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ORIGINAL PAPER

Mercury speciation in environmental samples associated with artisanal small-scale gold mines using a novel solid-phase extraction approach to sample collection and preservation

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Abstract In artisanal small-scale gold mines (ASGM), mercury (Hg) is known to pollute nearby river waters and sediments where it can be methylated to the highly bioavailable methylmercury (MeHg). The assessment of Hg speciation in water samples has been challenging for many years, with recommended procedures often not adequately allowing for analysis of samples in a suitable timeframe. Using a novel solid-phase extraction (SPE) method for sampling and preservation of Hg species, representative speciation data can be safely and easily collected and retained for up to 4-weeks (MeHg = 115 ± 8% refrigerated and 109 ± 13% unrefrigerated storage; Hg^{2+} = 100 ± 14% refrigerated and 94 ± 12% unrefrigerated storage). Concentrations of MeHg in environmental water samples and drinking water were below detection limit across two ASGM sites in western Kenya and concentrations of Hg^{2+} were below drinking water guidelines; however, drinking water sources contribute 20–30% of the tolerable weekly intake of Hg, indicating a need to minimise exposure of Hg from dietary sources to prevent Hg poisoning. Sediments from receiving rivers at ASGM sites showed total Hg concentrations above guideline limits (0.08–1.84 mg kg^{-1} total Hg) along the length of the river; however, MeHg concentrations fluctuated dependent on the stagnation of the river due to damns and ponds (5.9 ± 14.3 µg kg^{-1} MeHg). The findings show that SPE can be used as a robust sample collection and preservation approach for Hg speciation, which can better inform mitigation measures, understand ecological and human health implications, and improve environmental monitoring.

Keywords Mercury · ASGM · SPE · Speciation · Water · Sediment

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Introduction

Mercury (Hg) is one of the most toxic metals present in the environment and is of great concern to human and environmental health (WHO, 2005). Assessment of Hg pollution typically concerns total Hg concentrations in environmental matrices, but neglecting

Fig. 1 An ore washing pond at Rosterman, Kenya (left) and its runoff, which enter the nearby river (right) used for domestic waters (laundry, cleaning, etc.) and crop irrigation

speciation may lead to an underestimate of hazards associated with Hg pollution. The two main species of interest in the aquatic environment are inorganic mercury (Hg^{2+}), the most prevalent species, and methylmercury (MeHg), a highly bioavailable species known to be neurotoxic and long-lasting in the human body (Kim & Zoh, 2012). Inter-species conversion of Hg occurs in sediments when bacteria convert Hg^{2+} to MeHg under anaerobic conditions (Driscoll et al., 2013; Kim & Zoh, 2012). The MeHg introduced to the sediment can be displaced by bioturbation and sediment transport into rivers, where it dissolves into the water column. Thus, the methylated species is distributed through the aquatic environment and can bioaccumulate and biomagnify up trophic levels, leading to pollution of the environment and potential toxicity to biota and human populations from low initial concentrations. This is especially problematic in vulnerable areas such as artisanal small scale gold mines (ASGM) where Hg is used to extract gold from crushed gold ore, resulting in sediment, water, soil and air pollution with Hg and increasing exposure of the local populations to the toxic metal (Ondayo, et al., 2023a, 2023b). In these areas, nearby rivers are used for ASGM activities and waste disposal, as a domestic water source e.g. for laundry and cleaning, for drinking water, agricultural irrigation, aquaculture, and others. Figure 1 demonstrates typical points of pollution at ASGM sites and Fig. 2 shows Hg used for amalgamation of the crushed ore to extract gold, typically conducted at rivers at or nearby mine sites that may also be used by the local population. Monitoring Hg and its species in ASGM sites is vital to ensure Hg exposure routes are minimised and human and environmental health is maintained.

Previous literature investigated concentrations of Hg species in river water at which no negative effects were estimated for the environment or human health, and reported this to be 0.0065 μg L^{-1} MeHg (Du et al., 2015). In contrast, this estimated concentration for Hg^{2+} was reported as 0.39 μg L^{-1}, because the species is less bioavailable than MeHg. These estimated concentrations are significantly lower than the environmental guideline limit for total Hg in waters (0.77 μg L^{-1} Hg), suggesting environmental harm may occur at concentrations much lower than

Fig. 2 Mercury (Hg) used for amalgamation of gold from crushed ore, usually handled with limited or no protective equipment

current guideline values, and there is limited guidance on species specific threshold limits in river and lake water (EPA, 1995). To measure Hg pollution in the environment, sediment and tissue samples are preferred as the species are more stable when bound to a solid matrix (Richard et al., 2016). However, without representative data from water samples, many direct exposure routes to both human health and the environment are not assessed.

The poor stability of Hg species in water samples is well known (King et al., 2023; Louie et al., 2012; Parker & Bloom, 2005; Yu & Yan, 2003), with near complete loss of total Hg within 1 week. Individual species require different methods of preservation (USEPA, 1996, 2002) which increases the difficulty of sample collection, particularly in challenging environments such as ASGM sites. It is recommended to ship samples to a lab within 48 h of collection in polytetrafluoroethylene (PTFE) or glass containers for preservation, with acidification of separate samples using hydrochloric acid for Hg^{2+} or sulphuric acid for preventing ethylation of MeHg (USEPA, 2002). However, glass may be prone to breaking, PTFE containers are expensive, and handling acids in uncontrolled environments can result in accidental acid burns (King et al., 2023). In addition, it is recommended to collect larger sample volumes to minimise analyte losses and individual analysis of species. Literature into sampling and analysis of mixed species samples has focused on the use of solid-phase extraction (SPE) techniques (Blanco et al., 2000; King et al., 2023; Wang et al., 2022; Yin et al., 2010), but storage times are rarely assessed beyond 1 week.

Blanco et al. (2000) reported the development of an SPE-cartridge functionalised using diethydithiocarbamide to achieve total sorption and recovery of Hg^{2+} and MeHg from river waters over 1-week, but after a further 7 days recovery dropped to 70% Hg^{2+} and 65% MeHg in refrigerated conditions (4 °C), and 40% Hg^{2+} and 35% MeHg in unrefrigerated (15 °C) conditions. Unpolluted and Hg-polluted river waters from Spain were used to validate the developed method by spiking with up to 0.15 µg L^{-1} Hg species, recovering 90% Hg^{2+} and 89% MeHg (Blanco et al., 2000).

A dithizone-functionalised SPE method by Yin et al. (2010) and Wang et al. (2022) demonstrated total sorption of both Hg^{2+} and MeHg with recoveries of > 85% after 10 days, but no recoveries beyond this timescale were reported (Wang et al., 2022; Yin et al., 2010). These studies assessed speciation of Hg in river, lake and seawater as a preparation method for HPLC-ICP-MS analysis. Yin et al. (2010) reported recoveries of > 85% MeHg in tap, river, sea and coal-washing waste water from 0.05 µg L^{-1} MeHg spiked samples and > 85% $Hg^{2+}$from tap, river and sea water from 0.2 µg L^{-1} Hg^{2+} spiked samples. Meanwhile, Wang et al. (2022) reported similar recoveries of 95% Hg^{2+} and 100% MeHg waters from 0.001 µg L^{-1} Hg^{2+} and 0.001 µg L^{-1} MeHg spiked sea, river and lake water, concentrations expected of unpolluted waters. Previous work using a dithizone-functionalised SPE cartridge for total Hg preservation reported representative recoveries of > 75% up to 4-weeks from collection from river waters associated with ASGM activities (King et al., 2024). This is summarised in Table 1. Further optimisation for Hg species preservation allows for improvements in Hg monitoring by ensuring representative concentrations of MeHg can be determined after moderate storage times.

Table 1 Storage times and recovery of Hg from environmental water samples from relevant literature

Study	Storage time reported	Recovery from environmental water samples
Blanco et al. (2000)	1 week	90% Hg^{2+}, 85% MeHg
Yin et al. (2010)	10 days	85% Hg^{2+}, 85% MeHg
Wang et al. (2022)	10 days	95% Hg^{2+}, 100% MeHg
King et al. (2024)	4 weeks	85% total Hg

The analysis of Hg species is conducted using online-separation techniques, either chromatographic techniques such as HPLC-ICP-MS (Blanco et al., 2000; Wang et al., 2022; Yin et al., 2010) or cold vapour methods such as CV-AAS (USEPA, 1996, 2002; Yu & Yan, 2003). These detection methods are suitable for water samples and extractant solutions from solid matrices such as sediments, with detection limits as low as 1 µg L^{-1} Hg for CV-AAS and 0.005 µg L^{-1} for HPLC-ICP-MS. Recent literature has focused on the development of HPLC-ICP-MS analysis methods, using a functionalised solid-phase to immobilise and separate individual Hg species (Balarama Krishna et al., 2010; Favilli et al., 2022; Wang et al., 2022; Yin et al., 2010), due to the robustness of the technique and detection limits suitable for unpolluted environmental samples, usually around 0.002 to 0.1 µg L^{-2} Hg^{2+} and 0.001 to 0.005 µg L^{-1} MeHg. These concentrations are below the estimated concentrations reported by Du et al. (2015), and thus enable the assessment of potential hazard posed by MeHg and Hg^{2+} in environmental water samples.

These established methods using HPLC-ICP-MS to measure Hg species in sediments along with new development in field preservation techniques using SPE for Hg species in water samples associated with ASGM practices enable a robust assessment of environmental pathways for Hg used at ASGM sites incorporating both water and sediment matrices to better understand the source-pathway of Hg. Therefore, the aim of this study was to undertake the characterisation of Hg speciation in environmental samples associated with ASGM sites using novel methodologies. To achieve this, the objectives were:

1) optimisation of a functionalised cartridge for the field preservation of Hg^{2+} and MeHg in water samples, to enable field application in remote locations and robustness to complex water matrices associated with ASGM.
2) measurement of Hg species in waters and sediments at ASGM sites in the Kakamega and Vihiga gold belt, western Kenya, including full elemental characterisation for a broader context and understanding of the chemistry of these matrices.

Methodology

Cartridge functionalisation

Functionalisation of cartridges was previously described in King et al. (2024). Briefly, 5 mL methanol (SigmaAldrich, UK) was put through a C18 cartridge (Bond Elut Jr, Agilent, UK), followed by 5 mL 0.5 M sodium formate-formic acid buffer (pH 4, SigmaAldrich, UK), 1 mL 0.002 M dithizone solution (AlfaAesar, UK), followed by 3 mL of 0.5 M sodium formate-formic acid buffer (pH 4) and finally 3 mL 0.05 M sodium formate-formic acid buffer (pH 4, SigmaAldrich, UK).

HPLC-ICP-MS

An aqueous-C18 column (Poroshell aqueous C18, 5 µm, 4.6 mm i.d. × 50 mm length, Agilent, UK) was selected for HPLC separation, using a mobile-phase of 2-mercaptoethanol solution (0.5% v/v, SigmaAldrich, UK). The pump unit was an Agilent 1290II infinity (Agilent, UK). The HPLC unit was coupled to an Agilent 8900 ICP-MS (Agilent, UK). Conditions for ICP-MS analysis are described in Supplementary Table 1. All samples were analysed by HPLC-ICP-MS for Hg speciation using masses of ^{201}Hg for analysis of Hg species in water samples using the SPE method, and using masses of ^{199}Hg and ^{201}Hg for isotope dilution analysis of MeHg in sediment samples. An example chromatogram is shown in Fig. 1. Analysis method detection limits for Hg^{199} and Hg^{201} were determined as 3 times the standard deviation of 9 blank replicates as summarised in Supplementary Table 2, and an example chromatogram of Me(^{199}Hg), Me(^{201}Hg), ^{199}Hg and ^{201}Hg is shown in Fig. 3.

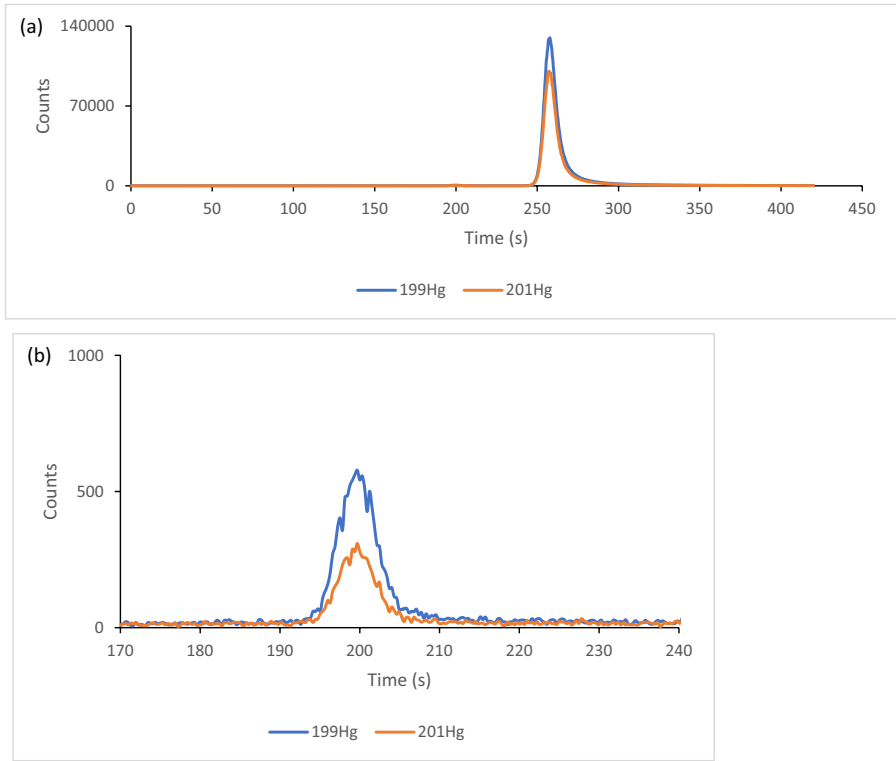


Fig. 3 **a** An example chromatogram of MeHg (190–210 s) and Hg^{2+} (240–280 s) and **b** a magnification of the MeHg peak

Quality control in cartridge optimisation work

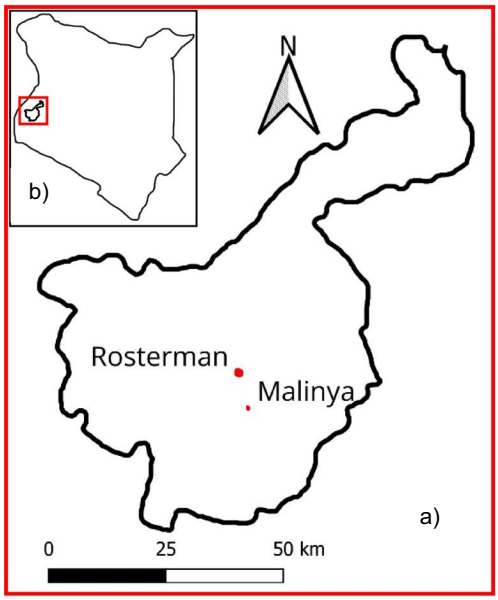
Due to the known instability of Hg species, certified reference materials for aqueous matrices are not readily available. During HPLC-ICP-MS analysis, a 0.25 µg L^{-1} Hg quality control solution and a 0.04 µg L^{-1} MeHg quality control solution were periodically analysed (every 10 samples) to determine accuracy and precision. A synthetic river water was used as a matrix in experimental work, to ensure the performance of the cartridge would not be impacted by major anion and cation chemistry found at ASGM study sites, such as those reported by Ondayo et al. (2023a) (Supplementary Table 3) (Ondayo et al., 2023b). A method for manufacturing the matrix is described in previous work (King et al., 2023; Smith et al., 2002). The matrix was spiked to 0.5 µg L^{-1} Hg^{2+} and 0.05 µg L^{-1} MeHg to represent Hg species concentrations. The use of a standard hard water matrix across all experimental work demonstrates the robustness of the method under expected matrix conditions and the effects of common major ionic constituents (Fig. 4).

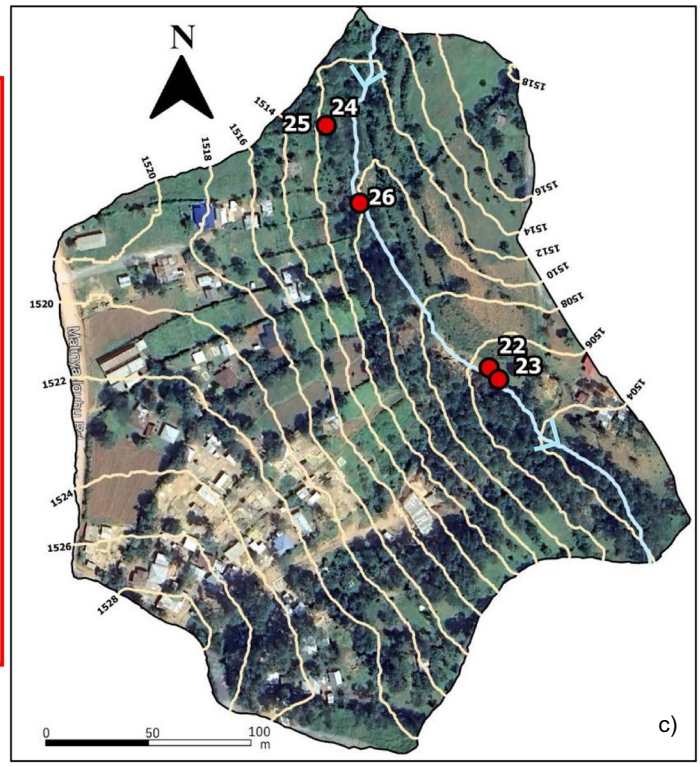
Matrix conditions

River and lake water matrices are complex and vary in geochemical characteristics, such as pH, salinity and organic matter. These different matrix conditions may affect the sorption and recovery of Hg species from the functionalised cartridge. To determine the effect of matrix components, pH (pH 5 and 8 (adjusted using 1 M ammonium hydroxide, SigmaAldrich, UK and concentrated nitric acid, Romil, UK), salinity (0.1 and 1.9% w/v chloride (using sodium chloride, SigmaAldrich, UK), and humic acid content (5 and 20 mg L^{-1} humic acid (SigmaAldrich, UK)) were assessed. A 30 mL aliquot of each spiked matrix was passed through a functionalised cartridge and was eluted with 30 mL 2-mercaptoethanol solution (1% v/v, SigmaAldrich, UK). The eluent was collected for analysis.

4-week preservation

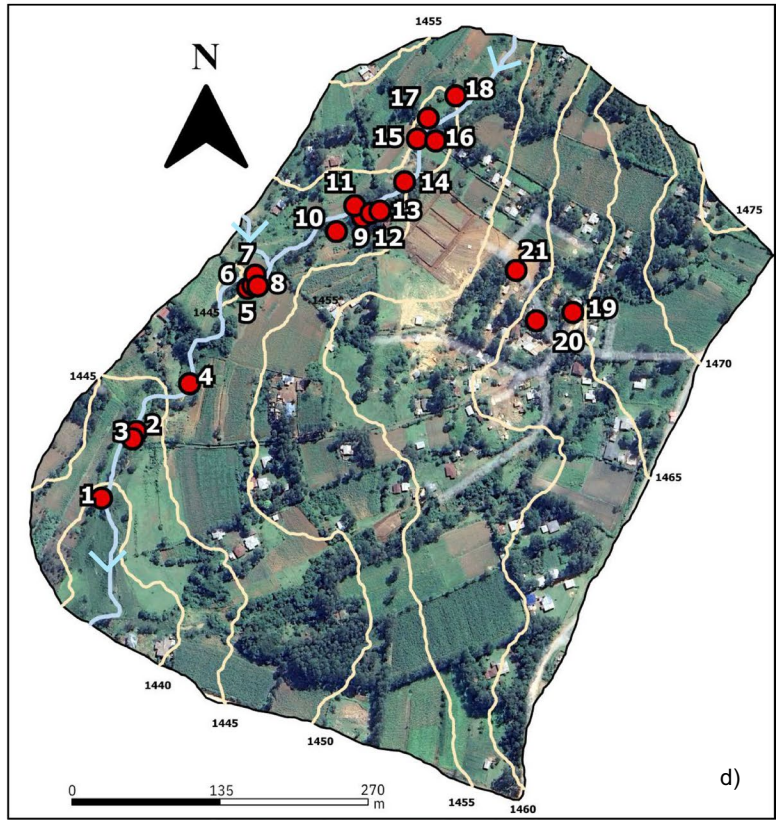
It is vital to determine the stability of Hg species on the functionalised cartridge, to ensure its usefulness as a sampling method. To assess this, 30 mL aliquots





River
Contour lines
Sampling point

1	Hg-001
2	Hg-002
3	Hg-003
4	Hg-004
5	Hg-005
6	Hg-006
7	Hg-007
8	Hg-008
9	Hg-009
10	Hg-010
11	Hg-011
12	Hg-012
13	Hg-013
14	Hg-014
15	Hg-015
16	Hg-016
17	Hg-017
18	Hg-018
19	Hg-019
20	Hg-020



◂**Fig. 4** Sampling points (26) for Hg, water and sediment samples at ASGM sites in **a** Kakamega gold belt, **b** Kenya: **c** Malinya mine and **d** Rosterman mine

of the Hg^{2+} and MeHg spiked solution was passed through functionalised cartridges, and were subsequently stored for 0, 1, 2, 3 and 4 weeks in either refrigerated (4 °C, dark) and unrefrigerated (16 °C, dark) conditions. After the specified storage period, the cartridges were eluted with 30 mL 2-mercaptoethanol (1% v/v) which was collected for analysis.

Case study of artisanal gold mines, Kenya

Water and sediment samples were collected from ASGM sites in the Kakamega gold belt, Kenya; Rosterman mine site (0° 15 ′35 .6 "N 34° 43 ′12 .0 "E) and Malinya mine site (0° 11 ′22 .4 "N 34° 44 ′10 .3 "E). Activities at these sites are well known (King et al., 2023; Ondayo, et al., 2023a, 2023b) and are representative of ASGM sites with river systems used by the miners and the local community. The rivers are small tributaries of the River Yala and Nzoia which ultimately drain into Lake Victoria, Kenya. Twenty-five water samples including preserved Hg samples, and twenty-one sediment samples were collected at intervals along the river passing through the mine site, ore washing ponds, and drinking water sources. Water samples were collected in HDPE containers (15 mL and 8 mL) and were filtered (0.45 µm hydrophilic HDPE filters) and stored at 4 °C until they could be acidified in a laboratory setting (1% $HNO_3 + 0.5\%$ HCl). A 30 mL aliquot of river water was filtered and passed through functionalised cartridges at a rate of approximately 10 mL min^{-1}, and the cartridges were stored at 4 °C in the dark until elution and analysis. Sediment samples were collected with a stainless-steel scoop and stored in LDPE zip-lock bags, and transported to a laboratory within 48 h of collection where the samples were then freeze-dried (−70 °C) until constant weight. Sediment samples were then hand-milled and sieved to < 250 µm particle size. Flow charts showing relationships between sampling points and run off are shown in Supplementary Figs. 1a and 1b.

Water analysis for Hg species

Functionalised cartridges for Hg species were stored at 4 °C in the dark until in a laboratory setting for elution and analysis. An aliquot of 2-mercaptoethanol solution (30 mL, 1% v/v) was passed through each cartridge and collected for immediate analysis by HPLC-ICP-MS, as per the matrix and 4-week preservation experiments.

Total Hg analysis in sediment samples

Total Hg concentrations were determined in all sediment samples using a DMA-80 total Hg analyser (Milestone, UK). Sediment samples (0.05 g) were weighed into metal sample boats (Milestone, UK), which were then subjected to thermal decomposition and quantification using DMA-80 for direct Hg analysis. Certified reference materials (HR-1, NRCC and TH-2, NRCC, Canada) were analysed periodically (every 10 samples) to determine accuracy and precision of data (Supplementary Table 4).

Isotope dilution and microwave extraction in sediment samples

To determine Hg speciation in sediments, samples underwent isotope dilution and microwave extraction. By using an isotopic spike, more accurate Hg concentrations can be determined from sediment extractions by comparison of the ratio of the isotopically enriched spike isotope (^{199}Hg) and a reference isotope (^{201}Hg). The concentration of MeHg in sediment samples (C_x) is calculated following Eq. 1:

Equation 1: $$C_x = C_s \frac{M_x}{M_s} \frac{W_s}{W_x} \frac{(A_s - RB_s)}{(RB_x - A_x)}$$

Where C_s is the concentration of MeHg (^{199}Hg) in the spike solution, M_x represents the relative atomic mass of Hg in the sample, M_s represents the relative atomic mass of Hg in the spike solution, W_s represents the mass of the spike solution added, W_x represents the mass of the sample, R represents the ratio of reference isotope to spike isotope in the spiked sample as determined through measurement of isotopic abundance by ICP-MS analysis. A_s represents the abundance of the reference isotope in the spike solution, B_s represented the abundance of the spike

isotope in the spike solution, A_x represents the abundance of the reference isotope in the sample, and B_x represents the abundance of the spike isotope in the sample. The measured ratios were corrected for instrument mass bias by comparison of the expected ratio of reference isotope and the natural isotope to an unspiked reference material.

Reverse isotope dilution

To determine the concentration of the MeHg (^{199}Hg) spike solution, a reverse isotope dilution method was employed. Briefly, 0.0994 g of a naturally isotopically distributed MeHg standard (1000 µg L^{-1} MeHg, Alfa Aesar, US) was added to 0.303 g of the isotopically enriched MeHg (^{199}Hg) solution (reported as 52 µg L^{-1} MeHg, ISC Science, Spain). The mixture was analysed by HPLC-ICP-MS and the concentration of the isotopic spike was calculated using Eq. 1.

Microwave assisted extraction and HPLC-ICP-MS analysis

An extractant solution was made to 3 M HCl (Romil, UK) and 0.2 M citric acid (SigmaAldrich, UK) with deionised water. 0.5 g of sediment sample was spiked with 0.5 mL MeHg isotopically enriched solution (75 µg L^{-1} MeHg, ^{199}Hg, ISC Science, Spain) and left to equilibrate. 10 mL of the extractant solution was added to each sediment sample, which were subsequently microwaved for 5 min at 500 W (temperature regulated to 45 °C). The solution was filtered and analysed by HPLC-ICP-MS for $Hg^{2+}$and MeHg. An estuarine reference material, EU ERM®-CC58, was extracted and analysed alongside the sediment samples, to ensure adequate extraction (Supplementary Table 4).

Characterisation of water and sediment for multi-elemental analysis

Both sediment and water samples were analysed at the British Geological Survey for total elemental concentration by ICP-MS (Agilent 8900, Agilent, UK). Water samples were preserved by acidification with HCl (0.5%) and HNO_3 (1%) upon delivery to the laboratory. Sediment samples were dissolved for analysis using a mixed acid digestion approach (HF/HNO_3/$HClO_4$) with a programmable hot-block, as described in previous literature (Watts et al., 2008, 2013, 2019). Briefly, sediment samples were weighed (0.25 g) into PFA vials, acids added and heated on a temperature programmable graphite hot-block (80 °C for 8 h, 100 °C for 2 h, 120 °C for 1 h, 140 °C for 3 h, 160 °C for 4 h). Once digested and evaporated, the sample was taken up in 2.5 ml of concentrated nitric acid, heated at 50 °C for 30 min and then treated with 30% (v/v) hydrogen peroxide, before being made up to volume (25 ml) with deionised water to give a final solution of 5% nitric acid for analysis by ICP-MS. Certified reference materials (CRM), USGS BCR-2, NRCC LKSD-1, NRCC LKSD-3, and NRCC MESS-4, were digested and analysed alongside the sediment samples, with acceptable CRM recoveries of > 85% and standard deviation of < 10% (Supplementary Table 6). Duplicate sample analysis demonstrated percentage differences of < 25% for all assessed elements.

Results and discussion

Matrix conditions

To determine the efficiency of the functionalised cartridge in varied water matrices, the effect of three major matrix components on retention and recovery of spiked Hg species from the functionalised cartridge were assessed: pH, chloride concentration and humic acid concentration. A synthetic river water was used through the experiment, spiked with 0.5 µg L^{-1} Hg^{2+} and 0.05 µg L^{-1} MeHg (n = 5). The pH of the matrix was adjusted to pH 5 and 8, typical of water found

Table 2 Percentage recovery (%) of Hg2 + and MeHg from the spiked environmental waters to test influence of pH, chloride concentration and humic acid concentration (n = 5)

Characteristic assessed	Percentage recovery of Hg^{2+} (%)	Percentage recovery of MeHg (%)
pH 5	100 ± 1%	90 ± 1%
pH 8	98 ± 7%	84 ± 7%
0.1% Cl^-	101 ± 3%	98 ± 4%
1.9% Cl^-	99 ± 1%	96 ± 4%
5 mg L^{-1} Humic acid	100 ± 1%	92 ± 6%
25 mg L^{-1} Humic acid	105 ± 3%	91 ± 5%

near ASGM sites. Chloride concentrations were adjusted to 0.1% and 1.9% (w/v) chloride, typical of non-saline and saline water sources. Humic acid concentrations were adjusted to 5 and 25 mg L^{-1}, typical of varying amounts of organic matter entering the water body. Under all assessed conditions, recoveries of Hg^{2+} and MeHg were > 84% with a standard deviation of < 8% (Table 2).

The recoveries of both Hg^{2+} and MeHg, summarised in Table 1 for all matrices, demonstrated collection of representative concentrations is possible from waters with varied chemical characteristics, with a standard deviation of < 8% shows robustness of the cartridge method performance from the assessed conditions. This aligns with previous literature and similarly functionalised cartridges. Blanco et al. (2000) reported negligible effects on recovery from sample pH between pH 3 to 8 using a diethyldithiocarbamate functionalised cartridge, while Wang et al. (2022) noted an influence on species specific recovery of Hg^{2+} but maintained a > 70% recovery for all species using a similar dithizone functionalised phase to this study. This work demonstrates negligible effects of pH (5 and 8) on both Hg^{2+} and MeHg sorption and recovery. Chloride concentrations were also demonstrated in this study to have no effect on recovery, with > 96% MeHg and > 99% Hg^{2+} found at salinities representative of both river water and seawater. This is supported by Yin et al. (2010) and Wang et al. (2022), who both reported > 80% recovery of both Hg^{2+} and MeHg using a similar dithizone functionalised phase to this study when in the presence of up to 3% (v/v) Cl^{-},(Wang et al., 2022; Yin et al., 2010) and thus demonstrates usability of the functionalised cartridge for sampling in highly saline environments. Yin et al. (2010) found significant decrease in recovery of Hg^{2+} in solutions containing greater than 5 mg L^{-1} humic acid from the use of a 12.5 µg dithizone functionalised cartridge, potentially due to disruption of Hg sorption to the dithizone functionalised solid phase from thiol-containing humic acid molecules. In this study, functionalisation using 50 µg dithizone provided good performance for more complex matrices associated with ASGM of 100% recovery of $Hg^{2+}$and > 91% recovery of MeHg, suggesting the functionalised cartridge is suitable for a wide concentration of organic matter (King et al., 2024). These results indicate a dithizone functionalised SPE cartridge is a suitable method for adsorption and recovery of Hg species from a variety of river and lake water samples, and is not affected by major ion concentrations, pH, salinity or humic acid concentrations expected in environmental river waters. It is important to note while dithizone is insoluble in water, it can dissolve at a pH > 10 and mobilise through the cartridge. Thus, assessment of the water for sampling should be undertaken prior to sample loading to ensure an adequate pH of < 8, to minimise risk of re-mobilising the solid-phase and subsequent inability to suitably retain Hg concentrations.

4-week preservation

The stability of both Hg^{2+} and MeHg on the functionalised column is vital to ensure data are representative after transport from the field. The recommended procedure of shipping samples to laboratories within 48 h of collection (USEPA, 1996, 2002) is impractical in remote and challenging environments. Therefore, Hg recovery data from a synthetic river water (n = 5) for both Hg^{2+} (0.5 µg L^{-1} Hg^{2+}) and MeHg (0.05 µg L^{-1} MeHg) across 4-weeks of storage in the absence of light, in both unrefrigerated (16 °C) and refrigerated (4 °C) conditions is shown in Fig. 5. The mean recovered concentrations of MeHg over 4-weeks of storage was 109 ± 13% in unrefrigerated and 115 ± 8% in refrigerated conditions. Similarly, recovery of Hg^{2+} was between 93 ± 12% in unrefrigerated conditions and 100 ± 14% in refrigerated conditions. Recoveries of > 100% are likely due to the methyl- group enhancing signal in the ICP-MS detection.

In both refrigerated (4 °C, absence of light) and unrefrigerated (16 °C, absence of light) conditions, both Hg^{2+} and MeHg were found in high recoveries (96 ± 5% Hg^{2} and 112 ± 11% MeHg) after 0, 1, 2, 3, and 4 weeks of storage with acceptable deviation (Fig. 5). The recoveries are improved from previous literature, where Hg species were suggested to be eluted just 1-week from collection using a diethyldithiocarbamate functionalised cartridge with reported recoveries beyond this decreasing to just 65% MeHg and 70% Hg^{2+} in refrigerated conditions (Blanco et al., 2000). Wang et al. (2022) reported recoveries of 97% Hg^{2+} and 86% MeHg after 10-days storage using a dithizone functionalised cartridge but did not explore the usefulness of the cartridge beyond this timescale. In this study, Hg^{2+} recovery was 95 ± 10%

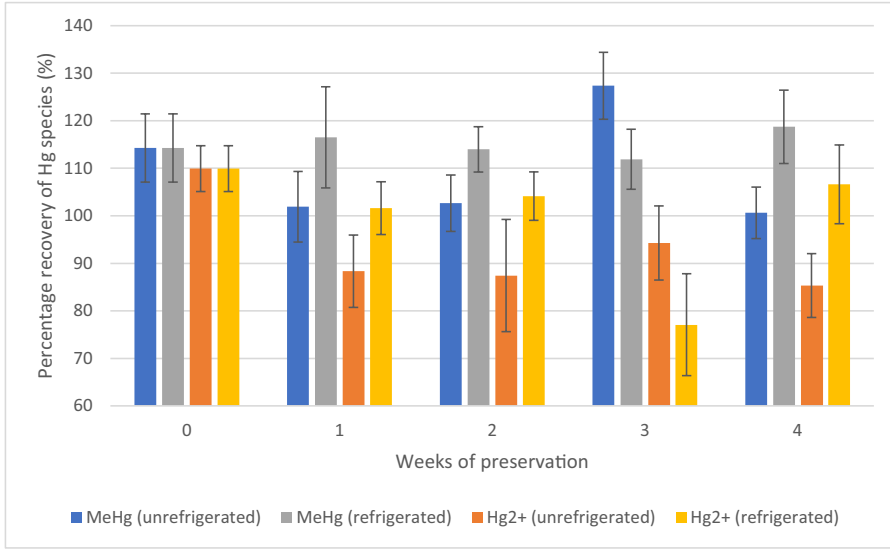


Fig. 5 Percentage recovery (%) of Hg^{2+} and MeHg after 0, 1, 2, 3, and 4 weeks of storage in refrigerated (4 °C, absence of light) and unrefrigerated (16 °C, absence of light) conditions, demonstrating suitable recoveries for a field sampling method (recovery 130% > x > 75%) across all assessed time periods (n = 5)

and MeHg recovery was 109 ± 12% after 1 weeks of storage, and 96 ± 13% Hg^{2+} and 108 ± 8% MeHg recovery after 2 weeks of storage, vastly improving on the preservation reported by previous literature. At 4 weeks of storage, recoveries were 96 ± 14% Hg^{2+} and 110 ± 12% MeHg, remaining representative of the original sample. A 4-week preservation of both Hg^{2+} and MeHg allows for improved speciation studies in challenging environments, such as ASGM sites, where adequate storage conditions are difficult to maintain and transportation to appropriate laboratories may be difficult.

At 3 weeks of storage, a recovery of 77 ± 12% Hg^{2+} was obtained from refrigerated storage conditions. This is likely decreased compared to the other results due to residual moisture in the cartridge due to inadequate drying. This can affect sorption of Hg^{2+} to the functionalised phase and thus cartridges should have air passed through after water samples are passed through the cartridge, minimising residual moisture and ensuring full sorption of Hg species to the cartridge.

Hg speciation in ASGM waters

Water samples from two ASGM sites in Kenya (Rosterman and Malinya mine sites, Kakamega gold belt) were collected and analysed for $Hg^{2+}$and MeHg (Table 3). General water chemistry is shown in Supplementary Table 5. Across both Rosterman and Malinya mine sites, MeHg concentrations were below the detection limit of 0.007 µg L^{-1} MeHg. For Hg^{2+}, concentrations ranged from 0.06–0.67 µg L^{-1} Hg^{2+} at Rosterman mine, with the highest concentration of 0.67 µg L^{-1} Hg^{2+} found in water from an ore washing pond (Hg-021). At a disused alluvial mining points (Hg-011, Hg-016 and Hg-017), Hg^{2+} concentrations were 0.06–0.31 µg L^{-1}, greater concentrations than river water samples away from direct Hg inputs (Hg-001 to Hg-003) between 0.06 and 0.1 µg L^{-1} Hg^{2+}. At Malinya mine, Hg^{2+}concentrations were 0.115–0.606 µg L^{-1} Hg^{2+} (Hg-022 to Hg-026), with the highest concentrations (0.41–0.61 µg L^{-1} Hg^{2+}) being found in water from a drinking water source originating from a pump buried downhill from sites of mining activities (Hg-024 and Hg-025). Water passing through mine tailings was found to contain 0.57 µg L^{-1} Hg^{2+} (Hg-026), and water leaving the site was found to contain 0.12–0.26 µg L^{-1} Hg^{2+} (Hg-022 and Hg-023).

While there is no safe exposure to Hg, the Hg^{2+} concentration found at each sampling point was below the WHO drinking water guideline of 1 µg L^{-1} total Hg (WHO, 2005) and the USEPA environmental chronic exposure guideline of 0.77 µg L^{-1} total Hg (EPA, 1995). It should be noted that the aquatic Hg concentrations may fluctuate with increasing alluvial Hg work throughout a day and with changes to the seasonal conditions throughout the year, thereby suggesting that environmental and human exposure may

Table 3 Speciation of MeHg and Hg^{2+} in river water samples at ASGM sites in the Kakamega Gold Belt, western Kenya (Rosterman mine and Malinya mine)

Sample point	Sampling point description	MeHg µg L^{-1}	Hg^{2+} µg L^{-1}	Cr µg L^{-1}	As µg L^{-1}	Ni µg L^{-1}	Cd µg L^{-1}	Pb µg L^{-1}
	Rosterman							
Hg-001	River water leaving mine site	< 0.007	0.070	0.40	0.64	1.39	0.006	0.06
Hg-002	River water leaving mine site	< 0.007	0.106	0.29	0.61	1.41	< 0.005	< 0.02
Hg-003	River water leaving mine site	< 0.007	0.059	0.20	2.23	4.34	< 0.005	< 0.02
Hg-004	River water leaving mine site, point of use for domestic activities	< 0.007	0.229	0.31	0.51	1.39	< 0.005	< 0.02
Hg-005	River water after mixing point	< 0.007	0.272	0.35	0.55	1.46	< 0.005	< 0.02
Hg-006	Mixing point of river from Rosterman mine and minor tributary from nearby villages	< 0.007	0.405	0.37	0.21	1.24	< 0.005	0.02
Hg-007	Minor tributary from nearby villages	< 0.007	0.165	0.37	0.14	1.09	< 0.005	0.02
Hg-008	Rosterman mine river water prior to mixing point	< 0.007	0.058	0.41	2.13	2.69	< 0.005	< 0.02
Hg-009	River water upstream of drinking water reservoir	< 0.007	0.119	0.41	1.34	3.31	< 0.005	< 0.02
Hg-010	River water downstream of mining activities	< 0.007	0.090	0.39	2.04	2.98	< 0.005	0.03
Hg-011	River water downstream of drinking water reservoir	< 0.007	0.105	0.46	1.10	3.70	< 0.005	< 0.02
Hg-012	Small pond formed from drinking water reservoir outlet	< 0.007	0.059	0.39	0.76	2.73	< 0.005	0.10
Hg-013	Drinking water reservoir	< 0.007	0.055	2.07	0.86	3.26	< 0.005	0.21
Hg-014	River water downstream of nearby disused ore washing pond	< 0.007	0.103	0.50	1.08	3.40	< 0.005	< 0.02
Hg-015	River water immediately after disused alluvial mining pond	< 0.007	0.062	0.53	0.80	3.52	< 0.005	< 0.02
Hg-016	Disused alluvial mining pond	< 0.007	0.095	0.56	0.49	3.39	< 0.005	< 0.02
Hg-017	River prior to disused alluvial mining pond, drinking/domestic water collection point	< 0.007	0.315	0.75	0.32	3.15	0.006	0.02
Hg-018	River water prior to entering mine site	< 0.007	0.293	0.43	0.37	2.94	< 0.005	< 0.02
Hg-019	Ore washing pond	< 0.007	0.075	0.09	10.4	17.0	0.090	0.09
Hg-020	Mine water, used as drinking water	< 0.007	0.144	1.54	1.51	2.77	0.012	0.04
Hg-021	Ore washing pond	< 0.007	0.671	0.56	11.2	7.65	0.011	0.04
	Malinya							
Hg-022	River water exiting mine site, nearby alluvial working point	< 0.007	0.216	0.19	3.38	1.57	< 0.005	< 0.02
Hg-023	River water exiting mine site	< 0.007	0.115	0.22	5.42	10.6	0.007	0.05
Hg-024	Drinking water from underground pump	< 0.007	0.413	0.87	0.59	17.6	0.032	0.05
Hg-025	Pond formed from drinking water pump	< 0.007	0.606	0.12	1.98	15.9	0.016	0.03
Hg-026	River water flowing through mine tailings	< 0.007	0.574	0.40	1.98	1.45	< 0.005	0.06
WHO	Guideline limits (WHO, 2003, 2005, 2006, 2017, 2021)	-	0.77	50	70	10	3	10

increase or decrease with external factors. The concentrations of Hg^{2+} in drinking water sources (Hg-004 Hg-012, Hg-013, Hg-20, Hg-024 and Hg-025) were assessed against a tolerable weekly intake of 0.7 µg kg^{-1} (body weight) $week^{-1}$ Hg (Table 4), to determine the contribution to weekly exposure, assuming water consumption of 3 L per day to consider the water consumption of the workers in the mines and thus worst-case exposure to Hg, and 2 L per day for an average consumption of water, and the average Kenyan bodyweight to be 60 kg. At the drinking water pump in Malinya (Hg-024), the exposure was found to be 0.15 µg (Hg) kg^{-1} (body weight) $week^{-1}$ at 3 L water consumption per day. This contributes 20% to the tolerable weekly intake and thus minimising exposure from all sources, including

Table 4 Assessment of Hg consumption from drinking water sources compared to the tolerable weekly intake of 0.7 µg(Hg) kg(body weight)$^{-1}$ week^{-1}

Sample point	Sampling point description	2 L water per day Hg^{2+} consumption	3 L water per day Hg^{2+} consumption	Percentage of tollerable weekly intake
		(µg kg(bw)$^{-1}$ week^{-1})	(µg kg(bw)$^{-1}$ week^{-1})	(%)
	Rosterman			
Hg-004	River water leaving mine site, point of use for domestic activities	0.05	0.08	17
Hg-012	Small pond formed from drinking water reservoir outlet	0.09	0.02	4
Hg-013	Drinking water reservoir	0.04	0.02	4
Hg-020	Mine water, used as drinking water	0.01	0.05	11
	Malinya	0.06		
Hg-024	Drinking water from underground pump	0.02	0.15	31
Hg-025	Pond formed from drinking water pump	0.02	0.21	45

waters, is vital to mitigate human exposure to the Hg species and reduce the risk of Hg toxicity.

While Hg and its species are of great concern, activities at ASGM sites pollute other potentially toxic elements to the water systems and thus should be considered when assessing environmental and human exposure. Elevated chromium (Cr), arsenic (As), nickel (Ni), cadmium (Cd), and lead (Pb) were previously reported in waters at ASGM sites (Ondayo, et al., 2023a), and so the concentrations of these elements were determined alongside Hg to examine environmental exposure to these potentially toxic elements from ASGM activities. The Cr concentrations in water at Rosterman and Malinya were between 0.12 and 2.07 µg L^{-1} Cr, with the highest concentrations, 2.04 and 1.54 µg L^{-1} Cr, found in the Rosterman drinking water reservoir (Hg-012) and Rosterman mine shaft water (Hg-020) respectively. The As and Ni concentrations at Rosterman and Malinya were 0.14–11.2 µg L^{-1} As and 1.09–17.6 µg L^{-1} Ni respectively, with the highest As concentrations found in the Rosterman ore washing ponds (Hg-019) (10.4–11.2 µg L^{-1} As), and highest Ni concentrations found in the Rosterman ore washing ponds (17.0 µg L^{-1} Ni) and the Malinya drinking water from an underground pump (15.6–17.6 µg L^{-1} Ni). Both Cd and Pb were mostly below detection limit (< 0.006 µg L^{-1} Cd and < 0.02 µg L^{-1} Pb) at Rosterman; however, sample from Malinya contained detectable Cd (0.007–0.032 µg L^{-1} Cd) and Pb (0.03–0.06 µg L^{-1} Pb) with the highest concentrations of both elements in the drinking pump water and subsequent pond. Concentrations of Cr, Ni, and As were reported in similar concentrations to this study (Ondayo, et al., 2023a), and are below the WHO drinking water guideline values of 50 µg L^{-1} Cr, 70 µg L^{-1} Ni, and 10 µg L^{-1} As (WHO, 2022). The concentrations of Pb and Cd are lower than in previous work (0.18 µg L^{-1} Pb and 0.05 µg L^{-1} Cd) (Ondayo, et al., 2023a) which may be due to seasonal environmental changes, such as rainfall, or reduction in pollution of these elements by anthropogenic means.

Total Hg and MeHg in ASGM sediments

Sedimentary total Hg concentrations (Table 5) were found between < 0.05 and 1.72 mg kg^{-1} total Hg at Rosterman mine and < 0.05–1.84 mg kg^{-1} at Malinya mine. The highest total Hg concentrations were found near disused alluvial mining ponds in Rosterman (Hg-015, 1.72 mg kg^{-1} total Hg and Hg-017 1.01 mg kg^{-1} total Hg) and near mine tailings in Malinya (0.377 mg kg^{-1} total Hg). Concentrations of MeHg were found between < 0.1 and 8.2 µg kg^{-1} MeHg at Rosterman mine, with the highest concentration found downstream of the drinking water reservoir (Hg-011). The pond formed by the drinking water reservoir (Hg-012) showed 3.3 µg kg^{-1} MeHg. At Malinya mine, sediment MeHg concentrations were between 1.0 and 36.8 kg^{-1} MeHg, with the highest concentration found in sediment from a pond

Table 5 Total Hg and MeHg concentration river sediment samples at ASGM sites in the Kakamega Gold Belt, western Kenya (Rosterman mine and Malinya mine)

Sample Point	Sampling point description	MeHg μg kg^{-1}	Total Hg mg kg^{-1}	Total Cr mg kg^{-1}	Total Ni mg kg^{-1}	Total As mg kg^{-1}	Total Cd mg kg^{-1}	Total Pb mg kg^{-1}
	Rosterman							
Hg-001	River water leaving mine site	< 0.1	0.086	1370	196	10.7	0.07	15.9
Hg-002	River water leaving mine site	< 0.1	0.107	747	216	13.6	0.12	21.8
Hg-003	River water leaving mine site	< 0.1	0.076	945	204	18.1	0.10	22.1
Hg-004	River water leaving mine site, point of use for domestic activities	< 0.1	0.257	657	146	16.1	0.08	16.4
Hg-005	River water after mixing point	< 0.1	0.090	1710	203	17.2	0.09	20.2
Hg-006	Mixing point of river from Rosterman mine and minor tributary from nearby villages	< 0.1	0.107	1830	237	12.0	0.10	22.4
Hg-007	Minor tributary from nearby villages	< 0.1	0.112	1940	230	7.94	0.11	17.7
Hg-008	Rosterman mine river water prior to mixing point	< 0.1	0.249	1270	209	51.2	0.07	31.4
Hg-009	River water upstream of drinking water reservoir	2.8	1.06	781	250	33.0	0.11	43.4
Hg-010	River water downstream of mining activities	< 0.1	0.325	798	202	52.1	0.12	41.5
Hg-011	River water downstream of drinking water reservoir	8.2	0.138	1200	300	21.5	0.09	24.0
Hg-012	Small pond formed from drinking water reservoir outlet	3.3	0.379	482	162	10.4	0.17	29.5
Hg-014	River water downstream of nearby disused ore washing pond	4.9	0.517	865	272	40.0	0.10	21.2
Hg-015	River water immediately after disused alluvial mining pond	0.9	1.72	681	187	77.4	0.08	24.8
Hg-017	Disused alluvial mining pond	4.8	0.721	588	151	69.4	0.08	33.7
Hg-016	River prior to disused alluvial mining pond, drinking/domestic water collection point	3.8	1.01	1010	345	16.0	0.09	18.7
Hg-018	River water prior to entering mine site	< 0.1	0.082	942	358	8.18	0.12	16.9
	Malinya							
Hg-022	River water exiting mine site, nearby alluvial working point	2.2	0.170	295	98.7	66.9	0.10	18.5
Hg-023	River water exiting mine site	6.1	0.170	316	113	52.2	0.13	31.1

Table 5 (continued)

Sample Point	Sampling point description	MeHg µg kg^{-1}	Total Hg mg kg^{-1}	Total Cr mg kg^{-1}	Total Ni mg kg^{-1}	Total As mg kg^{-1}	Total Cd mg kg^{-1}	Total Pb mg kg^{-1}
Hg-025	Pond formed from drinking water pump	36.8	1.84	275	64.2	345	0.32	38.2
Hg-026	River water flowing through mine tailings	1.0	0.377	301	73.8	28.5	0.04	18.3
USEPA	Guideline limits (EPA, 2006)	-	0.18	43.4	22.7	9.8	0.99	35.8

formed by an underground drinking water pump. The river at Malinya mine passes through mine tailings and waste material (Hg-022), thus total sediment Hg was 0.377 mg kg^{-1} but sediment MeHg was just 1.0 µg kg^{-1}. Similar findings are reported in sediments at Kenyan ASGM sites by Odumo et al. (2014), where total Hg was found to be 0.43 mg kg^{-1}. Ondayo, et al. (2023a) reported higher sediment concentrations of 2.4–6.1 mg kg^{-1} Hg at Rosterman mine, which may indicate a decrease in ASGM and alluvial activities since publication. Eleven out of twenty-one sediment samples exceeded the US EPA guideline limit for sediment Hg concentration of 0.18 mg kg^{-1} total Hg (EPA, 2006) highlighting significant environmental harm may occur within the river system. General chemistry of the sediments is in Supplementary Table 6.

Prior to entering Rosterman mine site (Hg-018), the river sediment concentration was found to be just 0.08 mg kg^{-1} Hg total with MeHg below the detection limit of < 0.1 µg L^{-1} MeHg, indicating minimal Hg transfer from potential upstream sources. This is similar to concentrations in river water determined in Ghana, where the total Hg concentration was significantly lower upstream of ASGM work (0.2 mg L^{-1} total Hg) than during and after ASGM activities (2.3 mg L^{-1} total Hg) (Samuel et al., 2018). It should be noted that no sampling procedure for water samples is reported. The most elevated Hg concentrations at Rosterman mine, 0.72–1.72 mg kg^{-1} total Hg, were found at a recently disused alluvial mining pond (Hg-015, Hg-016 and Hg-017), which acts as a dam for sediment material and thus retains much of the Hg-particulate matter. These ponds are frequently used for direct alluvial work, where metallic Hg is directly dispersed to the river by amalgamation activities with crushed gold ore to extract the precious metal, and use run off from other mining activities uphill, which also introduces Hg from other ASGM-related processes. The MeHg concentration at these sampling points is 0.9–4.8 µg kg^{-1}, decreasing as the river flows through the pond. Nearby vegetation and biotic matter in combination with the lower mobility of Hg-bound sediments provide an adequate environment for methylation of Hg to occur, thus introducing MeHg to the aquatic environment. The total Hg concentration decreased to 0.52 mg kg^{-1} total Hg (Hg-014) with MeHg concentration at 4.9 µg kg^{-1} MeHg, indicating the transfer of sedimentary materials with the river flow. As the river continues, total Hg concentrations remain consistent; however, MeHg concentrations fall below detection limit (< 0.1 µg kg MeHg) as the river flows past natural vegetation (Hg-004, Hg-006, Hg-008 to Hg-010, approximately 100 m), which take up Hg compounds such as MeHg (Schwesig & Krebs, 2003). At Malinya, the concentrations of total Hg at the point of the river leaving the site (Hg-022 and Hg-023) are just 0.17 mg kg^{-1} total Hg, similar to concentrations found at Rosterman mine site from samples at distance from mining activities (0.09–0.25 mg kg^{-1}), with MeHg concentrations at 2.2–6.1 µg kg^{-1}. As the river passes through mine tailings at Malinya (Hg-026), the concentration was found to be 0.377 mg kg^{-1} total Hg, potentially caused by the residual Hg in artisanal mine waste material. The MeHg concentration at Hg-026 was just 1.0 µg kg^{-1} MeHg, likely due to the more mobile flow of the river and constant supply of new sediment matter reducing the likelihood of Hg methylation. At a pond formed by an underground drinking water pump, total Hg concentration was 1.84 mg kg^{-1} total Hg and MeHg was the highest concentration

found at 36.8 µg kg^{-1} MeHg. Due to the non-mobile nature of the sediment matter in the pond, the conditions are likely ideal for bacterial methylation of Hg. Concentrations of iron (Fe) in the water samples collected from this pump are much higher than the other samples collected (1250–1900 µg L^{-1} Fe at the pump, 0.7–1150 µg L^{-1} Fe at the other sampling points) which supports bacterial methylation of Hg, as Fe is a key component in the methylation process (Flemming et al., 2006; Kerin et al., 2006).

Despite concentrations of Hg being lower than guideline limits, other elements must be considered for their potentially toxic nature; Cr, As, Ni, Cd, and Pb are potentially toxic elements commonly measured in sediments taken from ASGM sites to assess pollution from mining activities and subsequently environmental health. Concentration of Cr at Rosterman ranged between 482 and 1940 mg kg^{-1} Cr, far exceeding the US EPA guideline limit of 43.4 mg kg^{-1} Cr (EPA, 2006), with the highest concentration in the minor tributary originating from other nearby villages (Hg-007) and the point at which the rivers mix (Hg-006). At Malinya, Cr concentrations were typically lower than Rosterman, at 275–316 mg kg^{-1} Cr, but still above guidance limits. Concentrations of As showed an inverse trend to Cr with more elevated concentrations at Malinya (28.5–349 mg kg^{-1} As) than Rosterman (7.94–77.4 mg kg^{-1} As). Nineteen out of twenty-one sediment samples were above the US EPA guideline limit of 9.8 mg kg^{-1} As (EPA, 2006), one of which was prior to the river entering the mine site (Hg-018). This implies the mining activities introduce an environmentally significant amount of Cr to the river, which is transported downstream and further pollutes the aquatic environment. Concentration of Ni, Cd and Pb were consistent across both mine sites, at 64.2–358 mg kg^{-1} Ni, 0.04–0.32 mg kg^{-1} Cd and 15.5–41.5 mg kg^{-1} Pb. All samples exceeded the US EPA guideline limit for Ni of 22.7 mg kg^{-1} (EPA, 2006), three samples (Hg-010, Hg-001 and Hg-025) exceeded the guideline limit of 35.8 mg kg^{-1} Pb including the pond formed by the drinking water pump at Malinya mine, but no samples exceeded the 0.99 mg kg^{-1} Cd guideline limit. The concentrations of Cr, As, and Ni highlight the need to monitor toxic elements in addition to the focus on Hg around ASGM sites, to mitigate pollution to the environment.

Conclusion

The monitoring of Hg species in water samples has been a challenge to many environmental studies, often limiting the collection and use of Hg speciation data from some of the most vulnerable areas. The development of a novel SPE-based method for sampling and preservation of MeHg and Hg^{2+}, achieving recoveries of $96 \pm 5\%$ Hg^{2+} and $112 \pm 11\%$ MeHg, will allow for representative concentrations to be determined from a variety of water matrices, with representative data obtainable up to 4-weeks after collection. Previous literature surrounding water pollution in ASGM has either not included Hg speciation, or often does not report sampling and preservation methods used, thus the optimised SPE-based method demonstrates robust Hg speciation sampling and preservation capability for use in complex water matrices. Salinity, humic matter and pH between pH 5–8 show negligible impact on the sorption and recovery of Hg species from the functionalised cartridge. It is important to note that high pH ($pH > 8$) may remobilise the dithizone phase, thus significantly impacting retention of Hg species. This method was applied to a challenging environment (ASGM sites in western Kenya), for the assessment of Hg species in environmental samples. Waters at both Rosterman and Malinya mine site were below chronic environmental guideline limits, and thus ambient water concentrations do not pose an immediate environmental risk. Drinking water sources may pose a risk to human health when consumed as part of a diet with other Hg-contaminated substances, as waters make up 20–30% of the tolerable weekly intake of Hg. This outcome is important to guide future monitoring of ASGM activities to ensure they are assessed using representative data—often due to the use of inappropriate sample preservation, the problem of Hg exposure from drinking water associated with ASGM is underestimated. Sediment concentrations demonstrate a less time-dependent method of assessing Hg pollution due to being less mobile than waters, and the majority of collected samples exceeded safety thresholds for Hg, As, Cr, Ni and Pb, highlighting the need for to consider potentially toxic elements besides Hg. The cartridge method applied in this study demonstrates a novel, robust

approach to preservation of Hg species, to provide a better understanding of ecological health, monitoring of aquaculture for food safety (Aura et al., 2024; Marriott et al., 2023), and human and environmental exposure routes (Ondayo et al., 2024) in the challenging environments of ASGM sites. Thus, regulatory authorities can obtain representative Hg pollution data to construct appropriate policies and take informed approaches to implementing Hg-free alternative to protect human and environmental health.

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Data Availability No datasets were generated or analysed during the current study.

Declarations

Conflict of interests The authors declare no competing interests.

References

Aura, C. M., Humphrey, O. S., Marriott, A. L., Watts, M. J., Ongore, C. A., Mwamburi, J. M., Osano, O., & Coffey, T. J. (2024). Assessing the spatial distribution of elemental concentrations in surface sediments of Lake Victoria Kenya: Implications for ecological health and management. *Environmental Geochemistry and Health, 46*, 137. https://doi.org/10.1007/s10653-024-01930-7

Balarama Krishna, M. V., Chandrasekaran, K., & Karunasagar, D. (2010). On-line speciation of inorganic and methyl mercury in waters and fish tissuesusing polyaniline microcolumn and flow injection-chemical vapourgeneration-inductively coupled plasma mass spectrometry (FI-CVG-ICPMS). *Talanta, 81*, 462–472.

Blanco, R. M., Villanueva, M. T., Sánchez Urıa, J. E., & Sanz-Medel, A. (2000). Field sampling, preconcentration and determination of mercury species in river waters. *Analytica Chimica Acta, 419*, 137–144.

Driscoll, C. T., Mason, P. R., Chan, H. M., Jacob, D. J., & Pirrone, N. (2013). Mercury as a global pollutant: Sources, pathways, and effects. *Environmental Science and Technology, 47*, 4967–4983.

Du, M., Wei, D., Tan, Z., Lin, A., & Du, Y. (2015). Predicted no-effect concentrations for mercury species and ecological risk assessment for mercury pollution in aquatic environment. *Journal of Environmental Sciences (China), 28*, 74–80.

EPA, U. (1995). *1995 Water Quality Criteria*.

EPA, U. (2006). *Freshwater Sediment Screening Benchmarks*.

Favilli, L., Giacomo, A., Malandrino, M., Inaudi, P., Diana, A., & Abollino, O. (2022). Strategies for mercury speciation with single and multi-element approaches by HPLC-ICP-MS. *Frontiers in Chemistry, 10*, 1082956.

Flemming, E. J., Mack, E. E., Green, P. G., & Nelson, D. C. (2006). Mercury methylation from unexpected sources: molybdate-inhibited freshwater sediments and an iron-reducing bacterium. *Applied and Environmental Microbiology*. https://doi.org/10.1128/AEM.72.1.457-464.2006

Kerin, E. J., Gilmour, C. C., Roden, E., Suzuki, M. T., Coates, J. D., & Mason, R. P. (2006). Mercury methylation by dissimilatory iron-reducing bacteria. *Applied and Environmental Microbiology, 72*(12), 7919.

Kim, M. K., & Zoh, K. D. (2012). Fate and transport of mercury in environmental media and human exposure. *Journal of Preventive Medicine and Public Health, 45*(6), 335–343. https://doi.org/10.3961/jpmph.2012.45.6.335

King, D. C. P., Watts, M. J., Hamilton, E. M., Mortimer, R., Kilgour, D. P. A., & Di Bonito, M. (2023). The present and potential future of aqueous mercury preservation: a review. *Environmental Science Processes Impacts, 3*, 351.

King, D. C. P., Watts, M. J., Hamilton, E. M., Mortimer, R. J. G., Coffey, M., Osano, O., Ondayo, M. A., & Di Bonito, M. (2024). Field method for total mercury in waters, including those associated with artisanal scale gold mining. *Analytical Methods*. https://doi.org/10.1039/d3ay02216a

Louie, H., Wong, C., Huang, Y. J., & Fredrickson, S. (2012). A study of techniques for the preservation of mercury and other trace elements in water for analysis by inductively

coupled plasma mass spectrometry (ICP-MS). *Analytical Methods, 4*(2), 522–529. https://doi.org/10.1039/c2ay05182f

Marriott, A. L., Osano, O. F., Coffey, T. J., Humphrey, O. S., Ongore, C. O., Watts, M. J., & Aura, C. M. (2023). Considerations for environmental biogeochemistry and food security for aquaculture around Lake Victoria Kenya. *Environmental Geochemistry and Health*. https://doi.org/10.1007/s10653-023-01585-w

Odumo, O. B., Carbonell, G., Angeyo, H. K., Patel, J. P., Torrijos, M., & Martín, J. A. R. (2014). Impact of gold mining associated with mercury contamination in soil, biota sediments and tailings in Kenya. *Environmental Science and Pollution Research*. https://doi.org/10.1007/s11356-014-3190-3

Ondayo, M. A., Watts, M. J., Hamilton, E. M., Mitchell, C., Mankelow, J., & Osano, O. (2023a). Artisanal gold mining in Kakamega and Vihiga counties, Kenya: Potential human exposure and health risk. *Environmental Geochemistry and Health, 45*, 6543–6565.

Ondayo, M. A., Watts, M. J., Mitchell, C., King, D. C. P., & Osano, O. (2023). Review: Artisanal gold mining in Africa—Environmental Pollution and Human Health Implications. *Exposure and Health, 16*, 1067.

Ondayo, M. A., Watts, M. J., Humphrey, O. S., & Osano, O. (2024). Public health assessment of Kenyan ASGM communities using multi-element biomonitoring, dietary and environmental evaluation. *Ecotoxicology and Environmental Safety, 277*, 116323.

Parker, J. L., & Bloom, N. S. (2005). Preservation and storage techniques for low-level aqueous mercury speciation. *Science of the Total Environment, 337*(1–3), 253–263. https://doi.org/10.1016/j.scitotenv.2004.07.006

Richard, J. H., Bischoff, C., Ahrens, C. G. M., & Biester, H. (2016). Mercury (II) reduction and co-precipitation of metallic mercury on hydrous ferric oxide in contaminated groundwater. *Science of the Total Environment, 539*, 36–44.

Samuel, W., Buamah, R., Essandoh, H., & Darkwah, L. (2018). Mapping, characterization and dispersion of metallic pollutants within a catchment of illegal gold mining activities in Ghana. *American Journal of Engineering and Applied Sciences*. https://doi.org/10.3844/ajeassp.2018.111.120

Schwesig, D., & Krebs, O. (2003). The role of ground vegetation in the uptake of mercury and methylmercury in a forest ecosystem. *Plant and Soil, 253*, 445.

Smith, E. J., Davidson, W., & Hamilton-Taylor, J. (2002). Methods for preparing synthetic freshwater. *Water Research, 36*, 1286–1296.

USEPA. (1996). *Method 1669 Sampling ambient water for trace metals at EPA water quality criteria levels*.

USEPA. (2002). *Method 1631, Revision E: Mercury in water by oxidation, purge and trap, and cold vapour atomc fluorescence spectrometry*.

Wang, Y., Zhu, A., Fang, Y., Fan, C., Guo, Y., Tan, Z., Yin, Y., Cai, Y., & Jiang, G. (2022). Dithizone-functionalized C18 online solid-phase extraction-HPLC-ICP-MS for speciation of ultra-trace organic and inorganic mercury in cereals and environmental samples. *Journal of Environmental Sciences (China), 115*, 403–410. https://doi.org/10.1016/j.jes.2021.08.013

Watts, M. J., Barlow, T. S., Button, M., Sakar, S. K., Bhattacharya, B. D., Aftab Alam, M., & Gomes, A. (2013). Arsenic speciation in polychaetes (Annelida) and sediments from the intertidal mudflat of Sundarban mangrove wetland India. *Environmental Geochemistry and Health, 35*, 13.

Watts, M. J., Button, M., Brewer, T. S., Jenkin, G. R. T., & Harrington, C. F. (2008). Quantitative arsenic speciation in two species of earthworms from a former mine site. *Journal of Environmental Monitoring, 10*, 753–759.

Watts, M. J., Middleton, D. R. S., Marriott, A. L., Humphrey, O. S., Hamilton, E. M., Gardner, A., Smith, M., McCormack, V. A., Menya, D., Munishi, M. O., Mmbaga, B. T., & Osano, O. (2019). Source apportionment of micronutrients in the diets of Kilimanjaro Tanzania and Counties of Western Kenya. *Science Reports, 9*, 14447.

WHO. (2003). *Guidelines for safe recreational water environments: coastal and fresh waters (Vol. 1).*

WHO. (2005). *Mercury in Drinking-water*.

WHO. (2006). *Guidelines for safe recreational water environments. (Vol. 2).*

WHO. (2017). *Guidelines for drinking-water quality: First addendum to the fourth edition*.

WHO. (2021). *Guidelines for safe recreational water environments: Coastal and fresh waters (Vol. 1).*

WHO. (2022). *Guidelines for Drinking Water Quality*.

Yin, Y.-G., Chen, M., Peng, J.-F., Liu, J.-F., & Jiang, G.-B. (2010). Dithizone-functionalized solid phase extraction–displacement elution-high performance liquid chromatography–inductively coupled plasma mass spectrometry for mercury speciation in water samples. *Talanta, 81*, 1788–1792.

Yu, L.-P., & Yan, X.-P. (2003). Factors affecting the stability of inorganic and methylmercury during sample storage. *TrAC Trends in Analytical Chemistry, 22*(4), 245–253. https://doi.org/10.1016/s0165-9936(03)00407-2