The Development of Preservation Methods for Mercury and its Species in Water Samples

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Copyright Statement

The work presented in this thesis has not been submitted for a comparable academic award.

Abstract

Some of the most toxic compounds in the environment are mercury (Hg) and its species. Monitoring the pollution of Hg is of paramount performance in all spheres of the environment, and in particular in vulnerable areas such as artisanal and small-scale gold mines (ASGM). These areas produce the majority of anthropogenic Hg emissions, yet currently, recommended sampling and preservation methodologies in the scientific literature are not appropriate for the determination of Hg and its species from water sources in these and other such challenging environments. These waters are fully integrated into the local communities, providing drinking water, domestic, agricultural, aquacultural and industrial uses, among others.

In water samples, Hg species are known to be highly unstable, with total loss of Hg within a few days if unpreserved. Recommended procedures for preserving Hg in water samples involve the acidification of waters, which can pose significant risk to field workers in uncontrolled environments. Therefore, it is recommended to collect at least 500 mL of water in glass or PTFE containers, which are fragile and expensive, respectively, and require immediate shipment of samples to a laboratory for analysis. This is inadequate for use in challenging environments, such as remote locations or ASGM sites, due to the difficulty in sample transport, particularly when collecting multiple samples for total Hg and different Hg species, as well as the lack of accessible laboratories with appropriate facilities to preserve and analyse Hg, often found in environmental and polluted waters at $\mu g\ L^{-1}$ to $ng\ L^{-1}$ ranges.

Therefore, the aim of this thesis was to develop a sampling method for Hg and its species in water samples, that can preserve Hg speciation for an adequate time from field-to-laboratory and is applicable to supporting the study of ASGM activities and their impact on environmental and human health.

To achieve this, a literature survey of Hg preservation methods and solid-phase extraction (SPE) methods was conducted and thus a dithizone-functionalised SPE methodology was developed as a sampling technique for the preservation of Hg for up to 4-weeks from waters associated with ASGM activities. This timescale was chosen as a practical time for transport of samples from field to laboratory, including where international transportation is required. Total Hg concentrations are used for guideline concentrations and therefore it is vital to ensure the method can reliably produce representative data from the field, and so the functionalised cartridge demonstrated 85 ± 10% recovery of total Hg from water samples after 4-weeks of storage. The method was also applied in ASGM sites to demonstrate the robustness

of the method in an appropriate environment, showing total Hg concentrations below guideline limits (6 µg L^{-1} in drinking water and 0.77 µg L^{-1} in environmental waters), between 0.01 to 0.35 µg L^{-1} Hg across river, mineshaft, and spring water, and ore washing ponds.

The functionalised cartridge was further assessed for Hg species sampling and preservation (inorganic mercury, Hg^{2+}, and methylmercury, MeHg) from waters associated with ASGM activities. The functionalised cartridge showed recovery of 115 ± 8% (4°C, absence of light) and 109 ± 13% % (16°C, absence of light) MeHg and 100 ± 14% % (4°C, absence of light) and 94 ± 12% % (16°C, absence of light) Hg^{2+} over 4 weeks of storage.

The cartridges were then used in ASGM sites in western Kenya alongside collection of river sediment samples, to obtain Hg speciation data and assess the relationship between waters and sediments, in the context of human health exposure. All sampled water sources provided no detectable MeHg and 0.06 to 0.67 µg L^{-1} Hg^{2+}, below environmental guideline limits (0.77 µg L^{-1} Hg). Drinking water sources showed Hg^{2+} concentrations making up approximately 30% of the total weekly Hg intake. At the same drinking water sources, sediments measured up to 2 mg kg^{-1} total Hg and 64.8 µg kg^{-1} MeHg, showing contamination from the mining activities and significant methylation of the Hg species. Total Hg concentrations in sediments were between 0.09 to 1.72 mg kg^{-1} total Hg, with the most elevated concentrations from sampling points near to active alluvial work and ponds previously used for alluvial activities. The case study highlighted relationships between sediment and river water Hg^{2+} and MeHg concentrations, where areas of stagnation show greater concentrations of both Hg species.

The results of this work contribute to the knowledge of Hg sampling and preservation, demonstrating the application of SPE as an in-field sampling and preservation method for Hg and its species. The project outcome will enable the robust monitoring of Hg species and consequences of their exposure to environment and human health, enabling a more holistic approach to environmental Hg pollution studies. Thus, this can pave the way for both local communities and policymakers to be better informed of the impact from Hg pollution originating from ASGM activities, building the case to empower change to Hg-free alternatives.

List of Publications

King, D.C.P., Watts, M.J., Hamilton, E.M., Mortimer, R.J.G., Kilgour, D.P.A., Di Bonito, M., 2023, The present and potential future of aqueous mercury preservation: a review, *Environ. Sci. Process. Impacts.* https://doi.org/10.1039/D2EM00409G

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 preservation of total mercury in waters, including those associated with artisanal scale gold mining, *Anal. Methods*, https://doi.org/10.1039/D3AY02216A

King, D.C.P., Watts, M.J., Hamilton, E.M., Mortimer, R.J.G., Coffey, M., Osano, O., Di Bonito, M., (*In-Print)*, Mercury speciation of environmental samples associated with artisanal small-scale gold mines using a novel solid-phase extraction approach to sample preservation, *Environ. Geochem. Health.*

Ondayo, M.A., Watts, M.J., Mitchell, C.J., King, D.C.P., 2023, Review: Artisanal Gold Mining in Africa—Environmental Pollution and Human Health Implications. *Expo Health*, https://doi.org/10.1007/s12403-023-00611-7

List of Conference Attendance

King, D.C.P., Watts, M.J., Hamilton, E.M., Mortimer, R.J.G., Kilgour, D.P.A., Di Bonito, M., (2022), 'The Preservation of Total Dissolved Mercury from Water Samples using Functionalised C18 Solid-Phase Extraction' [Poster], Biennial National Atomic Spectrommetry Symposium 2022, Manchester, UK, June 28th – June 29th.

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List of Abbreviations

Symbol	Meaning
AAS	Atomic absorption spectroscopy
Al^{3+}	Aluminium (II) ion
AMA	Advanced mercury analyser
As	Arsenic
A_s	Abundance of the reference isotope in the spike solution
As(III)	Arsenic (III) oxidation state
As(IV)	Arsenic (IV) oxidation state
ASGM	Artisanal and small-scale gold mining
Au	Gold
AuNP	Gold nanoparticles
A_x	Abundance of the reference isotope in the sample
Å	Ångstrom
BGS	British Geological Survey
B_s	Abundance of the spike isotope in the spike solution
B_x	Abundance of the spike isotope in the sample
C18	Octadecyl bonded silica gel
Ca^{2+}	Calcium ion
Cd	Cadmium
Cd^{2+}	Cadmium (II) ion
Cl-	Chloride ion
cm	centimeter
C_s	Concentration of methylmercury in spike solution
Cu^{2+}	Copper (II) ion
CV	Cold vapour
C_x	Concentration of methylmercury in sample
DGT	Diffusive gradient thin-film
DI water	Deionised water
°C	Degrees Celcius
$EtHg^{+}$	Ethylmercury ion
FAAS	Flame atomic absorption spectroscopy
Fe	Iron
Fe^{2+}	Iron (II) ion
Fe^{3+}	Iron (III) ion
GBP	Great British Pound sterling
h	hours
H^{+}	Hydrogen ion or Proton
H_2SO_4	Sulphuric acid
HA	Humic acid
HCl	Hydrochloric acid
$HClO_4$	Perchloric acid
HDPE	High density polyethylene
HF	Hydrofluoric acid
Hg	Mercury

$Hg(OH)_2$	Mercuric hydroxide
$Hg(OH)_2^{2+}$	Mercuric hydroxide ion
$Hg_{(p)}$	Particulate bound mercury
$Hg(SH)_2$	Dithiomercury
$Hg(SH)S^-$	Mercury thiol-compounds
Hg^0	Elemental mercury
Hg^{2+}	Mercuric mercury
Hg_2^{2+}	mercurous mercury
$Hg_3S_2Cl_2$	Corderoite
$HgCl_2$	Chloromercury
$HgCl_3^-$	Trichloromercury
$HgCl_4^{2-}$	Tetrachloromercury
Hg-N	Mercury-nitrogen bond
HgS	Cinnabar and mercury-sulphur minerals
Hg-S	Mercury-sulphur bond
$HgSb_4S_8$	Livingstonite
$HgSH^+$	Thiomercury ion
HNO3	Nitric acid
HPLC	High performance liquid chromatography
HPLC-ICP-MS	High performance liquid chromatography - inductively coupled plasma - mass spectrommetry
i.d.	Internal diameter of column
ICP-MS	Inductively coupled plasma - mass spectrometry
ID-ME	Isotope dilution - microwave extraction
kg	kilogram
km	kilometer
L	Litre
LC	Liquid chromatography
LC-AFS	Liquid chromatorgraphy - atomic fluorescence spectroscopy
LC-ICP-MC	Liquid chromatography - inductively coupled plasma - mass spectrometry
LD10	Lethal Dose (10)
LDPE	Low density polyethylene
M	Molarity
m	meter
Me_2Hg	Dimethylmercury
MeHg	Methylmercury
$MeHg^+$	Methylmercury ion
mg	Milligram
mg kg^{-1}	Milligram per kilogram
mg L^{-1}	Milligram per litre
Mg^{2+}	Magnesium ion
min	Minute
mL	Millilitre
mL min^{-1}	Millilitre per minute
mM	Millimolar
mmol	Millimoles

mol	Moles
mol L^{-1}	Moles per litre
ms	Milliseconds
M_s	Relative atomic mass of spike solution
M_x	Relative atomic mass of sample
µg	Microgram
µg kg^{-1}	Microgram per kilogram
µg L^{-1}	Microgram per litre
µL	Microlitre
µm	Micrometer
µS cm^{-1}	Microsiemens per centimeter
n	Number of replicates/samples
N.d	Not determined
Na	Sodium
Na+	Sodium ion
NaCl	Sodium chloride
ng	Nanogram
ng L^{-1}	Nanogram per litre
Ni	Nickel
nm	Nanometer
NO_3^+	Nitrate ion
NRCC	National Radiological Centre of Canada
NTU	Nottingham Trent University
PANi	Polyaniline
Pb	Lead
PE	Polyethylene
PET	Polyethylene terephthalate
pg	Picogram
pg L^{-1}	Picogram per litre
PhHg	Phenylmercury
PP	Polypropylene
PTFE	Polytetrafluoroethylene
PVA	Polyvinylacetate
PVC	Polyvinylchloride
R	Ratio of reference isotope to spike isotope in the spiked sample
S	Sulphur
s	seconds
Sn	Tin
SO_4^{2-}	Sulphate ion
SPE	Solid-phase extraction
3σ	3 times the standard deviation
Total Alk	Total alkalinity
US EPA	United States Environmental Protection Agency
USD	United States Dollar
USGS	United States Geological Survey

V	Vanadium
V(IV)	Vanadium (IV) oxidation state
V(V)	Vanadium (V) oxidation state
v/v	Volume per volume
w/v	Weight per volume
WHO	World Health Organisation
W_s	Mass of spike solution
W_x	Mass of sample
Zn	Zinc
Zn^{2+}	Zinc (II) ion
β-activity	Beta particle activity

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Chapter 1 – Mercury, environmental and human exposure, and toxicity

The aim of this thesis is to develop an in-situ sampling method for mercury (Hg) in environmental waters, the overall structure is detailed in Section 1.6 Thesis Structure; briefly, the issue of Hg as an environmental pollutant is detailed and the problems surrounding preservation and sampling. This is followed by development of a sampling method for total Hg in water samples, and then further assessment for Hg species. Speciation analysis of environmental samples from vulnerable areas was then conducted. The main outcomes of the work are then summarised.

1.1. Mercury

Mercury (Hg) is a toxic, naturally occurring transition metal (WHO, 2005, Kim and Zoh, 2012) with atomic number 80 and a standard atomic mass of 200.59. As a metal, it is uniquely a liquid at standard temperature and pressure. There are three main oxidation states of Hg: Hg^0 (elemental or metallic Hg), Hg_2^{2+} (mercurous Hg), and Hg^{2+} (mercuric Hg), of which Hg_2^{2+} is generally unstable and will disproportionate to Hg^0 and Hg^{2+} (O'Driscoll, et al., 2005). There are seven stable isotopes of Hg in the environment: ^{196} Hg (0.15%), ^{198}Hg (10.1%), ^{199}Hg (17.0%), ^{200}Hg (23.3%), ^{201}Hg (13.2%), ^{202}Hg (29.6%), and ^{204}Hg (6.7%). A notable characteristic of Hg is the ability to form stable amalgams with many other metals, such as gold (Au), zinc (Zn), tin (Sn), sodium (Na), etc. There is no known health benefit to Hg and the toxicity of all Hg species is well documented, with the WHO classifying Hg and its species as one of the top ten chemicals posing a major public health concern (WHO, 2005, WHO, 2022). Despite this, Hg is present in all spheres of the environment as a result of both natural processes and anthropogenic activity (Gworek et al., 2016, Kim and Zoh, 2012, Ondayo et al., 2023b).

1.2. Environmental exposure and toxicity

1.2.1. The environmental Hg cycle

Once released to the environment, global distribution of Hg is facilitated by atmospheric species (Lyman et al., 2020). Elemental Hg vapour, Hg^0, is the predominant species in the atmosphere at >98% of atmospheric Hg (Sommar et al., 2020, Fu et al., 2016) and has a long atmospheric lifetime of 0.8-72 years depending on atmospheric location (Saiz-Lopez et al., 2018, Slemr et al., 2018, Sommar et al., 2020), allowing for global transportation of the vapour before deposition (Driscoll et al., 2013). Atmospheric Hg^0 is oxidised to inorganic Hg species (Hg^{2+}) through various, not-well understood, pathways, which can then be deposited to the aquatic

and terrestrial environment through wet- and dry-deposition events (Sommar et al., 2020, Kim and Zoh, 2012, Beckers and Rinklebe, 2017). Thus, terrestrial and aquatic environments have a concentration of Hg of 60 µg kg^{-1} in soils and sediments, 0 – 5 ng L^{-1} Hg in marine waters and 5 – 30 ng L^{-1} Hg in river, estuarine and coastal waters, even when there is no nearby release of the metal (Lyman et al., 2020, Beckers and Rinklebe, 2017, Kim and Zoh, 2012). Once in the aquatic environment, Hg^{2+} may bind to particulate matter and sediments where further transformation to mineralogical or organomercurial species can occur. The metal can bind irreversibly to S in sediments and form stable, insoluble Hg-S minerals. Labile Hg bound to sediments can be methylated by S-reducing bacteria, resulting in synthesis of organomercurial chemicals such as methlmercury (MeHg) (Kim and Zoh, 2012, Nogara et al., 2019). These compounds can bioaccumulate in fish and seafood, increasing up trophic levels and resulting in up to 10^6 times the concentration of Hg in predatory fish tissue when compared to the aquatic environment (Leopold et al., 2010, Leopold et al., 2009). The long-lasting nature of the element in the environment results in pollution significantly affecting nearby soils, sediments and waters (Ondayo et al., 2023a, Ondayo et al., 2023b). Thus, there is a need to monitor Hg in environmental matrices, to manage and mitigate exposure to the environment and humans.

1.2.2. Natural emissions of Hg

Hg is found in the Earth's crust at 0.05 mg kg^{-1}, typically as the mineral cinnabar (HgS minerals) (Rudnick and Gao, 2003). Mineralogically, it occurs as cinnabar (HgS), corderoite ($Hg_3S_2Cl_2$), and livingstonite ($HgSb_4S_8$), as well as others (USGS, 2024). The abundance of Hg-S minerals is due to the strong binding forces found between Hg, a soft acid, and S, a soft base, which also allow HgS to form in a range of pH conditions (Paquette and Helz, 1995). Natural Hg emissions to the environment are predominantly from volcanic activity, with degassing from Hg deposits and weathering contributing to emissions, and have been estimated to be ~1 kilotonne $year^{-1}$; anthropogenic emissions far outweigh this and have been estimated as large as 4.6 kilotonnes $year^{-1}$ (Outridge et al., 2018, UNEP, 2008).

1.2.3. Anthropogenic emissions of Hg

The anthropogenic use of Hg stretches back thousands of years, with civilisations from as 4000 BC using cinnabar as a bright red pigment (Beckers and Rinklebe, 2017, Young and Kaplan, 2023). Other historical uses of Hg include alchemically as an ingredient for synthesising gold (Au), medicinal purposes as a treatment for skin conditions, syphilis and other ailments, and felting in hat-making. More recent uses

include as thermometers, calomel probes, in batteries and electronics, as dental amalgams for fillings, as industrial catalysts for chemical synthesis, and as fungicides and pesticides. In many of these examples, Hg use has been phased out for less toxic approaches.

A major anthropogenic use of Hg is for the extraction of gold by amalgamation, a process developed hundreds of years ago and still used today in the artisanal and small-scale gold mining (ASGM) sector. The ASGM sector is currently considered the largest pollutant of Hg to the environment, accounting for nearly 40% of total anthropogenic Hg emissions (Figure 1.1) (UNEP, 2018).

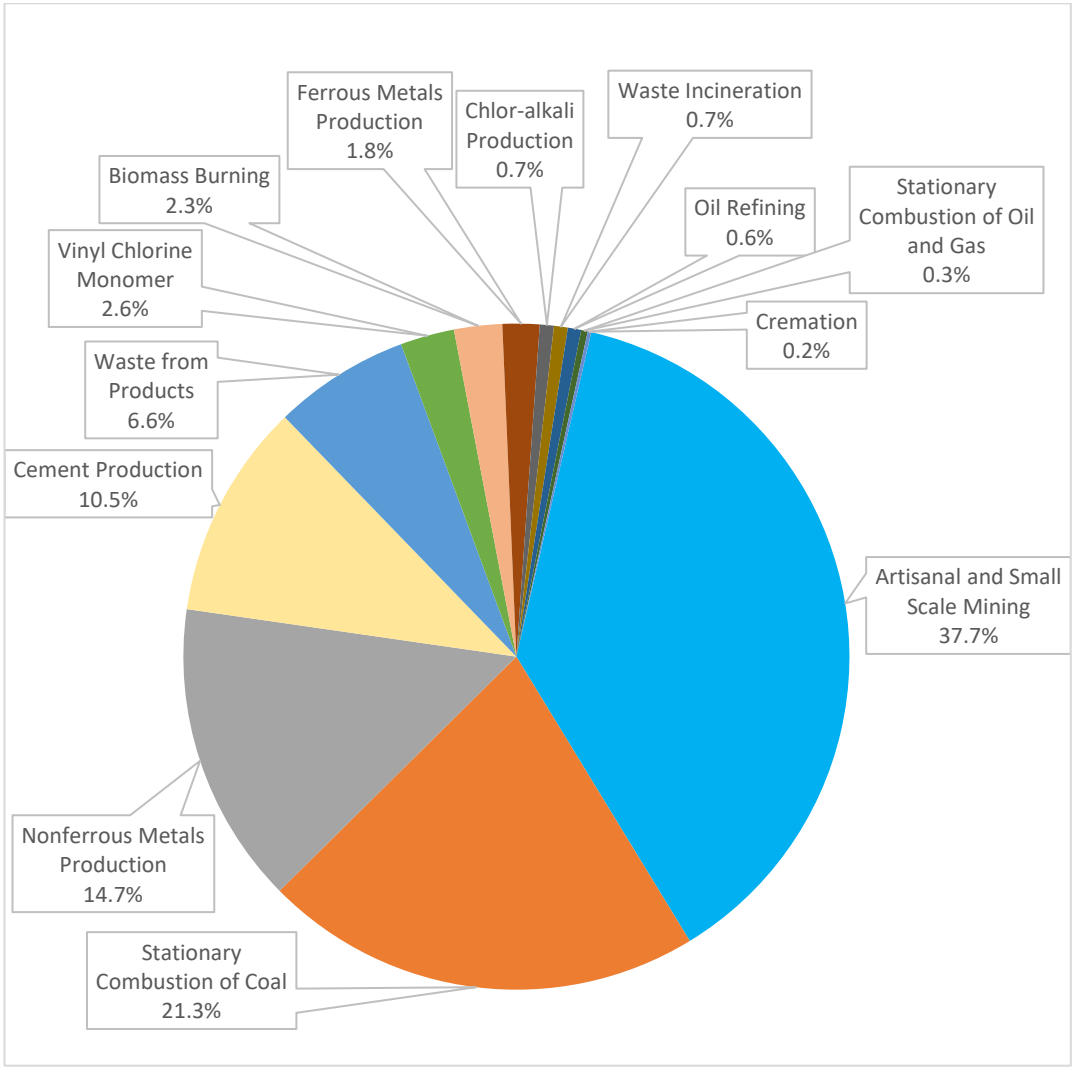

Figure 1.1 Anthropogenic emission sources, as reported by the UNEP (2018)

1.2.4. Artisanal and Small-Scale Gold Mining (ASGM)

The ASGM sector provides income for over 45 million workers directly and supports a further >150 million people globally (IGF, 2018, Ondayo et al., 2023b). These activities are most commonly performed in lower income countries, such as in Sub-

Saharan Africa, South America, and Asia, where gold veins are present (Ondayo et al., 2023b). These gold deposits have often been previously exploited for conventionally mineable gold or are not at a level commercially viable for traditional mining, and so small-scale practices are set up by local communities.

In ASGM activities, gold ore is mined from disused commercial mines, where it is then crushed and sluiced to a fine powder (IGF, 2018, Ondayo et al., 2023b). Due to the ability of Hg to form stable amalgams with many precious metals through metal-metal bonding, the liquid metal is mixed with the crushed ore to amalgamate the gold particles from the sluiced sedimentary material. This process is often conducted in nearby rivers and waterways, thus polluting the metal directly to the aquatic environment (IGF, 2018, Ondayo et al., 2023a,b). The resulting amalgam is then burned to produce small quantities of gold by releasing the bonded Hg to the atmosphere. Photos of the ASGM process are shown in Appendix 1.

Discussion with the miners revealed safety precautions at ASGM sites are rudimentary and often neglected for both chemical and physical hazards. Helmets and gloves are often not worn when working, and lighting conditions in the mines are poor. Explosives are sometimes used to mine the ore, resulting in potential mine shaft collapse and harm to the workers. The alluvial process of amalgamation is conducted with bare hands in rivers, upstream of drinking, domestic, agricultural and aquacultural waters. The resulting amalgam is burned with poor ventilation or air filtration, resulting in Hg vapours being inhaled directly by workers or released to the nearby environment. These processes lead to significant Hg pollution and exposure to human health (Ondayo et al., 2023a, Ondayo et al., 2023b, Beckers and Rinklebe, 2017, Kim and Zoh, 2012, Kristensen et al., 2014, Ngure et al., 2017, Olgola et al., 2001).

At Kenyan ASGM sites, Ondayo et al. (2024) reported Hg urinary concentrations of up to 178 μg L^{-1} Hg and hair concentrations of up to 12.8 mg kg^{-1} Hg, far exceeding those of non-ASGM residents with <0.14 μg L^{-1} urinary Hg and 0.14 mg kg^{-1} Hg in hair. All eleven active ASGM workers the study investigated reported symptoms of chest pain, headaches, strokes, epilepsy and fertility problems, which are all associated with Hg-poisoning (USEPA 2024) although the authors did not define a cause for the symptoms presented due to needing further study.

Replacement of Hg in the ASGM process is difficult. While alternative extraction methods to Hg, such as cyanide leaching or borax smelting, are more efficient, they

are often more complex and the cost is impractical for installation and operation (Manzila, 2022).

1.3. Mercury poisoning and bioaccumulation

In organisms, Hg compounds have an affinity to bind with sulfhydryl group found in proteins (WHO, 2005, WHO, 2003, Kim and Zoh, 2012, Liu et al., 2011). This binding results in bioaccumulation of the toxic metal, being found to persist in biological tissues long after exposure, with a biological half-life of approximately 50 days (US, 2000). This longevity and bioaccumulation often result in high concentrations of Hg in marine and aquatic organisms, particularly those living in contaminated waters (Muñoz et al., 2005). Contaminated tissue consumed by other organisms results in biomagnification of mercury up the food chain; predatory fish can have 10^6 times higher Hg than the surrounding waters, with 95% being MeHg (Leopold et al., 2010). This results in exposure to much higher concentrations of Hg than from ambient exposure (Liu et al., 2011, Ekino et al., 2007). Constant exposure has been modelled showing intake of Hg equalling excretion of Hg at roughly 1 year, accumulation being found to be 100 times the average daily intake (WHO, 1990).

Table 1.1 Mercury species, their major exposure routes, and their toxicological information

Species	Major exposure route	Target organs	Biological reaction	Other relevant information
Hg^0	Inhalation, dermal adsorption (minor)	Brain, central nervous system, liver and kidneys	Oxidised to Hg^{2+}, by hydrogen peroxide – catalase reaction	Low bioaccessibility through consumption, but much higher bioaccessibility through inhalation.
Hg^{2+}	Consumption	Liver and kidneys	N/A	Low bioaccessibility, so
MeHg	Dermal adsorption, consumption of fish and seafood	Brain and central nervous system	Demethylation to Hg^{2+} in target organ	Highly bioaccessible, easily entering bloodstream. Easily crosses placental barrier.

1.3.1. Elemental mercury (Hg^0)

The metallic species of Hg is the most predominant species found in the atmosphere. The major exposure to human health is through inhalation, where approximately 80% of Hg^0 vapours can be absorbed by the respiratory system (Langford and Ferner, 1990). Bioavailability through oral consumption or dermal contact of Hg^0 is poor, and the oral lethal dose (LD_{10}) is 1429 mg kg^{-1} Hg^0 (Langford and Ferner, 1990). Metallic Hg is highly lipophilic; thus once in the bloodstream, Hg^0 will readily cross the blood-

brain barrier and placental barrier, and result in Hg poisoning and Minamata disease (Clarkson and Magos, 2008). Therefore, exposure of open wounds should be avoided. Once in the bloodstream and target organs, Hg^0 is oxidized to Hg^{2+}, which results in severe negative health effects. The biological half-life of Hg^0 is approximately 10 days in blood and 2 days in the respiratory system, with excretion typically occurring via the renal system (Sandborgh-Englund et al., 1998). Symptoms of exposure to Hg^0 include: tremors, emotional changes, insomnia, muscle weakness, headaches, and decreased mental function (USEPA, 2024).

1.3.2. Inorganic mercury (Hg^{2+})

Inorganic mercury, Hg^{2+}, is the most prevalent species found in aquatic media (WHO 2005; Beckers, Rinklebe 2017; Stoichev et al. 2006; Liu et al. 2012). Complexes of this species are controlled by the matrix and physiochemical conditions, with $HgCl_2$ and $Hg(OH)_2$ being the most common complexes in freshwater; $HgCl_3$- and $HgCl_4^{2-}$ are the most common species in saline waters due to the higher chloride concentrations than freshwater sources (Liu et al. 2012). The presence of sulphide will result in preferential formation of soluble bi- and polysulfide complexes, such as $HgSH^+$, $Hg(SH)_2$, $Hg(SH)S^-$. Inorganic Hg^{2+} compounds are more soluble and stable in water samples than other species, and preservation methods typically exploit this by increasing anionic concentrations and promote formation of Hg^{2+} complexes (Yu and Yan, 2003). As the most abundant species in waters, total Hg limits are often more closely related to Hg^{2+}concentrations, for example the drinking water guideline for total Hg is defined as 6 µg L^{-1} by the WHO (WHO, 2022). Therefore, guidelines and limits may not accurately reflect the hazard posed by other Hg species, namely organomercurial compounds.

In the human body, sorption of Hg^{2+} through oral consumption is inefficient, as these species are often hydrophilic and so are poorly bioavailable through the digestive system. Thus, within a few days up to 85% of ingested Hg^{2+} is excreted (Cernichiari et al., 2007). The poor lipophilicity of Hg^{2+} species also reduces the ability of the species to pass through the blood-brain barrier, thus resulting in accumulation in the liver and kidneys (WHO, 1990). Health effects of Hg^{2+}exposure include: damage to the gastrointestinal tract and excretory systems, rashes, decreased mental function, and muscle weakness (USEPA, 2024).

1.3.3. Methylmercury (MeHg) and organomercurials

In anaerobic conditions, S-reducing bacteria in aquatic bodies will methylate and occasionally ethylate Hg to organomercurial compounds (WHO, 1990, Ji et al., 2023,

Sánchez Uría and Sanz-Medel, 1998). In the aquatic environment, organomercurials are synthesised by bacterial methylation of inorganic Hg during the reduction of sulphur, thus the methylmercury (MeHg) in the environment is linked to the quantity of Hg^{2+} and biotic presence. In freshwater, MeHg, is the predominant organomercurial compound and is typically found in concentrations of 0.02 – 0.1 ng L^{-1} in surface waters and >4 ng L^{-1} in anoxic bottom waters of freshwater lakes (Ullrich et al. 2001). In deep ocean water, dimethylmercury, $(CH_3)_2Hg$, is the predominant species produced from labile inorganic mercury complexes.

Organomercurial compounds, such as MeHg and ethylmercury (EtHg), are much more bioaccessible than inorganic Hg species. The likelihood bioaccessibility and long biological lifetime result in significant bioaccumulation in fish and seafood, thus creates a potential human exposure route through oral consumption. Exposure to organomercurial compounds occurs not only through concentrations found in fish and shellfish, but also through dermal contact due to the lipophilicity of organomercurial compounds (Kim and Zoh, 2012). These compounds bioaccumulate in the central nervous system and have been considered the main cause of mercury poisoning in humans (WHO, 2022, WHO, 1990). Symptoms of MeHg exposure include: visual impairment, impairment of speech, hearing and walking, muscle weakness, poor coordination, and parasthesia (USEPA, 2024).

The biological half-life of MeHg is approximately 57 days in blood and 64 days in hair samples (Yaginuma-Sakurai et al., 2012). Methylmercury has been observed to pass through the placental barrier and cause birth defects and delays in developmental milestones, such as memory, cognitive thinking, fine motor skill, and others, when pregnant women are exposed to Hg (Clarkson and Magos, 2008, Ekino et al., 2007).

Major incidents of Hg poisoning in the past 70 years have been as a result of organomercurial compounds, spurring investigation into the hazards posed by MeHg in particular. In the 1960's in Minamata, Japan, release of industrial waste from acetone and polyvinyl chloride production introduced Hg and MeHg into local river systems. This pollution affected food and water sources, including crops and fish, especially through bioaccumulation, and caused exposure of hazardous quantities of Hg to nearby villages and towns (Ekino et al., 2007, Eto et al., 2010, Gonzalez-Raymat et al., 2017). No exact number of deaths was given as a result of the poisoning event, but over 2000 people were confirmed to have Minamata disease and over 10,000 people were estimated to be affected by the pollution event. Another major Hg poisoning event occurred in Iraq in 1972, where Hg-containing fungicides

were applied to wheat crops, resulting in the hospitalization of over 6000 people (Bakir et al., 1973).

1.3.4. Minamata convention

The Minamata convention (2013), outlining policies and regulations for reduction of Hg use, is signed by 148 countries, with the ultimate goal of ending anthropogenic Hg emissions (UN, 2013). It highlights the immediate need to cease Hg pollution to protect environmental and human health, by replacing Hg use in industrial processes, monitoring Hg concentrations in the environment and potential human exposure routes, and regulating the ASGM sector to ensure sustainable income to workers without release of the harmful metal.

1.4. Instability of Hg species in water

It is important to accurately measure Hg concentrations that are representative of the sampled water bodies, especially in vulnerable areas such as ASGM sites where Hg pollution directly affects local communities (Ondayo et al., 2023a,b). Current literature relating to Hg concentrations in waters at ASGM sites is often unsuitable, due to Hg instability and poor preservation methods (King, et al, 2024). Many studies fail to adequately state preservation methods used, casting doubt on the reported concentrations. In water samples, concentrations of Hg and its species are prone to volatilisation, sorption to container walls, and interspecies conversion to less stable species such as Hg^0 (Yu and Yan, 2003, Parker and Bloom, 2005, King et al., 2023). This results in a significant decrease in concentration over just a few days and often prevents research from assessing and monitoring Hg in water sources (Table 1.2.).

Table 1.2 Typical losses of Hg from water samples in different container materials, adapted from (Yu and Yan, 2003)

Sample	Hg species	Container	Hg loss over time	Reference
Distilled water	Hg^{2+}	PE	75% over 150h	(Rosain and Wai, 1973)
		PVC	90% over 150h	
		Soft glass	85% over 350h	
Distilled water	Hg^{2+}	Linear PE III	97% over 8 days	(Heidan, 1983)
		Linear PE IV	26% over 8 days	
		Conventional PE	52% over 8 days	
		PP	81% over 8 days	
Deionized water	Hg^{2+}	PE	87% over 12 days	(Leermakers et al., 1994)
		Pyrex	86% over 20 days	

Deionized water	MeHg	PE	80% loss over 12 days, 94% loss over 30 days	(Leermakers et al., 1994)
		Teflon	Stability retained over months	
		Glass	40% over 12 days, 80% over 30 days	
Potable water	Hg^{2+}	PET and glass	40% over 10 days	(Copeland et al., 1996)
Distilled and deionized water	MeHg	PTFE	Stability retained up to 6 months	(Lansens et al., 1990)
		Glass	50% over 14 days	
Distilled water	Hg^{2+}	Glass	Stability retained up to 5 months	(Feldman, 1974)
		PE	Stable for 10 days	

The recommended procedure for Hg sampling is to ship samples to a laboratory overnight, for preservation by acidification in appropriate facilities (USEPA, 2002, USEPA, 1996). This methodology has been successfully used (Guedron et al. 2014, Cobbina et al. 2015) but this is not always possible, particularly in challenging environments and so sampling and in-field preservation of Hg has long been considered difficult for widespread monitoring and toxicology studies, despite the potential to be a major exposure route for environmental and human health (Ondayo et al., 2023a,b, King et al., 2024). Regulatory authorities do not have adequate information to make relevant and appropriate policies to mitigate Hg pollution, and local communities lack the information on the impact of Hg pollution to the environment on a local and global scale. Thus, there is a need to develop a sampling method for Hg and its species, to adequately assess Hg concentrations in the aquatic environment, to better understand and protect human and environmental health.

1.5. Aim and Objectives

The aim of this research project is to develop a method for sampling Hg and its species from water samples, including those found in challenging environments such as ASGM sites where previous literature has been unable to effectively monitor the pollutant. To achieve this, the following objectives were met:

1a) Assess current literature for recommended sampling and preservation methods for Hg and its species in water samples, including in challenging environments where Hg monitoring is most needed;

1b) Assess current literature of SPE methods for Hg analysis, including preconcentration, as a sample introduction system, analyte separation, etc. Then, identify a suitable method for optimisation as a sampling method that can be applied in-field for Hg preservation;

2) Optimise the identified method for preservation of total Hg from water samples associated with challenging environments. This is then validated by deployment in ASGM sites in western Kenya, for assessing waters affected by the mining activities;

3a) Further optimise the SPE method for sampling and preservation of Hg species from waters associated with challenging environments.

3b) Collect environmental samples (waters and sediments) from rivers around ASGM sites in western Kenya, to assess Hg pollution and speciation from mining activities including at sections of the rivers used for domestic and drinking water collection.

1.6. Thesis structure

During this project, portions of the work were submitted for publication and peer-review. Chapters 2 and 3 are published, and chapter 4 is undergoing peer-review. All chapters are provided as published, with addenda where necessary. Author contributions are stated at the beginning of each chapter.

Chapter 1 – General Introduction

The first chapter is a general introduction into Hg as an environmental pollutant. It discusses the toxicity of Hg and its species, anthropogenic and natural emissions, and the instability of Hg species. The aim and objectives of the research are specified.

Chapter 2 – Present and Potential Future of Aqueous Mercury Preservation; A Review"

The second Chapter assessed current literature on Hg preservation and sampling techniques, including methods recommended by governing bodies. The use for solid-phase extraction (SPE) for Hg sampling is examined, and some potentially suitable SPE methods are identified.

Chapter 3 – Field method for preservation of total mercury in waters, including those associated with Artisanal Scale Gold Mining

The third chapter is an investigation into the use of an SPE method, as identified in Chapter 2, for sampling and preservation of total Hg in water samples. The method is optimised for waters from challenging environments such as ASGM sites, and is validated at ASGM sites in western Kenya.

Chapter 4 – Mercury species preservation and sediment concentrations

The fourth chapter further develops the SPE method, used in Chapter 3, for Hg species sampling and preservation. The method was applied in a case study of ASGM sites in western Kenya, alongside analysis of river sediments for Hg species analysis.

Chapter 5 – General discussion and conclusion

The fifth chapter concludes the work, demonstrating the benefit of the sampling method for total Hg and speciation. In addition, it highlights potential future work and developments that may improve the performance and useability of the method.

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Chapter 2 – Present and Potential Future of Aqueous Mercury Preservation; A Review

This chapter was submitted to the journal *Environmental Sciences: Processes and Impacts*, and was published in February 2023. The formatting has been changed for uniformity with the rest of the thesis, and clarifying text has been added to section 2.1.

This chapter explored current literature surrounding sampling and preservation methods for Hg and its species in water samples, the difficulties faced when collecting Hg samples from environmental water sources, and recommended procedures that are the current standard. Solid-phase extraction methods, routinely used for preconcentration, separation and laboratory-based speciation in Hg analysis, were considered as potential sampling methodologies and suitable sorbents were highlighted for further optimisation as field-based sampling methods.

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2A. Abstract

Mercury is considered to be one of the most toxic elements to human health. Due to pollution from industry and artisanal gold mining, mercury species are present globally in waters used for agriculture, aquaculture, and as drinking water. This review summarises methods reported for preserving mercury species in water samples and highlights the associated hazards and issues with each. This includes the handling of acids in an uncontrolled environment, breakage of sample containers, and the collection and transport of sample volumes in excess of 1 L, all of which pose difficulties to both *in-situ* collection and transportation. Literature related to aqueous mercury preservation from 2000 – 2021 was reviewed, as well as any commonly cited and relevant references. Amongst others, solid-phase extraction techniques were explored for preservation and preconcentration of total and speciated mercury in water samples. Additionally, the potential as a safe, *in-situ* preservation and storage method for mercury species was summarised.

The review highlighted that the stability of mercury is increased when adsorbed on a solid-phase and therefore the metal and its species can be preserved without the need for hazardous reagents or materials in the field. The mercury species can then be eluted upon return to a laboratory, where sensitive analytical detection and speciation methods can be better applied. Developments in solid phase extraction as a preservation method for unstable metals such as mercury will improve the quality of representative environmental data, and further improve toxicology and environmental monitoring studies.

2.1. Introduction

2.1.1. Background (Mercury in the environment)

Mercury (Hg) is ubiquitous in the environment and is one of the most toxic elements to human health, being described as one of the 13 priority hazardous substances under the Water Framework Directive. Concentrations in drinking water are restricted to just 6 µg L^{-1} total Hg for acute poisoning (WHO, 2005, WHO, 2022) and a tolerable intake set at 2 µg total Hg kg^{-1} body weight per day (Risher, 2003). Both acute and long-term exposure to the metal can result in severe, irreversible neurological and developmental complications, commonly referred to as Minamata disease. It is therefore one of the most widely studied and monitored environmental pollutants. There are three main species of Hg found in natural waters: inorganic mercury (Hg^{2+}), elemental mercury (Hg^{0}), and organic species such as methylmercury ($MeHg^{+}$) and dimethylmercury (Me_2Hg). These species are often highly mobile (Kim and Zoh,

2012) and their most significant environmental interactions are shown in Figure 2.1. Making up 1-40 % of the total Hg fraction, $MeHg^{+}$ is considered the most toxic Hg species. Organic Hg species are highly bioaccessible and prone to bioaccumulation. For example, predatory fish can have up to 10^{6} times higher concentrations of total Hg than the surrounding waters, with 95 % of this being methylmercury (USEPA, 1999). Total aqueous Hg concentrations are usually less than 10 ng L^{-1} Hg in uncontaminated natural freshwaters, with polluted waters generally being defined as higher than 100 ng L^{-1} and even being reported at over 50 µg L^{-1} Hg (WHO, 2005, Bloom, 1994, Driscoll et al., 2007, Ngure et al., 2014). Monitoring total Hg and speciation data is vital to prevent human and environmental exposure to harmful concentrations of the metal. Due to the naturally low concentrations of total and speciated Hg, sample pre-treatment and preparation are vital to ensure accurate and precise measurement with appropriate detection limits. Many preservation and preparation techniques for Hg recommend filtration of the water sample. Dissolved Hg concentrations may adsorb to particulate matter over time, altering the measurable dissolved concentration and posing different analytical challenges (USEPA, 1996, USEPA, 2002, Parker and Bloom, 2005). The potential for toxicity of particulate-bound Hg is not well researched (Bosch et al., 2009, Gworek et al., 2016), with toxicity studies focusing on dissolved species concentrations. For speciation analysis, sample pre-treatment methods should generally avoid inter-species conversion, to provide representative species data of the sample.

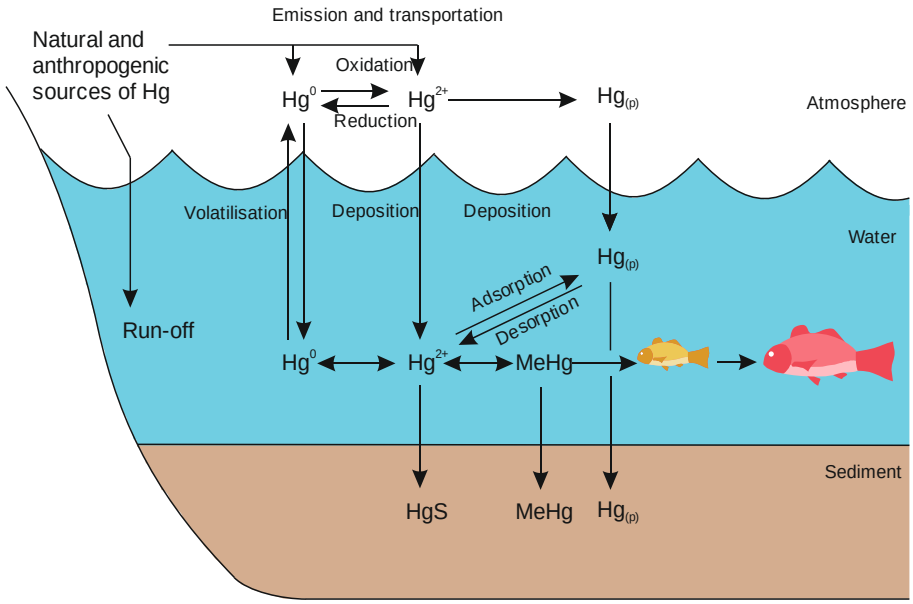

Figure 2.1. Biogeochemical cycle of mercury in the environment. Species descripted: Elemental mercury, Hg^0; inorganic mercury, Hg^{2+}; methylmercury, MeHg; particulate bound mercury, $Hg_{(p)}$; mercury sulfide, HgS (Adapted from Kim and Zoh (2012))

2.1.2. Preservation and storage: importance and challenges

Preventing losses of Hg from water samples has been an ongoing problem for many years (Yu and Yan, 2003, Leopold et al., 2010, Zhang et al., 2020). Safely preserving and storing Hg concentrations in water samples is particularly difficult. Many species are unstable in water, with total Hg concentrations can show losses of >70 % within 1 week (Yu and Yan, 2003, Zhang et al., 2020). Elemental Hg^0 is volatile in solution and so readily escapes from uncapped samples or into any container headspace (Yu and Yan, 2003, Beckers and Rinklebe, 2017). Inorganic Hg^{2+} is the most stable species in solution but is still prone to losses through sorption to the container walls or reduction to less stable species (Yu and Yan, 2003, Lo and Wai, 1975, Leermakers et al., 1994). Methylmercury and other organomercurials can undergo photolytic reduction to Hg^0 and can adsorb to container walls, as well as minor losses occurring from coagulation with humic acids (Yu and Yan, 2003, Leermakers et al., 1994, Riscassi et al., 2014). Any loss of Hg from the sample or contamination of the sample will produce erroneous results and limit the usefulness of the data, especially for samples used in environmental monitoring and human health studies.

Wall-sorption of Hg has been extensively studied in the past (Parker and Bloom, 2005, Yu and Yan, 2003, Zhang et al., 2020, Lo and Wai, 1975, Carr and Wilkniss, 1973, Bothner and Robertson, 1975, Hammerschmidt et al., 2011) and the choice of sample container material has been noted as a major factor in mitigating this. Glass and PTFE containers are preferred for sampling Hg in waters, as wall sorption is greatly reduced in these materials (USEPA, 2002). However, PTFE is relatively expensive when compared to other materials such as polyethylene, as demonstrated in Table 2.1.

Table 2.1. Approximate price comparison of container materials from online suppliers (sourced from FischerScientific on 4th March 2022)

Container material	**Approximate price per 100 mL sample bottle***
High density polyethylene (HDPE)	$1.50
Polypropylene (PP)	$2
Borosilicate glass	$1
Polytetrafluoroethylene (PTFE)	$40

* Prices are obtained from online vendors (SigmaAldrich, FischerScientific) and converted from GBP (£) to USD ($) (4th March 2022)

Glass containers are heavier and will become hazardous should breakage occur. Polyethylene (PE) and polypropylene (PP) are relatively more affordable alternatives than PTFE or glass, are often used in water sampling (USEPA, 1996) and are more robust than glass containers, but can pose a significant risk of Hg loss if the sample is untreated (Zhang et al., 2020, Hammerschmidt et al., 2011).

2.1.3. Scope of the Review and Methodology

The aim of this review was to critically assess the current literature related to the sampling, preservation and storage of Hg samples during transport from field to laboratory, to improve the likelihood of obtaining representative concentration and speciation data.

For the current preservation and pre-treatment methods, key benefits were highlighted and limitations associated with the different methods were considered. Using the WorldCat Library database, NERC library services and ScienceDirect literature database, peer-reviewed, published literature from 2000 to 2021 was reviewed using the search terms “mercury”, “water” or “aquatic”, and “preservation”, “storage” or “speciation”, for example “mercury aquatic preservation”, “mercury water speciation”, etc. Literature that involved the preservation and storage of aqueous Hg was reviewed. Any papers that were frequently cited throughout the reviewed literature were also assessed and included if relevant. The benefits of the preservation methods were explored, and any hazards associated with *in-situ* use and transportation were highlighted. The current applications of solid-phase extraction (SPE) in Hg analysis were examined and the potential benefits of the sorbents for sampling and preservation were explored. For solid-phase methods, the search terms “mercury”, “preconcentration” or “speciation” or “removal” or “recovery”, “water” or “aquatic” were used, for example “mercury preconcentration in water”, and similarly any common and relevant references were assessed and included. Some key considerations of the reviewed SPE methods were: the retention of the target analyte, the recovery of the analyte upon desorption, and the compatibility of the desorption method with analysis techniques.

A summary of literature on the preservation of Hg and its species by addition of a reagent is given in Appendix 2 Supplementary table A2.1. and a summary of literature on the solid-phase extraction of Hg and its species is given in Appendix 2 Supplementary table A2.2. The extent of the literature found in this review should be considered. The search terms used provided a comprehensive list of literature related to the development and use of preservation methods for Hg in water samples, but

may not have included all relevant articles demonstrating successful use of current in-field sampling methods.

2.2. Current trends for preservation and solid-phase extraction methods

2.2.1. Field measurements

Analysis of analytes and their species *in-situ* eliminates the need for preservation and reduces the risk of inter-species conversion and loss of analyte over time, providing a more accurate representation of real-world chemistry. However, there is a greater risk of sample contamination and a greater difficulty maintaining appropriate analytical conditions, due to a lower control over the environment when compared to a laboratory.

Commercially available field probes have been developed for *in-situ* Hg^{2+} stripping voltammetry analysis. These are reported to measure Hg^{2+} in the field with detection limits of 5 µg L^{-1}, as well as other trace and transition metals. For pristine waters, these probes will likely struggle to produce an accurate quantitative result as mercury concentrations can be as low as 1 ng L^{-1} Hg^{2+}. Rocha et al. (2019) reported detection limits of 5 µg L^{-1} Hg^{2+} in river water using a portable analyser, unsuitable for the WHO guideline value of 6 µg L^{-1} Hg (WHO, 2022) but may be useful for indicating severe Hg pollution. A similar detection limit was reported by Bhardwaj et al. (2020) in pond and drain water. Gold nanoparticle electrodes have been developed by Hwang et al. (2021) for Hg^{2+} determination, achieving detection limits of 1.7 µg L^{-1} Hg^{2+} with a linear response between 10-100 µg L^{-1} in landfill leachates. This highlights the ability for these technologies to be used in difficult matrices, however detection limits are currently unsuitable for speciation analysis or for Hg concentrations found in most natural waters.

Other methods of Hg field analysis use headspace Hg vapor analysers, relying on the evolution of elemental Hg vapour from water samples (Kriger and Turner, 1995). These probes have been reported to achieve detection limits of approximately 0.09 µg L^{-1} Hg^{0}, but are designed specifically for Hg concentration in air; preparative chemicals are required for other matrices to evolve Hg vapour and the data must be converted to other units for comparison to other analysis methods and water studies (WHO, 2005, WHO, 2022, Bloom, 1994, Driscoll et al., 2007, Ngure et al., 2014).

2.2.2. Acidification

As field measurement of Hg in natural waters in not currently possible, preservation of the dissolved metal is vital for Hg analysis. Recommended methods for

preservation of Hg in natural water samples generally follow the guidance of other trace metals, namely acidifying water samples with nitric (HNO_3), hydrochloric (HCl), or sulphuric acid (H_2SO_4) (USEPA, 2002, USEPA, 1996, Parker and Bloom, 2005). The choice of acid is important for Hg stability; the use of HNO_3 has been found to still be susceptible to large losses of Hg from water samples through both volatilisation and sorption to container walls (Yu and Yan, 2003, Zhang et al., 2020, Lo and Wai, 1975, Krivan and Haas, 1988).

Hydrochloric acid is recommended as a suitable preservation method for dissolved Hg species in freshwater samples (Carr and Wilkniss, 1973). Inorganic Hg^{2+} can complex with chloride ions to form the stable $HgCl_2$ complex, and tri- or tetra-chloromercury complexes if the chloride concentration is further increased (Bothner and Robertson, 1975, Louie et al., 2012). These are more stable in solution than other species and are not co-precipitated by metal oxides and hydroxides (Addis Lockwood and Chen, 1973, Inoue and Munemori, 1979, Hylander et al., 2000). A 1 % (v/v) HCl solution was reported to prevent loss of Hg^{2+} over 55 days in 500 mL HDPE containers (Louie et al., 2012). The lower pH and the presence of chloride ions increases the stability of Hg^{2+}, as demonstrated by the preservative abilities of HNO_3 (6% v/v HNO_3) for Hg in seawater reported by Gardner and Gunn (1997) and 20 mg L^{-1} NaCl + 0.15% (v/v) HNO_3 for Hg in deionised water reported by Louie et al. (2012).

These conditions also increase the stability of MeHg in water samples. In a 0.5% (v/v) HCl solution stored in Teflon containers at 1-4 °C in the dark, MeHg is reported to be stable for up to 250 days in both freshwater and seawater (Parker and Bloom, 2005). Sulphuric acid has also been recommended for the preservation of aqueous MeHg in saline media, as hydrochloric acid (>0.4% v/v) may result in the artificial formation of monomethyl mercury during the distillation and ethylation process typically used for MeHg speciation (Parker and Bloom, 2005).

When considering speciation, Bloom (1994) reported that acidification may alter labile Hg^{2+} resulting in desorption from particulates in unfiltered samples, oxidation of Hg^0 or coagulation of dissolved organic carbon and humic acids which can precipitate Hg from solution.

2.2.3. Solid-phase extraction

Solid-phase extraction offers the potential for reagent-free field sampling of Hg from water samples. Sorbents and solid-phase methods are frequently used in the analysis of dissolved Hg for sample preparation and pre-treatment, such as preconcentration (Blanco et al., 2000, Sánchez et al., 2000, Pohl and Pruisisz, 2004, Balarama Krishna

et al., 2005, Vermillion and Hudson, 2007, Leopold et al., 2009a, Basadi et al., 2020), speciation (Sánchez et al., 2000, Balarama Krishna et al., 2005, Vermillion and Hudson, 2007, Cairns et al., 2008, Balarama Krishna et al., 2010, Yin et al., 2010, Zhang et al., 2016) and removal of Hg (Vermillion and Hudson, 2007, Ritter and Bibler, 1992, Chiarle et al., 2000, Monteagudo and Ortiz, 2000, Wang et al., 2009, Zhang et al., 2010, Rajasimman and Rajamohan, 2017). Solid-phase extraction was previously studied for retention and stabilisation of heavy metals for analysis at a later date (Blanco et al., 2000, Hanhauser et al., 2020). Adsorption of Hg to a solid-phase mitigates the risk of loss from wall sorption; there is less chance of contact between an analyte bound to a solid-phase and the container walls when compared to an analyte in an aqueous-phase. This approach has been investigated in the past (Leopold et al., 2010, Blanco et al., 2000, Schlathauer et al., 2019), but is not in widespread use.

2.2.3.1. Thiol- functionalised resins

A common approach to Hg-selectivity in sorbents is to exploit the affinity for Hg of thiol-containing compounds; diphenylthiocarbazone (dithizone) (Yin et al., 2010, Mahmoud et al., 2000, Wang et al., 2022), 2-mercaptoethanol (Margetínová et al., 2008), diethyldithiocarbamate (Blanco et al., 2000), and other compounds have been used to either functionalise resins or for complexation with aqueous Hg. These reagents provided recoveries of inorganic, methyl-, ethyl- and phenyl- species of Hg of over 70 %, with preconcentration factors suitable for aqueous Hg concentrations of between 0.1 – 50 µg L^{-1} (Blanco et al., 2000, Yin et al., 2010, Margetínová et al., 2008, Mahmoud, 1999). While most work focuses on a deionised water matrix, Margetínová et al. (2008) successfully extracted Hg from natural freshwaters, by complexing Hg with 2-mercaptoethanol before passing samples through C18 columns. The use of 2-mercaptoethanol as a complexing agent comes with separate risks as the reagent is volatile, has a strong odour, and the concentrated solution is highly toxic, so was reportedly diluted to a 5 mM 2-mercaptoethanol solution before use. The high organic concentration of the methanol eluent solution limits the analytical techniques available to this method, relying on HPLC/CV-AAS for speciation analysis.

Blanco et al. (2000) achieved a similar extraction by immobilising diethyldithiocarbamate onto homemade C18 microcolumns. The C18 immobilised diethyldithiocarbamate column used a 50 mL sample volume to achieve recoveries of >70 % for Hg^{2+} and >65 % for $MeHg^{+}$ from freshwater river samples, with detection

limits of 0.2 µg L^{-1}. The diethyldithiocarbamate modified C18 columns showed potential as an in-field preservation method, as samples could be readily passed through the microcolumns in the field and inorganic Hg^{2+} is stablised for approximately 2 weeks. The detection limits are unsuitable for typical concentrations in many unpolluted natural water samples, primarily being suitable for areas of moderate to high Hg pollution. There was a substantial decline in recovery of MeHg after 2 weeks of storage, even when held at 4°C in the dark. Over 85 % of the spiked MeHg could be recovered within 7 days of extraction to the cartridge, declining to <50 % by 14 days. This trend was also seen with Hg^{2+}, albeit much less significant with a recovery of 80 % Hg^{2+} after 30 days storage. This method was applied to LC-ICP-MS, allowing for a fraction of the sample volume required from the technique reported by Margetínová et al. (2008). In addition, the eluent composition (0.5% v/v HCl + 5% w/v sodium thiosulphate) is compatible with a wide variety of analytical techniques, so is suitable for a broader scope of laboratories.

Dithizone functionalised C18 columns were developed by Yin et al. (2010) to effectively recovery Hg species from tap water samples (Hg^{2+}, $MeHg^{+}$, $EtHg^{+}$). The method used just 3 mL 100 mmol L^{-1} sodium thiosulphate solution to elute the retained species, from sample volumes of 100 mL. This eluent choice allows a wide variety of analytical techniques to be applied but may show limitations where acidification or oxidation of the sample is required, due to the formation of solid sulphur which may decrease Hg concentrations by formation of solid Hg_2S. Using HPLC-ICP-MS analysis, detection limits of 3 ng L^{-1} Hg were reported from sample injection volumes of just 20 µL. This work supported by Wang et al. (2022), using a 1% (v/v) 2-mercaptoethanol eluent for elution of Hg species. This eluent is still compatible with a wide range of analytical techniques with some careful adjustments, such as use of organic introduction systems for ICP-MS analysis for routine use.

2.2.3.2. Commercially available chelating resins

Commercially available ion exchange resins have been developed for the removal of Hg from industrial wastewaters; Duolite GT-73™ and AmberSep GT-74™ are examples of these. The recovery and preconcentration of Hg in solution using these resins has been explored as diffusive gradient thin-film cartridges (Pohl and Pruisisz, 2004, Chiarle et al., 2000, Pelcova et al., 2014). Pelcova et al. (2014) reported that both Duolite GT-73™ and AmberSep GT-74™ can remove inorganic, methyl-, ethyl-, and phenylmercury from both tap and river waters with limits of detection between 30 – 50 ng L^{-1} Hg concentrations. The loading capacities for Hg are often high, >70

mg Hg g^{-1} resin (Fondeur et al., 2002), as these resins were designed for the treatment of wastewaters with high Hg concentrations, often greater than 500 µg L^{-1}. These efficiently and selectively extract Hg^{2+} from a variety of water matrices but some studies reported difficulty in recovery from the resins by elution (Pohl and Pruisisz, 2004, Pelcova et al., 2014), instead resorting to either digestion of the resin prior to analysis or direct absorption spectrometry measurement of the resin. From solutions containing up to 100 µg L^{-1} Hg^{2+}, over 92 % of the total Hg concentration could be readily recovered by digestion of the Duolite GT-73 resin™, with negligible losses in the digestion step (Pohl and Pruisisz, 2004). Duolite GT-73™ is now out of production, but AmberSep GT-74™ and other variations of this resin are still available (Pelcova et al., 2014, Pelcova et al., 2015).

2.2.3.3. Cationic exchange resins

As Hg species are predominantly cationic in the aquatic environment, cation exchange resins offer a method to remove these from solution. These resins are effective at removing cationic Hg species such as $Hg(OH)_2^{2+}$ complexes, but there may be issue with the sorption of uncharged complexes and species, such as $HgCl_2$ or MeHgCl. The conditioned resins are often washed with deionised water prior to extraction, as this decreases the likelihood of forming uncharged or negatively charged Hg complexes in the resin which impede sorption (Bothner and Robertson, 1975, Addis Lockwood and Chen, 1973).

A commercially available resin, Dowex 50W X4™, was found to remove inorganic Hg and some organic Hg species from a variety of natural water matrices whilst allowing for elution using 0.1 % thiourea and 8 % hydrochloric acid (Krata et al., 2003). Gomez et al. (2014) used the commercially available Dowex Marathon™ cation exchange resin as comparison to activated carbon and treated and non-treated coals. They reported the cationic exchange resin had the best sorption capacity, 98 µg Hg g^{-1} resin, and recoveries, >95 % Hg^{2+}, of the studied sorbents. These experiments used 50 mL sample volume but used a high Hg concentration, between 0.1 – 998.4 mg L^{-1}.

Cationic exchange columns have been used for online preconcentration of Hg species in sea waters, achieving detection limits of 42 pg L^{-1} when using HPLC-ICP-MS analysis (Jia et al., 2012). This indicates suitability for the analysis of low Hg concentrations in waters. Ion exchange sorbents, columns and cartridges are commercially available and often relatively inexpensive. Some technical knowledge and training are required for field use, but use of hazardous materials is limited in field

applications. Other cations present may compete for the active sites of the resin, but high loading capacities would overcome this. Issues may also arise in samples with high chloride concentrations due to the formation of uncharged complexes that would not be retained by the sorbent and pass through to the effluent (Bothner and Robertson, 1975). Metals are eluted from these columns using strong acids, such as hydrochloric acid. While this is compatible with many analysis techniques, the concentrations of acid may require a dilution and thus reduce the overall sensitivity of the method.

2.2.3.4. Polyaniline

Polyaniline is a readily available polymeric sorbent that can be used for removal of metals from aqueous solutions and preconcentration of trace metals. Studies have primarily examined inorganic Hg species by addition of a bulk resin to a water sample, but separation of methylmercury is also possible (Balarama Krishna et al., 2005, Balarama Krishna et al., 2010). Mercury analyses using polyaniline for preconcentration have achieved 2-3 ng L^{-1} Hg limits of detection using CV-AAS and FAAS, suitable for uncontaminated natural water and drinking water; these matrices usually show Hg concentrations below 10 ng L^{-1} (Leopold et al., 2009b). Mercury has been successfully preconcentrated with polyaniline from a variety of matrices including bottled water, lake and groundwaters, seawater, and even fish tissue using 100 mL sample and 10 mL 0.3 % HCl + 0.5 % thiourea eluent (Balarama Krishna et al., 2005, Balarama Krishna et al., 2010). The eluent is suitable for a wide range of analytical detection methods, due to the relatively low organic compound concentration and acid concentration.

Some polyaniline composites have also been examined for Hg removal, to improve resin stability, Hg selectivity and efficiency of sorption. Polyvinyl alcohol (Vafaei Molamahmood et al., 2018), humic acid (Zhang et al., 2020), polystyrene (Gupta et al., 2004), and other reagents have been used to create polyaniline composites, usually with an optimal pH range of 4-7. The predominant Hg species at this pH range is $Hg(OH)_2$ species, which will form Hg-N bonds with the polyaniline units and other bonds such as Hg-S with the composite molecules. At pH <4 the polyaniline nitrogen may be protonated, reducing the number of possible Hg-N bonds that can be formed.

2.2.3.5. Magnetic SPE

In the past decade, developments for solid-phase extraction technologies have incorporated the use of magnetic particles. By functionalising magnetic particles, the sorbent can be added to a sample to sorb Hg and then be readily removed by applying

a magnetic field. The selectivity and efficiency of the sorbent is dictated by the functionalisation; some previously used compounds include 1,2-ethanedithiol (Chen et al., 2021), 3-mercaptopropyltrimethoxysilane (Zhang et al., 2016), 1,5-diphenylcarbazide (Zhai et al., 2010), and other task-specific monoliths (Song et al., 2021a). These have achieved 0.1-100 ng L^{-1} Hg^{2+} detection limits of inorganic Hg in real-world aquatic matrices such as lake, river water and for spiked tap water. The 1,2-ethanedithiol functionalised particle also adsorbed cadmium (Cd) and lead (Pb) ions from solution with 0.82 ng L^{-1} Cd and Pb limits of detection (Chen et al., 2021), making the method more desirable commercially and for heavy metal pollution studies. Song et al. (2021b) synthesised a task specific monolith with vinylboronic anhydride pyridine complex for functionalising the magnetic particles. This was synthesised for magnetic solid-phase extraction microextraction of inorganic Hg^{2+}, methyl-, ethyl- and phenyl- Hg. Using the chelating sorbent, recoveries of up to 94 % and detection limits of 20-160 ng L^{-1} could be achieved.

Magnetic solid-phase particles can be readily removed from solution with a magnetic field and can be used to achieve detection limits of <100 ng L^{-1} Hg. Speciation of Hg^{2+} and MeHg can be achieved and changing functional groups on the sorbent can allow for multi-elemental extraction. The sorbent can be applied to a water sample collected in a container, allowed to sit in the sample for an appropriate length of time, and then the enriched sorbent can be removed using a magnet. Elution from magnetic sorbents is generally achieved using HCl or HNO_3 and thiourea in relatively low concentrations. Analysis is typically conducted using ICP-MS techniques, however the composition of eluent and the preconcentration of the metal make the methods compatible with less sophisticated techniques such as AAS. The main limitation for magnetic SPE is the multi-step synthesis required to produce the sorbent, as the methods used often produce approximately 1 g of sorbent and scaling-up the synthesis has not yet been explored (Chen et al., 2021). The product must also be characterised before use to ensure a homogenous and effective sorbent.

2.2.3.6. Gold-based SPE

Many metals form amalgams with Hg and this property is frequently exploited for solid-phase extraction of Hg vapour. Cold vapour (CV) methods use gold to amalgamate reduced Hg vapour, trapping the analyte in place and allowing release of the preconcentrated Hg by thermal desorption. As the amalgam is formed on the solid particles, problems arising from interferents are often negligible and so can be readily applied to environmental matrices. This has been examined and exploited in

the form of gold nanoparticle columns (Leopold et al., 2009a, Panichev et al., 2014, Stock et al., 2021), greatly increasing the surface area when compared to a bulk solid. Similarly, columns made using gold nanosheets offer a relatively simple method of extracting and preconcentrating aqueous Hg with very good sensitivity, as low as 80 pg L^{-1} Hg^{2+} (Zierhut et al., 2010). Schlathauer et al. (2019) developed a dipstick of immobilised gold nanoparticles, allowing for a simple field-sampling method that can achieve levels of sensitivity suitable for pristine waters and sea waters. As a simple dipstick, this method is easily conducted in the field without the need for extensive training or technical competency, as well as posing little hazard to the operator or during transport. While technically capable, the cost and complexity of manufacturing the dipstick alongside the need for annealing at 600 °C before each measurement currently prevents this from being easily reproduced. Additionally, gold-based SPE typically uses thermal desorption to liberate Hg from the solid-phase. While this effectively eliminates potential interferences, the detection method becomes limited to those suited for gases and vapors, such as CV-AAS.

2.2.4. Critical review of sample preservation and solid-phase extraction

While acidification of water samples for Hg analysis is commonly recommended, the handling and transportation of acids is becoming increasingly more regulated, particularly where controls over health and safety are more difficult, i.e. handling concentrated acids outside of a laboratory setting, and where limitations are imposed for international transportation of acids (Figure 2.2). Any handling of acids comes with inherent hazards and risks due to their corrosive nature. This makes preservation methods that require concentrated acids particularly difficult to conduct in the field, as well as for transportation of acids and acidified samples particularly transport by air where regulations are becoming stricter (IATA, 2022). Historically, oxidising agents were also recommended for Hg preservation (Feldman, 1974) by oxidising the Hg species to the stable Hg^{2+}, however this destroys speciation data and so has fallen out of favour. Other reagent-based methods for preservation show potential, but have not been fully explored (Yu and Yan, 2003). For example, increasing the ionic strength of solution by addition of ionic salts, i.e. NaCl, allows for a less hazardous method to preserve Hg (Zhang et al., 2020), but literature primarily focuses on Hg^{2+} in spiked and synthetic matrices with little assessment of un-spiked water environmental samples. In addition, elevated chloride concentrations can co-precipitate in MeHg distillation and ethylation procedures (Parker and Bloom, 2005), making the procedure unsuitable for traditional speciation analysis.

Due to the importance of Hg speciation analysis for toxicity studies, there is a concern for interspecies conversion and loss of sample integrity during preparation and storage of water samples (Colman et al., 1997, Hintelmann, 1999, Snell et al., 1999, Hammerschmidt and Fitzgerald, 2001). Individual samples are often taken for each desired species and preserved using different methods (Mladenova et al., 2012). This approach allows the operator to collect speciation data for Hg, but vastly increases same volumes required and limits the environments in which Hg studies can be conducted. For example, studies in developing countries and remote area must ship samples internationally for analysis (Bonzongo et al., 1996, Kotnik et al., 2007). If samples are shipped unpreserved, then speciation data may not be considered as representative of the sampled environment.

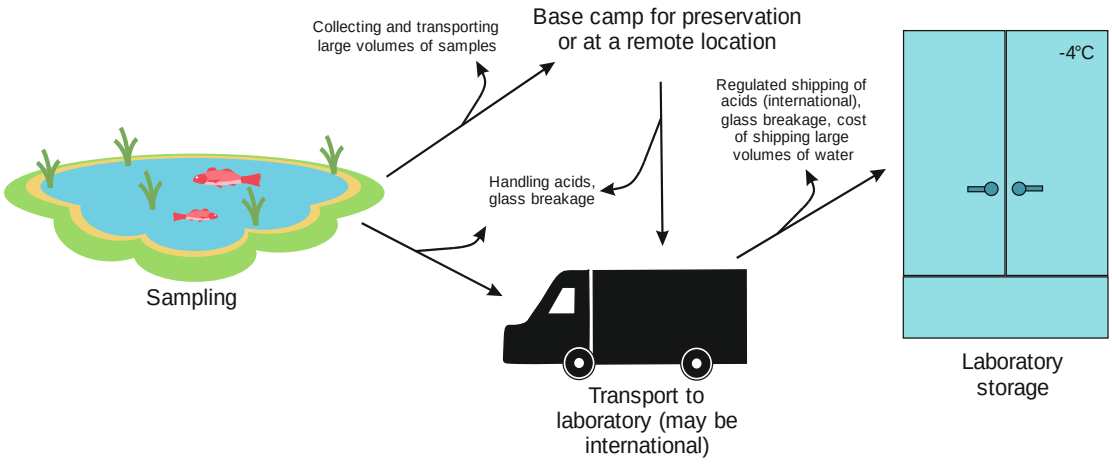

Figure 2.2. The sampling and preservation process, with key challenges highlighted

Recent developments in SPE for Hg analysis have focused on preparative methods such as online-speciation and -preconcentration; hyphenating a chromatographic separation to the detection method to enrich the analyte, improving detection limits and analytical sensitivity. The SPE preparation methods often operate in a broad pH range, usually optimal at pH 4-7 so suitable for many natural waters. These methods are often developed for mass spectrometry techniques (Blanco et al., 2000, Chen et al., 2021, Song et al., 2021b) and atomic absorption and fluorescence techniques (Blanco et al., 2000, Basadi et al., 2020, Mahmoud et al., 2000, Margetínová et al., 2008, Black et al., 2007, Duval et al., 2020), due to their comparatively high sensitivity for environmental metal analysis.

Ion exchange resins sorbents and columns are commercially available and have been shown to sorb both inorganic and organic Hg species from aquatic media. Chelating resins are the more prominent choice for Hg sorption in the literature, as the affinity for sulphur allows for selective extraction of Hg. Some chelating resins, however,

require synthesis or processing to create columns and cartridges for field use. Commercially available resins are available but have shown difficulty in eluting retained Hg species, requiring digestion processes to liberate the adsorbed Hg which may affect speciation data through oxidation of the retained species. Cation exchange resins only require an acid, HCl in this case, and thiourea to efficiently elute Hg, but these may be more susceptible to competition with other cations in the sample. Typically, ion exchange resins use dilute acids and weak organic concentrations to elute immobilised Hg species. The eluent composition allows for analysis using a wide range of instruments and can therefore be applied in most laboratories.

Functionalised magnetic sorbents can be added to a collected water sample and then readily removed by application of a magnetic field, either an electro-magnet or a strong, permanent magnet. These sorbents are relatively simple to use in the field, with minimal training requirements and a high Hg extraction efficiency. The eluent can typically be used with a wide variety of analysis techniques, although some developed sorbents require methanol (Song et al., 2021a) which will limit the compatibility of the methods. The synthesis of these sorbents is often more complicated than other sorbent materials, and may require work to scale-up synthesis to be viable as a widespread sampling procedure.

Gold-based sorbents are some of the most selective and efficient sorbents available for Hg extraction and preservation, but come with a considerable cost, due to the raw materials price. Therefore, a high reusability is necessary to offset the cost. Desorption of the retained Hg is conducted via thermal desorption, which limits the compatible analysis techniques to those which can measure Hg vapour, such as AAS.

Another technology, diffusive gradient thin-film (DGT), was investigated for the sorption and storage of Hg in the field (Pelcova et al., 2014, Gao et al., 2011), with analysis after transport to the laboratory. These are usually deployed into a water source, for example a river or waste treatment water tank, for 4 – 24 hours, where the Hg species become bound to the resin (Gao et al., 2014, Pelcova et al., 2015). For some DGT resins, Hg is irreversibly bound and so must be digested before analysis or alternative analysis methods must be used (Pelcova et al., 2014, Pelcova et al., 2015). For other resins, Hg species are elutable with either thiourea or HCl the latter being preferable for many analysis techniques and when ethylation of the Hg species is required for MeHg quantification (Gao et al., 2014).

2.3. Future perspectives

2.3.1. The future of Hg field analysis

Emerging technologies use nanoparticles and colorimetric methods to determine Hg^{2+} in natural waters. Fluorescence probes have been developed for Hg^{2+} determination, for example Kaewnok et al. (2021) developed a [5]helicene-based probe highly selective for Hg^{2+} which can be used as a test-strip with a detection limit of 6.5 µg L^{-1} Hg^{2+}. This work requires development for the in-field screening of Hg^{2+} in environmental samples. Rhodamine nanoparticles have been developed for smartphone-based colorimetric analysis, as a method to detect Hg^{2+} in pristine water matrices (Zhao et al., 2020, Aydin and Yilmaz, 2021). These nanoparticles are highly selective for Hg^{2+}, showing little interference with other metals. Recoveries of over 95 % were reported for both drinking water and dam water with a limit of detection of 1.3 µg L^{-1} Hg^{2+} (Aydin and Yilmaz, 2021), and 0.1 µg L^{-1} Hg^{2+} in spiked deionised water with recoveries >80 % Hg^{2+} in river and lake water (Zhao et al., 2020). Lopreside et al. (2021) reported colorimetric smartphone detection of Hg^{2+} using an orthogonal paper biosensor. Using three different biotic "reagents", Hg^{2+} concentrations and toxicity can be evaluated simultaneously. The sensors each determine Hg over different periods of time, between 17 – 60 minutes, and with varying limits of detection, 0.58 – 17 µg L^{-1} Hg^{2+}. This allows for either quantitative or semi-quantitative analysis, if required. The use of multiple sensors reduces the chance of interference by other compounds and elements in the matrix, however silver and cadmium reportedly inhibit the activity of the sensors.

With the prevalence of smartphones and simplicity of use, colorimetric methods are likely to become a mainstay in field analysis methods for trace metals in the future. The biggest challenges to analysis in the field are the limit of detection in relevant matrices, determination of different Hg species and contamination of the sample. While good practice can overcome sample contamination issues, the sensitivity of portable instruments is currently not suitable for mercury concentrations less than the WHO guideline limit of 6 µg L^{-1} total Hg, or for speciation analysis. Current field analyses of Hg species are unable to achieve appropriate sensitivity, as Hg species are often found in concentrations <10 ng L^{-1} particularly in unpolluted sites. The portable instruments tend to favour analysis of Hg^{2+}, neglecting the determination of MeHg and other relevant species. This limits their usefulness for toxicological and monitoring studies, as organic Hg species data is vital for assessing the health impact of Hg concentrations in waters. With these current restraints, analysts must weigh up improved analysis and sensitivity in laboratory measurements versus representative but less accurate data measured in the field.

2.3.2. Solid-phase extraction as a sampling and preservation method

Solid-phase extraction shows potential as future reagent-free sampling methods for Hg in natural water samples, as columns and microcolumns (Blanco et al., 2000, Leopold et al., 2009a, Yin et al., 2010, Margetínová et al., 2008, Krata et al., 2003, Zierhut et al., 2010), as DGT cartridges (Pelcova et al., 2014, Pelcova et al., 2015), particles added directly to samples (Zhang et al., 2016), or as a dipstick (Schlathauer et al., 2019). The ability to extract Hg from solution and retain the metal on a solid bed reduces the likelihood of Hg loss over time; volatilisation is reduced due to strong interactions with the stationary phase and wall sorption is reduced as the analyte is immobilised on a solid phase with little interaction with the container walls. By immobilising the analyte to a solid phase, the likelihood of chemical changes is reduced, and speciation data can be preserved (Blanco et al., 2000, Zhang et al., 2016, Wang et al., 2022). Most column-based SPE methods are relatively simple to conduct and, once prepared, can be used in the field to extract dissolved Hg without the need for additional reagents. The use of columns and cartridges also eliminate the need for glass containers, reducing hazards from breakages.

One promising SPE methods for Hg preservation, gold-nanoparticle dipsticks, are effective at removing Hg from natural water samples (Schlathauer et al., 2019). The dipsticks can be simply dipped into a water sample, with little knowledge required for field-use, few possible interferents and is a relatively quick method at only 10 – 20 minutes per sample. The dipstick must be annealed at 600 °C to ensure gold nanoparticle formation and the synthesis was reported to require a system for depositing a gold vapour to a defined area on the stick. This limits the ability to scale-up production of the dipstick for routine use, however the article reported excellent reusability at 145 cycles of sampling and annealing without performance loss. While offering superb extraction and recovery, the gold-based sorbents are limited to techniques for analysing Hg vapours, such as AAS, due to the requirement for thermal desorption. This may limit the overall usefulness of the technique, as other analysis methods cannot be used as readily.

Other sorbents, such as thiol-functionalised sorbents or functionalised magnetic solid-phases, are typically compatible with a wider variety of analytical techniques due to eluent composition. Often, the eluents used are a dilute acid (Blanco et al., 2000, Balarama Krishna et al., 2005, Yin et al., 2010, Krata et al., 2003, Vafaei Molamahmood et al., 2018, Chen et al., 2021) or a low organic compound concentration (Blanco et al., 2000, Balarama Krishna et al., 2005, Yin et al., 2010, Zhang et al., 2016, Krata et al., 2003, Chen et al., 2021), so are not as restricted as thermal desorption. In addition, inorganic and methyl- Hg species were retained and

stored on diethyldithiocarbamate immobilized C18 microcolumns for up to 2-weeks and 1 week respectively before elution (Blanco et al., 2000), highlighting the potential for reagent-free field sampling while preserving speciation. These columns, microcolumns, and cartridges are simple to use in the field, with minimal training requirements and little-to-no risk to the operator. With high recoveries and readily incorporating preconcentration of Hg species, SPE methods offer the ability to collect and preserve representative Hg concentration and speciation data, while being suitable to many analysis techniques and laboratory settings.

The cost of the SPE methods is often higher than that of sample acidification but the reduction in storage space and sample volume, as well as reduced risk to the operator and simpler field-application, offset the cost (Table 2.2). Preconcentration, usually via SPE, may be required for samples only treated by acidification, so sorbents may already be required. A currently unexplored risk of columns and cartridges is the accumulation of Hg from ambient storage conditions; this is likely mitigated by choice of casing material and appropriate storage, but further work on this is needed. However, avoiding storage in areas of with Hg vapour contamination may make this concern negligible.

Another important, yet unresolved issue is the lack of validated *in-situ* analytical techniques that can accurately measure Hg species concentrations. Without the determination of species concentration at the point of collection, interspecies conversion during storage and transportation cannot be fully validated for environmental samples and so speciation data determined in the laboratory may not be representative of the real-world concentrations.

Table 2.2. Approximate cost-per-sample of some suggested Hg preservation and solid-phase extraction methods

Preservation/ preparation method	**Relevant species**	**Main costs**	**Approximate reagent cost per sample***	**Sample holding time**	**Application in the field**	**Cost-benefit**
Acidification (HCl and HNO_3)	Total Hg, Hg^{2+}, MeHg	HCl and HNO_3 divided by samples at 1 %	$2.50 per 500 mL sample	6-12 months	Addition of 1% (v/v) acid to a collected water sample, either at a base-camp or in a laboratory after shipping	+ Relatively cheap per sample + Recommended − Separate samples are usually required for speciation techniques (i.e. distillation for MeHg) − Needs to be added to samples when at a base-camp or laboratory, to ensure safety measures − Potential difficulties and regulations in transportation of samples
Thiol-functionalised ion exchange cartridges	Hg^{2+}, MeHg	Price of cartridges and approximate price of functionalising reagents	$5 per sample	Not investigated beyond 2 weeks	Water samples are passed through homemade microcolumns or cartridges in the field, transported to a laboratory for elution	+ Highly selective for Hg and can be filled with water prior to sampling, reducing the amount of hazardous waste in-field. + Can be re-used and regenerated several times, improving cost-effectiveness − Preservation and storage of Hg species has not been fully explored − A time-cost must be considered for preparation of SPE-phases, albeit relatively labour intensive

Commercially available resins	Total Hg, Hg^{2+}	Initial price of resin	$10 per sample	Not investigated	Water samples passed through preprepared DGT cartridges, or applied as a batch sorbent	+	Selective for Hg in water samples, usually applied for Hg removal in waste-water
						+	High analyte capacity relative to other resins and sorbents
						−	Resins are often expensive (~$500 for 250g resin)
						−	For a column/cartridge, column loading and preparation time must be considered
Gold sorbents	Total Hg, Hg^{2+}	Price of gold metal for synthesising sorbent	$5 for sample	Not investigated	A dipstick, which can be dipped into a water sample, or passing water through preprepared microcolumns or cartridges	+	These offer high levels of reusability (>145 cycles), and Hg desorption can be conducted thermally
						+	Minimal interference/competition for the sorbent, due to selective amalgamation
						−	Synthesis requires controlled sputtering and vapour deposition technologies, which may not be readily available
						−	Gold is an expensive reagent, not readily available in most laboratories for preparation of the columns/dipsticks, therefore has a high initial cost

*Prices are obtained from online vendors (sigmaaldrich.com, fishersci.co.uk, accessed 21 August 2022) converted from GDP (£) to USD ($)

2.5. Conclusion

While the mechanisms for dissolved Hg loss have become more well defined over the years, safe methods for preservation, storage and transportation of samples to the laboratory for measurement of both total Hg and individual Hg species still remains a challenge. Different species require separate preservation methods, hazardous or expensive materials, and large sample volumes to improve detection limits for trace and ultra-trace Hg analysis and speciation analysis. This makes routine Hg studies and monitoring impractical in challenging environments such as remote locations or lower- and middle-income countries. Current literature on preserving Hg species in water samples has shown minimal developments on limiting the use of hazardous materials, instead highlighting the need for rapid transportation to a laboratory for preservation. In remote areas and uncontrolled environments, the use of concentrated acids can pose a significant risk to the operator and increase the challenges of transporting samples to laboratories in a timely manner.

Solid-phases methods and sorbents are already used in Hg analysis for preconcentration, removal, and speciation of Hg, immobilising the chemical species without altering the chemical forms. Mercury-specific functionalized sorbents, in particular functionalisation with diethyldithiocarbamate or diphenylthiocarbazone, have shown effective extraction of Hg^{2+} and some organic Hg species from natural water samples and suitable recovery after 1 week of storage. From a literature survey, there is a lack of literature on the concentrations of adsorbed Hg species after 1-week of storage and though the limitations of the search terms may not have fully captured all work on the storage timescales of these developed methodologies, the literature assessed is representative of the wider body of work on preservation and storage of Hg and its species. Therefore research into the long-term storage of Hg-species, particularly MeHg, is necessary for the development of SPE as a preservation method. However, SPE is a relatively inexpensive and safe method for *in-situ* sampling and preserving Hg species from natural water samples for transport from field-to-laboratory and obtaining representative dissolved Hg data.

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Chapter 3 – Field method for preservation of total mercury in waters, including those associated with Artisanal Scale Gold Mining

This chapter was submitted to the journal *Analytical Methods* and was published in February 2024. The formatting has been changed for uniformity with the rest of the thesis.

This chapter assessed an identified solid-phase extraction method, a dithizone functionalised cartridge, for the in-field sampling and preservation of total Hg in environmental water samples. The work employed a synthetic matrix with similar major ion chemistry to waters found at artisanal gold mining sites and validated by collection of samples at ASGM sites in Western Kenya.

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3A. Abstract

Analysis of mercury (Hg) in natural water samples has routinely been impractical in many environments, for example, artisanal and small-scale gold mines (ASGM), where difficult conditions make monitoring of harmful elements and chemicals used in the processes highly challenging. Current sampling methods require the use of hazardous or expensive materials, and so difficulties in sample collection and transport are elevated. To solve this problem, a solid-phase extraction-based method was developed for the sampling and preservation of dissolved Hg in natural water samples, particularly those found around ASGM sites. Recoveries of 85% ± 10% total Hg were obtained during 4-weeks of storage in refrigerated (4°C, dark) and unrefrigerated (16 °C, dark) conditions, and from a representative river water spiked to 1 µg L^{-1} Hg^{2+}, 94% ± 1% Hg recovery was obtained. Solid-phase extraction loading flow rates were tested at 2, 5, and 10 mL min^{-1} with no breakthrough of Hg, and sorbent stability showed no breakthrough of Hg up to 2-weeks after functionalisation. The method was deployed across five artisanal gold mines in Kakamega gold belt, Kenya, to assess Hg concentrations in mine shaft water, ore washing ponds, and river and stream water, including drinking water sources. In all waters, Hg concentrations were below the WHO guideline limit value of 6 µg L^{-1}, but drinking water sources contained trace concentrations of up to 0.35 µg L^{-1} total Hg, which may result in negative health effects from long-term exposure. The SPE method developed and deployed here is a robust sampling method that can therefore be applied in future Hg monitoring, toxicology, and environmental work to provide improved data that is representative of total dissolved Hg in water samples.

Keywords: Mercury, Artisanal gold mining, Sampling, Preservation, Solid-Phase Extraction

3.1. Introduction

3.1.1. Mercury (Hg) as a pollutant

Mercury (Hg) is a highly toxic element that is ubiquitous in the environment, arising from both natural and anthropogenic processes (Risher, 2003). Due to its ability to bioaccumulate in animals and biomagnify up the food chain (Kim and Zoh, 2012), this element can be persistent and problematic for wildlife, where exposure to low concentrations of just 0.03 µg L^{-1} Hg in the aquatic environment can result in considerable accumulation up the various trophic levels and top predators in the environment (Morcillo et al., 2017). Consumption of contaminated fish, a key protein source, may subsequently result in severe negative human health effects in the

neurological, renal, cardiovascular, immunological, and reproductive systems (Svobodova et al., 1993, DHHS, 2022). Unpolluted concentrations for environmental water sources, which may affect human exposure routes, are usually 0.01 µg L^{-1} Hg but can be as high as 0.1 µg L^{-1} Hg (Svobodova et al., 1993). However, guideline values for the protection of aquatic wildlife from chronic Hg exposure are 0.77 µg L^{-1} Hg as defined by the US EPA (EPA, 1995). while drinking water guidelines are defined at 6 µg L^{-1} Hg by the WHO (WHO, 2022).

To prevent anthropogenic pollution of Hg, the 2013 Minamata Convention limits the use of Hg in all sectors of society, but in most middle- and lower-income countries the metal is relied upon for many industries. The most prevalent use of Hg is in artisanal and small-scale gold mining (ASGM) (Kristensen et al., 2014, Esdaile and Chalker, 2018), where Hg is used to amalgamate gold in mined and pulverised ores and is subsequently released by burning to obtain gold. Through the amalgamation and burning processes, surrounding soil and atmosphere is polluted with Hg (Kristensen et al., 2014). This practice occurs globally and has been routinely investigated over the past 20 years (Hentschel et al., 2002, Li et al., 2009, Ondayo et al., 2023a, Ondayo et al., 2023b). Across over 40 African countries, approximately 10 million people work in ASGM sites (Grynberg et al., 2022) and are potentially exposed to large quantities of potentially harmful elements, including Hg. In a review of ASGM across Africa (Ondayo et al., 2023b) concentrations of Hg in waters (rivers, drinking water sources, and ore washing ponds) ranged from 0.01 to 8040 µg L^{-1} Hg, with two-thirds of studies (18 out of 27) reporting Hg water concentrations above the 0.77 µg L^{-1} Hg chronic exposure limit. This underscores the need for monitoring and possible intervention, to limit environmental harm. The most extreme concentrations, up to 8040 µg L^{-1} Hg, were reported in Kenya (Olgola et al., 2001); however, sample collection and preservation methods are not specified and so may cast doubt on the reported values. In addition, this study reported data from an acid digestion of the sample, which may release Hg bound to particulate matter that would otherwise have been filtered off in standard procedures. This potentially lead to a report of a Hg concentration above what the aquatic environment would be exposed, as this will release strongly bound Hg that may not otherwise be released to the water. Drinking water sources may be treated prior to consumption, i.e. through boiling, chlorination, etc., but may be consumed untreated and thus particulate bound Hg can contribute to human exposure.

Since 2003, it is estimated that up to 100 tonnes of Hg have been imported to Kenya for ASGM activities (Esdaile and Chalker, 2018), but the ultimate fate of this metal is

unknown due to difficulties in sampling and analysis. Workers, communities, and environments near ASGM sites in Migori and Kakamega gold belts, Kenya, are reported to be adversely exposed to Hg (Olgola et al., 2001, Odumo et al., 2011, Ondayo et al., 2023a), with soils and sediments reported to contain up to 150 mg kg^{-1} Hg (Odumo et al., 2011), residential soils containing 1.07 mg kg^{-1} Hg and fish pond sediments containing 2.4 mg kg^{-1} Hg (Ondayo et al., 2023a), all above WHO, USEPA, and European Union standards for Hg in environmental media. Concentrations of Hg in water sources at these sites are not reported and therefore the true extent of Hg exposure of the local populations is not determined due to difficulties with sampling and preservation of the metal in the field.

3.1.2. Sampling methods and their challenges

Instability of Hg dissolved in water samples is a well-known challenge when monitoring the metal; sorption to container walls and loss through volatilisation are the main mechanisms of loss from a water sample (Yu and Yan, 2003, Parker and Bloom, 2005). Conventional sampling processes for dissolved Hg often have attributes of risk and difficulty, exacerbated by the need for use in challenging environments such as ASGM sites. It is recommended to send samples overnight to a laboratory for preservation (USEPA, 1996), however, when sampling waters at ASGM sites, samples often must be shipped internationally, which increases the time between sampling and analysis and thus potentially results in considerable Hg losses. Acidification of samples with hydrochloric acid stabilises the metal but can result in spillage and acid burn accidents if not carefully handled, and increased restrictions on international transportation of acids limits the shipping of samples (IATA, 2022), Collection of >500 mL sample volume can mitigate loss of Hg by wall sorption due to a decreased volume-to-surface area ratio and allows for preconcentration methods except, where multiple samples must be collected, as transportation and storage may become impractical due to the large total sample volumes. This also increases the cost of collection due to bottle material costs. Glass and PTFE containers are recommended to minimize sorption of Hg to the container walls, but glass is fragile while PTFE is expensive ($100 per 500 mL PTFE bottle (Camlab, 2023)). If unpreserved or inadequately preserved, considerable losses of Hg are noted within few days of sampling and 90% of total Hg can be lost after just 1 week (Yu and Yan, 2003, Parker and Bloom, 2005, Louie et al., 2012). In-field monitoring of Hg in water sources is particularly challenging, due to analysis requiring sensitive detectors, which currently do not achieve adequate detection limits (King et al., 2023). Some emerging technologies demonstrate appropriate sensitivity but are not currently

widespread or commercially available (Zhao et al., 2020, Karuk Elmas et al., 2021, Lopreside et al., 2021).

Solid-phase extraction offers a method to stabilise dissolved Hg in water for a substantial amount of time (Kim and Zoh, 2012). Through immobilisation to a solid-phase, the risk of analyte loss is minimised and representative data can be collected, particularly in challenging environments where there is difficulty in transporting materials and samples. Previous work for the preservation of other metals (arsenic, As and vanadium, V) has shown the usefulness of SPE techniques in sampling and stabilisation of metals in water samples (Watts et al., 2008, Watts et al., 2010, Al Rawahi and Ward, 2017). In water samples, significant losses of V data are reported over less than 1 week of storage, which can limit sample collection for many studies. An optimised SPE-based sampling method was developed, improving sample storage to up to 2 weeks with recoveries of 95-101% V(IV) and V(V) from river water samples (Al Rawahi and Ward, 2017). For As concentrations, there is a known instability of the metal species in a 1 mg L^{-1} Fe water matrix, often found in rivers and lakes (Stetson et al., 2021). To avoid this issue, an SPE-based sampling method was developed by Watts et al. (2008) and O'Reilly et al. (2010) to preserve As(III), As(V), monomethylarsonic acid, and dimethylarsinic acid. This method uses anionic and cationic SPE cartridges to allow in-field separation and preservation of As for up to 4-weeks with 100% recovery of each species. Parameters were optimised based upon field conditions. For example, the flow rate for sample loading was determined by the physical ability of the operator to push water through the cartridges. A 4-week storage time was chosen as a typical time from field to laboratory analysis, accounting for a field sampling campaign and potential international travel. Previous research into the SPE method for Hg sampling and preservation methods have shown >90% recovery of total Hg from river and lake water samples over a 1-week period (Al Rawahi and Ward, 2017, Stetson et al., 2021), but extending this preservative timeframe is essential for an effective field method that can be used in all environments.

3.1.3. Considerations for a field-method

For SPE to be used as a field-based sampling method, it is crucial to consider practical characteristics that affect the immobilisation of dissolved Hg and stability of the metal once retained (Figure 3.1). Initially, the cartridges must be stable from the point of functionalisation to sampling in the field. Degradation of the functionalised phase may result in a decrease in immobilised Hg during the loading process and therefore underrepresent the dissolved Hg concentration in the environmental

samples. This is a characteristic not often explored in the literature, as typical speciation and preconcentration methods apply the cartridge immediately after functionalisation (King et al., 2023), thus not requiring a storage time for the solid phase. However, to ensure representative data collection, the functionalised solid-phase must be stable during transfer from laboratory to sampling location, approximately 2-weeks between functionalisation and application in the field. Flow rates for loading samples onto the cartridges must be appropriate for field-use, to ensure feasible sampling times and thus overall cost-effectiveness. If flow rates are too slow, the time required to load a sample onto a cartridge will not be appropriate for field studies. This is generally not explored in the literature, as SPE methods are often developed for on-line laboratory speciation or preconcentration, and so flow rate is controlled by optimised chromatographic flow rates. The quantity of functionalising agent immobilised on the solid-phase is also important, as quantities below the optimum will fail to retain sufficient Hg concentrations, while quantities above the optimum may impede elution processes and result in partial elution of Hg. Once immobilised, Hg must be retained to the phase with adequate time for transportation to a laboratory. This may range from hours to weeks depending on the sampling environment and location, so Hg must be eluted with good recovery after at least a 4-week period. Similar characteristics were used in previous literature related to SPE as a sampling technique (Watts et al., 2008, Watts et al., 2010, Al Rawahi and Ward, 2017), which is representative of necessary parameters for a functional sampling and

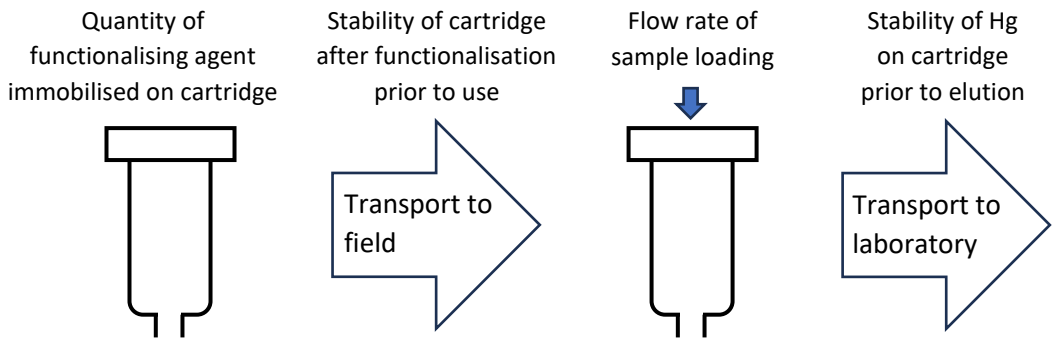


Figure 3.1. Main considerations of an SPE-based field sampling method, including quantity of functionalising agent applied, stability of the functionalised cartridge prior to use, flow rate of sample loading, and stability of Hg on the cartridge after immobilisation.

preservation method for dissolved Hg. The development of an SPE field method for sampling and preserving Hg from water samples will allow for the determination and monitoring of Hg in waters that affect some of the most vulnerable populations.

3.1.4. Aim and Objectives

There is an identified gap in the literature to safely and reliably sample total dissolved Hg from water matrices in challenging environments (King et al., 2023). Therefore, the aim of this study was to develop a robust method for the sampling and accurate measurement of Hg in water matrices based on SPE techniques. To achieve this aim, the objectives were to: (1) develop a practical functionalised SPE method to retain and preserve Hg from water matrices and for elution on return of samples to a controlled laboratory environment; (2) optimise parameters of sorption and desorption of Hg to the stationary phase for subsequent measurement; (3) demonstrate robustness of method in water matrices.

3.2. Method

Based on literature methods (Yin et al., 2010, Wang et al., 2022), a dithizone-functionalised C18 cartridge was assessed for the extraction and recovery of Hg from 30 mL of water, eluted using a 2-mercaptoethanol solution.

The effect of flow rate of the sample through the cartridge was assessed, as well as the stability of the functionalised cartridge prior to use in the field, approximately 2-weeks from functionalisation to use. The stability of Hg immobilised on the cartridge was assessed over a 4-week period. This is an expected time from sampling to analysis for projects conducted in challenging environments such as ASGM sites, including time needed for international shipping of samples, and ensures preservation over shorter time periods, i.e., hours or days.

The workflow of functionalisation and field-use of SPE cartridges are illustrated in Figure 3.2.

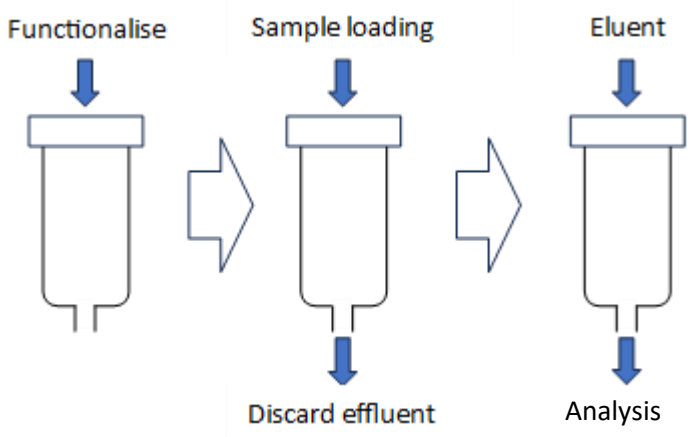


Figure 3.2. The functionalisation of SPE cartridges using 50 µg dithizone, loading of water samples in the field at 10 mL min^{-1} to immobilise Hg, and elution of Hg using 30 mL of a 1% 2-mercaptoethanol solution

The functionalisation of the cartridges was conducted following methods reported by Yin et al. (2010), Wang et al. (2022). Briefly, C18 cartridges (Bond Elut Jr, Agilent, UK) were flushed with 5 mL of methanol followed by 5 mL of sodium formate-formic acid (0.5 mol L^{-1}, pH 4; SigmaAldrich, UK) to condition the resin bed. A dithizone solution (1 mL, 0.5 mg L^{-1} dithizone; AlfaAesar, UK) was immobilised on the cartridge. The resin bed was then conditioned by passing through a 3 mL sodium formate-formic acid solution (0.5 mol L^{-1}, pH 4; SigmaAldrich, UK) and 3 mL of sodium formate-formic acid solution (0.05 mol L^{-1}, pH 4; SigmaAldrich, UK).

Parameters and conditions for ICP-MS analysis are given in Appendix 3 Supplementary Table A3.2. A method detection limit was calculated as 3σ from measurements of a blank solution (n = 10) (Appendix 3 Supplementary Table A3.3.). Other water chemistry of real samples was analysed by ion chromatography, pH and alkalinity analysis, and conductivity probe at the British Geological Survey, Nottingham, UK (Appendix 3 Supplementary Table A3.4.).

3.2.1. Quality control

Due to the instability of dissolved Hg in water samples, certified reference materials for Hg in environmental waters are not readily available. Commercially available certified reference materials are often preserved using BrCl solutions, (ORMS-5, NRCC, Canada) (Willie et al., 2011). During analysis, a 0.25 µg L^{-1} Hg^{2+} quality control sample was periodically analysed (every 15 samples) to ensure accuracy and precision between experiments. To ensure the quality and performance of the cartridge would not be impacted by major anion and cation chemistry commonly found at ASGM sites (Ondayo et al., 2023a) (Appendix 3 Supplementary Table A3.1.), a synthetic hard water was used as a matrix in experimental work. The matrix was made following a method described by Smith et al. (2002). To represent total dissolved Hg, it was spiked to 1 µg L^{-1} inorganic Hg^{2+}, which is the most common form of Hg in water. 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.

3.2.2. Quantity of dithizone immobilised onto the cartridge

An important factor for ensuring the sorption and stability of Hg on the solid phase is the quantity of dithizone immobilised on the cartridges. To assess this, dithizone was loaded onto C18 cartridges in quantities of 25, 50, 100, and 200 µg dithizone. A 1 µg L^{-1} Hg^{2+} synthetic freshwater solution (30 mL) was passed through the functionalised cartridges. The effluent was collected and acidified to 1% HNO_3 and 0.5% HCl. A 2-

mercaptoethanol eluent solution (1% v/v, 30 mL) was then passed through the cartridges and collected for analysis.

3.2.3. Sample flow rate and sorbent stability

It is necessary to establish an optimum sample flow rate for immobilisation of Hg that is achievable in field conditions. To ensure adequate flow rates could be achieved, a 1 µg L^{-1} Hg^{2+} synthetic freshwater solution (30 mL) was passed through the functionalised cartridges at a flow rate of 2, 5, or 10 mL min^{-1} using a vacuum box. Prior to use, cartridges were functionalised with a 50 µg dose of dithizone (1 mL, 0.05 mg L^{-1} dithizone solution). The cartridge effluents were collected and acidified to 1% HNO_3 and 0.5% HCl for analysis.

The stability of the functionalised cartridge was investigated over 2-weeks. Cartridges were functionalised and stored for 0, 7 and 14 days (16 °C, in the dark and 4 °C, in the dark), to simulate potential storage conditions for transfer from the laboratory to the field. A 1 µg L^{-1} Hg^{2+} solution (30 mL) was loaded onto functionalised cartridges at a flow rate of 10 mL min^{-1}, and the effluent was collected and acidified to 1% HNO_3 + 0.5% HCl.

3.2.4. Recovery of immobilised Hg over time

The stability of Hg on the solid-phase must be established to determine the efficacy of SPE as an in-field sampling method. A 1 µg L^{-1} Hg^{2+} spiked synthetic freshwater matrix (30 mL) was passed through functionalised C18 cartridges. These cartridges were stored in the dark at either 4 °C or 16 °C, temperatures of cooled and non-cooled storage respectively, for up to 4-weeks, a typical timescale between sampling in challenging environments and analysis in a laboratory. Retained Hg was eluted following the previously described method at 0, 1, 2, 3, and 4 weeks after extraction of Hg.

3.2.5. Sampling sites

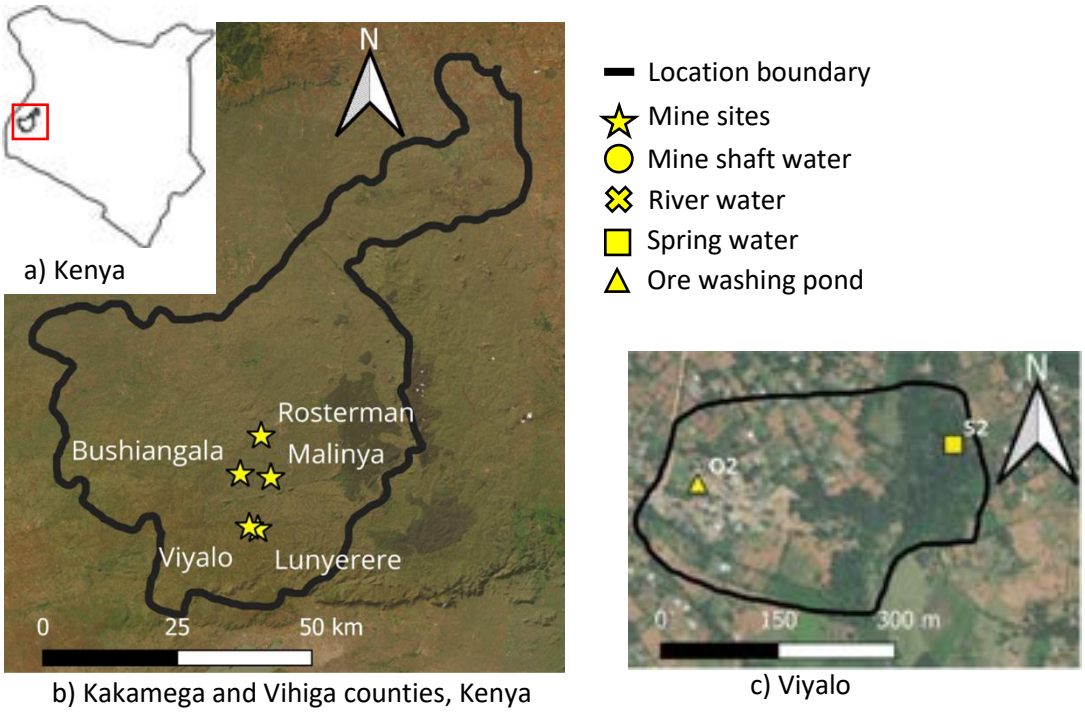
3.2.5.1 River Trent

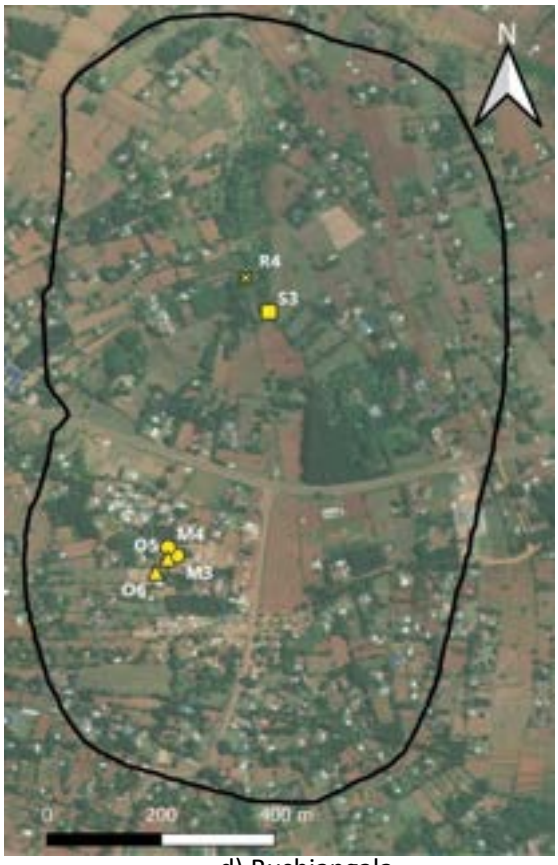
Once the SPE sampling method was developed, application with natural water samples was conducted using the River Trent, Nottingham, UK (52°56'08.4"N 1°08'19.1"W) to reproduce the matrix of a complex water system. Thus, a bulk water sample was collected from the River Trent. This major river is known to have hard water with elevated cationic concentrations and significant pollution due to industry along its length (DEFRA, 2019). This matrix is more complex than distilled water and

comparable to water bodies found around mine sites (Appendix 3 Supplementary Table A3.1.), and therefore is more representative of a natural water sample than deionised water. The bulk water was collected using a 1 L Nalgene container and 500 mL was spiked with Hg to 1 µg L^{-1} to represent significant Hg pollution.

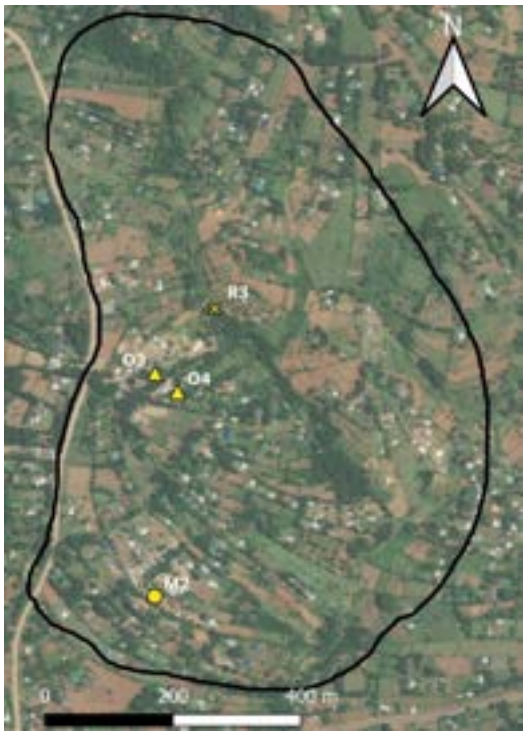
3.2.5.2 ASGM mines

Within the Kakamega gold belt fwhich spans Kakamega and Vihiga Counties, ASGM sites are present and active, polluting local rivers and streams with mine waste such as Hg, which confers health risks to the miners and surrounding community (Ondayo et al., 2023a, Ondayo et al., 2023b). The river and stream water is relied upon by the local population for watering livestock, drinking water, irrigation for agriculture, aquaculture, and domestic uses such as cleaning and laundry. Therefore, mine sites in this area were considered to be good test sites for this study to assess the deployment of the developed method under field conditions. Five mines were selected for testing: Lunyerere (0°06'06.1"N 34°42'52.1"E) and Viyalo (0°06'21.4"N 34°42'01.0"E) in Vihiga county, Kenya, Bushiangala (0°11'42.9"N 34°41'06.4"E), Malinya (0°11'22.4"N 34°44'10.3"E) and Rosterman (0°15'35.6"N 34°43'12.0"E) in Kakamega county, Kenya, as typical ASGM sites and based on environmental chemistry obtained from a public health study (Ondayo et al., 2023a) (Figure 3.3.). Samples were collected from local river and stream systems, as well as mine waters, ore washing ponds, and drinking water sources.

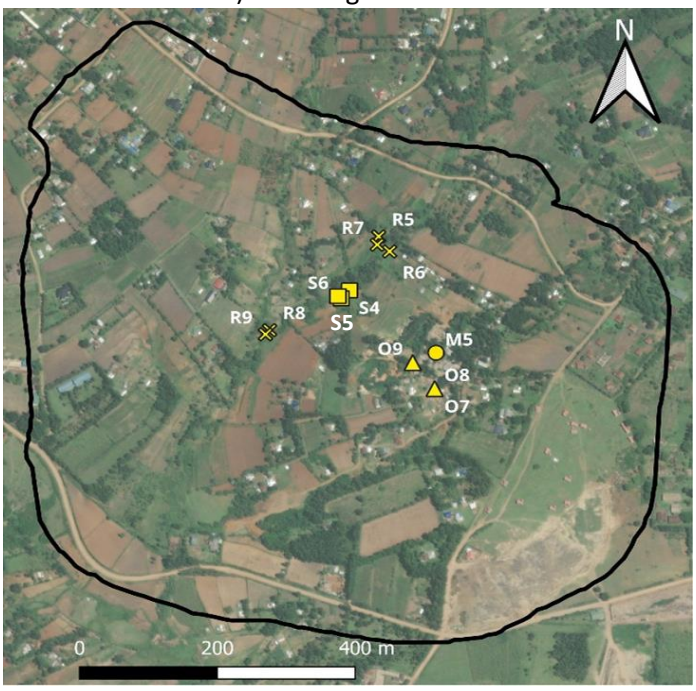




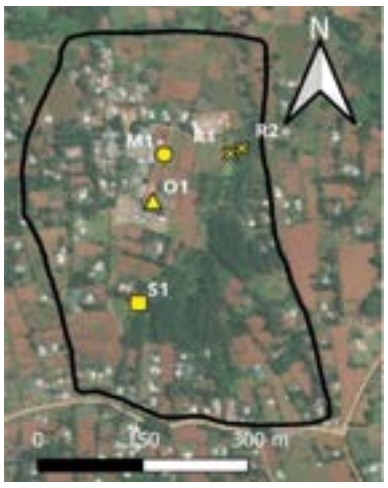
d) Bushiangala



e) Malinya



f) Rosterman



g) Lunverere

Figure 3.3. Map of b) Kakamega and Vihiga counties, a) Kenya, the ASGM sites sampled: c) Viyalo, d) Bushiangala, e) Malinya, f) Rosterman, and g) Lunyerere mine sites, and sampling locations

3.3. Results and Discussion

3.3.1 Dithizone load quantity

Concentrations of Hg measured in the effluent were below the 0.008 ug L^{-1} detection limit across 50-200 µg dithizone, indicating total immobilisation of Hg to the resin bed. At 25 µg dithizone, breakthrough of the metal at 0.01 µg L^{-1} Hg^{2+} was observed. This is a potential issue for polluted water sources (>1 µg L^{-1} Hg), such as those found by Olgola et al. (2001) and Ngure et al. (2017) where Hg concentrations were found up to 8 and 19 µg L^{-1} Hg respectively and thus are likely to exceed the capacity of the functionalised cartridge at 25 µg dithizone. Complex matrices which contain increased quantities of particulate matter and mine-related elements, may also compete for active sites on the solid-phase, and therefore may decrease the sorption of Hg thus requiring a dithizone quantity greater than 25 µg.

Elution from all assessed dithizone quantities showed Hg recoveries above 85%, with the most optimal recovery of 92% at 50 µg dithizone, indicating suitable recovery for representative data collection (Figure 3.4). Using a cartridge functionalised with a lower quantity of dithizone, 12.5 µg dithizone, Wang et al. (2022) reported high recoveries (95% ± 8% Hg), however, this study focused on trace unpolluted concentrations of Hg, <0.2 µg L^{-1} Hg. The lower quantity of dithizone may not appropriately immobilise higher concentrations of Hg such as those reported by Ngure et al. (2014) in the Migori gold belt, Kenya, 0.36 – 52 µg L^{-1} Hg. The comparable recoveries achieved by this work, 92% (± 3 %) Hg, demonstrate the suitability of a 50 µg dithizone quantity for the extraction and recovery of Hg, and can fully immobilise Hg from polluted water sources up to 1 µg L^{-1}. A 50 µg dithizone load was used for all other experiments.

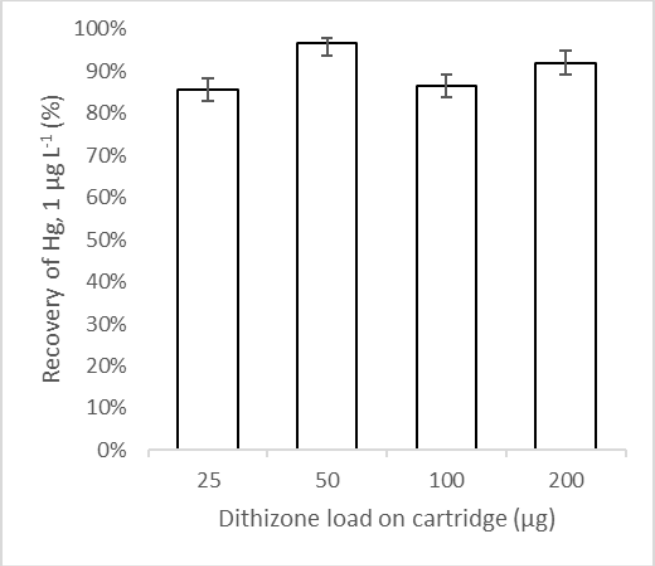


Figure 3.4. Recovery and standard deviation of Hg^{2+} from SPE cartridges with various quantities of immobilised dithizone (25, 50, 100 and 200 µg) (n=5), indicating a load of 25 µg dithizone or greater is suitable for immobilisation of Hg^{2+} from water samples with 50 µg showing the most optimal performance.

3.3.2 Sample flow rate and sorbent stability

Two key factors of a viable field sampling SPE method are the maximum flow rate of sample loading and the stability of the cartridge prior to use. A moderate sample flow rate is necessary to ensure sampling does not require unreasonable lengths of time to conduct when in the field. At loading speeds of 2, 5, and 10 mL min^{-1}, all effluent samples had Hg concentrations below the detection limit (0.008 µg L^{-1} Hg^{2+}), showing total sorption of Hg to the solid-phase. This is approximately the speed that can be achieved when manually pushing solution through a filter and cartridge using a Luer-lock syringe, and so typically, this is what may be achieved by operators in the field with minimal equipment requirements.

The stability of the cartridges prior to use was also investigated. Cartridges were stored for up to 14 days after functionalisation, and an extraction was conducted. Across the 14-day period, Hg in the effluent samples was consistently below the detection limit of 0.008 µg L^{-1} Hg. This means that a 30 mL sample of 1 µg L^{-1} Hg completely absorbed into the stationary phase after being stored in the dark for up to two weeks at a temperature of 4 or 16 °C. Prepared cartridges can therefore be stored prior to use with no breakthrough of Hg, even at significant concentrations of Hg pollution.

3.3.3 Four-week preservation

Stability of the sorbed Hg was investigated over 0–4 weeks in the dark in either refrigerated (4 °C) or unrefrigerated (16 °C) conditions. As illustrated in Figure 3.5, recovery of Hg^{2+} was consistently high at both 4 °C and 16 °C in the dark across 4 weeks. At either 4°C or 16°C in the dark, average recoveries of Hg were 88% ± 8% and 85% ± 10%, respectively, showing good reproducibility and demonstrating adequate Hg preservation for typical transportation and storage times from field to laboratory. The lowest recorded recovery was 68% after 3-weeks of storage at 16 °C in the dark, which is still considered an acceptable recovery for SPE techniques (EPA, 2007). Blanco *et al.* (2000) used a diethyldithiocarbamate functionalised microcolumn for dissolved Hg preservation but noted a significant decrease in recovery from 102% to just 40% after just 1 week of storage (Blanco et al., 2000). A similar dithizone-based microcolumn (Wang et al., 2022) was reported to stabilise dissolved Hg from waters over 10 days of storage, which may not be an adequate timeframe in many environments.

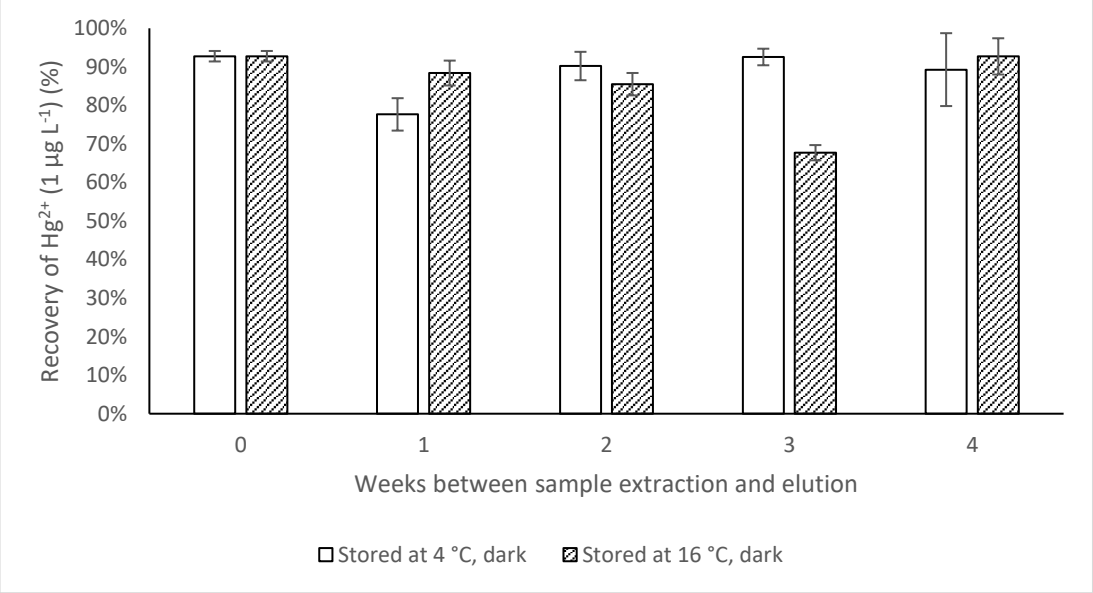


Figure 3.5. Recovery (%) and standard deviation (n=5) of Hg (30 mL, 1 µg L-1 Hg2+ solution) immobilised on a functionalised cartridge over 4-weeks storage and eluted with a 2-mercaptoethanol solution (1% v/v, 30 mL).

The recorded improvement using the dithizone functionalised cartridges allows for representative data to be collected and analysed with a reasonable time limit from field to laboratory with minimal losses over time. A comparison of the performance of the developed cartridge and literature methods is shown in Table 3.1.

Table 3.1. Performance, as %recovery, of functionalised cartridges loaded with 50 µg dithizone in comparison to previous literature using 12.5 µg dithizone load

Study	Functional agent	Hg spike concentration assessed (µg L^{-1} Hg^{2+})	Sample volume (mL)	Eluent volume (mL)	Recovery (%)	Effective storage time	Replicates
This work	Dithizone (50 µg)	1	30	30	92 ± 3	4-weeks	5
(Blanco et al., 2000)	Diethyldithio-carbamate	0.05-0.15	50	0.5	70 ± 3	1-week	3
(Yin et al., 2010)	Dithizone (200 µg)	0.2	100	3	83 ± 4	4-days	3
(Wang et al., 2022)	Dithizone (12.5 µg)	0.005	1.5	N/A (online elution)	95 ± 8	1-week	7

3.3.4 Natural water samples water

3.3.4.1 River Trent, Nottingham, UK

To validate the developed method for use on real water samples, the procedure was applied to natural water samples collected from freshwater streams and rivers. Reference materials for these matrices are not available due to the instability of Hg (King et al., 2023), so spiked waters are a common substitute for test waters in the literature (Margetínová et al., 2008, Yin et al., 2010, Wang et al., 2022). A bulk water sample was collected from the River Trent, Nottingham, using a 1 L Nalgene bottle and transported back to the laboratory. The water was filtered and a portion (500 mL) was spiked to 1 µg L^{-1} Hg^{2+}, to represent a water source with polluted levels of dissolved Hg. Both the spiked and unspiked samples (30 mL) were passed through functionalised cartridges at a flow rate of 5 mL min^{-1}, and the effluent was collected and acidified (1% v/v HNO_3 + 0.5% v/v HCl). A 2-mercaptoethanol eluent (1% v/v in deionised water, 30 mL) was passed through the cartridges and collected. The effluent and eluent samples were analysed by ICP-MS analysis.

Mercury was not detected in the effluent samples, indicating total sorption of Hg to the solid-phase from the river water matrix. Recoveries from the spiked water samples

were >90%, which is comparable to literature recoveries of typical preservation methods such as acidification of the samples (Table 3.2.) (Parker and Bloom, 2005, Louie et al., 2012).

Table 3.2. Comparison of recovery (%) of Hg from river waters between those reported in literature and this work.

Reference	Sample	Hg concentration (μg L^{-1})	Sample volume (mL)	Elution volume (mL)	Spike (μg L^{-1})	Recovery (%)	Relative Standard deviation
This work	River water (River Trent)	N.d.	30	30	1.004 Hg	94	1%
(Blanco et al., 2000)	River water	N.d.	50	0.5	0.05-0.15 Hg^{2+}	90	6.7%
(Yin et al., 2010)	River water	0.025	100	3	-	86	Not reported

3.3.4.2 ASGM sites in Kakamega and Vihiga counties, Kenya

Mercury concentrations were measured across 5 ASGM sites in the Kakamega gold belt, Kenya, for different water matrices (Table 3.3). The concentrations found in waters across all 5 sites were below the limit for chronic exposure to aquatic wildlife, <0.77 μg L^{-1} Hg (EPA, 1995), between 0.010 – 0.348 μg L^{-1} Hg, with the most contaminated samples collected from mine shaft water at the Bushiangala mine site. Samples collected from drinking water sources were below the WHO guideline maximum value of 6 μg L^{-1} (WHO, 2022), containing Hg concentrations of 0.023 – 0.166 μg L^{-1} in spring and river drinking water sources. Water from mine shafts is also used as a drinking water source by miners and these samples were typically higher in concentration than spring- and river-based drinking water sources, varying between 0.041 – 0.348 μg L^{-1} Hg. While this implies no significant immediate hazard to the environment, it is important to note that samples were collected before alluvial mining and gold amalgamation occurred, so they are representative of ambient Hg concentrations in waters and does not capture Hg concentrations immediately after release to the water bodies. Exposure from these waters is below the tolerable weekly intake of 0.7 μg (Hg) kg^{-1} (body weight) $week^{-1}$ (Kuras et al., 2017), assuming a 3L water intake per day and an approximate 60 kg average Kenyan bodyweight (WHO,

2022), but the mine shaft water consumed by the mine workers makes up 17% of the tolerable weekly intake and so may pose a health hazard in conjunction with dietary and other external exposure routes, for example inhalation during burning.

The measured Hg concentrations are lower than previously reported values from ASGM sites in Kenya by Olgola et al. (2001), 8040 µg L^{-1} Hg, however, methods of sampling, filtration, or preservation were not reported for waters, so the data should be considered with this in mind. The analysis method from this literature study also included a HNO_3 digestion step, which may digest particulate matter-bound Hg from unfiltered samples and therefore increase the measured concentration of Hg. A study by Ngure et al. (2017) reported Hg concentrations of 0.06 – 19 µg L^{-1} Hg in drinking water in the Migori gold belt, Kenya, where the most elevated concentrations were found at sampling points closer to the mouth of the river system and indicating potential pollution from ASGM activities along the rivers length. The lower concentration samples, 0.06 and 0.92 µg L^{-1} Hg, are comparable to those found in this study. In both the literature study and this work, the sample locations are similarly positioned in the Kenyan gold belts with respect to potential nearby ASGM sites.

For a more accurate assessment of associated health risks, Hg speciation should be taken into consideration due to differing bioavailability and toxicity. Future work on SPE-based sampling methods should examine the potential for species sampling and preservation to better understand Hg speciation and transformations in the environment.

Table 3.3. Hg concentrations determined from water samples at ASGM sites across the Kakamega gold belt, Kenya.

Sample	Water type	Location	Hg concentration (µg L^{-1})	Weekly exposure (µg(Hg) kg^{-1}(body weight) $week^{-1}$
M1	Mine shaft water	Lunyerere	0.179	0.063
M2	Mine shaft water	Malinya	0.255	0.089
M3	Mine shaft water	Bushiangala	0.348	0.122
M4	Mine shaft water	Bushiangala	0.041	0.014

M5	Mine shaft water	Rosterman	0.045	0.016
R1	River water (near mine shaft)	Lunyerere	0.166	0.058
R2	River water (near mine shaft)	Lunyerere	0.148	0.052
R3	River water (drinking water)	Malinya	0.023	0.008
R4	River water (mineshaft water released to river)	Bushiangala	0.084	0.029
R5	River water (upstream of new alluvial mining operation)	Rosterman	0.02	0.007
R6	River water (downstream of new alluvial mining operation)	Rosterman	0.032	0.011
R7	River water (new alluvial mining operation)	Rosterman	0.058	0.02
R8	River water (from a separate mining village)	Rosterman	0.141	0.049
R9	River water (downstream of ASGM site)	Rosterman	0.028	0.01
S1	Spring water (drinking water)	Lunyerere	0.132	0.046
S2	Spring water (drinking water)	Viyalo	0.043	0.015
S3	Spring water (drinking water)	Bushiangala	0.036	0.013
S4	Spring water (drinking water)	Rosterman	0.055	0.019
S5	Spring water (upstream of ore washing ponds)	Rosterman	0.01	0.004
S6	Spring water (downstream of ore washing ponds)	Rosterman	0.043	0.015
O1	Ore washing pond	Lunyerere	0.151	0.053
O2	Ore washing pond	Viyalo	0.079	0.028

O3	Ore washing pond	Malinya	0.03	0.011
O4	Ore washing pond	Malinya	0.046	0.016
O5	Ore washing pond	Bushiangala	0.162	0.057
O6	Ore washing pond	Bushiangala	0.102	0.036
O7	Hg amalgamation pan for ore washing	Rosterman	0.277	0.097
O8	Ore washing pond	Rosterman	0.162	0.057
O9	Ore washing pond	Rosterman	0.032	0.011
	(WHO Guideline value)		1.000	
	(US EPA no effect guideline – aquatic wildlife)		0.770	

3.4. Conclusion

Due to poor stability of Hg in water samples, previous literature assessing Hg concentrations in waters in challenging environments may not accurately report representative values, likely as a result of inherent difficulties in the sampling, preservation, and subsequent analysis methods. This study successfully assessed an SPE-based method for the sampling and preservation of Hg in water samples, providing a robust method applicable in a wide variety of environments. Total dissolved Hg can be completely extracted from a natural water sample and preserved over a 4-week period with a >75% recovery, an improvement over previous literature preservation of just 1-week. The method was applied at Kenyan ASGM sites, finding Hg concentrations between 0.020 and 0.348 µg L^{-1} Hg, with water sources used as drinking waters found to not pose a significant health risk individually but do contribute up to 17% of the total weekly intake limit. The improved sampling method presented in this work will allow the collection of representative data at concentrations below the guidance limit for drinking water and thus improve future Hg monitoring schemes and toxicological studies, notably in vulnerable areas which currently cannot be assessed effectively. The assignment of such guidance limits is often constrained by the analytical chemistry detection limit capability and availability of equipment to reliably measure potentially toxic elements in environmental matrices at low concentrations that may better inform chronic exposure.

3.5 References

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Chapter 4 – Mercury speciation of environmental samples associated with artisanal small-scale gold mines using a novel solid-phase extraction approach to sample collection and preservation

This chapter was submitted to the journal *Environmental Geochemistry and Health*, and was published October 2024. The formatting has been changed for uniformity with the rest of the thesis, and a clarifying statement is added to Figure 4.2.

In this chapter, the sampling and preservation method developed in Chapter 3 is further assessed for extraction of Hg species (Hg^{2+} and MeHg). Experimental work used a synthetic river water to assess the performance in samples with chemistry similar to waters at ASGM sites, with suitable recoveries of >94% for both species with storage up to 4-weeks. Environmental samples were collected at two ASGM sites in western Kenya to demonstrate the performance of the developed method in challenging environments, and to consider the environmental and human health implications of Hg pollution at these sites.

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4A. 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 dams 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.

4.1 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 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,b). 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 4.1. demonstrates typical points of pollution at ASGM sites and Figure 4.2. 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.

Figure 4.1 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.

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 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.

Figure 4.2 Mercury (Hg) used for amalgamation of gold from crushed ore, usually handled with limited or no protective equipment.

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 hours 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 4.1. 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 4.1 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.

4.2. Methodology

4.2.1. 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 5mL 0.5M sodium formate-formic acid buffer (pH 4, SigmaAldrich, UK), 1 mL 0.002 M dithizone solution (AlfaAesar, UK), followed by 3mL of 0.5 M sodium formate-formic acid buffer (pH 4) and finally 3mL 0.05 M sodium formate-formic acid buffer (pH 4, SigmaAldrich, UK).

4.2.2. 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 Appendix 4 Supplementary Table A4.1. 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 Figure 4.3. Analysis method detection limits for ^{199}Hg and ^{201}Hg were determined as 3 times the standard deviation of 9 blank replicates as summarised in Appendix 4 Supplementary Table A4.2, and an example chromatogram of Me(^{199}Hg), Me(^{201}Hg), ^{199}Hg and ^{201}Hg is shown in Figure 4.3.

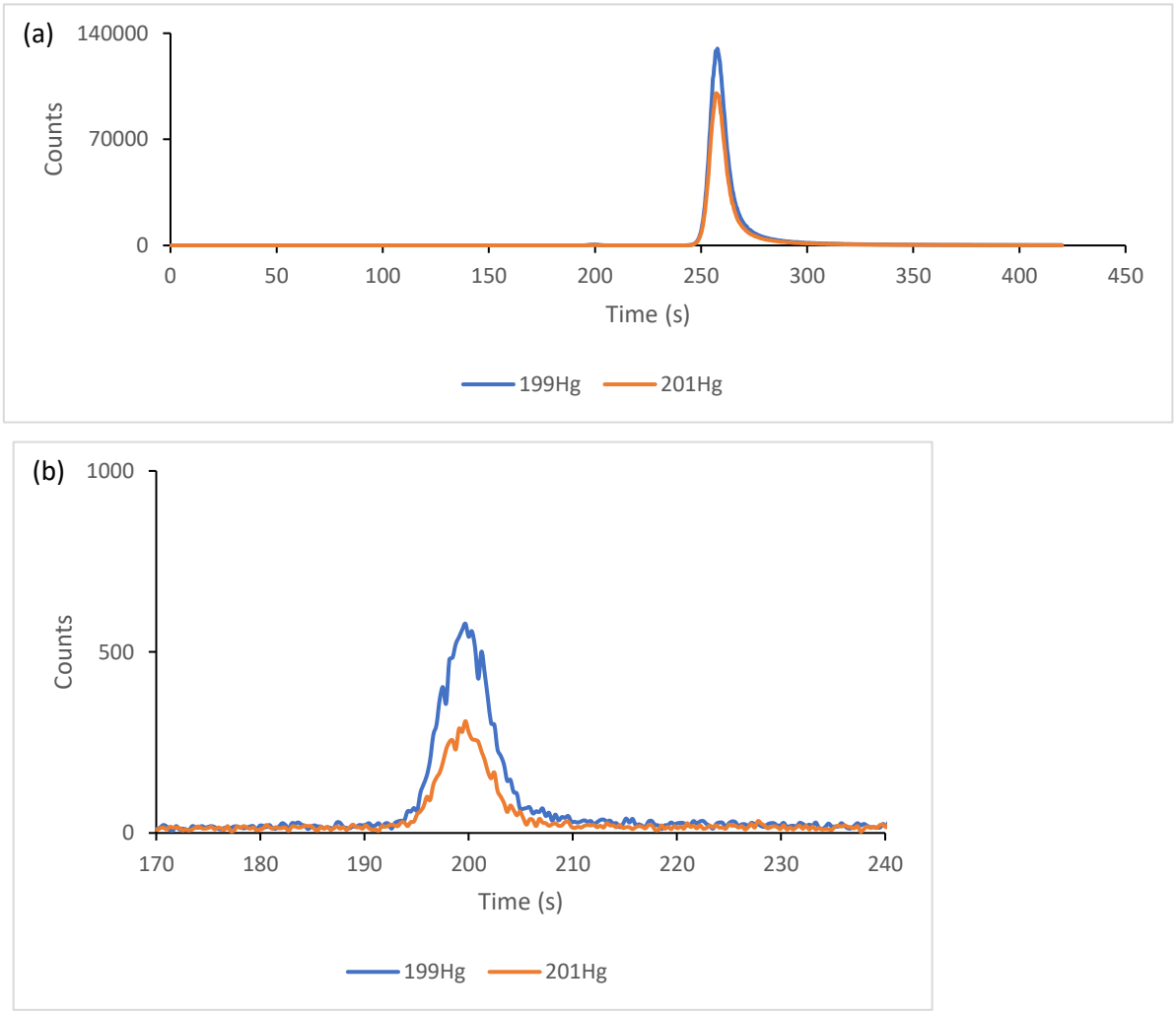

Figure 4.3 (a) An example chromatogram of MeHg (190-210 s) and Hg^{2+} (240-280 s) and (b) a magnification of the MeHg peak

4.2.3. Quality control

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) (Appendix 4 Supplementary Table A4.3) (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.

4.2.4. 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.

4.2.5. 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 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.

4.2.6. Case study of artisanal gold mines, Kenya

Maps of Case study sites are shown in Figure 4.4.

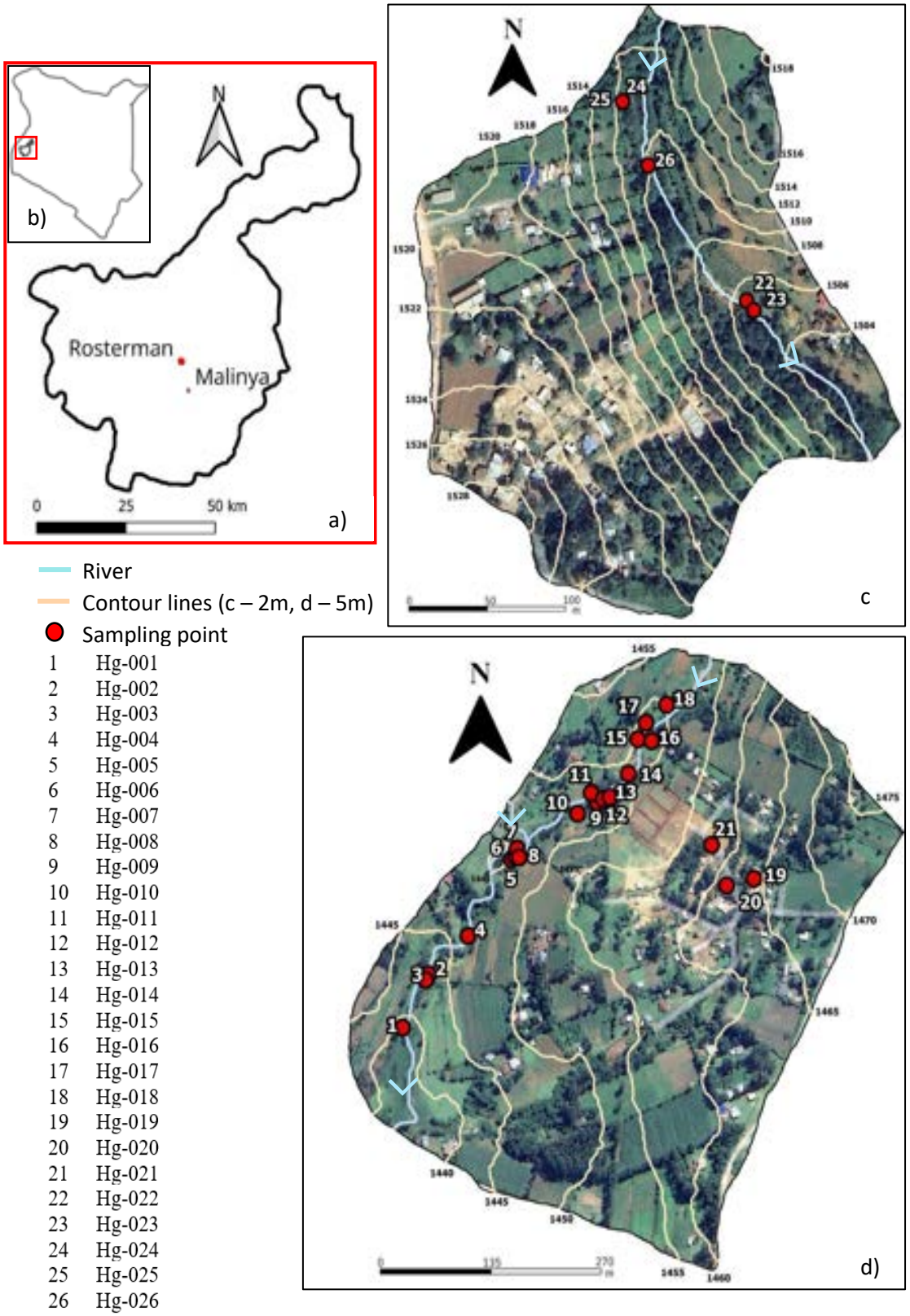

Figure 4.4 Sampling points (26) for Hg, water and sediment samples at ASGM sites in (a) Kakamega gold belt, (b) Kenya: (c) Malinya mine and (d) Rosterman mine. Rivers and contour lines are included for topography

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,b) 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 hours 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 Appendix 4 Supplementary Figures A4.1a and A4.1b.

4.2.7. 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.

4.2.8. 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, TH-2, NRCC, and PACS-2, NRCC, Canada) were analysed periodically (every 10 samples) to determine accuracy and precision of data (Appendix 4 Supplementary Table A4.4).

4.2.9. 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 Equation 1 (Hamilton et al, 2020):

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.

4.2.9.1. 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 Equation 1.

4.2.9.2. 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 minutes at 500W (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 (Appendix 4 Supplementary Table A4.4).

4.2.10. 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 (0.25g) 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% (Appendix 4 Supplementary Table A4.6). Duplicate sample analysis demonstrated percentage differences of <25% for all assessed elements.

4.3. Results and Discussion

4.3.1. 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 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 4.2).

Table 4.2 Percentage recovery (%) of Hg2+ and MeHg from varied environmental characteristics (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%

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.

4.3.2. 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 hours 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 Figure 4.5. 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.

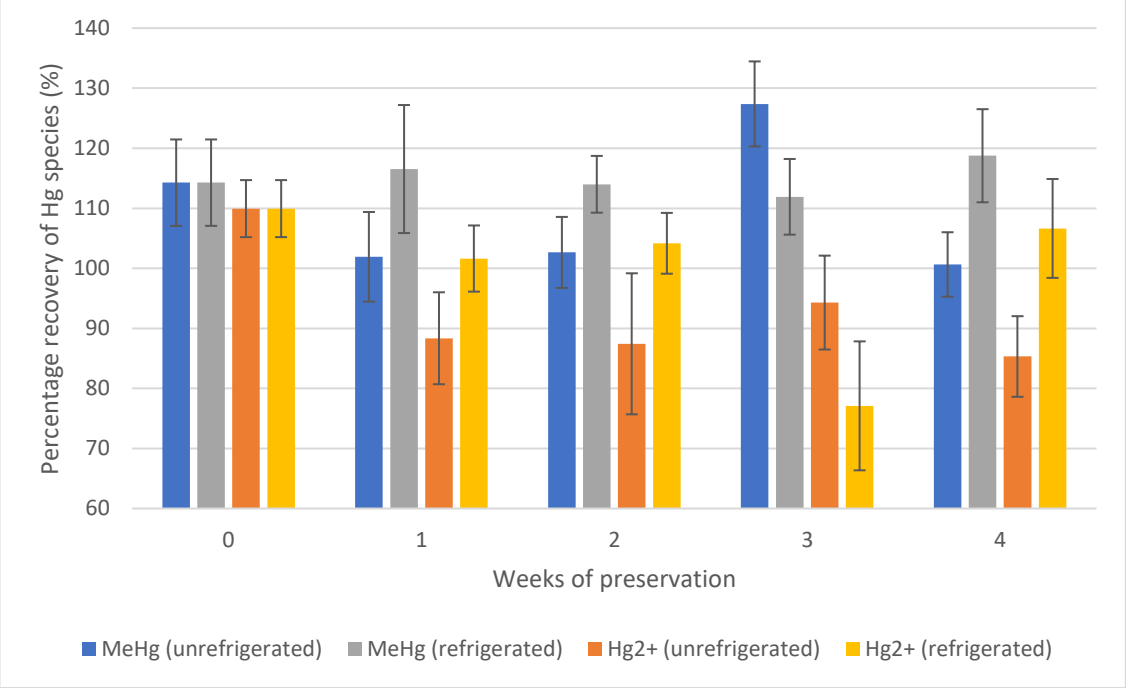

Figure 4.5 Percentage recovery (%) of Hg^{2+} and MeHg after 0, 1, 2, 3, and 4 weeks of storage in refrigerated (4 °C, absence of light) and unrefrigerated (16 °C, absence of light) conditions, demonstrating suitable recoveries for a field sampling method (recovery 130%> x >75%) across all assessed time periods (n=5).

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 (Figure 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% 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.

4.3.3. 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 4.3). General water chemistry is shown in Appendix 4 Supplementary Table A4.5. 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 – 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).

Table 4.3 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

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

Table 4.4 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 (µg kg(bw)$^{-1}$ week^{-1})	3 L water per day Hg^{2+} consumption (µg kg(bw)$^{-1}$ week^{-1})	Percentage of tolerable weekly intake (%)
	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

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 – 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.

4.3.4. Total Hg and MeHg in ASGM Sediments

Sedimentary total Hg concentrations (Table 4.5) were found between <0.05 – 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 – 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 – 36.8 kg^{-1} MeHg, with the highest concentration found in sediment from a pond 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 Appendix 4 Supplementary Table A4.6.

Table 4.5 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
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

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 100m), 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 – 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.

4.4. 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 ± 5%

Hg^{2+} and 112 ± 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 (Marriott et al. 2023; Aura et al. 2024), 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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Chapter 5 – General discussion and conclusion

5.1. Overview

The aim of this project was to develop a sampling method for total Hg and species in water samples, that can be used in challenging environments and preserve Hg for an adequate amount of time from field to laboratory. Hg is considered one of the most toxic elements posing a threat to humans and needs monitoring to mitigate pollution of the metal to the environment. The known instability of Hg species in waters has prevented adequate assessment of Hg concentrations in many studies, particularly in challenging environments such as artisanal and small-scale gold mining (ASGM) sites. The recommended sampling procedure for Hg in water was to collect at least 500 mL water in glass or polytetrafluorine (PTFE) containers, and to ship them within 24 hours to a laboratory for acidification or immediate analysis (USEPA, 1996). This was not possible in many locations or challenging environments, such as remote locations and ASGM sites, due to accessibility issues, lack of appropriate laboratories and facilities, and increased difficulty for international sample transportation. Thus, literature relating to pollution, and environmental and human health is limited regarding Hg concentrations in waters. This work aimed to address this issue by developing a water sampling method using solid-phase extraction (SPE) techniques that is readily applicable in the field.

The outcome of this project provides a novel, robust methodology for collecting representative Hg species data in environments where this has not previously been possible, such as ASGM sites or remote locations with potential Hg pollution, and other water sources where Hg is of interest. For the first time, reliable measurements of in-situ Hg exposure to poorly protected ASGM communities were obtained. This will facilitate toxicology and Hg monitoring studies, providing representative data on Hg species in waters with human and environmental exposure routes and inform policies and mitigation measures for Hg exposure. Historic and current guidelines for Hg in waters have been determined based on instrumental limitations and the challenges faced during in-field sampling (WHO, 2022). The developed sampling methodology has improved the collection of Hg from water samples by overcoming the instability of Hg species, cost of sample collection from material requirements, storage space and transport requirements due to the portable nature of the developed SPE methodology, and minimising hazards to the operator and environment, and thus research into Hg in the aquatic environment. This can improve our understanding of environmental pathways of Hg, the effect of toxic metal in nature, assist with our

understanding of both human and ecological health, and inform guidelines for Hg pollution and monitoring globally.

In addition, as sample collection times are quicker than other methods, such as collecting and filtering 0.5 – 1 L water in PTFE containers, a greater number of samples can be collected over a defined time period and thus larger scale collection is possible. This is also supported by the portable nature of the SPE cartridges, requiring significantly less storage space than traditional methods and pose minimal challenges when transporting samples both domestically and internationally.

To achieve this, the following objectives were met: 1) identifying a suitable method for sampling Hg from water samples, including assessing SPE methods for their potential as a sampling method; 2) Optimising the identified method as a sampling method for total Hg in water samples, including from challenging environments; 3) Further optimising the method for sampling and preservation of Hg speciation in water samples; and 4) Assessing the developed method for Hg speciation by deployment in ASGM sites in western Kenya, collecting water and sediment samples to examine pollutants. The results of the work are considered in context with their potential impacts and recommendations for further work.

5.2. Review of literature on Hg preservation and SPE

A review of literature surrounding Hg preservation methods highlighted a gap in knowledge of adequate in-field sampling methods that stabilise Hg and its species beyond 1-week of storage without the use of hazardous chemicals or expensive materials (King et al., 2023). Current preservation methods include acidification with nitric or hydrochloric acid, storage in glass or PTFE containers, and collecting in excess of 500 mL of water sample (USEPA, 1996, Yu and Yan, 2003). This comes with difficulties if used in-field, such as acid burns from spills, breakage of fragile containers, increased cost requirements for PTFE containers, and difficulties in transport of chemicals and storage of large sample volumes.

Previous work on SPE methods for preconcentration and separation of Hg often reported the useability of these methods for storage of Hg after extraction (Blanco et al., 2000, Margetínová et al., 2008, Wang et al., 2022, Yin et al., 2010). These methods require minimal training to use, pose minimal risk to the operator when used in the field, and were reported to produce representative data after at least 1 week of storage. The previous use of SPE methods in species separation of Hg indicated suitability for extracting and preserving Hg species from water samples, and thus make SPE ideal for further optimisation as an in-field sampling and preservation

method, particularly in challenging environments. A dithizone-functionalised C18 cartridge was identified as a stabilising approach for online-ICP-MS Hg speciation that can preserve Hg species from water samples (Wang et al., 2022, Yin et al., 2010).

5.3. Total Hg preservation

The second objective was met by assessing a dithizone-functionalised SPE cartridge for total Hg extraction from water samples and determining the ability of the cartridge for storage of Hg for a practical time period, treated as 4-weeks in this work. The development of a preservation method for total Hg in water samples was necessary to ensure representative data from environmental waters, especially from challenging environments where concentrations of Hg in waters have not previously been effectively assessed (Olgola et al., 2001, Ondayo et al., 2023b). Total Hg informs guideline limits for human and environmental Hg exposure, and so a method to ensure representative total Hg concentrations from water samples was necessary. The use of SPE methods for preservation of metals, such as Hg, As and V, from water samples has shown promise in the literature, but exploration for use as a sampling method for Hg has failed due to less practical storage times (Blanco et al., 2000) or use of expensive reagents (Leopold et al., 2010, Schlathauer et al., 2019, Zierhut et al., 2010). In challenging environments, such as ASGM sites, suitable laboratories for preservation and analysis may not be readily available and so samples may require transportation within country and international shipment. Thus, ensuring total Hg data are representative after 4-weeks of storage and ensuring the sampling methods is cost-effective is essential for application of the method in challenging environments.

To achieve the objective, the work conducted in Chapter 3 reports a 50 µg quantity of dithizone immobilised to a C18 cartridges (Bond Elut Jr – Agilent, UK) provided suitable extraction and preservation of total Hg from a synthetic river water sample. A synthetic matrix, representative of water sources at ASGM sites such as rivers, allowed for control over the major chemistry of the sample and thus could more accurately represent environmental waters to efficiently assess the performance of the functionalised cartridges. Hg is immobilised onto the functionalised phase, thus volatilisation is mitigated and the likelihood of interspecies conversion to less stable species reduced. Recoveries of total Hg using the cartridge were reported at 85 ± 10% (n=5) total Hg during 4-weeks of storage in both refrigerated (4°C, in the absence of light) and unrefrigerated (16°C, in the absence of light) conditions. The recoveries were similar to previous literature, but demonstrated stability of Hg over a greater time-period, of 4-weeks versus just 1-week, and so effective as a sampling method

with preservation capability. Essential factors for field methods, such as flow rate of loading sample to the functionalised phase and sorbent stability prior to use, were investigated, with no breakthrough of Hg, demonstrating the applicability as a field method. While flow rate is often investigated when developing a speciation, separation, and sample preparation method, stability of the sorbent is often overlooked as it is typically used immediately after functionalisation. This factor is key for sampling methods as the cartridges must be functionalised in a laboratory prior to use in the field, which may take weeks due to transportation time. The cartridges were stable for at least 2-weeks prior to use in the field and showed no breakthrough at a flow rate achievable by an operator pushing the sample through a syringe, a filter, and the cartridge, thus demonstrating the potential as a field method. This was validated by use in five ASGM sites in western Kenya, where water samples were collected and the total Hg determined to be between 0.02 and 0.38 μg L^{-1}. The sampling required minimal training and presenting little-to-no hazard to the operator. This is especially vital for environments such as ASGM sites, where there is inherent risk in conducting research.

Samples were collected from water sources throughout the day, with no aim to avoid any particular times or ASGM activities. Work involving Hg occurs throughout the day at ASGM sites, with an increase in Hg burning and alluvial activities later in the afternoon as more prepared ore is produced. Thus, Hg concentrations may fluctuate in the surrounding environment with changes to the types of work. Changes in Hg concentrations in rivers throughout the day with work and river-use has not been thoroughly assessed, and thus would be a good basis for future work to better understand human exposure from domestic activities and collected drinking water, and environmental health.

The ASGM sites used in this work have characteristics similar to other sub-Saharan ASGM sites, such as quantity and type of work conducted, socioeconomic backgrounds, and geochemical characteristics (Ondayo et al. 2023b). However, they may not be fully representative of global ASGM sites or sites of interest, which will differ in climate, scale, geology, socioeconomic status, and other factors that may affect Hg pollution to the environment. Assessment of the developed SPE cartridge in other environments may be necessary for widespread use of the methodology.

5.4. Hg species preservation

While total Hg concentrations are used for guideline limits of human and environmental Hg exposure (DHHS, 2022, WHO, 2022, EPA, 1995), assessment of

Hg species provides a more holistic understanding of the risk posed from Hg in waters. In order to determine Hg speciation, recommended procedures suggest collecting individual samples for each required species (US EPA 1996). Different species require different preservation methods, for example Hg^{2+} acidification with nitric or hydrochloric acid to stabilise the metal, whereas MeHg requires acidification with sulphuric acid due to the analysis methods required (Yu and Yan, 2003). This greatly increased the volume of sample required and necessary storage space, as well as the difficulty in transportation and overall analysis time. This limited the ability to monitor harmful Hg species that pose a greater risk, such as MeHg.

By assessing the SPE sampling technique, previously developed in Chapter 3, for Hg species, these issues can be greatly reduced as the cartridges are more portable than previously required >500 mL sample volumes, and thus more manageable for transportation and storage. By using chromatographic techniques for analysis, such as HPLC-ICP-MS, species can be analysed simultaneously and thus reduce overall analysis time. This was used to meet objective 3, to further optimise the method for sampling and preservation of Hg speciation in water samples.

The developed SPE method was assessed for performance using a synthetic river water matrix spiked with both Hg^{2+} and MeHg, to determine the potential recovery of both species from waters similar to those found in ASGM sites. The cartridges demonstrated recovery of 115 ± 8% (4°C, absence of light) and 109 ± 13% % (16°C, absence of light) MeHg and 100 ± 14% % (4°C, absence of light) and 94 ± 12% % (16°C, absence of light) Hg^{2+} over 4 weeks of storage. These recoveries and errors are similar to previously reported recoveries in the literature (Blanco et al., 2000, Wang et al., 2022, Yin et al., 2010). Overestimation of the MeHg recovery may be a result of the organic nature of the species, but recoveries are still within the range of previous work (Chen et al., 2005). As with the total Hg analysis, the synthetic river water provided a matrix that resembled the chemistry of environmental waters from ASGM sites, which are a key target environment for this work.

5.5. A case study of ASGM sites

The performance of the developed SPE speciation sampling method was validated by use in the field at two ASGM sites in western Kenya, which was reported in Chapter 4 forming part of the objective 4. The useability of the cartridges at these sites demonstrated the ability to quickly and safely collect Hg speciation samples at ASGM sites. These sites represent some of the most vulnerable communities in the world and areas of the highest anthropogenic Hg pollution (UNEP, 2008, UNEP, 2018,

Ondayo et al., 2023a, Ondayo et al., 2023b), while being some of the most challenging areas to study for Hg pollution to waters. Thus, the need for a robust sampling and preservation method for Hg in waters is vital to ensure exposure to environmental and human health is minimised. The rivers and water sources around these sites are fully integrated in the everyday lives of the local communities, with uses as drinking water, domestic use, for irrigation, agriculture, and aquaculture (Grynberg et al., 2022, Ondayo et al., 2023b). Local communities are often concerned of the impact on the environment from ASGM activities, but are unable to determine the effects with limited data and resources available. In addition to assessing the performance of the SPE sampling method, environmental samples were collected from rivers flowing through ASGM sites.

In waters, no MeHg was detected above the detection limit of 0.007 µg L^{-1} MeHg while Hg^{2+} ranged from 0.06 – 0.67 µg L^{-1} Hg^{2+}. This shows the risk posed to human and environmental health minimal from MeHg exposure. However, the potential harm from Hg^{2+} exposure should not be ignored, as chronic exposure can result in significant negative health effects and thus should be carefully monitored. A drinking water pump installed by the local community showed Hg concentrations elevated above the river waters, resulting in approximately 45% of the tolerable weekly intake of Hg which demonstrates a real risk to the workers and communities. This also indicates potential contamination of the groundwater from the uphill mining activities which should be further investigated. This is supported by sediment Hg concentrations, as areas of elevated Hg^{2+} in water showed elevated total Hg in sediment samples. Sediment Hg concentrations ranged from <0.05 – 1.84 mg kg^{-1} total Hg and <0.1 – 36.8 µg kg^{-1} MeHg, implying notable bacterial activity in stagnant sections of the water bodies or those with limited water flow. where conversion of sediment-bound Hg to the methylated species can occur.

In these mine sites, routine monitoring of Hg is not currently undertaken. This is predominantly due to the difficulties surrounding sampling of waters for Hg and subsequent analysis (Ondayo et al., 2023b). Discussions with the workers and leaders of the mines revealed an interest from both regulatory authorities and the local population to understand the impact of the mining activities and Hg pollution to the environment. The cost of implementing Hg-free alternative methods, such as cyanidation and thiosulphate leaching, is not feasible and often poses other toxicological hazards, such as the release of cyanide (Manzilla, 2022). As the developed sampling methodology requires minimal training, the local officials/extension officers with minimal training would be able to assist with sample

collection which may improve representativeness of samples for human exposure studies. For example, samples could be collected when drinking or domestic water is collected, or after use of any treatment methods. Currently, subsequent analysis of the eluted Hg does not incorporate any preconcentration of the sample, however future work aims to address this to ensure the method is viable for a wide variety of analytical techniques and laboratory capabilities – lower concentration measurements will be possible with analytical instrumentation less sophisticated than ICP-MS and will better inform hazard-toxicological assessments.

5.6. Summary and impact

The outcome of this thesis demonstrates the unique ability of SPE methods to preserve Hg and its species from the field to analysis in some of the most challenging environments to collect samples. This has been an issue plaguing Hg pollution and health studies for decades, and the importance of a suitable, robust method for determining representative data cannot be understated. This can improve monitoring of Hg and its species, thus better informing and empowering policy makers and affected communities for public and environmental health outcomes. Previously, these parties have relied on data from solid matrices such as sediments or fish tissues as an indicator of Hg pollution, while neglecting the direct pollution to water due to difficulty in sampling and preservation. Analysis of waters provides a “snapshot” of concentrations at the time of sampling and so can be applied repeatedly to assess polluting inputs to rivers over short-term timescales. By assessing both the long-term pollution using solid matrices and the short-term, direct pollution using water samples in tandem will provide a more holistic approach to Hg pollution assessment, and better describe the human and environmental risks associated with Hg-containing activities, such as ASGM.

While there is still substantiative work that can be undertaken to further develop the sampling method, this thesis has drawn some important conclusions:

1) Solid-Phase Extraction methods demonstrate significant potential as in-field sampling methods for both total Hg and its species, with relatively cost-effective equipment, minimal training requirements, and virtually no risk to the operator;
2) A dithizone-functionalised cartridge can successfully extract and preserve Hg species for at least 4 weeks, allowing representative total Hg data to be collected from waters in challenging environments;

3) The same cartridge can be used for the collection of Hg speciation data for the most environmentally and human health relevant species, Hg^{2+}, due to it being the most abundant species, and MeHg, due to the high bioaccessibility and significant heath risks associated with organomercurial compounds;
4) Both total Hg and species data can be successfully determined in areas with some of the most vulnerable populations in the world, such as ASGM communities, to better inform regulatory authorities, who currently do not have adequate data to effectively regulate Hg use and pollution to mitigate health and environmental risks. This will also help advise ASGM communities about the impacts of the use of Hg, to empower the development and transition to Hg-free alternatives. The results collected represent the first use of an SPE as a sampling method for Hg species in ASGM sites in Kenya.

5.7. Future developments

While the developed Hg sampling technique provides a novel, robust method for collecting representative data, further investigation and optimisation can make the method more attractive for widespread deployment in pollution and health studies.

1) The assessment of ASGM sites in western Kenya demonstrate the usefulness of the developed method and potential for monitoring studies in challenging environments globally. Further studies at other Hg-affected environments will prove the applicability of the method in differing environments and conditions; ASGM sites in western Kenya are representative of sub-Saharan ASGM sites, but may not fully represent South American and Asian ASGM sites due to differences in climate, socioeconomic status, geography, geology, hydrogeochemistry, etc. The application across these environments can demonstrate the full effectiveness and limitations of the sampling method, while also informing local communities and policy makers of the risk posed by ongoing Hg use.

2) Additionally, preconcentration of Hg may be necessary in some environments and remote locations, to achieve detectable quantities of the metal and its species. This may be achievable through modification of the elution process, which currently employs a 1% 2-mercaptoethanol solution to preferentially bond to, and thereby remobilise, the extracted Hg. Modifications to this step may include changing the concentration of 2-mercaptoethanol to reduce the volume required for elution, using solvents such as methanol or alkaline solutions to remobilise and elute the dithizone-Hg complex, or other chelating compounds such as sodium diethyldithiocarbamate. Investigation into alternative eluents may provide both a preconcentration of the

extracted Hg species and a “greener” waste for a more environmentally friendly method. This may also be developed for use on less sensitive analytical methods. Techniques such as Flame Atomic Absorption Spectroscopy, which are more readily available in lower income countries, may not be suited to eluents with organic concentrations, and therefore the optimisation of an alternative eluent may prove beneficial to improve scientific capacity in such laboratories and promote local monitoring of Hg and its species.

3) Previous work with S-functionalised columns for SPE techniques have shown promise for the extraction of other toxic elements, including Pb and Cd, which are also considered some of the most important elements to monitor in the environment (Chen et al., 2021, Song et al., 2021). Both Pb and Cd exhibit similar preferential binding to thiol-containing molecules and thus may warrant further investigation into the usefulness of the developed method for multi-element sampling and preservation. This will diversify the usability of the cartridge and to improve cost-effectiveness of the sampling method for toxic element, pollution, and health studies. Thus, the developed method will be more attractive for widespread application in pollution monitoring studies, including in the most vulnerable areas with the greatest need for human and environmental health assessment.

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Appendices

Appendix 1

Figure A1.1 Gold mine shafts, active (left) and dis-used (right) in Rosterman, Kenya

Figure A1.2. Gold ore, mined at Rosterman, Kenya

Figure A1.3. Gold ore being sifted manually from mined rock, at Lunyerere, Kenya

Figure A1.4. An ore crusher in operation, typically used at ASGM sites for crushing the mined ore to a fine powder. Taken at Rosterman, Kenya

Figure A1.5. Sluicing of the crushed ore using cloth and water, often along riverbanks (left, at Rosterman, Kenya) or in purpose built ponds (right, at Lunyerere, Kenya)

Figure A1.6. ASGM workers sluicing crushed ore into concrete ore washing ponds, in Bushiangala, Kenya

Figure A1.7. An ore washing pond after a few weeks drying for disposal of waste tailings, at Rosterman, Kenya

Figure A1.8 Hg is manually mixed with the gold-containing fine particles, to form an amalgam

Figure A1.9. Simplistic huts are set up for both amalgam burning to obtain sponge-gold and for the sale of the produced gold

Figure A1.10. Amalgam burning is conducted with little-to-no safety, directly exposing workers to Hg via inhalation and releasing the vapours to the envrironment. This stage is often conducted by women and children

Figure A1.11. ARABIC 22 Sponge-gold produced by burning the Hg-Au amalgam. This is sold for further processing to purer gold products

Figure A1.12. An example of food crops grown nearby ASGM activities, often withing 5-10m from activities, or nearby polluted water sources

Figure A1.13. The author using the developed SPE method for sampling river water at Malinya mine, Kenya.

Figure A1.14. Hg handled by ASGM workers for alluvial mining, conducted with bare hands and in rivers relied upon by many villages and communities for domestic, industrial, agricultural and aquacultural activities

Appendix 2

Supplementary information

Supplementary Table A2.1 Reagent-based preservation methods for Hg, reported storage times, analytical merit and challenges

Reagent	Mercury species	Storage time	Sample volume reported	Analytical merit and challenges	References
Acidification				● Recommended for many metals and trace elements ● In an uncontrolled environment, there is a risk of acid burns and skin irritation from spills ● A pH <4 promotes stable chloro-mercury complexes in the presence of chloride ions	
Hydrochloric acid (0.4-1 %)	Hg^{2+} in freshwater	6-12 months (>90 % recovery)	0.5-1 L in polytetrafluoroethylene (PTFE) or glass containers	● >90 % recovery over long term storage (12 months) ● Recommended container materials are either hazardous (glass, risk of cuts from breakage) or expensive (PTFE, £15-30 per bottle)	(USEPA, 1996, USEPA, 2002, Parker and Bloom, 2005, Louie et al., 2012)
Nitric (1 %) and hydrochloric acid (0.005 %)	Hg^{2+} in deionised water	53 days (>90 % recovery)	500 mL in high-density polyethylene (HDPE) containers	● Recoveries of >90 % Hg^{2+} can be obtained with small quantities of acid ● A nitric and hydrochloric acid mixture is a common matrix for ICP-MS analysis, which is sensitive enough for Hg analysis	(USEPA, 1996, USEPA, 2002, Louie et al., 2012)
Sulphuric acid (0.2 %)	MeHg in seawater	6 months	Not specified as a series of different experiments – PE container material	● Mitigates formation of artificial MeHg during speciation by distillation and ethylation ● This speciation method is less prevalent nowadays, due to the development of HPLC speciation	(Parker and Bloom, 2005)

Oxidising agent				• Oxidises species to inorganic Hg^{2+} (comparatively more stable than Hg^0) • Risk of oxidising atmospheric Hg^0 and artificially increasing Hg concentration (particularly for trace Hg concentrations of relatively high atmospheric Hg^0 concentrations) • Oxidising chemicals are often harmful, risk of spillage and harm to the operator	
Potassium permanganate -persulphate digestive solution	Hg^{2+} in deionised water	560 days (negligible losses)	50 mL in polypropylene (PP) containers	• Negligible losses by adsorption for 0.2 – 1 µg L^{-1} Hg^{2+} • Improves cost-effectiveness by allowing use of polypropylene containers • Increased risk of spills and harm to the operator in an uncontrolled environment	(Zhang et al., 2020)
Potassium dichromate solution (0.05 % w/v acidified)	Hg^{2+} in deionised water	21 days (>95 % recovery)	100 mL in borosilicate glass and PE containers	• Effectively preserves Hg for up to 21 days, further investigation has not been conducted • As a strong oxidising agent, there is a risk to the operator, especially in an uncontrolled environment	(Lo and Wai, 1975, Feldman, 1974)
Increased ionic strength				• Mitigates wall sorption by reducing the number of active sites in container walls • Formation of $Hg(OH)_x^{2-x}$ can be limited with the introduction of different complexing agents	
Sodium nitrate (3 %)	Hg^{2+} in deionised water	90 days (95 % recovery in PP, >95 % recovery in glass)	50 mL in PP or glass containers	• An initial loss of 5 % Hg is seen over 7 days (likely stabilisation and losses to with container walls) • Below 3 % sodium nitrate, the recovery of Hg drops to <90 % in PP containers	(Zhang et al., 2020)

Sodium chloride (1-3 g L^{-1})	Hg^{2+} in deionised water	180 days (>95 % recovery)	50 mL in PP containers	• Chloro-mercury complexes are promoted, improving stability of the metal • The initial loss of Hg is decreased to <2 % over 7 days	(Zhang et al., 2020, Louie et al., 2012)
Sodium chloride (20 mg L^{-1}, acidified)	Hg^{2+} in deionised water	35 days (>98 % recovery)	500 mL in PP containers	• Low pH and increased chloride concentrations further promotes chloro-mercury complexes • Stability was only recorded up to 35 days, but further preservation is likely possible	(Louie et al., 2012)

Supplementary Table A2.2 Solid-phase extraction sorbents for Hg, reported recoveries, detection limts, analyical merits and difficulties

Sorbent/Solid-phase functionalization	Hg species	Matrix	Eluent/digestion	Detection limit	Recovery	Mesh/pore/particle size	Analytical merits and difficulties	References
Thiol functionalised resins								
Dithizone functionalised silica (for preconcentration)	Hg^{2+}, MeHg, EtHg	DI water, tap water (**filtered** waters)	100 mmol L^{-1} sodium thiosulphate or 1% 2-mercaptoethanol	3 ng L^{-1} by HPLC-ICP-MS	83.4 % Hg^{2+} 93.7 % MeHg 71.7 % EtHg	Storage capacity: not investigated, dependent on load of functional reagent, 20 µg L^{-1} Hg tested with a 12.5 µg dithizone load Pore size: 60 Å Particle size: 45 µm	+ Selective for Hg and its species + Extraction efficiency and elution were not affected after 7 days storage at 4 °C − Must be functionalised before use, so time between functionalisation and sampling needs to be accounted for − Optimised for pH 4 samples, higher and lower pH values may affect performance − High chloride concentration lowers Hg adsorption	(Yin et al., 2010, Wang et al., 2022)

2-mercaptophenol-Hg complex on C18 microcolumn (for preconcentration)	Hg^{2+}, MeHg, EtHg, PhHg	Sediments, zoobenthos, river water (**filtered** and acidified to pH3 with HCl)	Solid samples were microwave digested with HCl (3 mol L^{-1}), citric acid (0.2 mol L^{-1}), methanol (50%) (10 mL). Elution with methanol	Hg^{2+}=0.9 µg L^{-1}, MeHg=4.3 µg L^{-1}, EtHg=1.4 µg L^{-1}, PhHg=0.8 µg L^{-1} by AMA mercury analyser and HPLC/CV-AFS	>90 %	Storage capacity: up to 155 µg Hg (500 mL sample) with 20 mL 14 mmol L^{-1} complexing agent Pore size: 80 Å Particle size: 5 µm	+ + − −	No functionalisation of resin is required, instead a complexing agent (2-mercaptophenol) is added to the sample ~95 % extraction efficiency was reported with preconcentration factors of up to 1000 using 500 mL sample 2-mercaptoethanol has a strong, unpleasant odour and is harmful in high concentrations and must be diluted before use Any iron may compete with Hg for complexation with the 2-mercaptoethanol and interfere with extraction	(Margetínová et al., 2008)
Diethyldithiocarbamate functionalised C18 microcolumn (for preservation and storage)	Hg^{2+}, MeHg	River water (**filtered** waters)	5% (v/v) thiosulphate + 0.5% (v/v) HCl	Hg^{2+}=5.2 ng L^{-1}, MeHg=5.6 ng L^{-1} by LC-ICP-MS	97 %	Storage capacity not investigated, but 30 ng Hg successfully recovered Unspecified pore and particle sizes.	+ + − −	The sorbent operates under a wide range of pH values, so suitable for a variety of matrices Spiked river waters showed >80 % Hg^{2+} recovery reported for up to 30 days storage at 4°C in the dark (80 % MeHg recovery up to 7 days storage) Must be functionalised before use, so time between functionalisation and sampling must be accounted for MeHg recovery significantly decreases after 7 days to 50 % recovery at 14 days	(Blanco et al., 2000)

Commercially available resins									
Duolite GT-73™ as a diffusive gradient thin film (for preconcentration and removal)	Hg^{2+}	DI water, tap water, river water (**filtered** in the DGT apparatus)	Thermal decomposition (total Hg)	0.05 µg L^{-1} by LC-AFS	95 %	Storage capacity: 2.4 mg per DGT disk Particle size: sieved to <150 µm	+ +	The resin has a high sorption capacity and is selective for Hg Can be synthesised as a diffusive thin film, so can be immersed directly into the water source	(Pelcova et al., 2014)
							− −	The resin is no longer commercially available, but alternatives are available Desorption of Hg is difficult, so the resin must be digested using a nitric and hydrochloric acid mixture, eliminating the possibility of re-use	
AmberSep GT-74™ as a diffusive gradient thin film (for preconcentration and removal)	Hg^{2+}, MeHg EtHg PhHg	DI water, tap water, river water (**filtered** in the DGT apparatus)	Thermal decomposition (total Hg), microwave extraction with 6 mol L^{-1} HCl	Hg^{2+}=13 ng L^{-1}, MeHg=38 ng L^{-1}, EtHg=34 ng L^{-1}, PhHg=30 ng L^{-1} by Advanced Mercury Analyser (AMA245) for absorption spectroscopy	>95 %	Storage capacity: 3.8 mg per DGT disk Particle size: sieved to <150 µm	+ +	The resin has a high sorption capacity and is selective for Hg Can be synthesised as a diffusive thin film, so can be immersed directly into the water source	(Pelcova et al., 2014, Pelcova et al., 2015)
							− −	The resin is no longer commercially available, but alternatives are available The resin is reluctant to release Hg, so other analysis methods are required such as thermal desorption and atomic absorption	
Cationic exchange resins									

Dowex 50W X4™ as a microcolumn (for preconcentration and removal)	Hg^{2+}	Mineral, spring and tap-water	0.1% thiourea + 8% HCl	27 ng L^{-1} by CV-AAS	>79 %	Storage capacity: Not investigated, but 860 µg Hg was totally adsorbed by 0.5 g sorbent Particle size: 150-300 µm	+ + − −	Recoveries of >79 % were reported for natural water samples spiked to 10 µg L^{-1} Hg^{2+} As a non-specific sorbent, multi-elemental analysis is possible Copper and iron significantly decrease the recovery of Hg by direct determination and selenium (IV) cause signal suppression in CV-AAS analysis A sequential elution is required to remove interfering ions	(Krata et al., 2003)
Polyaniline									
Polyaniline (PANi) as a microcolumn (for preconcentration and removal)	Hg^{2+}, MeHg	Lake water, groundwater, seawater, fish tissue	0.3% HCl (for MeHg) and 0.3% HCl + 0.02% thiourea (for Hg^{2+})	0.05 ng L^{-1} by CV-AAS	>96 %	Storage capacity: 100 mg Hg^{2+} g^{-1} resin, 2.5 mg MeHg g^{-1} resin Particle size: 100-150 µm	+ + − −	The resin has a high sorption capacity (100 mg g^{-1} Hg^{2+} and 2.5 mg g^{-1} MeHg) A 100 mL sample can be used to achieve preconcentration factors of 120 for Hg^{2+} and 60 for MeHg from lake water, ground water and seawater Lower pH values (<2) significantly affect the Hg removal ratio to <20 %, higher pH values (>6) also decrease the Hg removal ratio (<80 %) A pH of 4-5 is preferred to ensure nitrogen atoms are not fully protonated and can bind to Hg from solution	(Balarama Krishna et al., 2005)

							−	The sorbent must be synthesised and homogenised before use	
Polyaniline-polyvinyl alcohol (PANi-PVA) as batch sorbent applied to solution (for removal)	Hg^{2+}	DI water	0.1 mol L^{-1} HNO_3	FAAS. No detection limit was reported	90 % Hg removal after 30 mins contact time	Storage capacity: 7.5 mg Hg g^{-1} resin No specification for particle or pore size	+	The sorbent has a high sorption capacity (>90 % removal from a 35 mg L^{-1} Hg^{2+} solution at pH 6)	(Vafaei Molamahmood et al., 2018)
							+	Desorption of Hg was reported with 0.1 M HNO_3, although this required 90 minutes to achieve 90 % recovery	
							−	Chloride at 30 mmol L^{-1} significantly decreases adsorption of Hg to <15 %, likely due to chloro-mercury complexation	
							−	The sorbent must be synthesised and homogenised before use	
Polyaniline-humic acid (PANi-HA) as batch sorbent applied to solution (for removal)	Hg^{2+}	DI water	No desorption investigated	AFS of supernatant. No detection limit was reported	95 %	Storage capacity: 671 mg Hg g^{-1} resin Particle size: 50-60 nm	+	The addition of humic acid widens the operable pH range to between 4 – 7, with Hg removal at >90 %.	(Zhang et al., 2010)
							+	The additional humic acid stabilises PANi by mitigating aggregation of the sorbent	
							+	The sorption capacity is high (671 mg g^{-1} Hg^{2+}) and most other ions in samples do not significantly affect Hg removal	

								−	Chloride concentrations of 50 mg L^{-1} significantly decrease removal of Hg from samples to <20 %
								−	Desorption from the composite has not been explored, nor stability of retained Hg species over time
								−	Humic acid is a broad term to define many naturally occurring organic molecules, so extraction efficiency may vary between batches of synthesised sorbent
Polyaniline-polystyrene (PANi-PE) as batch sorbent applied to solution (for removal)	Hg^{2+}	DI water	DI water, no desorption noted	β-activity by an end window Geiger-Müller counter. No detection limit was reported	67 %	Storage capacity: 0.15 mg Hg g^{-1} resin No particle size specified	+	The stability of the sorbent is improved, as polyethylene prevents aggregation of PANi	(Gupta et al., 2004)
							+	The composite was reported to adsorb 79 % Hg from a 10 ng L^{-1} Hg^{2+} solution	
							−	Mercury is not readily desorbed from the composite. 47 % was desorbed with 0.1 M HNO_3 after 30 minutes	
							−	Interfering ions and characteristics have not been explored	

Magnetic SPE

1,2-ethanedithiol as batch sorbent applied to solution (for preconcentration)	Hg^{2+}	DI water, lake water (**filtered** waters)	0.1 mol L^{-1} HNO_3 + 2% (w/v) thiourea	0.82 ng L^{-1} by ICP-MS	>90 %	Storage capacity: 254 mg Hg g^{-1} resin Particle size: 400 nm	+	>95 % adsorption of Hg over pH 2 – 8 and >95 % recover using a 4 % thiourea, 0.5 M HNO_3 eluent solution	(Chen et al., 2021)
							+	Common cations and anions do not significantly impact the adsorption or recovery of Hg at 5000 mg L^{-1} Ca^{2+}, Mg^{2+}, Cl^-, SO_4^{2-}, Na^+, NO_3^+, 10 mg L^{-1} Al^{3+}, Zn^{2+}, $Fe^{2+ \text{ or } 3+}$, and 1 mg L^{-1} Cu^{2+}	
							+	The sorbent has a high sorption capacity (254 mg g^{-1} Hg) and can extract lead and cadmium, so is potentially suitable for multi-elemental analysis	
							+	Recoveries of >93 % were reported for river water and lake waters spiked with 50 – 1000 ng L^{-1} Hg^{2+}	
							−	The multi-step synthesis has a yield of <50 % and may not be readily scaled up	
							−	The stability of the sorbent and of Hg retained on the sorbent has not been investigated	
							−	Speciation analysis has not been investigated for this sorbent, so the usefulness for toxicity studies and monitoring is not fully defined	

3-mercaptopropyl-trimethoxysilane as batch sorbent applied to solution (for removal)	Hg^{2+}, MeHg	DI water, lake water, tap water, sea water (**unfiltered** waters)	0.5 mol L^{-1} HCl + 1% thiourea	0.1, 0.3 ng L^{-1} by HPLC-ICP-MS	>90 %	Storage capacity: Not investigated, focused on trace Hg (10 ng L^{-1} Hg, 500 mL sample) No particle size specified	+	A >90 % recovery of Hg^{2+} and MeHg using 500 mL of a 20 ng L^{-1} Hg spiked tap, sea and lake waters	(Zhang et al., 2016)
							+	The sorbent can be freely added to the sample and removed with a magnet after 10 minutes stabilisation time	
							+	The sorbent can be reused up to 5 times without significant degradation of performance	
							–	The sorbent requires a multi-step synthesis and characterisation, which may not be readily scaled up	
							–	Common ions and interferences were not investigated	
1,5-diphenylcarbazide as batch sorbent applied to solution (for preconcentration)	Hg^{2+}	DI water, river water, tap water, (oxidised with H_2O_2, acidified to pH1 with HCl, **filtered**) vegetation (microwave digested with HNO3)	Regeneration with 0.5 mol L^{-1} HNO_3 (No elution assessed)	0.16 µg L^{-1} by CV-AAS	>95 %	Storage capacity: 44 mg Hg g^{-1} resin Particle size: 60-80 nm	+	Common ions in water (Ca^{2+}, Mg^{2+}, Cl^-) have no significant effect on Hg adsorption up to 4 mg L^{-1} interferent	(Zhai et al., 2010)
							+	Other metals (Cu^{2+}, Zn^{2+}, Cd^{2+}) have no significant effect on Hg adsorption up to 50 µg L^{-1} interferent	
							+	The sorbent can be reused up to 8 times without noticeable degradation in performance	
							+	The sorbent is stable up to 6 months	
							–	The sorbent is sensitive to pH, with adsorption of Hg decreasing from 95 % at	

								pH >6 to <40 % at pH <5 (active sites become protonated at lower pH values) − Speciation and MeHg sorption were not investigated	
Task-specific monolith with vinylboronic anhydride pyridine complex as a microcolumn (for preconcentration)	Hg^{2+}, MeHg, EtHg, PhHg	DI water, seawater, river water, lake water (**filtered** and acidified to pH4 with HCl)	Methanol	102, 22, 28, 162 ng L^{-1} by HPLC-diode array detector	>80 %	Storage capacity: Not investigated, 100 ng Hg per prepared column fully sorbed Pore size: 230 nm	+	>94 % of Hg^{2+}, MeHg, ethylmercury and phenylmercury can be recovered under optimised conditions + Recovery of Hg from river, sea, lake and tap water is >80 % Hg − A complexing agent is required to promote Hg extraction, which may be a source of contamination and is not readily useable in an uncontrolled environment − A pH of 4 is necessary, as lower pH values promote H^+-complexing agent formation and higher pH values may hydrolyse Hg species	(Song et al., 2021a)
Gold-based SPE									

Gold nanoparticles as a microcolumn (AuNP) (for preconcentration)	Total Hg	DI water, river water (**filtered** and acidified with 0.5% v/v HCl)	Thermal desorption	180 pg L^{-1} by AFS	>90 %	Storage capacity: Not investigated, 0.007 ng Hg fully recovered from 1 g sorbent Particle size: 100-350 µm Pore size: 60 Å	+ + + + + + −	Highly selective for Hg due to Au-Hg amalgamation A >99 % Hg recovery can be achieved from river waters No additional reagents are required for extraction of Hg Extracted Hg is stable for at least 2 days with no significant losses Speciation analysis is possible, as Hg^{2+} thermally desorbs at approximately 550 °C and MeHg thermally desorbs at approximately 20 °C (columns should be stored at 4 °C in the dark to mitigate loss of MeHg) The column can be reused 180 times without loss of precision or sensitivity Synthesis of column should be done in Hg vapour-free conditions and reagents require purging before use	(Leopold et al., 2009a)
Gold nano-sheets as a column (for preconcentration)	Hg^{2+}, MeHg	DI water, seawater, river water, lake water (**filtered** and acidified with 0.5% v/v HCl)	Thermal desorption	0.08 ng L^{-1} by AFS	>96 %	Storage capacity: Not investigated, work focused on trace Hg, 0.002 ng Hg adsorbed per column "Mesh size": 1024 meshes cm−2	+ +	The columns are highly selective for Hg with a high tolerance for interfering matrix components >95 % recovery Hg can be achieved from river, sea and lake waters at trace concentrations (0-3 ng L^{-1} Hg^{2+})	(Zierhut et al., 2010)

							−	The columns were developed for online preconcentration, so may not be suitable for offline extraction and storage	
							−	Reusability of the column was not fully explored	
Immobilised gold nanoparticle dipstick (for preservation and storage)	Total Hg	DI water, river water, seawater (**unfiltered** water, acidified with 0.5% v/v HCl)	Thermal desorption	0.06 ng L^{-1} by AFS	>94 %	Storage capacity: Not investigated, work focused on trace Hg, 0.0015 ng Hg per dipstick Particle size: 38 nm (14 µg Au cm^{-2})	+	The dipstick is highly selective for Hg with little interferences	(Schlathauer et al., 2019)
							+	Hg is thermally desorbed form the stick, so limits the opportunity for contamination	
							+	Low variation was reported between individually prepared dipsticks	
							+	The dipsticks can be reused 145 times without significant degradation to Hg recovery	
							−	Specialised sputtering technology is required to synthesise the dipsticks, so synthesis may not be readily scaled up	
							−	The dipsticks must be annealed at 600 °C before each use, which may be challenging in the field and so must be stored in Hg-free conditions during transport to and from the field	

Appendix 3

Supplementary information

Synthetic matrix recipe

A synthetic matrix was used throughout developmental experiments, to take into consideration the ionic characteristics of natural water samples. This matrix was created following Smith et al. (2002), and ionic concentrations are shown in Supplementary Table A3.1. alongside major ion chemistry from experimental data at ASGM sites in Kenya (Ondayo et al 2023).

Supplementary Table A3.1.: Comparison of major ion chemistry in a synthetic hardwater recipe (Smith et al (2002)), and measured concentrations from ASGM sites in Kakamega gold belt, Kenya (Ondayo et al (2023))

Ion	Synthetic matrix (mg/L) (Smith *et al.* (2002))[33]	Natural water (mg/L) (Ondayo *et al.* (2023))[11]
Sodium	5.748	7.72
Magnesium	1.458	6.64
Calcium	10.621	12.5
Potassium	0.977	1.87
Chloride	9.926	2.98
Carbonate	23.491	73.5
Sulphate	11.046	9.39

Supplementary Table A3.2.: ICP-MS parameters and conditions used in experimental analysis

Parameter	Value
ICP-MS	Agilent 8900
Nebulizer	MicroMist 100µm
Isotope	^{201}Hg, ^{202}Hg
RF Power	1550W
Replicates	3
Plasma gas flow rate	15 L min^{-1}
Auxiliary gas flow rate	0.9 L min^{-1}
Nebulizer gas flow rate	1.00 L min^{-1}
Makeup Gas	0.2 L min^{-1}
Dwell time	100 ms

Supplementary Table A3.3.: Measurements of a blank solution applied to the developed method to calculate a method detection limit (3σ)

Number (n=10)	**Measurement (µg L^{-1} Hg)**
1	-0.001
2	0.004
3	-0.003
4	0.002
5	0.001
6	0.002
7	0.000
8	0.004
9	0.005
10	0.006
LoD (3σ)	0.008

Supplementary Table A3.4.: Water geochemistry of samples from ASGM sites across Kakamega gold belt (Bushiangala, Lunyerere, Malinya, Rosterman, and Viyalo mine sites)

Sample	Location	Field pH	Field Conductivity µS cm^{-1}	Conductivity µS cm^{-1}	pH	Hg µg L^{-1}	Ca mg l^{-1}	Mg mg l^{-1}	Na mg l^{-1}	K mg l^{-1}	Total Alk mg l^{-1} HCO_3^-	Cl^- mg l^{-1}	SO_4^{2-} mg l^{-1}	NO_3^- mg l^{-1}
M1	Lunyerere	7.50	370	434	8.01	0.179	28.9	5.79	37.5	5.01	115	11.2	44.0	70.8
M2	Malinya	6.00	160	207	7.77	0.255	17.0	9.09	8.4	2.12	112	5.11	2.51	11.4
M3	Bushiangala	6.20	60	68.7	7.08	0.348	5.2	2.18	2.4	1.70	38.8	1.59	0.786	4.52
M4	Bushiangala	6.20	30	79.2	7.29	0.041	7.4	2.94	2.1	1.39	43.9	0.823	1.09	7.55
M5	Rosterman	6.60	220	280	8.12	0.045	27.4	13.1	7.5	1.17	164	6.97	6.25	4.04
R1	Lunyerere	6.30	80	101	7.35	0.166	7.5	2.90	5.0	1.74	36.2	4.22	2.56	17.7
R2	Lunyerere	6.20	30	101	7.06	0.148	7.8	2.91	5.1	1.68	36.0	4.22	2.85	18.1
R3	Malinya	6.60	110	143	7.72	0.023	10.9	6.20	8.0	1.30	77.6	5.28	1.08	7.44
R4	Bushiangala	5.80	50	70.9	7.08	0.084	7.2	2.12	2.2	0.80	39.7	1.12	1.33	5.51
R5	Rosterman	6.80	160	207	7.98	0.020	14.3	14.0	5.3	1.57	132	3.51	0.725	0.865
R6	Rosterman	6.90	150	211	7.96	0.032	15.4	13.1	5.3	1.72	134	3.60	0.796	0.951
R7	Rosterman	7.00	100	138	7.65	0.141	10.3	4.89	7.8	1.24	69.4	5.13	1.71	7.71
R8	Rosterman	7.00	110	154	7.77	0.028	11.3	6.64	7.4	1.35	83.0	4.78	1.56	6.34
S1	Lunyerere	5.40	70	95.0	6.75	0.132	7.0	2.90	4.4	1.79	28.4	4.64	2.34	19.7
S2	Viyalo	6.70	260	332	7.54	0.079	36.0	6.99	10.0	6.32	31.2	7.66	86.4	11.3
S3	Viyalo	5.40	70	94.3	7.66	0.043	5.4	3.84	4.2	1.55	31.4	3.22	2.12	22.6
S4	Bushiangala	5.50	60	85.9	7.27	0.036	9.5	2.63	2.3	0.69	49.8	0.835	1.07	5.89
S5	Rosterman	6.10	160	209	7.80	0.055	14.4	12.2	7.7	0.84	120	3.70	1.57	9.07
S6	Rosterman	6.60	150	203	7.97	0.010	14.0	13.5	5.1	1.54	129	3.52	0.717	0.861
S7	Rosterman	6.70	150	204	8.03	0.043	14.3	13.4	5.2	1.72	128	3.56	0.874	1.02
O1	Lunyerere	7.70	240	299	7.96	0.151	26.6	7.89	15.7	4.06	94.2	9.92	44.8	17.3
O2	Malinya	7.00	190	229	7.93	0.030	17.7	8.38	11.6	3.93	109	10.4	12.2	4.35

O3	Malinya	6.70	60	86.5	6.52	0.046	4.3	1.95	5.8	2.03	15.8	11.6	6.53	5.80
O4	Bushiangala	6.70	140	175	6.99	0.162	14.2	1.68	9.4	7.76	30.3	8.79	27.9	19.1
O5	Bushiangala	6.20	70	94.7	7.24	0.102	5.4	2.59	5.4	5.80	53.8	1.21	1.42	5.83
O6	Rosterman	6.70	270	349	7.93	0.277	29.4	10.4	15.9	8.88	119	19.6	45.3	1.29
O7	Rosterman	6.50	270	351	7.92	0.162	29.0	10.3	15.6	8.58	119	19.6	45.0	1.30
O8	Rosterman	6.60	180	232	7.87	0.032	23.6	6.91	6.7	4.84	113	7.47	14.2	2.94
O9	Rosterman	7.10	150	209	8.00	0.058	14.6	13.6	5.2	1.61	132	3.57	0.750	0.952

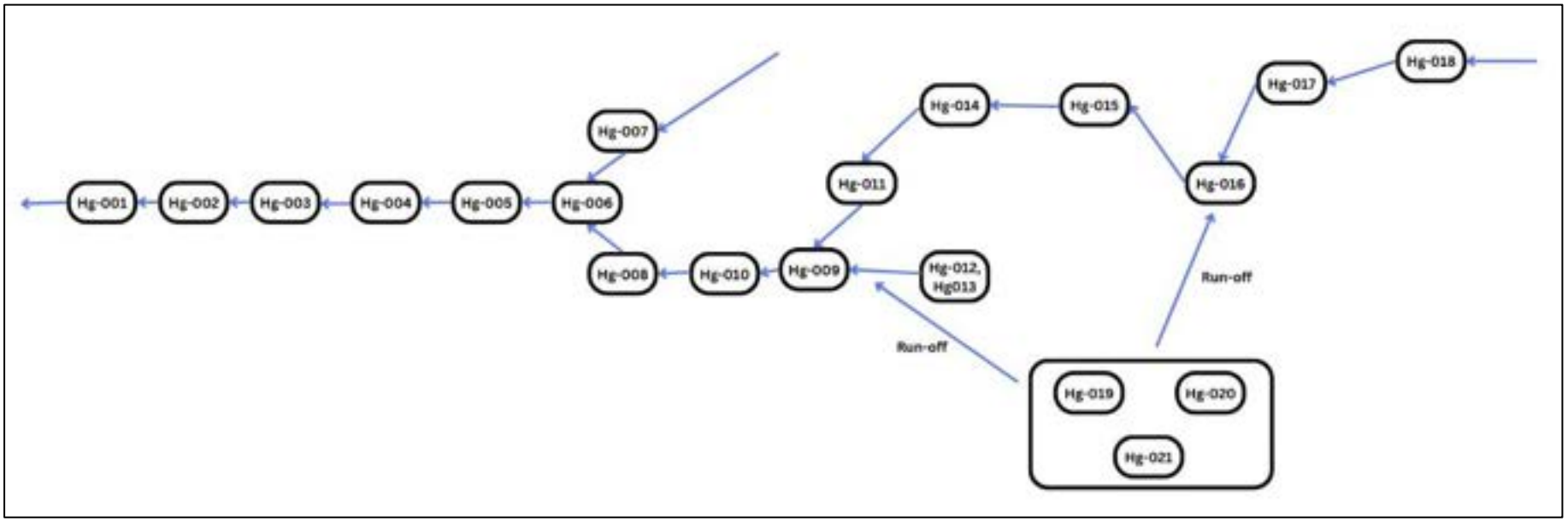
Appendix 4

Supplementary information

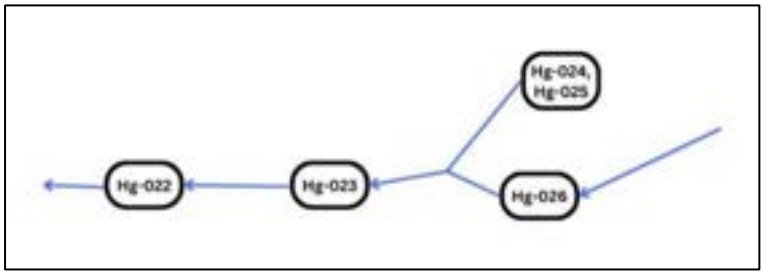
Supplementary Table A4.1.: HPLC-ICP-MS parameters and conditions used in experimental analysis

Parameter	Value
HPLC	
HPLC pump	Agilent Infinity 1290II
HPLC column	Poroshell aqueous C18 [dimensions]
Mobile phase	1% (v/v) 2-mercaptoethanol (in deionised water)
Flow rate	1.5 mL min^{-1}
Injection volume	100 µL
ICP-MS	
ICP-MS	Agilent 8900
Nebulizer	MicroMist 100µm
Isotope	^{201}Hg for water analysis, ^{199}Hg, ^{201}Hg, for sediment isotope dilution analysis
RF Power	1550W
Replicates	3
Plasma gas flow rate	15 L min^{-1}
Auxiliary gas flow rate	0.9 L min^{-1}
Nebulizer gas flow rate	1.00 L min^{-1}
Makeup Gas	0.2 L min^{-1}
Dwell time	100 ms

Supplementary Information



Supplementary Figure A4.1a: Flow chart of samples along river at Rosterman mine, Kenya, with arrows depicting the direction of river flow



Supplementary Figure A4.1b: Flow chart of samples along river at Malinya mine, with arrows depicting the direction of river flow

Supplementary Table A4.2.: limits of detection in HPLC-ICP-MS for MeHg and Hg^{2+} defined as three times standard deviation (3σ) of nine replicate blanks

Blank	MeHg199 µg/L	Hg2+ 199 µg/L
1	0.001	0.001
2	0.006	-0.003
3	0.002	0.002
4	0.004	0.000
5	0.000	0.003
6	0.000	0.013
7	0.005	0.000
8	0.000	0.027
9	0.002	0.011
Standard deviation	0.002	0.009
Detection limit. (3σ)	0.007	0.027

Synthetic matrix recipe

A synthetic matrix was used throughout developmental experiments, to take into consideration the ionic characteristics of natural water samples. This matrix was created following Smith et al. (2002), and ionic concentrations are shown in Supplementary Table A4.3 alongside major ion chemistry from experimental data at ASGM sites in Kenya (Ondayo et al 2023a).

Supplementary Table A4.3.: Comparison of major ion chemistry in a synthetic hardwater recipe (Smith et al. (2002)), and measured concentrations from ASGM sites in Kakamega gold belt, Kenya (Ondayo et al. (2023a) and King et al. (2024))

Ion	**Synthetic matrix (mg/L) (Smith *et al.* (2002))[33]**	**Natural water (mg/L) (Ondayo *et al.* (2023))[11]**
Sodium	5.75	7.72
Magnesium	1.46	6.64
Calcium	10.6	12.5
Potassium	0.98	1.87
Chloride	9.93	2.98
Carbonate	23.5	73.5
Sulphate	11.0	9.39

Supplementary Table A4.4: Total Hg data for certified reference materials HR-1 (NRCC, Canada), TH-2 (NRCC, Canada), and PACS-2 (NRCC, Canada), and Isotope dilution-microwave extraction (ID-ME) of MeHg from a certified reference material (ERM-CC58, ERM)

CRM	HR-1 (Total Hg) mg kg^{-1}	TH-2 (Total Hg) mg kg^{-1}	PACS-2 (Total Hg) mg kg^{-1}	ERM-CC58 (MeHg by ID-ME) µg kg^{-1}
n	6	5	3	6
Target value	0.261	0.542	2.62	75
	0.259	0.480	2.64	44
	0.271	0.499	2.48	60
	0.243	0.500	2.45	57
	0.281	0.451		70
	0.217	0.483		63
	0.236			68
Recovery (%)	89%	96%	96%	84%
Relative standard deviation (%)	4%	9%	4%	14%

Supplementary Table A4.5.: General chemistry of water samples from ASGM sites (Rosterman and Malinya sites)

Sample Code	Date Sampled	Field pH	Field Conductivity	Ca	Mg	Na	K	Cl^-	SO_4^{2-}	NO_3^-	Cat1	Cat2	Br^-	NO_2^-	HPO_4^{2-}	F^-	NPOC	Total P	Total S
			µS cm^{-1}	mg l^{-1}	mg l^{-1}	mg l^{-1}	mg l^{-1}	mg l^{-1}	mg l^{-1}	mg l^{-1}	meq l^{-1}	meq l^{-1}	mg l^{-1}	mg l^{-1}	mg l^{-1}	mg l^{-1}	mg l^{-1}	mg l^{-1}	mg l^{-1}
Hg_001	02/02/2023	7.8	110	12.4	7.18	8.6	1.53	5.10	2.11	6.18	1.62	0.005	0.025	0.019	<0.01	0.091	2.32	0.005	0.84
Hg_002	02/02/2023	7.6	110	12.2	7.36	8.3	1.53	4.91	2.13	6.38	1.61	0.004	0.025	0.021	<0.01	0.161	1.86	<0.005	0.82
Hg_003	02/02/2023	7.2	160	18.3	10.1	8.4	1.96	5.23	0.769	0.077	2.16	0.068	0.045	0.020	<0.01	0.349	3.49	<0.005	0.33
Hg_004	02/02/2023	7.6	120	12.1	7.28	8.2	1.48	4.79	1.98	6.26	1.60	0.004	0.024	0.020	<0.01	0.155	2.62	<0.005	0.80
Hg_005	02/02/2023	7.5	120	12.3	7.44	8.8	1.51	5.07	2.03	6.42	1.65	0.004	0.023	0.023	<0.01	0.144	2.24	<0.005	0.80
Hg_006	02/02/2023	7.5	110	10.8	5.98	8.6	1.38	5.45	2.04	7.86	1.44	0.003	0.023	0.026	<0.01	0.174	2.84	<0.005	0.74
Hg_007	02/02/2023	7.6	110	10.9	5.85	8.8	2.58	7.32	2.20	8.58	1.47	0.004	0.026	0.027	<0.01	0.161	2.58	<0.005	0.78
Hg_008	02/02/2023	7.7	170	18.6	14.6	7.0	2.28	4.22	2.31	0.931	2.49	0.005	0.043	0.018	<0.01	0.296	2.84	<0.005	0.85
Hg_009	02/02/2023	7.3	180	17.9	15.5	6.7	2.65	3.35	1.06	1.16	2.53	0.004	0.036	0.014	<0.01	0.280	2.76	<0.005	0.49
Hg_010	02/02/2023	7.6	180	18.3	14.7	6.8	2.21	3.82	1.98	0.862	2.48	0.005	0.037	0.045	<0.01	0.276	2.82	<0.005	0.78
Hg_011	02/02/2023	7.6	160	19.3	16.3	7.4	1.90	3.50	1.11	1.33	2.67	0.005	0.038	0.015	<0.01	0.279	3.09	<0.005	0.48
Hg_012	02/02/2023	7.2	180	14.0	9.30	7.0	9.71	5.74	5.00	0.129	2.02	0.008	0.036	0.061	<0.01	0.207	5.26	0.106	1.76
Hg_013	02/02/2023	6.9	210	15.6	12.7	8.7	3.10	5.91	1.53	6.53	2.28	0.003	0.025	0.396	<0.01	0.228	10.2	0.076	0.64
Hg_014	02/02/2023	7.6	180	17.9	14.9	6.8	1.54	3.34	1.29	1.34	2.46	0.004	0.037	0.015	<0.01	0.272	4.82	<0.005	0.56
Hg_015	02/02/2023	7.4	170	18.4	15.1	6.7	2.99	3.56	1.62	1.52	2.53	0.004	0.039	0.017	<0.01	0.294	2.84	<0.005	0.62
Hg_016	02/02/2023	7.5	170	18.2	15.2	6.6	1.51	3.37	1.26	1.47	2.48	0.004	0.036	0.014	<0.01	0.276	2.46	<0.005	0.52
Hg_017	02/02/2023	7.0	190	18.4	16.1	7.1	1.56	3.38	1.10	1.29	2.59	0.004	0.037	0.014	<0.01	0.249	2.69	<0.005	0.47
Hg_018	02/02/2023	7.6	180	19.4	16.0	6.9	1.67	3.39	1.21	1.15	2.63	0.005	0.040	0.011	<0.01	0.260	2.54	<0.005	0.51
Hg_019	02/02/2023	7.4	410	45.5	17.9	13.4	4.33	13.4	125	2.75	4.44	0.009	0.103	0.012	<0.01	0.164	8.93	<0.005	42.5
Hg_020	02/02/2023	7.3	300	30.4	13.4	8.4	1.09	9.54	7.83	5.85	3.01	0.006	0.051	0.074	<0.01	0.211	12.4	0.020	2.73
Hg_021	02/02/2023	8.0	1100	136	70.2	48.8	14.5	40.9	535	0.038	15.1	0.029	0.521	0.010	<0.01	0.709	16.8	0.019	194
Hg_022	03/02/2023	7.5	120	11.9	7.08	8.6	2.57	5.72	1.21	7.41	1.62	0.006	0.036	0.041	<0.01	0.154	5.34	<0.005	0.52
Hg_023	03/02/2023	7.0	130	8.6	6.32	11.1	1.38	5.16	2.70	0.051	1.47	0.013	0.050	0.011	<0.01	0.202	7.14	<0.005	1.05
Hg_024	03/02/2023	6.4	130	11.7	6.39	8.8	1.98	5.58	2.84	6.06	1.54	0.107	0.041	0.023	<0.01	0.043	10.3	0.005	1.09
Hg_025	03/02/2023	6.5	120	11.5	6.24	8.8	2.16	5.94	2.90	4.95	1.53	0.072	0.041	0.065	<0.01	0.049	7.30	<0.005	1.04
Hg_026	03/02/2023	7.5	180	12.2	7.17	8.4	2.24	6.34	0.968	7.69	1.62	0.009	0.036	0.017	<0.01	0.144	11.7	0.010	0.42

Sample Code	Si	SiO_2	Ba	Sr	Mn	Fe	Be	B	Al	Ti	V	Cr	Co	Ni	Cu	Zn	Ga	As
	mg l^{-1}	mg l^{-1}	µg l^{-1}	µg l^{-1}	µg l^{-1}	µg l^{-1}	µg l^{-1}	µg l^{-1}	µg l^{-1}	µg l^{-1}	µg l^{-1}	µg l^{-1}	µg l^{-1}	µg l^{-1}	µg l^{-1}	µg l^{-1}	µg l^{-1}	µg l^{-1}
Hg_001	18.8	39.0	48.4	112	97.4	37.4	<0.08	<53	17.1	0.45	1.26	0.40	0.318	1.39	0.85	192	<0.04	0.64
Hg_002	13.5	40.1	47.7	110	94.6	7.3	<0.08	<53	2.9	0.08	1.19	0.29	0.346	1.41	0.81	0.7	<0.04	0.61
Hg_003	18.3	28.9	194	177	5181	1147	<0.08	<53	0.8	<0.06	0.07	0.20	13.7	4.34	0.18	1.2	<0.04	2.23
Hg_004	18.4	39.2	47.9	109	109	7.5	<0.08	<53	8.4	0.07	1.23	0.31	0.381	1.39	0.72	0.6	<0.04	0.51
Hg_005	22.3	39.3	47.6	112	69.3	5.5	<0.08	<53	3.6	0.13	1.14	0.35	0.250	1.46	0.96	0.8	<0.04	0.55
Hg_006	19.2	47.7	42.6	102	49.0	8.5	<0.08	<53	4.3	0.10	1.00	0.37	0.193	1.24	0.81	1.1	<0.04	0.21
Hg_007	15.6	41.2	42.6	103	40.7	10.3	<0.08	<53	6.0	0.14	1.00	0.37	0.159	1.09	0.72	1.7	<0.04	0.14
Hg_008	15.6	33.4	67.4	145	137	16.6	<0.08	<53	8.1	0.32	1.70	0.41	0.403	2.69	1.02	0.7	<0.04	2.13
Hg_009	16.0	33.3	63.7	141	219	6.1	<0.08	<53	2.2	0.08	1.72	0.41	0.613	3.31	0.82	1.1	<0.04	1.34
Hg_010	15.8	34.3	65.8	142	175	15.6	<0.08	<53	5.4	0.28	1.68	0.39	0.505	2.98	1.27	1.8	<0.04	2.04
Hg_011	23.0	33.7	65.4	149	217	5.9	<0.08	<53	3.2	<0.06	1.44	0.46	0.659	3.70	1.11	1.7	<0.04	1.10
Hg_012	34.7	49.3	70.0	111	153	89.9	<0.08	<53	2.3	0.07	5.39	0.39	1.56	2.73	0.37	1.6	<0.04	0.76
Hg_013	15.8	74.2	35.3	123	11.8	2.9	<0.08	<53	0.7	<0.06	11.9	2.07	0.129	3.26	0.12	3.3	<0.04	0.86
Hg_014	15.7	33.8	60.9	140	235	4.4	<0.08	<53	2.1	<0.06	1.48	0.50	0.727	3.40	0.78	0.6	<0.04	1.08
Hg_015	15.5	33.6	61.4	144	260	4.9	<0.08	<53	1.5	<0.06	1.10	0.53	0.770	3.52	0.79	0.7	<0.04	0.80
Hg_016	14.9	33.1	56.3	141	237	6.5	<0.08	<53	1.8	0.07	0.92	0.56	0.632	3.39	0.72	0.8	<0.04	0.49
Hg_017	15.6	31.9	51.6	144	147	7.3	<0.08	<53	2.4	0.06	0.83	0.75	0.403	3.15	0.69	0.5	<0.04	0.32
Hg_018	20.0	33.4	51.8	148	260	7.8	<0.08	<53	2.8	<0.06	1.09	0.43	0.582	2.94	0.69	1.4	<0.04	0.37
Hg_019	27.8	42.7	96.2	307	2975	2.4	<0.08	<53	6.2	<0.06	1.62	0.09	11.0	17.0	0.39	1.6	<0.04	10.4
Hg_020	13.3	59.5	68.4	202	175	3.6	<0.08	<53	2.7	<0.06	5.29	1.54	1.28	2.77	1.61	9.4	<0.04	1.51
Hg_021	21.5	28.5	224	1129	689	0.7	<0.08	<53	8.8	<0.06	4.50	0.56	1.54	7.65	5.50	0.9	<0.04	11.2
Hg_022	12.4	46.1	96.4	125	427	32.7	<0.08	<53	7.7	0.10	2.95	0.19	1.31	1.57	0.44	1.9	<0.04	3.38
Hg_023	22.8	26.6	183	103	1005	144	<0.08	<53	5.8	0.28	0.25	0.22	3.75	10.6	0.68	3.8	<0.04	5.42
Hg_024	22.6	48.8	115	149	771	1900	<0.08	<53	1.6	0.07	0.40	0.87	6.06	17.6	0.23	6.7	<0.04	0.59
Hg_025	23.1	48.4	117	145	841	1251	<0.08	<53	1.1	<0.06	0.34	0.12	7.76	15.9	0.27	8.7	<0.04	1.98
Hg_026	49.4	66.0	129	169	104	<7	<53	18.0	0.54	2.72	0.40	0.713	1.45	0.44	10.9	<0.04	1.98	0.08

Sample Code	Se	Rb	Y	Zr	Nb	Mo	Ag	Cd	Sn	Sb	Cs	La	Ce	Pr	Nd	Sm	Gd
	µg l^{-1}	µg l^{-1}	µg l^{-1}	µg l^{-1}	µg l^{-1}	µg l^{-1}	µg l^{-1}	µg l^{-1}	µg l^{-1}	µg l^{-1}	µg l^{-1}	µg l^{-1}	µg l^{-1}	µg l^{-1}	µg l^{-1}	µg l^{-1}	µg l^{-1}
Hg_001	0.08	3.42	0.047	0.065	0.01	0.5	<0.04	0.006	<0.08	<0.04	<0.04	0.075	0.081	0.012	0.053	0.008	0.012
Hg_002	<0.07	3.38	0.034	0.033	<0.01	0.5	<0.04	<0.005	<0.08	<0.04	<0.04	0.346	0.054	0.009	0.047	0.005	<0.005
Hg_003	0.08	7.39	0.028	0.031	<0.01	1.6	<0.04	<0.005	<0.08	<0.04	<0.04	0.080	0.035	0.004	0.016	<0.005	<0.005
Hg_004	<0.07	3.25	0.028	0.032	<0.01	0.5	<0.04	<0.005	<0.08	<0.04	<0.04	0.068	0.045	0.006	0.030	<0.005	<0.005
Hg_005	0.08	3.52	0.028	0.064	<0.01	0.5	<0.04	<0.005	<0.08	<0.04	<0.04	0.059	0.033	0.005	0.024	<0.005	<0.005
Hg_006	0.08	3.06	0.025	0.057	<0.01	0.4	<0.04	<0.005	<0.08	<0.04	<0.04	0.224	0.034	0.006	0.027	<0.005	<0.005
Hg_007	<0.07	2.89	0.024	0.047	<0.01	0.4	<0.04	<0.005	<0.08	<0.04	<0.04	0.686	0.035	0.006	0.023	<0.005	<0.005
Hg_008	0.10	5.17	0.046	0.053	<0.01	0.9	<0.04	<0.005	<0.08	<0.04	<0.04	0.049	0.064	0.009	0.038	0.006	<0.005
Hg_009	0.08	5.17	0.057	0.039	<0.01	1.0	<0.04	<0.005	<0.08	<0.04	<0.04	0.068	0.095	0.009	0.045	0.005	0.007
Hg_010	0.11	5.26	0.051	0.051	<0.01	0.9	<0.04	<0.005	<0.08	<0.04	<0.04	0.084	0.072	0.010	0.043	0.007	0.006
Hg_011	0.08	5.72	0.067	0.046	<0.01	1.0	<0.04	<0.005	<0.08	<0.04	<0.04	0.113	0.090	0.012	0.061	0.009	0.008
Hg_012	0.30	12.2	0.034	0.009	<0.01	0.2	<0.04	<0.005	<0.08	<0.04	<0.04	0.060	0.029	0.010	0.034	0.006	0.006
Hg_013	<0.07	0.46	0.007	<0.009	<0.01	<0.2	<0.04	<0.005	<0.08	<0.04	<0.04	0.090	0.010	<0.003	0.007	<0.005	<0.005
Hg_014	0.08	5.28	0.046	0.036	<0.01	1.0	<0.04	<0.005	<0.08	<0.04	<0.04	0.042	0.056	0.007	0.035	0.008	0.005
Hg_015	0.08	5.22	0.063	0.036	<0.01	1.0	<0.04	<0.005	<0.08	<0.04	<0.04	0.073	0.064	0.010	0.042	<0.005	0.008
Hg_016	0.08	5.35	0.063	0.035	<0.01	1.0	<0.04	<0.005	<0.08	<0.04	<0.04	0.057	0.071	0.011	0.048	0.006	0.008
Hg_017	0.08	5.44	0.061	0.036	<0.01	1.0	<0.04	0.006	<0.08	<0.04	<0.04	0.058	0.060	0.011	0.033	0.008	0.009
Hg_018	0.09	5.81	0.045	0.039	<0.01	1.0	<0.04	<0.005	<0.08	<0.04	<0.04	0.041	0.037	0.006	0.038	0.006	0.008
Hg_019	0.14	3.01	0.083	<0.009	<0.01	0.8	<0.04	0.090	<0.08	0.49	<0.04	0.076	0.156	0.011	0.040	0.006	0.010
Hg_020	0.10	0.98	0.029	0.009	<0.01	0.4	<0.04	0.012	<0.08	0.06	0.04	0.033	0.046	0.005	0.019	<0.005	<0.005
Hg_021	0.66	1.57	0.044	0.029	<0.01	7.3	<0.04	0.011	<0.08	1.50	<0.04	0.096	0.033	0.006	0.031	<0.005	<0.005
Hg_022	0.09	3.27	0.044	0.043	<0.01	0.3	<0.04	<0.005	<0.08	<0.04	<0.04	0.077	0.133	0.013	0.054	0.012	0.008
Hg_023	<0.07	7.75	0.070	0.039	<0.01	0.2	<0.04	0.007	<0.08	<0.04	<0.04	0.222	0.222	0.023	0.108	0.018	0.016
Hg_024	0.17	3.10	0.098	0.016	<0.01	<0.2	<0.04	0.032	<0.08	<0.04	<0.04	0.724	0.210	0.028	0.109	0.014	0.014
Hg_025	0.15	3.41	0.088	0.016	<0.01	<0.2	<0.04	0.016	<0.08	<0.04	<0.04	0.304	0.225	0.024	0.085	0.017	0.011
Hg_026	2.11		0.042	0.080	0.01	0.3	<0.04	<0.005	<0.08	<0.04	<0.04	0.206	0.149	0.016	0.060	0.010	0.008

Sample Code	Dy	Ho	Er	Tm	Yb	Lu	Hf	Ta	W	Tl	Pb	Bi	Th	U
	µg l^{-1}	µg l^{-1}	µg l^{-1}	µg l^{-1}	µg l^{-1}	µg l^{-1}	µg l^{-1}	µg l^{-1}	µg l^{-1}	µg l^{-1}	µg l^{-1}	µg l^{-1}	µg l^{-1}	µg l^{-1}
Hg_001	**0.003**	**0.003**	**0.003**	**0.003**	**0.004**	**0.003**	**0.006**	**0.006**	**0.06**	**0.02**	**0.02**	**0.08**	**0.03**	**0.009**
Hg_002	0.007	<0.003	0.004	<0.003	0.005	<0.003	<0.006	<0.006	<0.06	<0.02	0.06	<0.08	<0.03	0.053
Hg_003	0.003	<0.003	0.003	<0.003	0.004	<0.003	<0.006	<0.006	<0.06	<0.02	<0.02	<0.08	<0.03	0.054
Hg_004	<0.003	<0.003	<0.003	<0.003	<0.004	<0.003	<0.006	<0.006	<0.06	<0.02	<0.02	<0.08	<0.03	0.063
Hg_005	0.005	<0.003	0.004	<0.003	<0.004	<0.003	<0.006	<0.006	<0.06	<0.02	<0.02	<0.08	<0.03	0.056
Hg_006	0.003	<0.003	<0.003	<0.003	<0.004	<0.003	<0.006	<0.006	<0.06	<0.02	<0.02	<0.08	<0.03	0.058
Hg_007	0.003	<0.003	<0.003	<0.003	<0.004	<0.003	<0.006	<0.006	<0.06	<0.02	0.02	<0.08	<0.03	0.034
Hg_008	0.003	<0.003	0.003	<0.003	<0.004	<0.003	<0.006	<0.006	<0.06	<0.02	0.02	<0.08	<0.03	0.030
Hg_009	0.005	<0.003	0.004	<0.003	0.005	<0.003	<0.006	<0.006	<0.06	<0.02	<0.02	<0.08	<0.03	0.184
Hg_010	0.006	<0.003	0.005	<0.003	0.004	<0.003	<0.006	<0.006	<0.06	<0.02	<0.02	<0.08	<0.03	0.198
Hg_011	0.006	<0.003	0.005	<0.003	<0.004	<0.003	<0.006	<0.006	<0.06	<0.02	0.03	<0.08	<0.03	0.178
Hg_012	0.005	<0.003	0.007	<0.003	0.006	<0.003	<0.006	<0.006	<0.06	0.02	<0.02	<0.08	<0.03	0.196
Hg_013	0.004	<0.003	0.003	<0.003	<0.004	<0.003	<0.006	<0.006	<0.06	<0.02	0.10	<0.08	<0.03	0.017
Hg_014	<0.003	<0.003	<0.003	<0.003	<0.004	<0.003	<0.006	<0.006	0.14	<0.02	0.21	<0.08	<0.03	0.029
Hg_015	0.004	<0.003	0.004	<0.003	<0.004	<0.003	<0.006	<0.006	<0.06	0.02	<0.02	<0.08	<0.03	0.191
Hg_016	0.005	<0.003	0.004	<0.003	<0.004	<0.003	<0.006	<0.006	<0.06	<0.02	<0.02	<0.08	<0.03	0.185
Hg_017	0.007	<0.003	0.005	<0.003	0.004	<0.003	<0.006	<0.006	<0.06	<0.02	<0.02	<0.08	<0.03	0.187
Hg_018	0.006	<0.003	0.004	<0.003	<0.004	<0.003	<0.006	<0.006	<0.06	<0.02	0.02	<0.08	<0.03	0.197
Hg_019	0.004	<0.003	0.005	<0.003	<0.004	<0.003	<0.006	<0.006	<0.06	<0.02	<0.02	<0.08	<0.03	0.205
Hg_020	0.007	<0.003	0.006	<0.003	0.004	<0.003	<0.006	<0.006	0.16	<0.02	0.09	<0.08	<0.03	0.123
Hg_021	<0.003	<0.003	<0.003	<0.003	<0.004	<0.003	<0.006	<0.006	0.55	<0.02	0.04	<0.08	<0.03	0.101
Hg_022	0.003	<0.003	<0.003	<0.003	<0.004	<0.003	<0.006	<0.006	0.41	0.02	0.04	<0.08	<0.03	2.17
Hg_023	0.005	<0.003	0.004	<0.003	<0.004	<0.003	<0.006	<0.006	<0.06	0.03	<0.02	<0.08	<0.03	0.090
Hg_024	0.010	<0.003	0.006	<0.003	0.006	<0.003	<0.006	<0.006	<0.06	0.05	0.05	<0.08	<0.03	0.019
Hg_025	0.007	<0.003	0.008	<0.003	0.006	<0.003	<0.006	<0.006	<0.06	0.02	0.05	<0.08	<0.03	<0.009
Hg_026	0.008	<0.003	0.007	<0.003	0.006	<0.003	<0.006	<0.006	<0.06	0.02	0.03	<0.08	<0.03	0.023

Supplementary Table A4.6.: General chemistry of sediment samples from ASGM sites (Rosterman and Malinya sites)

Sample Code	Hg mg kg^{-1}	Li mg kg^{-1}	Be mg kg^{-1}	Na mg kg^{-1}	Mg mg kg^{-1}	Al mg kg^{-1}	P mg kg^{-1}	K mg kg^{-1}	Ca mg kg^{-1}	Ti mg kg^{-1}	V mg kg^{-1}	Cr mg kg^{-1}	Mn mg kg^{-1}	Fe mg kg^{-1}	Co mg kg^{-1}	Ni mg kg^{-1}
Detection limit	**0.02**	**0.3**	**0.002**	**17**	**27**	**78**	**0.4**	**50**	**596**	**6**	**0.3**	**0.6**	**3**	**80**	**0.05**	**0.3**
Hg_001	0.09	11.9	2.28	4484	13218	57909	502	11929	11443	3768	178	1365	1685	77039	72.6	196
Hg_002	0.11	16.5	1.93	4309	10822	83643	530	12889	8824	4919	173	747	2145	77206	78.2	216
Hg_003	0.08	15.8	2.45	4287	10874	79255	522	12545	8953	4503	193	945	1930	85492	75.1	204
Hg_004	0.26	13.0	2.07	7350	13458	84549	611	13766	15352	5108	191	657	1723	79573	62.1	146
Hg_004 Duplicate	0.29	14.8	2.21	7209	14386	83436	552	12943	15754	5092	197	808	1809	81083	63.4	151
Hg_005	0.09	12.2	2.38	3870	13186	50140	483	10563	10687	2803	197	1710	2321	89425	71.0	203
Hg_006	0.11	14.0	1.83	3078	12429	57665	496	8991	8679	3585	202	1831	3188	90253	81.7	237
Hg_007	0.11	12.0	2.46	2665	10877	52749	442	10153	7621	2851	204	1940	3098	95659	85.5	230
Hg_008	0.25	12.4	1.65	7099	14018	68699	716	11296	13110	4058	194	1272	2080	86965	65.8	209
Hg_009	1.06	16.7	1.94	5725	17529	77752	648	11805	12680	5176	170	781	1762	75395	67.3	250
Hg_010	0.32	12.6	2.27	6754	14892	66102	673	11242	12624	4338	161	798	1534	71221	55.5	202
Hg_011	0.14	13.9	2.36	6887	19367	70131	728	11923	13236	4185	177	1196	1335	79374	68.8	300
Hg_011 Duplicate	0.20	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Hg_012	0.38	16.5	2.94	4347	8121	89331	765	13773	6058	6677	203	482	1066	78243	60.7	162
Hg_012 Duplicate	n.a.	21.1	2.05	4419	8394	92397	768	14099	6347	7102	214	505	1125	81967	62.8	165
Hg_014	0.52	16.2	1.85	7387	21617	72108	575	12600	14160	4665	154	865	1461	65675	60.8	272
Hg_015	1.72	11.5	1.12	9079	19980	62434	545	13068	16431	3978	150	681	1020	59177	49.9	187
Hg_016	0.72	11.6	1.54	9133	15801	61140	572	11808	16709	4268	160	588	1042	59999	44.7	151
Hg_017	1.01	20.0	2.13	3813	16674	89881	636	11703	9153	5580	172	1007	3213	82270	78.4	345
Hg_018	0.08	19.1	1.92	3433	19168	92550	690	11077	9594	5867	168	942	2513	81038	74.2	358
Hg_022	0.17	11.7	1.80	10509	8992	58586	373	16760	5151	2958	101	295	925	43205	43.9	98.7
Hg_023	0.17	15.1	2.53	6887	8359	72194	499	13746	3859	3742	124	316	1066	53355	45.4	113
Hg_023 Duplicate	0.19	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Hg_025	1.84	10.8	1.98	8853	3952	64050	497	12121	5260	3687	127	275	445	48751	27.3	64.2
Hg_025 Duplicate	n.a.	11.9	2.27	9170	4102	66397	508	12156	5004	3699	128	286	456	49016	27.5	66.0

Hg_026	0.38	11.6	2.12	13949	7833	62471	410	18952	7504	2920	100	301	617	40282	33.1	73.8
Sample Code	Cu mg kg^{-1}	Zn mg kg^{-1}	Ga mg kg^{-1}	As mg kg^{-1}	Rb mg kg^{-1}	Sr mg kg^{-1}	Y mg kg^{-1}	Mo mg kg^{-1}	Cd mg kg^{-1}	Sb mg kg^{-1}	Cs mg kg^{-1}	Ba mg kg^{-1}	La mg kg^{-1}	Ce mg kg^{-1}	Pr mg kg^{-1}	Nd mg kg^{-1}
Detection limit	**0.2**	**0.9**	**0.03**	**0.07**	**0.2**	**0.5**	**0.03**	**0.2**	**0.007**	**0.02**	**0.02**	**0.8**	**0.05**	**0.09**	**0.02**	**0.05**
Hg_001	55.0	80.6	14.5	10.7	52.6	130	15.4	2.7	0.067	0.60	1.89	478	24.5	83.9	5.26	20.4
Hg_002	77.5	99.0	19.0	13.6	67.5	108	23.3	3.1	0.116	0.50	2.65	532	39.9	134	8.32	32.0
Hg_003	71.9	87.6	18.5	18.1	65.4	109	22.5	3.6	0.095	0.55	2.53	570	36.8	118	7.79	31.1
Hg_004	90.8	98.9	17.8	16.1	65.6	165	21.9	2.9	0.077	0.57	2.64	600	34.3	87.9	7.61	29.8
Hg_004 Duplicate	88.1	94.8	18.6	12.9	61.7	163	21.0	2.4	0.097	0.55	2.51	579	33.4	88.4	7.37	29.3
Hg_005	63.5	86.4	12.3	17.2	47.5	120	13.9	3.4	0.094	0.69	1.75	451	21.2	98.4	4.56	17.5
Hg_006	76.4	84.7	13.9	12.0	47.2	91.5	15.8	4.0	0.096	0.42	2.03	499	25.2	112	5.27	20.4
Hg_007	63.8	80.6	14.4	7.94	47.2	88.6	13.9	3.7	0.109	0.39	1.86	543	22.3	91.5	4.77	18.7
Hg_008	76.8	92.4	17.0	51.2	57.1	174	18.1	2.8	0.074	1.05	2.30	498	29.9	96.4	6.43	25.3
Hg_009	94.6	109	16.8	33.0	63.5	149	21.8	2.2	0.112	1.16	2.74	512	34.6	91.3	7.42	29.1
Hg_010	92.0	104	14.7	52.1	54.2	168	17.5	3.0	0.115	3.29	2.32	490	28.7	78.2	6.47	25.5
Hg_011	56.9	84.2	15.8	21.5	58.8	170	19.3	1.9	0.085	4.76	2.67	497	31.4	118	6.61	26.3
Hg_011 Duplicate	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Hg_012	72.9	117	21.2	10.4	79.3	104	24.2	2.7	0.165	0.84	2.83	512	44.9	137	9.06	34.5
Hg_012 Duplicate	75.8	121	20.6	11.6	82.8	105	25.1	2.9	0.126	0.83	2.81	528	46.9	143	9.55	35.9
Hg_014	62.6	87.4	16.6	40.0	59.3	180	19.0	1.8	0.097	5.07	2.73	520	31.2	82.9	6.74	26.0
Hg_015	47.8	73.6	13.8	77.4	54.4	208	16.3	1.3	0.077	0.56	2.09	539	27.9	72.9	6.25	24.7
Hg_016	69.2	71.7	13.0	69.4	49.8	194	16.6	1.4	0.078	0.84	1.98	506	27.1	73.2	5.96	23.6
Hg_017	69.1	94.2	20.7	16.0	62.9	117	29.6	2.9	0.087	0.45	3.38	497	48.2	135	10.1	39.2
Hg_018	75.9	102	20.2	8.18	66.7	106	27.5	2.8	0.120	0.48	3.65	498	44.5	100	9.21	35.7
Hg_022	31.9	87.3	13.2	66.9	62.3	145	14.3	2.3	0.104	0.78	1.65	627	26.0	60.1	5.66	21.9
Hg_023	43.3	102	17.0	52.2	64.1	106	19.5	2.1	0.130	0.79	1.89	560	37.2	86.9	7.80	29.9
Hg_023 Duplicate	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Hg_025	68.1	88.9	14.5	345	55.9	133	20.4	4.2	0.322	2.08	1.58	435	39.9	93.1	8.19	30.9
Hg_025 Duplicate	60.1	93.5	15.3	349	57.1	135	21.0	3.8	0.307	2.36	1.57	444	40.5	95.1	8.40	31.3
Hg_026	29.9	67.3	12.7	28.5	75.4	196	12.7	1.6	0.040	0.56	1.80	668	24.8	59.7	5.33	19.8

Sample Code	Sm mg kg^{-1}	Eu mg kg^{-1}	Gd mg kg^{-1}	Tb mg kg^{-1}	Dy mg kg^{-1}	Ho mg kg^{-1}	Tm mg kg^{-1}	Yb mg kg^{-1}	Lu mg kg^{-1}	Tl mg kg^{-1}	Pb mg kg^{-1}	Th mg kg^{-1}	U mg kg^{-1}
Detection limit	**0.02**	**0.02**	**0.02**	**0.02**	**0.02**	**0.02**	**0.02**	**0.01**	**0.02**	**0.006**	**0.06**	**0.02**	**0.02**
Hg_001	3.85	0.84	3.33	0.48	2.80	0.57	0.24	1.67	0.26	0.374	15.9	5.24	1.57
Hg_002	5.69	1.16	4.83	0.71	4.19	0.84	0.34	2.36	0.34	0.446	21.8	7.78	2.08
Hg_003	5.69	1.19	4.78	0.70	4.07	0.82	0.35	2.41	0.34	0.442	22.1	7.16	1.80
Hg_004	5.49	1.20	4.58	0.67	3.92	0.79	0.33	2.22	0.31	0.391	16.4	7.35	1.78
Hg_004 Duplicate	5.50	1.23	4.47	0.64	3.75	0.78	0.32	2.19	0.31	0.401	15.5	6.39	1.75
Hg_005	3.39	0.74	2.91	0.44	2.62	0.52	0.21	1.50	0.22	0.324	20.2	4.29	1.47
Hg_006	3.87	0.83	3.30	0.49	2.92	0.57	0.24	1.71	0.24	0.381	22.4	5.28	1.73
Hg_007	3.60	0.75	2.97	0.44	2.59	0.53	0.23	1.62	0.21	0.383	17.7	4.65	1.56
Hg_008	4.59	1.01	3.90	0.58	3.37	0.66	0.29	1.94	0.28	0.346	31.4	5.83	1.90
Hg_009	5.63	1.16	4.62	0.66	3.89	0.76	0.32	2.28	0.30	0.407	43.4	6.48	1.79
Hg_010	4.80	1.03	3.92	0.57	3.23	0.64	0.26	1.86	0.26	0.365	41.5	43.9	3.99
Hg_011	4.99	1.07	4.06	0.63	3.45	0.71	0.31	2.02	0.29	0.400	24.0	5.86	1.61
Hg_011 Duplicate	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Hg_012	6.12	1.21	5.09	0.74	4.36	0.91	0.39	2.60	0.40	0.488	29.5	9.90	2.69
Hg_012 Duplicate	6.49	1.24	5.18	0.75	4.45	0.90	0.39	2.72	0.39	0.486	30.4	10.6	2.78
Hg_014	5.03	1.10	4.21	0.57	3.39	0.67	0.28	1.97	0.28	0.369	21.2	5.86	1.53
Hg_015	4.58	1.05	3.75	0.53	3.01	0.57	0.25	1.64	0.23	0.295	24.8	4.72	1.25
Hg_016	4.47	0.99	3.59	0.52	2.89	0.60	0.24	1.66	0.23	0.280	33.7	4.69	1.26
Hg_017	7.32	1.48	6.14	0.90	5.25	1.05	0.43	2.96	0.42	0.517	18.7	8.38	2.29
Hg_018	6.67	1.41	5.68	0.81	4.84	0.98	0.41	2.83	0.42	0.549	16.9	8.44	2.35
Hg_022	3.91	0.86	3.05	0.44	2.56	0.54	0.22	1.56	0.22	0.432	18.5	6.39	1.90
Hg_023	5.59	1.07	4.22	0.59	3.53	0.70	0.30	1.96	0.28	0.467	31.1	7.86	2.24
Hg_023 Duplicate	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Hg_025	5.46	1.03	4.22	0.62	3.63	0.74	0.30	2.04	0.28	0.421	38.2	7.35	2.09
Hg_025 Duplicate	5.52	1.09	4.42	0.63	3.64	0.74	0.31	2.19	0.30	0.440	40.4	7.48	2.19
Hg_026	3.49	0.80	2.75	0.39	2.30	0.47	0.20	1.38	0.20	0.439	18.3	7.07	1.87

Supplementary Table A4.7.: %difference of analytes from duplicate sediment samples.

Duplicate % differences	Hg	Li	Be	Na	Mg	Al	P	K	Ca	Ti	V	Cr	Mn	Fe	Co
Hg_004	12%	12%	7%	2%	6%	1%	11%	6%	3%	0%	3%	19%	5%	2%	2%
Hg_011	30%	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Hg_012	n.a.	12%	7%	2%	6%	1%	11%	6%	3%	0%	3%	19%	5%	2%	2%
Hg_023	13%	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Hg_025	n.a.	9%	13%	3%	4%	4%	2%	0%	5%	0%	1%	4%	2%	1%	1%

Duplicate % differences	Ni	Cu	Zn	Ga	As	Rb	Sr	Y	Mo	Cd	Sb	Cs	Ba	La	Ce
Hg_004	4%	3%	4%	4%	25%	6%	1%	5%	21%	21%	4%	5%	4%	3%	1%
Hg_011	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Hg_012	4%	3%	4%	4%	25%	6%	1%	5%	21%	21%	4%	5%	4%	3%	1%
Hg_023	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Hg_025	3%	13%	5%	5%	1%	2%	1%	3%	11%	5%	12%	1%	2%	1%	2%

Duplicate % differences	Pr	Nd	Sm	Eu	Gd	Tb	Dy	Ho	Tm	Yb	Lu	Tl	Pb	Th	U
Hg_004	3%	2%	0%	2%	2%	5%	5%	1%	3%	1%	0%	2%	6%	15%	2%
Hg_011	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Hg_012	3%	2%	0%	2%	2%	5%	5%	1%	3%	1%	0%	2%	6%	15%	2%
Hg_023	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Hg_025	3%	1%	1%	6%	5%	2%	0%	0%	3%	7%	7%	4%	5%	2%	5%

Supplementary Table A4.8.: Recovery (%) and Standard deviation (%) of analytes in certified reference materials (BCR-2 (n=3), LKSD-1 (n=1), LKSD-3 (n=1), and MESS-4 (n=1))

	Li	Be	Na	Mg	Al	P	K	Ca	Ti	V	Cr	Mn	Fe	Co	Ni
BCR-2 Recovery (%)	82%	n.a.	99%	93%	92%	97%	95%	96%	99%	102%	89%	98%	98%	101%	n.a.
BCR-2 Relative standard deviation (%)	9%	n.a.	5%	6%	5%	7%	1%	2%	1%	2%	4%	2%	2%	2%	n.a.
LKSD-1 Recovery (%)	97%	n.a.	109%	102%	96%	76%	100%	100%	81%	98%	87%	85%	96%	94%	100%
LKSD-3 Recovery (%)	99%	n.a.	107%	100%	97%	118%	102%	104%	88%	97%	89%	90%	100%	103%	110%
MESS-4 Recovery (%)	89%	111%	101%	92%	92%	97%	101%	102%	79%	99%	99%	97%	100%	101%	96%

	Cu	Zn	Ga	As	Rb	Sr	Y	Mo	Cd	Sb	Cs	Ba	La	Ce	Pr
BCR-2 Recovery (%)	87%	108%	84%	n.a.	95%	85%	92%	103%	n.a.	n.a.	103%	93%	97%	96%	95%
BCR-2 Relative standard deviation (%)	1%	1%	6%	n.a.	2%	5%	3%	3%	n.a.	n.a.	3%	4%	4%	5%	5%
LKSD-1 Recovery (%)	93%	104%	n.a.	82%	96%	98%	100%	103%	n.a.	99%	45%	88%	85%	93%	n.a.
LKSD-3 Recovery (%)	93%	97%	n.a.	83%	96%	93%	88%	n.a.	n.a.	89%	96%	91%	88%	95%	n.a.
MESS-4 Recovery (%)	99%	104%	98%	91%	n.a.	89%	n.a.	n.a.	103%	103%	78%	96%	89%	84%	n.a.

	Nd	Sm	Eu	Gd	Tb	Dy	Ho	Tm	Yb	Lu	Tl	Pb	Th	U
BCR-2 Recovery (%)	101%	100%	96%	98%	98%	92%	94%	92%	95%	97%	n.a.	88%	88%	94%
BCR-2 Relative standard deviation (%)	4%	6%	6%	6%	5%	6%	2%	2%	3%	5%	n.a.	3%	2%	3%
LKSD-1 Recovery (%)	105%	98%	101%	n.a.	86%	89%	n.a.	n.a.	92%	64%	n.a.	109%	84%	88%
LKSD-3 Recovery (%)	98%	99%	89%	n.a.	80%	94%	n.a.	n.a.	n.a.	89%	n.a.	102%	88%	88%
MESS-4 Recovery (%)	n.a.	97%	81%	n.a.	n.a.	n.a.	n.a.	n.a.	82%	n.a.	96%	100%	80%	81%