tseng *et al.*[14] have described a procedure based on quantitative microwave-assisted leaching of MeHg

from standard reference sediments with 2 M HNO_3. In our study, a procedure using HNO_3 leaching was refined and adapted to the method for determination of MeHg in sediment/soils. The procedure was also critically evaluated by comparison with other procedures.

Certified reference sediments were generally used for evaluation of analytical performance. However, certified reference sediments may be physically different from wet samples such as organic-rich and clay sediment/soil samples. Although physical differences may not affect the results of total metal analyses, for speciation this would be a significant confounding factor. As certified reference sediments were dry and homogeneous fine powders, MeHg in these sediments would apparently be more easily leached and extracted. However, MeHg may be tightly tied and/or embedded in wet samples, especially in clay soil samples. This would protect MeHg from extraction by leaching solutions, resulting in incomplete leaching. Thus, an acid solution that may quantitatively leach MeHg from certified reference sediments may not necessarily work the same way for wet samples. As a result, this study uses both dry certified reference sediments and wet samples for evaluation of procedure performances.

EXPERIMENTAL

Samples used for the investigation

Nine geologically, physically, and chemically different samples (listed in Table 1) were used for the experiments performed in this study. Mercury concentrations identified with an asterisk in Table 1 are certified values; the others were reported by Cebam using HNO_3 leaching for MeHg[12] and the modified EPA Method 1631 for total mercury (THg).[15,16] The values reported by Cebam were obtained by analyzing wet samples within 20 days of sample collection. Since ongoing experiments were performed within 2 years of sample collection and storage at $4\,^{\circ}C$, MeHg concentrations were found to vary; however, this will not affect this investigation, because these samples were used for comparisons of analytical performance between different procedures and/or conditions. For this reason, the highest results were generally presented as 100% recovery in the comparison. Since MeHg concentrations in CAI-8 and CAI-9 sediments collected from Florida Bay were low, spiked samples with 2 ng g^{-1} MeHg were used for the experiments performed.

Procedures evaluated

$HNO_3/CuSO_4$ leaching, CH_2Cl_2 extraction (HNO_3 leaching)

0.5–1.0 g of sediment was weighed into a 30 ml Oak Ridge centrifuge tube. 2 ml of 1 M $CuSO_4$, 8 ml of 3 M HNO_3 and 10 ml of CH_2Cl_2 was added. The tube was capped tightly. The tube was shaken vigorously for 30 min with a shaker. The sample was centrifuged at 3000 rpm for 30 min. 5 ml of the CH_2Cl_2 layer was pipetted into a 50 ml polypropylene

Table 1. Samples used for investigation[a]

Sample ID	Type	Location	THg ($\mu g\ g^{-1}$)	MeHg (ng g^{-1} as Hg)	Status	Comment
IAEA 356	Estuarine sediment	Mediterranean	7.35*	5.46*	Dry	Certified
BCR 580	Estuarine sediment	Ravenna Lagoon	132*	69.5*	Dry	Certified
NIST 2704	River sediment	Buffalo River, NY	1.44*	4.28	Dry	Cebam reported MeHg
CAI-1	Gravel soil	TN, USA	6.85	7.52	Wet	Cebam reported
CAI-2	Bog sediment	B.C., Canada	4.24	2.91	Wet	
CAI-3	Compacted clay and gravel soil	GA, USA	5.26	2.68	Wet	Cebam reported
CAI-4	Bog sediment	B.C., Canada	4.39	2.46	Wet	Cebam reported
CAI-5	Bay algae sediment	FL, USA	0.117	0.207	Wet	Cebam reported
CAI-6	Bay algae sediment	FL, USA	0.023	0.145	Wet	Cebam reported

[a] Asterisks indicate certified values.

centrifuge tube. About 40 ml of double-deionized water (DDW) was added to the tube. A bamboo skewer was placed to the bottom of the tube to prevent solvent spattering prior to heating for solvent evaporation. The tube was heated in a water bath at 45 °C for solvent evaporation until no visible solvent was left in the tube. The temperature of the water bath was increased to 75 °C, and the sample was purged with nitrogen for 8 min to remove solvent residue. The volume of the sample was brought to 50 ml with DDW. An appropriate aliquot of the extract was pipetted into a bubbler for analysis by aqueous-phase ethylation, Tenax trap collection, GC separation and CVAFS detection.[7,8]

$H_2SO_4/CuSO_4/KBr$ leaching, CH_2Cl_2 extraction (H_2SO_4 leaching)

Samples were prepared according to a published procedure.[10] MeHg extracted in DDW was analyzed by the same procedure as for HNO_3 leaching described above.

KOH/CH_3OH leaching, CH_2Cl_2 extraction (alkaline leaching)

A published procedure[11] was used for sample preparation, followed by the same procedure as for HNO_3 leaching described above.

Distillation

Sediment and soil samples were distilled according to our previous procedure,[3] but special attention was paid to sample aliquots to be distilled. Only aliquots containing less than 2 μg of naturally occurring mercury(II) were taken for distillation.

RESULTS AND DISCUSSION

Comparison of MeHg results generated using different procedures

Three certified reference material (CRM) sediments and three wet sediment/soil samples (listed in Table 1) were

analyzed using distillation, HNO_3 leaching and another two procedures for MeHg. The results are illustrated in Fig. 1. To simplify the comparison, the highest result of each sample was presented as 100%. Deviations of results presented as 100% were found to be within ±7% of certified values for CRMs, and within ±28% of values listed in Table 1 for wet samples. The relatively higher deviations from the listed values for wet samples were due to these samples having been stored at 4 °C for more than a year when this experiment was performed. MeHg concentrations in these wet samples may have changed during the storage. Within an analytical batch, precisions for analyzing wet samples were similar to those for analyzing CRMs. Recoveries of HNO_3 leaching ranged from 83 to 100% for the various types of sediment and soil samples tested; for H_2SO_4 leaching, recoveries range from 29 to 90%, for alkaline leaching the range was from 66 to 96%, and for distillation the range was from 89 to 100%. It should be noted that the recoveries by distillation could be achieved only when aliquots containing less than 2 μg mercury(II) were taken for distillation. The reasons for this are detailed later in this paper.

Reasons of producing lower recoveries

Data in Fig. 1 indicate that all procedures generated quantitative recoveries for the three CRM sediments, but not for wet samples. Lowest recoveries were found by H_2SO_4 leaching for all wet sediment/soil samples analyzed. In this paper, therefore, H_2SO_4 leaching was used as a typical example for discussion of reasons responsible for producing lower recoveries. The wet samples used here were organic-rich and/or clay sediment/soil samples. One reason for this might be that MeHg was tightly embedded in the wet and clay samples. HNO_3 could destroy the embedded site and leach MeHg into the solution, whereas low concentrations of H_2SO_4 could not, resulting in incomplete leaching. This problem was not encountered for dried and powdered CRMs, therefore, H_2SO_4 leaching was able to generate acceptable results for these samples.
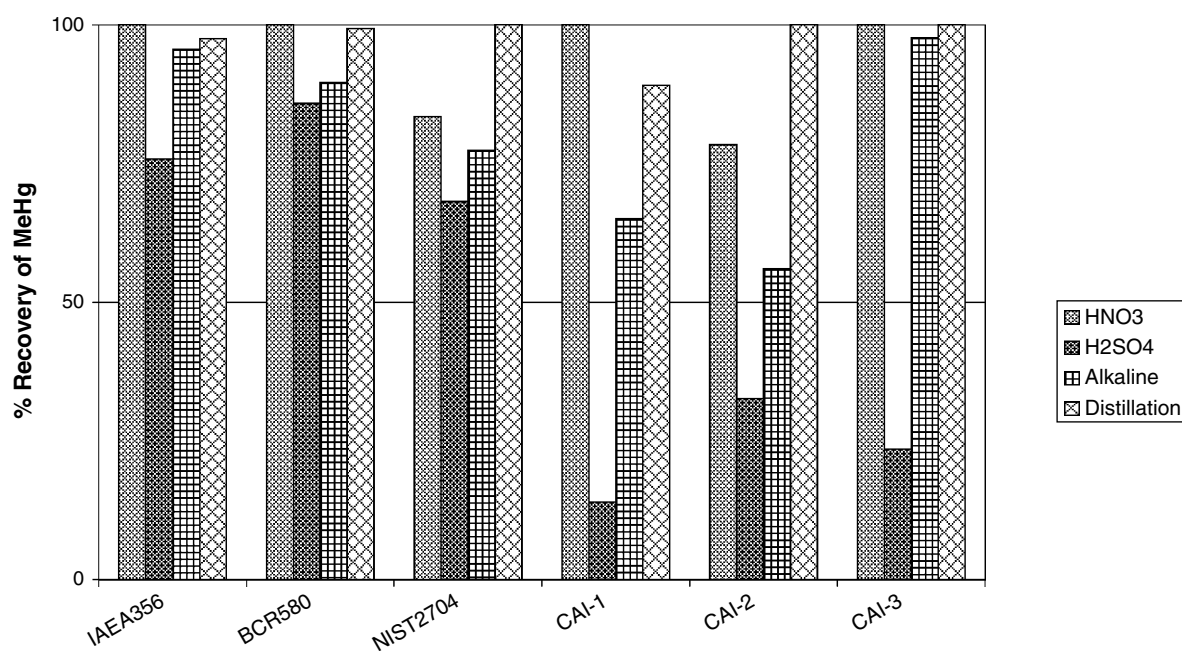
Figure 1. Comparison of MeHg results in typical sediments and soils using different isolation techniques. Highest value of each sample is presented as 100% in the figure.

Another possible reason for lower recoveries by H_2SO_4 leaching was considered to be back-adsorption of MeHg during leaching. Ideally, all MeHg released into acid solution can be immediately and completely extracted into solvents. However, a reverse process, back-adsorption, might also take place, resulting in lower results. To determine whether MeHg was back-adsorbed on sediment/soils during H_2SO_4 leaching, the following experiment was conducted. For comparison, HNO_3 leaching was also examined.

The experiment involved comparing spike recoveries between samples pre-soaked for 1 h prior to adding CH_2Cl_2 and samples not pre-soaked while shaking for extraction of MeHg. Except BCR580,[17] (the easiest sample to extract) eight of the samples listed in Table 1 were tested. When the HNO_3 leaching procedure was employed, no significant differences were found between the samples that were pre-soaked and those that were not (Fig. 2), suggesting that back-adsorption did not happen.

However, when using the H_2SO_4 leaching procedure, significant differences were observed for almost all samples as a result of pre-soaking (Fig. 2). Recoveries without pre-soaking were up to 27% higher than those with pre-soaking. This suggests that back-adsorption of MeHg standard spiked in samples did happen. However, this does not seem sufficient to account for the lower results generated by the procedure. For sample CAI-2 (an organic-rich and clay bog sediment), the difference arising from pre-soaking was found to be 27%, but the result generated for this sample by H_2SO_4 leaching was 29% of that by HNO_3 leaching only (Fig. 1). Therefore, back-adsorption may be a less important reason for producing

lower results, and the incomplete leaching described above seems to be more important.

The data in Fig. 2 also show that recoveries generated by H_2SO_4 leaching were lower than those obtained by HNO_3 leaching for all samples analyzed. Recoveries by H_2SO_4 could be improved by using higher concentrations of H_2SO_4 and then leaching while extracting from samples that are not pre-soaked, to thus minimizing back-adsorption of the analyte.

Formation of MeHg artifacts

The formation of MeHg artifacts during distillation has been observed (Liang and Horvat, unpublished results).[9,10] The data in Fig. 1 show that HNO_3 leaching generated results similar to those by distillation for the samples we tested. The question then arises as to whether results of HNO_3 leaching also contain artifacts.

The formation of artifacts during distillation was identified by analyzing samples spiked with mercury(II) standard (Liang and Horvat, unpublished results).[9,10] In this study, the same experiment was performed to examine the HNO_3 leaching procedure, while comparing with distillation. The sample IAEA 356 was spiked with mercury(II) standard and analyzed using two procedures. The results in Fig. 3 for distillation agree with those found in our previous work (Liang and Horvat, unpublished results) and the work of others.[9,10] Differences in MeHg values measured between spiked and non-spiked samples were identified as artifacts. As is shown in Fig. 3, the artifacts increased with increasing spiked mercury(II) standard. However, the results of HNO_3 leaching were found to be independent of spiked mercury(II)
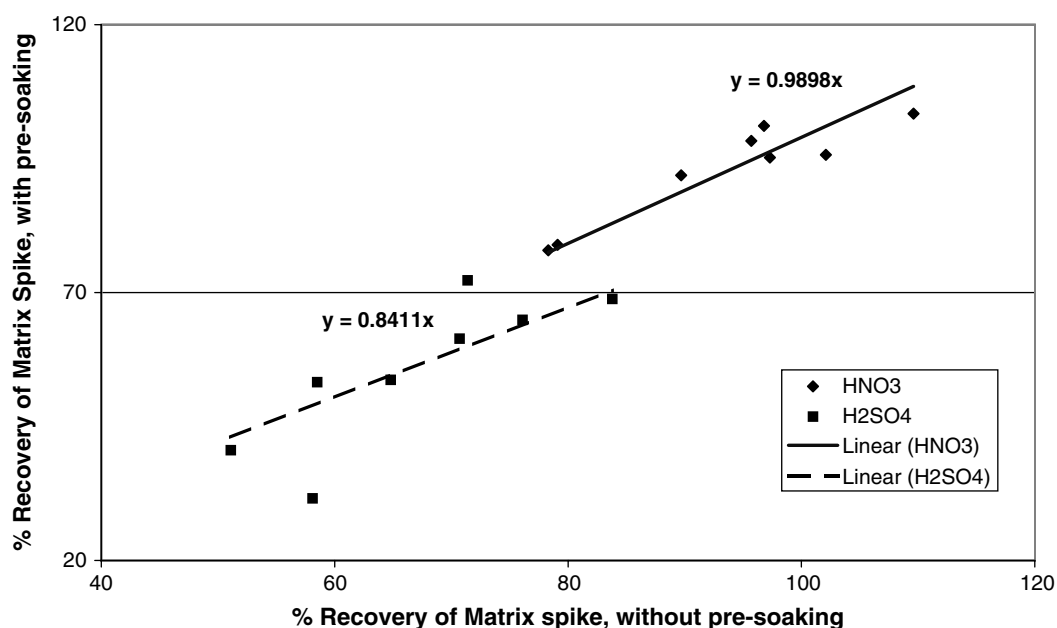
Figure 2. Comparison of matrix spike recoveries with and without pre-soaking samples in HNO_3 and H_2SO_4/KBr leaching solutions.

standard, suggesting no artifacts were produced by this method. Therefore, the results generated by distillation in Fig. 1 should also not include artifacts because they were similar to those observed by HNO_3 leaching for all the samples analyzed. This raises the questions of whether the behavior of spiked mercury(II) standard and naturally occurring mercury(II) in the formation of artifacts is different? If that is true, then analyzing mercury(II) standard spiked samples to identify the formation of artifacts is problematic.

In order to characterize whether naturally occurring mercury(II) produces artifacts similar to spiked mercury(II) standard, the following experiment was conducted. Two procedures, distillation and HNO_3 leaching, were compared in this experiment. The six geologically, physically, and chemically different sediment/soil samples in Table 1 were used. For each sample and each procedure, several aliquots in different weights from 0.01 to 5 g were taken, and its naturally occurring mercury(II) in each aliquot was calculated. All aliquots were analyzed for MeHg by the two procedures. The results are illustrated in Fig. 4. For comparison purposes, the highest MeHg results were presented as 100% for each sample in Fig. 4.

For HNO_3 leaching, MeHg measured was found to be independent of naturally occurring mercury(II) content in aliquots analyzed for all samples. However, the behavior is complex for distillation. Except for sample CAI-3, all samples in Fig. 4 showed similar patterns, which is contrary to the samples spiked with mercury(II) standards, like IAEA 356 in Fig. 3. The MeHg measured decreased, rather than increased, with increasing naturally occurring mercury(II). Data for sample CAI-3 show a similar potential to samples spiked with mercury(II) standard (Fig. 3), i.e. the MeHg measured
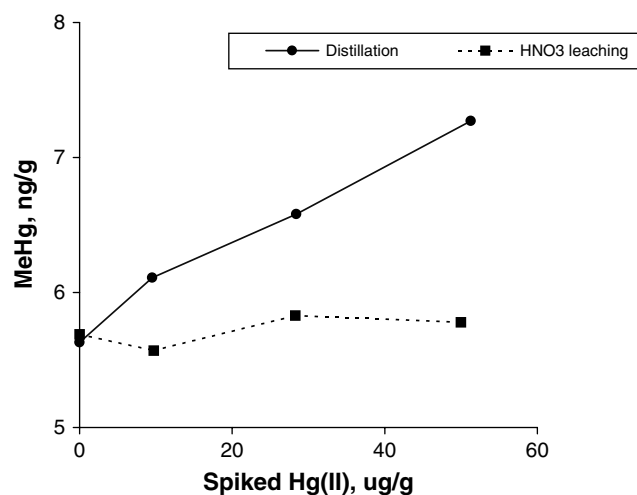

Figure 3. Effect of increasing spiked standard mercury(II) on MeHg results of IAEA 356 sediment by distillation and HNO_3 leaching/solvent extraction.

increases with increasing naturally occurring mercury(II). Thus raises questions about the nature of sample CAI-3. CAI-3 was a surface compacted clay and gravel soil from a disturbed floodplain. The sample was collected after the floodplain had been backfilled with clean top soil for remediation.

The most important explanation of the distillation results in Fig. 4 is that naturally occurring mercury(II) does not cause formation of artifacts, but it causes a negative bias in the MeHg results. This is contrary to the behavior of spiked mercury(II) standards. The magnitudes of bias vary for different individual samples with different
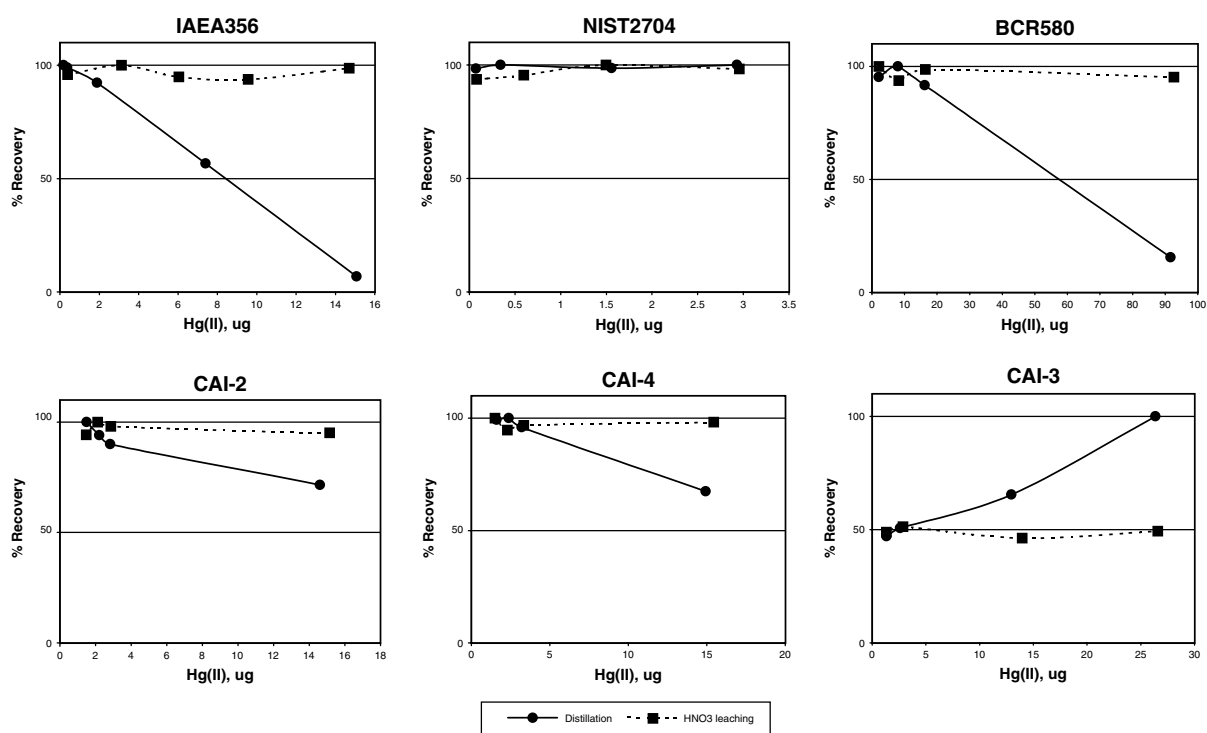
Figure 4. Effects of increasing naturally occurring mercury(II) by increasing sample sizes taken for preparation on recoveries of MeHg by distillation and HNO₃ leaching/solvent extraction. The highest result is presented as 100% for each sample.

naturally occurring mercury(II) concentrations. Generally, when aliquots contain less than 2 µg of naturally occurring mercury(II) the MeHg results are found to be identical to those obtained by HNO₃ leaching, and identical to certified values for CRMs, and also those listed in Table 1 for wet samples. This suggests that: (1) identifying the formation of MeHg by analyzing samples spiked with mercury(II) standard is problematic, because naturally occurring mercury(II) exhibits a different behavior from spiked mercury(II) standard; (2) distillation can generate accurate results provided that the aliquots distilled contain less than 2 µg mercury(II); and (3) results obtained by HNO₃ leaching are independent of the nature of individual samples and mercury(II) concentrations of both spiked standard and naturally occurring in samples. As a result, HNO₃ leaching should be recommended as the best choice among the procedures evaluated in this paper.

Overall, the data in Fig. 4 are important for both research and environmental survey activities related to MeHg in sediments/soils isolated by distillation. Because of its significant advantages,[3] distillation was commonly used for isolation of MeHg from matrices for analysis of sediment/soil samples until it was pronounced to form artifacts in the presence of high concentrations of mercury(II).[9,10] In this study, we found that based upon taking 1 g of wet sample for distillation, a THg concentration of less than 2 µg is not found to cause a positive or negative bias in the results. In fact, except in highly mercury-polluted areas, THg concentrations are far below this limit. As a result, it is still

viable to use distillation to obtain reliable results. When the THg concentration of a sample is known, or the sample origin is known, it should be easy to determine whether distillation can be used to generate accurate results for the sample.

Of course, Fig. 4 could also be drawn using different masses of aliquots distilled to replace mercury(II) contents, resulting in the same curves. Then another possible interpretation for the data would be matrix interference, as the data show that MeHg results decrease with increasing masses of aliquots distilled. However, it would be difficult to explain the results from the NIST 2704 and BCR 580 samples. For sediment NIST 2704, MeHg results do not drop at aliquot masses above 2 g, whereas for sediment BCR 580 the MeHg results start to drop at aliquot masses as small as around 0.1 g. However, it would be easy to explain if the drop in MeHg results is attributed to the content of naturally occurring mercury(II) in the aliquots distilled. 2 g of NIST 2704 sediment contains less than 3 µg of mercury(II), and 0.1 g of BCR 580 contains more than 13 µg of mercury(II).

The reason that MeHg measured decreases with increasing naturally occurring mercury(II) using distillation is unknown. Further research work is needed to answer this question. Here, the importance is to emphasize that spiked standard mercury(II) and naturally occurring mercury(II) are different in the formation of MeHg artifacts; therefore, identifying artifact formation by analyzing spiked samples with mercury(II) standard is not adequate.

CONCLUSIONS

Complete isolation of MeHg from matrices and the identification/minimization of artifact formation are still challenges for accurate determination of MeHg in sediment/soil samples. The procedure of HNO_3 leaching/CH_2Cl_2 extraction shows significant advantages over the other procedures evaluated in this paper. Distillation can still be used to generate reliable results provided that the sample aliquots to be distilled contain less than 2 µg mercury(II). Identification of MeHg artifacts by analyzing spiked samples with mercury(II) standard was found to be problematic. The question of artifact formation during sample preparation needs to be investigated further.

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