MERCURY ALKYLATION IN FRESHWATER SEDIMENTS FROM SCOTTISH CANALS

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\textbf{Abstract}

Mercury concentrations were investigated in freshwater sediment from two canals in Scotland, UK. High concentrations found in the Union Canal (35.3–1200 mg kg\textsuperscript{-1}) likely originate from historical munitions manufacture, with lower levels in the Forth & Clyde Canal (0.591–9.14 mg kg\textsuperscript{-1}). Concentrations of methylmercury (MeHg) were low – from 6.02 to 18.6 μg kg\textsuperscript{-1} (0.001–0.023\% of total Hg) in the Union Canal and from 3.44 to 14.1 μg kg\textsuperscript{-1} (0.11–0.58\% of total Hg) in the Forth & Clyde Canal – and there was a significant inverse relationship between total Hg concentration and %MeHg. Total Hg concentration was significantly negatively correlated with pH and positively correlated with Fe content (in the Union Canal only) but not with organic matter, S content or the proportion of clay present. The MeHg concentration was not correlated with any of the above sediment parameters. Ethylmercury was detected in the most highly contaminated sediments from the Union Canal.

\textbf{Keywords:} mercury, methylation, sediment, contamination, methylmercury, ethylmercury
1 Introduction

In the aquatic environment sediments adsorb and store both inorganic Hg and MeHg. However, adsorption may not be permanent. Mercury can be released through the formation of soluble complexes with sulfide or organic matter (OM) (Merrit and Amirbahman, 2007; Faganeli et al., 2003), or through the reduction of Fe\(^{III}\) and Mn\(^{IV}\) (oxy)hydroxide surfaces on which Hg species are adsorbed. The ease of Hg release from sediment varies depending on the species present, with inorganic Hg being released less readily than MeHg as a result of its stronger sorption. For example, Covelli et al. (1999) estimated that up to 25% of total sediment Hg content was released annually in sediments from the Gulf of Trieste, Italy, of which up to 23% could be in the form of MeHg.

Methylation is primarily an intracellular bacterial process, carried out mainly under anoxic conditions at the sediment-water interface where microbial activity is high, by certain species of sulfate- and iron-reducing bacteria (Compeau and Bartha, 1985; Fleming et al., 2006). It occurs only on dissolved inorganic Hg\(^{II}\) species since this is the form able to cross the cell membrane (Benoit et al., 1999; Mason et al., 1996). Bacteria either store the MeHg internally or excrete it into the water column. Potential exposure of other aquatic organisms to MeHg occurs mainly through direct uptake from sediment (Gagnon and Fisher, 1997) or the consumption of plankton by higher trophic level feeders. Bioaccumulation of MeHg increases with increasing trophic level (Mason et al., 1996; Watras et al., 1998; Campbell et al., 2005; Leopold et al., 2009). Consumption of high trophic-feeder fish is the main source of human exposure to Hg, with adverse neurological effects possible both in humans and the developing foetus above a reference dose of 0.1 μg kg\(^{-1}\) body weight day\(^{-1}\) (EPA, 2001).

The overall degree of Hg methylation is dependent not just on the rate of production but rather on the balance between rates of methylation and demethylation. Photodegradation is known to break down MeHg species (Sellers et al., 1996), as are two biotic processes: reductive demethylation and oxidative demethylation (Schaefer et
Reductive demethylation is mediated by Hg-resistant bacteria as part of their mercury resistance (mer) system in both aerobic and anaerobic environments (Merritt and Amirbahman, 2009; Barkey et al., 2003). In the presence of Hg, these bacteria express mer genes that encode for enzymes to degrade MeHg to Hg⁰ which may then be lost to the atmosphere (Barkey et al., 2003). Oxidative demethylation, which is also carried out by both aerobic and anaerobic bacteria, is not considered a detoxification process since Hg⁰ is formed, which is still available to bacteria (Hintelmann, 2010).

Typically, the proportion of the total Hg content in sediment that is methylated (%MeHg) is around 0.5% (Hines et al., 2000; Zelewski et al., 2001). Lower %MeHg has been observed in freshwater with higher total Hg concentration (Schaefer et al., 2004). A decrease in net methylation may be a result of lower microbial activity at high Hg concentrations (Ullrich et al., 2001). It has also been proposed (Schaefer et al., 2004) that, in more contaminated environments, mercury-resistance (mer) genes are expressed which regulate reductive demethylation, while lower levels of Hg are insufficient to effectively induce expression of these genes. In addition to Hg content, sediment parameters such as the presence of OM; sulfur and iron content and speciation; pH, redox potential and texture all influence methylation (Frohne and Rinklebe, 2013; Frohne et al., 2012; Ullrich et al., 2001). Due to its affinity for sulfur, Hg⁰ in sediment can bind to reduced S groups in OM, limiting its mobility and potential for methylation (Ravichandran, 2004). However, OM can also stimulate microbial activity and, as a consequence, MeHg production, (Drott et al., 2007). The ratio between dissolved organic carbon (DOC) and total dissolved Hg concentration has been shown (Frohne et al., 2012) to be a critical parameter influencing net Hg methylation in contaminated floodplain soils. Sulfide concentration also affects Hg methylation rates. While sulfate-reducing bacteria promote methylation, sulfides, produced from sulfate reduction, inhibit methylation due to the formation of insoluble HgS or soluble charged sulfide complexes such as HgS₂²⁻ and HgHS₂⁻ that cannot cross the cell membrane (Devai et al., 2007; Benoit et al., 1999). The influence of iron on methylation is also variable: while ferric iron is a substrate for iron-
reducing bacteria and may enhance methylation, iron may also limit Hg solubility and availability through the formation of iron-Hg complexes (Behra et al., 2001; Jeong et al., 2007). Sediment pH affects mercury speciation and particle surface charge (Sarkar et al., 1999) thus indirectly affecting Hg adsorption, bioavailability and consequently methylation. Similarly, variations in redox potential may indirectly influence methylation by affecting OM and sulfur and iron speciation and hence the adsorption and release of Hg species (Frohne et al., 2012). Further discussion of factors affecting Hg methylation can be found in Frohne et al. (2012).

The Forth & Clyde Canal runs from Bowling on the River Clyde, Scotland, UK, through Falkirk, to Grangemouth on the Firth of Forth, whilst the Union Canal runs from Falkirk to Edinburgh (Figure 1). Originally opened in 1790 and 1824, respectively, both canals were major routes for transport of goods before competition from the railways, beginning in the 1840’s, led to their gradual decline and eventual closure in the 1960’s (Haynes, 2015).

Substantial redevelopment and regeneration carried out under the Millennium Link Project saw both canals reopened as major leisure facilities in 2001–2002 and connected through a rotating boat lift, the Falkirk Wheel (Figure 1). However, Central Scotland was formerly a major hub for heavy industry and ‘legacy pollution’ from this period is a major concern in the area. In particular, the Union Canal has a history of Hg contamination arising from proximity to a munitions factory that manufactured detonators from 1876 to 1968, the main constituent of which was mercury fulminate (Smith and Lassiere, 2000). Despite dredging from the most contaminated section of the waterway and soil remediation on the former factory grounds carried out between 2000–2006, very high levels of Hg contamination still persist in the canal sediment (Cavoura et al., 2013).

This study investigated Hg concentrations and speciation in sediments from the Forth & Clyde Canal, and the Union Canal. Relationships between Hg, MeHg, OM, S and Fe content, sediment pH and texture were explored to gain insight into the factors affecting the distribution and fate of Hg species in freshwater systems.
2 Materials and methods

2.1 Sampling locations and method

Sampling points 1–10 were on the Union Canal between Falkirk and Polmont, sampling points 12–14 were on the Glasgow branch of the Forth & Clyde Canal and sampling points 15–20 were between Kirkintilloch and Falkirk on the Forth & Clyde Canal (Figure 1). The former munitions factory was located on both banks of the canal at site 7.

Sediment samples were collected by throwing a stainless-steel bucket attached to a rope across the width of the canal and slowly pulling it back along the canal bottom. This unconventional sampling method was adopted for several reasons. First, the canals are historic monuments and use of more conventional methods – grab samplers or corers – was not possible due to the risk of damaging the clay liner at the bottom of the canal. Second, the sediment layer is typically 10 cm in depth (overlain by ca. 2 m of water) and frequently re-suspended by passing boats. There is no long-term stratification or redox front present and so obtaining the entire sediment ‘column’ (as demonstrated by the presence of a minimal amount of clay liner on the lower edge of the bucket) was considered the most representative and reproducible sampling method possible under the circumstances. The sediment was placed in wide mouth glass bottles for transport.

On return to the laboratory, sediments were dried in a natural convection drying oven at 30 °C and sieved to < 2 mm before storage in glass bottles. Dried, sieved samples were coned and quartered to obtain representative test portions for analysis.

2.2 Analytical procedures

Glassware was soaked in 10% v/v HNO₃ overnight and rinsed with deionised (DI) water before use. Glass containers were used for storing Hg samples, standard solutions and reagents.

Moisture content was determined on dried, sieved test portions (BS, 2000) and then the OM content was estimated by loss on ignition (Schumacher, 2002).
Determination of pH was performed (EN, 2003) using approximately 5 g (dried) test portions and 0.01 M CaCl₂·2H₂O (25 mL). Particle size distribution was determined by sieving (BS, 2000b) and sedimentation (ASTM, 2007).

Determination of total Hg concentration in Union Canal sediment was performed in Athens, Greece, using cold vapour atomic absorption spectrometry (CVAAS) (PE, 2006). Briefly, after microwave assisted digestion of 0.5 g test portions with 10 mL HNO₃ in a Berghoff Speedwave MWS-2 system, 10 mL DI water was added, then digests were filtered and diluted to a final volume of 50 mL with further DI water. Analysis was performed following reduction with 3% NaBH₄ using a MHS-10 Hg/Hydride system (Perkin Elmer, Massachusetts, USA) operated in cold vapour mode. Determination of total Hg concentration in Forth & Clyde Canal sediment samples was performed in Glasgow, Scotland, using atomic fluorescence spectrometry (AFS). Test portions (1 g) were digested in 5 mL HNO₃ using a CEM MARSXpress™ microwave-assisted digestion system. Cooled vials were centrifuged (3000 rpm, 10 min) and a 2 g aliquot of the supernatant (accurately weighed) was removed and diluted 10-fold to give a 10% HNO₃ solution. Analysis was performed using AFS (10.025 Millennium Merlin, PS Analytical, Kent, UK) with 2% Sn(II)Cl₂ reductant. Determinations were carried out in triplicate.

The MeHg concentration was determined in fresh wet sediment by gas chromatography-inductively coupled plasma-mass spectrometry (GC-ICP-MS) after extraction with 4% w/w HCl (Bermejo-Barrera et al., 1999) and derivitization with NaBPr₄ (De Smaele et al., 1998). Briefly, 3 mL of 4% (w/w) HCl was added to approximately 1 g of sediment. The samples were shaken mechanically (2 min), centrifuged (3000 rpm, 10 min) and the supernatant transferred to a glass vial. The process was repeated with a further 2 mL of 4% (w/w) HCl to give a 5 mL combined extract. A 1 mL aliquot of this extract was transferred to a new glass vial and 5 mL of 0.1 M acetate buffer solution added. The pH was adjusted to 3.9 ± 0.1 using tetramethylammonium hydroxide (25% w/w aqueous solution) and acetic acid, then 1 mL of isooctane was added followed by 1 mL of 1% (w/w) sodium tetra propylborate (NaBPr₄). The samples were allowed to stand
for 30 min in order to ensure complete derivitization, then mechanically shaken for 5 min to extract derivitized species into the organic layer, before being centrifuged (3000 rpm, 5 min) and the organic layer removed into an amber GC vial for storage at −20 °C until analysis.

The GC-ICP-MS incorporated a Hewlett Packard HP 6850 gas chromatograph and a 7500c Series ICP-MS system (both from Agilent Technologies UK Ltd) connected via a heated (220 °C) Silcosteel transfer line. Manual sample injection (1 μL) in split-less mode was used. The GC temperature programme was: hold 50 ° C (1 min); ramp 50 °C min⁻¹; hold 250 °C (7 min) with He carrier gas (mL min⁻¹). A Tl isotope internal standard (25 μg L⁻¹ Tl in 1% HNO₃) was used for ICP-MS and quantification was based on the response for the most abundant Hg isotope, ²⁰²Hg (RSC, 2014). Sediment samples from the Forth & Clyde Canal were analysed in the same manner after spiking with appropriate amounts of a 20 ng g⁻¹ enriched Me-201 standard solution and the MeHg content quantified using Hg isotope dilution mass spectrometry.

2.3 Reagents
Reagents used were of analytical grade or higher. A stock standard Hg solution (10 mg L⁻¹ in 10% (v/v) HNO₃) was prepared from a 1000 mg L⁻¹ Hg standard solution (Hg(NO₃)₂, Certipur, Merck, Leicester, UK) stored at 4 °C and replaced monthly. Reagent-matched standard solutions with concentrations < 10 mg L⁻¹ were prepared daily as required. A stock solution containing 10 mg kg⁻¹ MeHg in methanol (AnalaR NORMAPUR BDH Prolabo–VWR International, Lutterworth, UK) was prepared from methylmercury(II)chloride powder (Pestanal analytical standard, Sigma-Aldrich Company Ltd. Dorset, UK). Standard solutions of lower concentrations were prepared from the stock solution as required in isooctane (≥ 99% ACS Reagent, Sigma-Aldrich Company Ltd. Dorset, UK). For the determination of pH a 0.01 M CaCl₂·2H₂O solution (pH = 5.45) was prepared by dissolving 1.47 g of CaCl₂·2H₂O (≥ 99%, ACS reagent, Sigma-Aldrich Company, Life Science Chemilab A.E., Athens, Greece) in distilled water and making up
to 1 L. The HCl (4% (w/w)) used in extraction of sediment samples was prepared from 30% HCl (for trace analysis) and the HNO₃ for washing glassware (> 65%, for trace analysis) were both from Sigma-Aldrich Company Ltd. Dorset, UK. The derivitizing agent NaBPr₄ (1% w/w) was prepared from NaBPr₄ (Chemos GmbH, Regenstauf, Germany) in DI water and stored at −20 °C until use. The NaBH₄ reductant, a 3% solution in 1% NaOH solution, was prepared daily using NaOH pellets (AR, Mallinckrodt, Dublin, Ireland) and NaBH₄ powder (GR for analysis, Merck KGaA, Darmstadt, Germany). The solution was filtered (glass fibre filters, Pall A/E Glass fibre filters 1.0 μm, 110 mm, Pall GmbH, Dreieich, Germany) into a MHS-10 reductant vessel (Perkin Elmer, Massachusetts, USA) before use. The SnCl₂ reductant, 2% in 10% HCl, was prepared from SnCl₂.2H₂O (98%, Alfa Aesar, Heysham, UK).

2.4 Limits of detection and quality control

The limit of detection (LOD) for Hg by CVAAS was 0.067 mg kg⁻¹. Recovery of Hg from CRM BCR 320 R Channel Sediment containing 0.85 ± 0.09 mg kg⁻¹ Hg (Geel, Belgium) (0.1 g test portions) was 116 ± 20.3% (n=3). For AFS, the LOD for Hg was 0.0484 mg kg⁻¹. Recovery from CRM ERM-CC580 Estuarine Sediment containing 132 ± 3 mg kg⁻¹ total Hg (Geel, Belgium) (0.02 g test portions) was 103% (average of 100%, 105%). For GC-ICP-MS the LOD for Me²⁰²Hg was 1.16 μg kg⁻¹. The recovery of MeHg from CRM ERM CC580 containing 75 ± 4 μg kg⁻¹ MeHg (0.1 g test portions) was 101% (average of 87.5%, 114%).

3 Results and discussion

3.1 General sediment characteristics

Information on the OM content, pH and particle size distribution of the sediment samples are shown in Table 1, together with total Fe and S concentrations, where available. The concentrations of OM in the Union Canal (5.1–13.9%) were lower than in the Forth & Clyde Canal (16.8–29.1%) whilst the pH ranges were similar. Sediment samples from the
Union Canal were generally coarser than those from the Forth & Clyde canal. The Forth & Clyde Canal was richer in Fe than the Union Canal, even at rural sites (15, 16) unaffected by past or present industrial activities. Total S content, determined only in the Union Canal, ranged from 0.07–0.40%, which is broadly similar to values previously reported in other locations where Hg contamination was present (Devai et al., 2005; Frohne and Rinklebe, 2013).

3.2 Total Hg, MeHg and EtHg concentrations in canal sediments

Total Hg and MeHg concentrations were determined and MeHg as a percentage of total Hg concentration (%MeHg) was calculated (Table 2). In the Union Canal EtHg was detected at sampling locations 5, 6, 7 and 8 (Figure 2). Since an EtHg standard was not available, an estimate of EtHg concentration and %EtHg was made based on the MeHg standard solutions (since the count-rate registered by the mass spectrometer reflected the response of the instrument to Hg ions, while the different species were identified by their retention times). Given the above, and in the absence of certified reference materials for EtHg to confirm the efficiency of the extraction/derivitization procedure, all concentrations reported herein should be considered approximate. At location five, where the highest Hg concentration was determined, Hg\(^0\) was also present (Figure 3). This has been detected previously, for example in sediments impacted by historic Hg mining (Biester et al., 2000) and chlor-alkali plant effluent (Reis et al., 2015) but is prone to loss during sample preparation due to its high volatility (Reis et al., 2015).

In the Union Canal, total Hg concentration ranged from 35.3 ± 7.3 to 1200 ± 180 mg kg\(^{-1}\) (n=3) with highest levels (> 500 mg kg\(^{-1}\)) found along the stretch close to the location of the former munitions factory site. This is considerably in excess of the probable effect level (PEL) value of 0.486 mg kg\(^{-1}\) for freshwater sediment (CCME, 1999). Based on the Dutch sediment pollution classification system, sediment containing over 10 mg kg\(^{-1}\) Hg is classified as very polluted (Kelderman et al., 2000) and this was the case at all sites sampled in the Union Canal including site 1 which is on a new stretch
of canal built at the beginning of the 21st century to provide a connection to the Falkirk Wheel. Further, the levels were greater than those found in previous surveys of the Union Canal conducted in 2010 and 2012 (Figure 3) which suggests re-supply of contaminated material is occurring.

The concentrations determined were similar to those reported recently in areas impacted by Hg mining: for example, Reichelt-Brushett et al. (2017) measured 8–82 mg kg⁻¹ Hg in sediments from an artisanal small-scale gold mining district in Indonesia, whilst Ruyamor et al. (2017) found Hg concentrations up to 946 mg kg⁻¹ in sediments from abandoned historical Hg mining-metallurgical sites in Spain (with soil concentrations as high as 3830 mg kg⁻¹).

Despite the large range of total Hg concentrations found in the Union Canal, MeHg concentrations did not vary greatly, ranging from 6.02 ± 2.0 μg kg⁻¹ to 18.6 ± 4.2 μg kg⁻¹, well below the Dutch target value of 0.3 mg kg⁻¹ for MeHg concentration in sediment (GESAMP, 2014). The MeHg concentration was not found to be significantly correlated to the total Hg content ($r^2 = 0.221$, $p > 0.05$) (Figure 4).

In the Forth & Clyde Canal total Hg concentration ranged from 0.591 to 9.14 mg kg⁻¹, above the PEL value for freshwater sediment (CCME, 1999) but roughly two orders of magnitude less than in the Union Canal. Concentrations were higher than levels determined in 1992 (BW, 1992) when Hg concentrations were found between 0.1 and 2.7 mg kg⁻¹. This increase, occurring after the construction of the Falkirk Wheel connecting the two canals, is probably due to the transfer of contaminated material from the Union Canal where Hg levels are higher, through the Wheel to the Forth & Clyde Canal. The highest Hg concentration in the Forth & Clyde Canal was determined at site 18, which is just downstream of the junction with the Falkirk Wheel, supporting the above hypothesis. The MeHg concentration in the Forth and Clyde Canal ranged from 3.44 to 14.1 μg kg⁻¹ and was not significantly correlated with total Hg concentration ($r^2 = 0.428$, $p > 0.05$) (Figure 4).
Net methylation has been found to increase with increasing total Hg concentration at low (background) concentrations. By monitoring the distribution of an isotopically enriched $^{202}$Hg spike in mesocosms, Orihel et al. (2006) found a positive correlation ($r^2 = 0.84$) between MeHg production and $^{202}$Hg$^{II}$ in sediment where background Hg sediment concentrations were 0.004–0.007 mg kg$^{-1}$. As shown in Table 3, positive correlation between total Hg and MeHg concentrations has also been found in the field, including in the Scheldt Estuary, Belgium (Muhaya et al., 1997); the Jiulong River Estuary, China (Wu et al., 2011); along the Fujian coast, also China (Zhang et al., 2013); and in sediments of the Vigo Ria, Spain (Canario et al., 2007). The latter study concluded that MeHg concentrations were constant for Hg concentrations in the range 0.75–2.5 nmol g$^{-1}$ (0.15–0.5 mg kg$^{-1}$) but the two variables were significantly positively correlated at higher concentrations.

At higher concentrations of Hg in sediment, different relationships with MeHg concentration have been observed. For example, in a contaminated coastal lagoon in Italy, Trombini et al. (2003) determined higher MeHg concentration in sediments with lower total Hg concentration, whilst no correlation was found between the two species in polluted sediments of the Lenga Estuary, Chile (Yanez et al., 2013).

Estimated concentrations of EtHg in the Union Canal sediment were up to 6.12 ± 1.0 μg kg$^{-1}$. Besides MeHg, EtHg is the only other monoalkyl Hg compound so far reported in the environment (Hintelmann, 2010). Unlike MeHg, it does not bioaccumulate (Zhao et al., 2012; Batsita et al., 2011) and is not persistent (Hintelmann, 2010). However, it may still play an important role in Hg cycling and so there is a need to improve understanding of its environmental behaviour. The species has been identified previously in industrially-contaminated sediments from the Kosseine River, Germany (Hintelmann et al., 1995) where its presence was attributed to discharge of wastewaters from a fungicide plant producing EtHg (Hintelmann et al., 1995). Two further studies have reported the presence of EtHg in sediment, in the absence of nearby point sources. Holmes and Lean (2006) found EtHg concentrations ranging from 0.3 ± 0.3 to 3.7 ± 0.5
μg kg⁻¹ in Canadian wetland sediments, where Hg concentrations ranged from 66.1 to 319 μg kg⁻¹. Cai et al. (1996) found that EtHg was widespread in the Florida Everglades, with total Hg concentrations from 26.6 to 433 μg kg⁻¹ and EtHg concentrations from < 0.01 μg kg⁻¹ to 4.91 μg kg⁻¹. Biotic ethylation is not known to occur (Hintelmann, 2010) and Cai et al. (1996) suggested that EtHg could have been produced by abiotic (chemical) alkylation, similar to the alkylation reported when high-octane gasoline containing tetraethyl lead (PbEt₄) was mixed with HgCl₂, producing ethylmercury chloride (EtHgCl).

There was a significant positive relationship between total Hg and EtHg concentration ($r^2 = 0.960, p < 0.05$) but it must be emphasised that this is based on just four data pairs and EtHg concentrations were estimates only. The data from the study of Cai et al. (1996) also yields a positive relationship between total Hg and EtHg concentrations ($r^2 = 0.534$) whereas that of Holmes and Lean (2006), indicates no relationship between total Hg concentration and EtHg concentration ($r^2 = 0.039$).

### 3.2 Relationships between total Hg concentration, %MeHg and %EtHg

In the Union Canal 0.001 to 0.023% of the Hg present was in methylated form. In the Forth & Clyde Canal, %MeHg was greater and ranged from 0.11 to 0.58% of the total Hg present. A strong negative correlation was found between total Hg concentration and %MeHg in each canal (Figure 4) and there was a significant negative relationship between total Hg concentration and %MeHg over all locations ($r^2 = 0.350, p < 0.05, n = 15$).

A low %MeHg has been reported in other contaminated environments. Schaefer et al. (2004) observed an inverse relationship between total Hg concentration and %MeHg ($r^2 = 0.804, p < 0.001$) in water at two freshwater sites, one affected by industrial inputs (where total Hg concentration ranged from 113 to 4200 ng L⁻¹ and MeHg concentration ranged from 0.08 to 1.6 ng L⁻¹) and one considered pristine (where total Hg concentration ranged from 0.3 to 5.4 ng L⁻¹ and MeHg concentration ranged from 0.03 to
0.34 ng L\(^{-1}\)). The presence of \textit{mer}A genes and a high rate of reductive demethylation (\(K_{\text{deg}} = 0.19 \text{ day}^{-1}\)) in the microbial community from the contaminated waters, compared to the absence of \textit{mer}A genes and a low rate of oxidative demethylation (\(K_{\text{deg}} = 0.01 \text{ day}^{-1}\)) in the microbial community from the uncontaminated waters, provided evidence that MeHg degradation was directly related to Hg\(^{II}\) concentration. It was proposed that, in highly contaminated waters, mercury-resistance (\textit{mer}) genes are expressed which regulate reductive demethylation and that these genes are not expressed to the same degree at lower levels of Hg.

An inverse relationship between total Hg concentration and \%MeHg has also been reported in freshwater sediment, both in spiked and natural sediment samples. Microbial assays of freshwater sediment using radiolabeled MeHg (as \(^{14}\text{CH}_3\text{HgI}\)) at levels between 15 and 2400 \(\mu\text{g kg}^{-1}\) indicated that demethylation rate increased with increasing Hg concentration (Marvin-DiPasquale \textit{et al.}, 2000). Similarly, in river sediments in Kazakhstan where total Hg concentration ranged from 9.95 to 306 mg kg\(^{-1}\) and MeHg accounted for < 0.1\% of this on average, a strong inverse relationship between total Hg concentration and \%MeHg was found (\(r = 0.761, p < 0.001\)) (Ullrich \textit{et al.}, 2007). It was proposed that, although MeHg production is controlled by total Hg concentrations where these are low, in contaminated sediment, net methylation is limited not through the inhibition of methylation but rather because bacterial demethylation is more efficient.

The presence of \textit{mer} genes has been confirmed in Union Canal sediments (Rodriguez-Gil \textit{et al.}, 2013) and it is possible that the very high levels of Hg present are limiting net methylation, leading to relatively low MeHg concentrations. Additional investigation of these extremely contaminated sediments would be of interest since it could provide further insight into microbial processes relevant to the Hg cycle.

The \%EtHg was low (around 0.0005\%) and roughly constant across the four sites in the Union Canal where this species was detected.

\textbf{3.3 Influence of sediment characteristics}
The OM content was not significantly correlated with MeHg concentration in either canal ($r^2 = 0.317$, $p > 0.05$ for the Union Canal and $r^2 = 0.125$, $p > 0.05$ in the Forth & Clyde Canal). Microbial activity can be stimulated by nutrient release from the degradation of OM and many studies have found a positive relationship between MeHg concentration and OM content in sediment (Hammerschmidt et al., 2008; Muhaya et al., 1997; Choi and Bartha, 1994). If, however, the organic compounds generated form complexes with Hg$^{II}$, the effect on methylation rate is variable. In some cases, bioavailability is reduced, yielding a negative relationship between OM content and methylation (Barkey et al., 1997) whilst, in others, it is increased (Schaefer and Morel, 2009). Indeed, even at a single location, an increase or a decrease in Hg methylation with OM content may be observed, depending on the time of year (Liang et al., 2013). A stronger correlation was found between EtHg concentration and OM content ($r^2 = 0.598$) based on the four locations within the Union Canal where this species was detected, however this was not significant ($p > 0.05$). Data from the study of Holmes and Lean (2006) show a similar correlation ($r^2 = 0.560$ for OM concentrations in the range 9–90%) but a weaker relationship ($r^2 = 0.183$ for sediments containing 9-94% OM) is indicated by the data of Cai et al., (1996).

The pH of the canal sediments is within the range where Hg$^{II}$ adsorption is favored (pH 4–10) (Lui et al., 2012). In the Union Canal, there was a significant negative correlation between total Hg concentration and increasing pH over the range 5.7–6.9 ($r^2 = 0.745$, $p < 0.05$) but no significant correlation between these parameters was observed in the Forth & Clyde Canal ($r^2 = 0.120$, for a pH range 5.4–6.6). No relationship between pH and MeHg concentration was observed in either the Union Canal or the Forth & Clyde Canal ($r^2 = 0.003$ and 0.009 respectively). A decrease in %MeHg with decreasing pH ($r^2 = 0.778$, $p < 0.05$) was observed in sediment of the Union Canal but correlation was weak in the Forth & Clyde Canal ($r^2 = 0.0716$). This decrease in %MeHg at lower pH in the Union Canal arises because the total Hg concentration increased, while MeHg concentration remained largely unaffected.
Changes in pH may affect methylation both directly and indirectly by affecting adsorption and partitioning. For example, in laboratory experiments, a decrease in methylation by over 65% was observed in lake sediment that had been spiked with isotopically-labelled Hg and acidified from an initial pH of 6.1 to pH 4.5 (Steffan et al., 1988). Partitioning of MeHg into the water column is also important since MeHg is more soluble at low pH (Miller and Akagi, 1979). Sediment pH and EtHg in the Union Canal were not significantly correlated ($r^2 = 0.305$, $p > 0.05$). Since ethylation is not microbially mediated, any effect of pH on EtHg is likely to result from an alternation in EtHg partitioning.

Despite the high affinity of Hg for sulfur, it seems to play a minor role in Hg adsorption in the Union Canal; neither total Hg nor MeHg concentrations were strongly correlated with sediment sulfur content ($r^2 = 0.140$, $p > 0.05$ and $r^2 = 0.181$, $p > 0.05$ respectively). Similarly, no relationship was found between S and total Hg content in contaminated floodplain soil from the Wupper and Saale River, Germany (Frohne and Rinklebe, 2013). In that case a significant, positive correlation ($r^2 = 0.45$, $p < 0.005$) was found between total Hg concentration and Fe content, which was also found in the Union Canal sediments ($r^2 = 0.809$, $p < 0.05$). No significant correlation was observed between iron and MeHg ($r^2 = 0.034$, $p > 0.05$) although a positive relationship has been observed between the two parameters in a biogeochemical microcosm system, using contaminated floodplain soil from the Wupper River ($r^2 = 0.08$, $p < 0.05$) (Frohne et al., 2012). Sulfur content was not measured in the Forth & Clyde Canal samples, but no significant relationships between total Hg and Fe, or between MeHg and Fe, were found ($r^2 = 0.118$, $p > 0.05$ and $r^2 =0.0137$, $p > 0.05$ respectively). No significant relationships were observed between the proportion of sediment particles in any of the size fractions and either total Hg or MeHg concentration in either canal.

4. Conclusions
The Union Canal and the Forth and Clyde Canal were both found to be impacted by Hg contamination, with levels in Union Canal sediment considerably in excess of indicator values for very polluted sediment (Kelderman et al., 2000) and increasing over the study period. Both EtHg and Hg⁰ were detected at the most contaminated sites. The MeHg concentrations were low and a significant negative relationship was observed between %MeHg and total Hg, supporting previous work (Shaefer et al., 2004; Ullrich et al., 2007) that suggested rates of reductive demethylation increase in highly contaminated sediments due to expression of merA genes. Sediment OM content, S concentration and the proportion of fine (clay fraction) particles present appeared not to influence either total Hg or MeHg concentrations in the sediments studied, nor were levels of Hg species significantly correlated with pH or Fe, except for an inverse relationship between total Hg and pH, and a direct correlation between total Hg and Fe, in the Union Canal only. There was a significant position relationship between total Hg and EtHg concentration. Further investigation is required to identify sources of (re)supply of Hg to the Union Canal and potential transfer routes for contaminated sediment, both within the Union Canal and to the Forth and Clyde Canal. The canals are mainly used for recreational activities such as boating, canoeing and fishing (with a ‘catch and release’ policy in operation whereby any fish caught must be return alive to the water). Any transport pathways leading to significant Hg exposure to canal users should be identified and appropriate steps taken to minimize risk. In the broader context, wider study is needed to improve understanding of mechanisms for occurrence of EtHg (in the absence of direct point sources) and of its behavior in environmental systems.

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References


Rodriguez-Gil, C., Cavoura, O., Davidson, C.M., Keenan, H.E. and Aspray, T.J., (2013), Presence, diversity and abundance of the mercuric reductase (merA) gene in sediment along a section of the Union Canal, Scotland, 7th Annual Environmental and Clean Technology Conference, Edinburgh, UK.


Figure 1 Location of sampling points on the Union Canal (sampling points 1–10) and Forth & Clyde Canal (sampling points 12–20), UK, and the Falkirk Wheel (which connects the two canals).
Figure 2 GC-ICP-MS chromatogram showing mercury species (as the propyl derivatives except for Hg⁰) detected at location five in the Union Canal, Scotland, UK.
Figure 3 Temporal trends in Hg concentration in the Union Canal (UC), Scotland, UK.
Figure 4 Relationships between total Hg concentration, MeHg concentration, and %MeHg in sediments from the Union Canal and the Forth & Clyde Canal, Scotland, UK.
Table 1 Characteristics of sediment samples from the Union Canal and Forth & Clyde Canal.

<table>
<thead>
<tr>
<th>Location</th>
<th>Latitude and Longitude</th>
<th>OM (%)</th>
<th>pH</th>
<th>Sand (%)</th>
<th>Silt (%)</th>
<th>Clay (%)</th>
<th>Fe (%)</th>
<th>S (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Union Canal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>55.996° N 3.830° W</td>
<td>7.6</td>
<td>6.9</td>
<td>66</td>
<td>19</td>
<td>16</td>
<td>2.72</td>
<td>0.13</td>
</tr>
<tr>
<td>5</td>
<td>55.984° N 3.787° W</td>
<td>13.9</td>
<td>5.7</td>
<td>59</td>
<td>12</td>
<td>29</td>
<td>4.10</td>
<td>0.36</td>
</tr>
<tr>
<td>6</td>
<td>55.984° N 3.774° W</td>
<td>5.1</td>
<td>6.1</td>
<td>71</td>
<td>12</td>
<td>18</td>
<td>3.26</td>
<td>0.21</td>
</tr>
<tr>
<td>7</td>
<td>55.983° N 3.746° W</td>
<td>11.4</td>
<td>6.4</td>
<td>57</td>
<td>18</td>
<td>25</td>
<td>3.26</td>
<td>0.07</td>
</tr>
<tr>
<td>8</td>
<td>55.984° N 3.735° W</td>
<td>13.7</td>
<td>5.9</td>
<td>40</td>
<td>20</td>
<td>40</td>
<td>3.78</td>
<td>0.40</td>
</tr>
<tr>
<td>10</td>
<td>55.983° N 3.715° W</td>
<td>7.6</td>
<td>6.4</td>
<td>68</td>
<td>16</td>
<td>16</td>
<td>3.15</td>
<td>0.30</td>
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<tr>
<td>Forth &amp; Clyde Canal</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>55.871° N 4.257° W</td>
<td>29.1</td>
<td>6.6</td>
<td>26</td>
<td>56</td>
<td>18</td>
<td>6.01</td>
<td>ND</td>
</tr>
<tr>
<td>13</td>
<td>55.877° N 4.261° W</td>
<td>17.1</td>
<td>6.2</td>
<td>19</td>
<td>61</td>
<td>20</td>
<td>5.51</td>
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<tr>
<td>14</td>
<td>55.887° N 4.292° W</td>
<td>23.0</td>
<td>6.2</td>
<td>20</td>
<td>56</td>
<td>24</td>
<td>5.58</td>
<td>ND</td>
</tr>
<tr>
<td>15</td>
<td>55.939° N 4.152° W</td>
<td>22.3</td>
<td>5.8</td>
<td>17</td>
<td>55</td>
<td>28</td>
<td>5.07</td>
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</tr>
<tr>
<td>16</td>
<td>55.973° N 4.024° W</td>
<td>23.7</td>
<td>5.8</td>
<td>22</td>
<td>47</td>
<td>31</td>
<td>5.33</td>
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</tr>
<tr>
<td>17</td>
<td>56.002° N 3.844° W</td>
<td>21.7</td>
<td>5.7</td>
<td>15</td>
<td>38</td>
<td>47</td>
<td>5.22</td>
<td>ND</td>
</tr>
<tr>
<td>18</td>
<td>56.001° N 3.840° W</td>
<td>17.9</td>
<td>5.4</td>
<td>15</td>
<td>54</td>
<td>31</td>
<td>5.86</td>
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<tr>
<td>19</td>
<td>56.000° N 3.816° W</td>
<td>16.8</td>
<td>5.5</td>
<td>13</td>
<td>58</td>
<td>29</td>
<td>5.60</td>
<td>ND</td>
</tr>
<tr>
<td>20</td>
<td>56.010° N 3.786° W</td>
<td>23.7</td>
<td>5.8</td>
<td>17</td>
<td>59</td>
<td>24</td>
<td>4.97</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND = not determined
Table 2 Concentrations of total Hg, MeHg and EtHg, %MeHg and %EtHg in Union Canal (mean ± SD, n = 3) and Forth & Clyde Canal sediment samples (mean, two replicate values).

<table>
<thead>
<tr>
<th>Location</th>
<th>Total Hg (mg kg⁻¹)</th>
<th>MeHg (μg kg⁻¹)</th>
<th>%MeHg</th>
<th>EtHg (μg kg⁻¹)</th>
<th>%EtHg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Union Canal</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>35.3 ± 7.3</td>
<td>8.17 ± 2.1</td>
<td>0.023</td>
<td>ND</td>
<td>NC</td>
</tr>
<tr>
<td>5</td>
<td>1200 ± 180</td>
<td>10.8 ± 2.9</td>
<td>0.001</td>
<td>6.12 ± 1.0</td>
<td>0.0005</td>
</tr>
<tr>
<td>6</td>
<td>571 ± 70</td>
<td>6.11 ± 2.1</td>
<td>0.001</td>
<td>2.49 ± 1.1</td>
<td>0.0004</td>
</tr>
<tr>
<td>7</td>
<td>742 ± 94</td>
<td>18.6 ± 4.2</td>
<td>0.003</td>
<td>4.11 ± 1.0</td>
<td>0.0006</td>
</tr>
<tr>
<td>8</td>
<td>787 ± 220</td>
<td>9.93 ± 1.2</td>
<td>0.001</td>
<td>3.73 ± 0.6</td>
<td>0.0005</td>
</tr>
<tr>
<td>10</td>
<td>71.7 ± 8.2</td>
<td>6.02 ± 2.0</td>
<td>0.008</td>
<td>ND</td>
<td>NC</td>
</tr>
<tr>
<td><strong>Forth &amp; Clyde Canal</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>2.25 (2.23, 2.26)</td>
<td>8.83 (8.99, 8.68)</td>
<td>0.39</td>
<td>ND</td>
<td>NC</td>
</tr>
<tr>
<td>13</td>
<td>5.96 (5.91, 6.01)</td>
<td>12.5 (12.1, 12.8)</td>
<td>0.21</td>
<td>ND</td>
<td>NC</td>
</tr>
<tr>
<td>14</td>
<td>4.39 (4.36, 4.42)</td>
<td>10.5 (8.63, 12.30)</td>
<td>0.24</td>
<td>ND</td>
<td>NC</td>
</tr>
<tr>
<td>15</td>
<td>0.591 (0.589, 0.592)</td>
<td>3.44 (3.20, 3.68)</td>
<td>0.58</td>
<td>ND</td>
<td>NC</td>
</tr>
<tr>
<td>16</td>
<td>2.02 (1.94, 2.09)</td>
<td>4.68 (4.76, 4.60)</td>
<td>0.23</td>
<td>ND</td>
<td>NC</td>
</tr>
<tr>
<td>17</td>
<td>5.77 (5.75, 5.78)</td>
<td>14.1 (15.1, 13.1)</td>
<td>0.25</td>
<td>ND</td>
<td>NC</td>
</tr>
<tr>
<td>18</td>
<td>9.14 (9.09, 9.19)</td>
<td>9.69 (9.24, 10.2)</td>
<td>0.11</td>
<td>ND</td>
<td>NC</td>
</tr>
<tr>
<td>19</td>
<td>3.38 (3.38, 3.38)</td>
<td>9.70 (8.14, 11.3)</td>
<td>0.29</td>
<td>ND</td>
<td>NC</td>
</tr>
<tr>
<td>20</td>
<td>4.18 (4.13, 4.22)</td>
<td>6.08 (5.98, 6.18)</td>
<td>0.15</td>
<td>ND</td>
<td>NC</td>
</tr>
</tbody>
</table>

ND: not detected; NC: not calculated since concentration < LOD.
Table 3: Concentrations of Hg and MeHg found in previous studies.

<table>
<thead>
<tr>
<th>Location</th>
<th>Hg (mg kg(^{-1}))</th>
<th>MeHg (μg kg(^{-1}))</th>
<th>Correlation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scheldt Estuary, Belgium</td>
<td>0.144 to 1.19</td>
<td>0.8 to 6</td>
<td>(r = 0.82), (p &lt; 0.01)</td>
<td>Muhaya et al., 1997</td>
</tr>
<tr>
<td>Jiulong River Estuary, China</td>
<td>0.170 to 0.620</td>
<td>0.23 to 0.87</td>
<td>(r = 0.558), (p &lt; 0.05)</td>
<td>Wu et al., 2011</td>
</tr>
<tr>
<td>Fujian coast, China</td>
<td>0.0011 to 0.087</td>
<td>0.011 to 0.29</td>
<td>(r = 0.84), (p &lt; 0.01)</td>
<td>Zhang et al., 2013</td>
</tr>
<tr>
<td>Vigo Ria Peninsula, Spain</td>
<td>0.5 - 2</td>
<td>0.076-1.6</td>
<td>(r = 0.91), (p &lt; 0.05)</td>
<td>Canario et al., 2007</td>
</tr>
<tr>
<td>Pialassa Baiona Lagoon, Italy</td>
<td>0.2 to 250</td>
<td>0.13 to 45</td>
<td>(r = -0.65)</td>
<td>Trombini et al., 2003</td>
</tr>
<tr>
<td>Lenga Estuary, Chile</td>
<td>0.5 to 129</td>
<td>11 to 53</td>
<td>(r^2 = 0.0003)</td>
<td>Yanez et al., 2013</td>
</tr>
</tbody>
</table>