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Effects of sample preservation and storage on mercury speciation in natural stream water

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Abstract

Despite an increasing focus on low level methods for determination of mercury species in water over the last decades, few studies have paid attention to direct effects of different sample preparation methods (i.e. preservation techniques) on natural freshwater samples. In this study we show how different preservation techniques give significantly different concentrations of total and methylmercury in freshwaters (9 and 14 % on average, respectively). Natural stream samples from a forested lake catchment were studied. Mean stream sample concentrations of total (3.6 ng/L) and methylmercury (0.06 ng/L) reflect levels typical for pristine humic boreal catchments. The main reason for the observed average differences in total and methylmercury concentrations is the use of one instead of two sample bottles and timing of sample acidification, respectively.

Keywords

Mercury speciation, total mercury, methylmercury, sample preservation, sample storage

Introduction

The detection limits (DL) of methods for determining mercury (Hg) species in water have been reduced significantly over the last few decades. The main reasons for this reduction are improved analytical methods [1], [2], and development of a more rigorous sample handling procedure [3]. Studies have also paid attention to sampling equipment, focusing on bottle material, e.g. glass and various types of Teflon [4], [5], preservation times for samples in different bottle types [5], [6], and cleaning procedures for equipment [1], [7]. However, less attention has been directed towards studying effects of sample preparation, i.e. preservation techniques, on analytical results. The fate and effect of mercury in pristine areas impacted by long-range Hg deposition is of growing concern [8] and a focus on the advantages and disadvantages of different sample preparation techniques is necessary.

Common routines for collecting water samples for determination of total Hg (TotHg) and methylmercury (MeHg) include the use of one sample bottle for both species, or alternatively two bottles, one for each species. Samples for MeHg analysis demand acid preservation [2], while samples where only TotHg is determined may be shipped to the laboratory unpreserved before oxidizing the sample [1]. Even if a sample is acid preserved, Hg may be lost to the bottle walls by adsorption through mechanisms of dissolved organic carbon coagulation or co-precipitation [5], [9]. The best way to avoid this is to add the oxidizing agent directly to the original sample bottle. If however, MeHg is also to be

determined from the same sample, an aliquot must be removed prior to the addition of the oxidizing agent [1].

The aim of this study was to study the effect of sample preparation techniques on measurement of Hg species in natural freshwater samples. Aqueous TotHg and MeHg were monitored in a forested catchment in Norway. Parallel samples were analysed over a period of 12 months in 2010 and 2011 using two sample preparation techniques. In the study, possible causes for observed differences in concentrations between the two sample preparation techniques are examined and evaluated. We hypothesized that the possible differences in TotHg and MeHg concentrations were related to either sampling procedure (i.e. bottle type and timing of sample acidification) or sample preparation (i.e. the use of one or two bottles for sampling).

Experimental

The present study was carried out at the Langtjern catchment, an acid-sensitive, forested lake catchment in southeast Norway (background water data in [10]). The Langtjern catchment has been an acid rain monitoring site since 1972. Samples were collected at three different locations; the two main inlets and at the lake outlet. To get sufficient data material to describe the yearly variation of TotHg and MeHg in the lake water, samples were collected monthly from October 2010 to December 2011. Samples for complete water chemistry were also collected, and the mean concentrations of pH and TOC for the three sample locations in the described sampling period were 5.0 ± 0.1 and 12.7 ± 1.7 mg/L (outlet), 5.1 ± 0.3 and 11.6 ± 2.2 mg/L (inlet 1) and 4.9 ± 0.3 and 12.0 ± 2.3 mg/L (inlet 2), respectively.

Sampling for Hg speciation provided a total of n = 39 samples that were taken in two different types of bottles and analyzed for both TotHg and MeHg utilizing two different sample preparation techniques. Additionally, 8 samples were taken from the same locations to

verify the influence of sample bottle type and volume on analysis results. Sampling followed the recommendations of USEPA Method 1669 [3].

The United States Environmental Protection Agency (USEPA) Method 1630 [2] was used for determining MeHg in water by distillation, aqueous ethylation, purge and trap, and cold vapor atomic fluorescence spectrometry (CVAFS). For TotHg, USEPA Method 1631 for determining Hg in water by oxidation, purge and trap and CVAFS was used [1]. The method detection limits (MDL) were respectively 0.02 ng/L for MeHg and 0.1 ng/L for TotHg (3 standard deviations of blanks). For both species automated systems were used for analysis (Brooks Rand Labs MERX automated systems with Model III Atomic Fluorescence Detector).

Quality assurance and quality control measures included method blanks, blank spikes, sample duplicates and matrix spikes. The relative standard deviation of sample duplicates was < 10 % for both MeHg and TotHg. Recovery of blank spikes and matrix spikes were within 80 – 120 % for MeHg and 90 – 110 % for TotHg. Calibration verification and calibration blanks were run every 10 samples.

According to the USEPA methods, the procedure used for determination of MeHg in water samples involves preservation with hydrochloric acid (HCl, 0.4 %), and for determination of TotHg the sample is oxidized with bromine monochloride (BrCl). Two sample preparation techniques are used. Technique A involves the use of one bottle (fluorinated ethylene propylene (FEP) 125 mL) for determining both MeHg and TotHg. These samples were preserved with HCl upon arrival at the laboratory (3-5 days after field sampling) and the analysis proceeded by the removal of a sample aliquot (25 mL) for determining MeHg first, before BrCl was added and the remainder of the sample used for determination of TotHg. Technique B involved the determination of MeHg and TotHg in two separate bottles (fluorinated polyethylene (FLPE), 250 mL). HCl was added to the MeHg

bottle just prior to sampling and BrCl to the TotHg bottle upon arrival to the laboratory. All samples were analysed unfiltered.

Results and discussion

The mean TotHg concentrations determined by sample preparation technique A and B are 3.9 ± 1.1 ng/L (mean ± 1 standard deviation) and 3.6 ± 0.9 ng/L, respectively. Corresponding values for levels of MeHg are 0.07 ± 0.02 ng/L and 0.06 ± 0.02 ng/L. Both concentrations of TotHg and MeHg obtained by the two techniques are significantly different (t-test on difference of paired samples, significance level $\alpha = 0.05$, Wilcoxon Signed Rank $p < 0.05$, samples < MDL excluded). For both species, the concentrations obtained by sample preparation technique A are significantly higher than the concentrations obtained by technique B. The averages of individual sample differences show that technique A give 9 % higher results for TotHg and 14 % higher for MeHg. The ratio of results obtained by the two sample preparation techniques is shown in Figure 1.

Possible explanations for the significant difference in concentrations of Hg species obtained by the two techniques include different bottle types and volumes. However, in investigating this, no significant differences were found (Technique B on 8 parallel samples using both bottle types, paired t-test, $\alpha = 0.05$, Wilcoxon Signed Rank $p = 0.18$). Mean TotHg concentrations for the 250 mL FLPE and 125 mL FEP bottles are 3.0 ± 0.7 ng/L and 3.2 ± 0.8 ng/L, respectively. This means that neither bottle material (FLPE/FEP) nor bottle volume (125 mL/250 mL) influences the final analysis result. This agrees with what has been shown previously by other studies using similar types of bottle material [6] and volumes from 125 – 1000 mL [1].

The likely cause for the observed higher TotHg concentration in technique A compared to B was thus not bottle type or sample volume, but the removal of the aliquot for

MeHg analysis in technique A. We propose that this is related to an increase in the surface area of the bottle relative to the remaining volume of sample, as suggested by Parker and Bloom [5] for filtered and spiked samples. When using only one bottle for both species, some Hg will adhere to the surface of the sample bottle after removing an aliquot for MeHg analysis, even when the sample is preserved with acid. When the oxidizing agent is added to the original bottle, this 'extra' Hg will be released into a smaller volume of sample, resulting in elevated concentrations. The higher MeHg concentrations for technique A is related to the time when acid was added to the sample bottles. Due to logistics, acid was added 3-5 days later for Technique A compared to B. The USEPA Method 1630 states that samples can be acid preserved within 48 hours after sampling if samples are taken in fluoropolymer bottles, with no head space, and samples maintained at 0-4 °C from collection until preservation [2]. Technique A did not follow the USEPA recommendation and based on our results, attention should be paid to stay within this limit to avoid methylation of Hg species in the sample bottle. Filtration and thereby removal of bacteria could possibly contribute to minimizing the in-bottle methylation of Hg. The advantages of determining the two Hg species in the same sample bottle includes lower costs, lower transportation volume, and the elimination of potential artefacts caused by having different samples. By using two sample bottles, more equipment is required, but the preservation of both Hg species is performed in the original bottle. The results of the present study show that significant and systematic differences in concentrations may occur between using a one or two sample bottle approach for the determination of MeHg and TotHg. Such differences in sample preparation techniques can lead to extra uncertainty in TotHg and MeHg concentrations obtained from different laboratories, despite the use of the same analytical methods.

Conclusions

151 The results of this study show that significant and systematic differences in
152 concentrations of TotHg and MeHg may occur depending on preservation technique prior to
153 analysis of natural stream water samples. This is due to the use of a one or two sample bottle
154 approach (TotHg) and timing of acid preservation (MeHg).
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157 **Figure**

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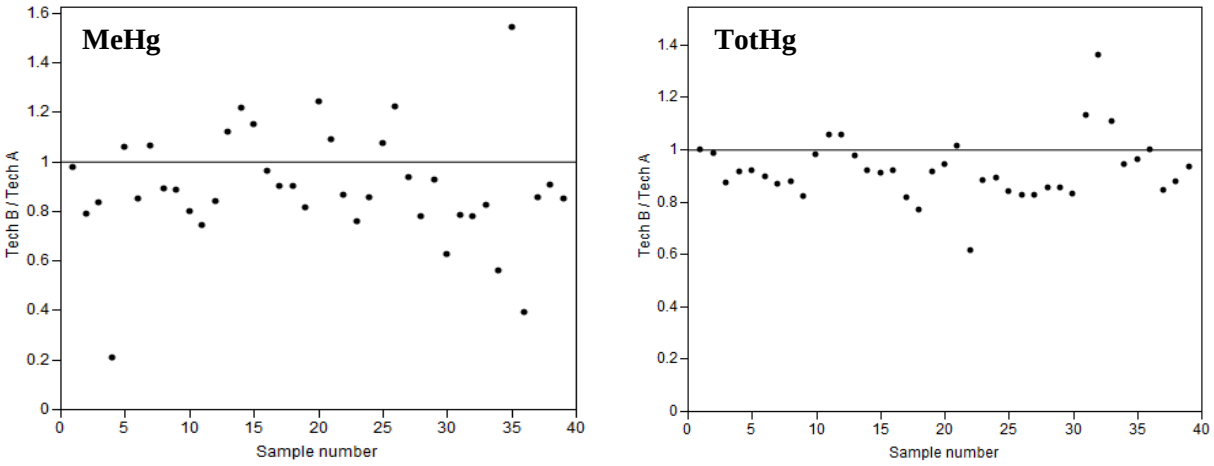

Figure 1 Levels of MeHg (left) and TotHg (right) as concentrations obtained by sample preparation technique B divided by concentrations obtained by technique A. Samples < MDL are set to concentration equal to MDL and included.

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