# MERCURY ALKYLATION IN FRESHWATER SEDIMENTS FROM SCOTTISH CANALS

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## 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 $kg^{-1}$) likely originate from historical munitions manufacture, with lower levels in the Forth & Clyde Canal (0.591–9.14 mg $kg^{-1}$). Concentrations of methylmercury (MeHg) were low – from 6.02 to 18.6 $\mu$g $kg^{-1}$ (0.001–0.023% of total Hg) in the Union Canal and from 3.44 to 14.1 $\mu$g $kg^{-1}$ (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.

**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 $\mu$g $kg^{-1}$ 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*

66 *al*., 2004). Reductive demethylation is mediated by Hg-resistant bacteria as part of their
67 mercury resistance (*mer*) system in both aerobic and anaerobic environments (Merritt
68 and Amirbahman, 2009; Barkey *et al.,* 2003). In the presence of Hg, these bacteria
69 express *mer* genes that encode for enzymes to degrade MeHg to $Hg^0$ which may then be
70 lost to the atmosphere (Barkey *et al*., 2003). Oxidative demethylation, which is also
71 carried out by both aerobic and anaerobic bacteria, is not considered a detoxification
72 process since $Hg^{II}$ is formed, which is still available to bacteria (Hintelmann, 2010).
73 Typically, the proportion of the total Hg content in sediment that is methylated
74 (%MeHg) is around 0.5% (Hines *et al*., 2000; Zelewski *et al*., 2001). Lower %MeHg has
75 been observed in freshwater with higher total Hg concentration (Schaefer *et al*., 2004). A
76 decrease in net methylation may be a result of lower microbial activity at high Hg
77 concentrations (Ullrich *et al.,* 2001). It has also been proposed (Schaefer *et al*., 2004)
78 that, in more contaminated environments, mercury-resistance (*mer*) genes are expressed
79 which regulate reductive demethylation, while lower levels of Hg are insufficient to
80 effectively induce expression of these genes. In addition to Hg content, sediment
81 parameters such as the presence of OM; sulfur and iron content and speciation; pH,
82 redox potential and texture all influence methylation (Frohne and Rinklebe, 2013; Frohne
83 *et al*., 2012; Ullrich *et al*., 2001). Due to its affinity for sulfur, $Hg^{II}$ in sediment can bind to
84 reduced S groups in OM, limiting its mobility and potential for methylation (Ravichandran,
85 2004). However, OM can also stimulate microbial activity and, as a consequence, MeHg
86 production, (Drott *et al.*, 2007). The ratio between dissolved organic carbon (DOC) and
87 total dissolved Hg concentration has been shown (Frohne *et al*., 2012) to be a critical
88 parameter influencing net Hg methylation in contaminated floodplain soils. Sulfide
89 concentration also affects Hg methylation rates. While sulfate-reducing bacteria promote
90 methylation, sulfides, produced from sulfate reduction, inhibit methylation due to the
91 formation of insoluble HgS or soluble charged sulfide complexes such as $HgS_2^{2-}$ and
92 $HgHS_2^-$ that cannot cross the cell membrane (Devai *et al*., 2007; Benoit *et al*., 1999). The
93 influence of iron on methylation is also variable: while ferric iron is a substrate for iron-

94 reducing bacteria and may enhance methylation, iron may also limit Hg solubility and
95 availability through the formation of iron-Hg complexes (Behra *et al*., 2001; Jeong *et al*.,
96 2007). Sediment pH affects mercury speciation and particle surface charge (Sarkar *et al.,*
97 1999) thus indirectly affecting Hg adsorption, bioavailability and consequently
98 methylation. Similarly, variations in redox potential may indirectly influence methylation
99 by affecting OM and sulfur and iron speciation and hence the adsorption and release of
100 Hg species (Frohne *et al*., 2012). Further discussion of factors affecting Hg methylation
101 can be found in Frohne *et al.* (2012).

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

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

118 This study investigated Hg concentrations and speciation in sediments from the
119 Forth & Clyde Canal, and the Union Canal. Relationships between Hg, MeHg, OM, S and
120 Fe content, sediment pH and texture were explored to gain insight into the factors
121 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 $HNO_3$ 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).

150 Determination of pH was performed (EN, 2003) using approximately 5 g (dried) test
151 portions and 0.01 M $CaCl_2.2H_2O$ (25 mL). Particle size distribution was determined by
152 sieving (BS, 2000b) and sedimentation (ASTM, 2007).

153 Determination of total Hg concentration in Union Canal sediment was performed
154 in Athens, Greece, using cold vapour atomic absorption spectrometry (CVAAS) (PE,
155 2006). Briefly, after microwave assisted digestion of 0.5 g test portions with 10 mL $HNO_3$
156 in a Berghoff Speedwave MWS-2 system, 10 mL DI water was added, then digests were
157 filtered and diluted to a final volume of 50 mL with further DI water. Analysis was
158 performed following reduction with 3% $NaBH_4$ using a MHS-10 Hg/Hydride system
159 (Perkin Elmer, Massachusetts, USA) operated in cold vapour mode. Determination of
160 total Hg concentration in Forth & Clyde Canal sediment samples was performed in
161 Glasgow, Scotland, using atomic fluorescence spectrometry (AFS). Test portions (1 g)
162 were digested in 5 mL $HNO_3$ using a CEM MARSXpress™ microwave-assisted digestion
163 system. Cooled vials were centrifuged (3000 rpm, 10 min) and a 2 g aliquot of the
164 supernatant (accurately weighed) was removed and diluted 10-fold to give a 10% $HNO_3$
165 solution. Analysis was performed using AFS (10.025 Millennium Merlin, PS Analytical,
166 Kent, UK) with 2% $Sn(II)Cl_2$ reductant. Determinations were carried out in triplicate.

167 The MeHg concentration was determined in fresh wet sediment by gas
168 chromatography-inductively coupled plasma-mass spectrometry (GC-ICP-MS) after
169 extraction with 4% w/w HCl (Bermejo-Barrera *et al*., 1999) and derivitization with $NaBPr_4$
170 (De Smaele *et al*., 1998). Briefly, 3 mL of 4% (w/w) HCl was added to approximately 1 g
171 of sediment. The samples were shaken mechanically (2 min), centrifuged (3000 rpm, 10
172 min) and the supernatant transferred to a glass vial. The process was repeated with a
173 further 2 mL of 4% (w/w) HCl to give a 5 mL combined extract. A 1 mL aliquot of this
174 extract was transferred to a new glass vial and 5 mL of 0.1 M acetate buffer solution
175 added. The pH was adjusted to 3.9 ± 0.1 using tetramethylammonium hydroxide (25%
176 w/w aqueous solution) and acetic acid, then 1 mL of isooctane was added followed by 1
177 mL of 1% (w/w) sodium tetra propylborate ($NaBPr_4$). 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 $\mu$L) 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 $\mu$g $L^{-1}$ Tl in 1% $HNO_3$) was used for ICP-MS and quantification was based on the response for the most abundant Hg isotope, $^{202}Hg$ (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) $HNO_3$) was prepared from a 1000 mg $L^{-1}$ Hg standard solution ($Hg(NO_3)_2$, 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 $CaCl_2.2H_2O$ solution (pH = 5.45) was prepared by dissolving 1.47 g of $CaCl_2.2H_2O$ (≥ 99%, ACS reagent, Sigma-Aldrich Company, Life Science Chemilab A.E., Athens, Greece) in distilled water and making up

206 to 1 L. The HCl (4% (w/w)) used in extraction of sediment samples was prepared from
207 30% HCl (for trace analysis) and the $HNO_3$ for washing glassware (> 65%, for trace
208 analysis) were both from Sigma-Aldrich Company Ltd. Dorset, UK. The derivitizing agent
209 $NaBPr_4$ (1% w/w) was prepared from $NaBPr_4$ (Chemos GmbH, Regenstauf, Germany) in
210 DI water and stored at −20 °C until use. The $NaBH_4$ reductant, a 3% solution in 1%
211 NaOH solution, was prepared daily using NaOH pellets (AR, Mallinckrodt, Dublin,
212 Ireland) and $NaBH_4$ powder (GR for analysis, Merck KGaA, Darmstadt, Germany). The
213 solution was filtered (glass fibre filters, Pall A/E Glass fibre filters 1.0 $\mu$m, 110 mm, Pall
214 GmbH,Dreieich, Germany) into a MHS-10 reductant vessel (Perkin Elmer,
215 Massachusetts, USA) before use. The $SnCl_2$ reductant, 2% in 10% HCl, was prepared
216 from $SnCl_2.2H_2O$ (98%, Alfa Aesar, Heysham, UK).
217
218 **2.4 Limits of detection and quality control**
219 The limit of detection (LOD) for Hg by CVAAS was 0.067 mg $kg^{-1}$. Recovery of Hg from
220 CRM BCR 320 R Channel Sediment containing 0.85 ± 0.09 mg $kg^{-1}$ Hg (Geel, Belgium)
221 (0.1 g test portions) was 116 ± 20.3% (n=3). For AFS, the LOD for Hg was 0.0484 mg $kg^{-1}$.
222 Recovery from CRM ERM-CC580 Estuarine Sediment containing 132 ± 3 mg $kg^{-1}$ total
223 Hg (Geel, Belgium) (0.02 g test portions) was 103% (average of 100%, 105%). For GC-
224 ICP-MS the LOD for $Me^{202}Hg$ was 1.16 $\mu$g $kg^{-1}$. The recovery of MeHg from CRM ERM
225 CC580 containing 75 ± 4 $\mu$g $kg^{-1}$ MeHg (0.1 g test portions) was 101% (average of
226 87.5%, 114%).
227
228 **3 Results and discussion**
229 **3.1 General sediment characteristics**
230 Information on the OM content, pH and particle size distribution of the sediment samples
231 are shown in Table 1, together with total Fe and S concentrations, where available. The
232 concentrations of OM in the Union Canal (5.1–13.9%) were lower than in the Forth &
233 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 $\mu$g $kg^{-1}$ to 18.6 ± 4.2 $\mu$g $kg^{-1}$, 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 $\mu$g $kg^{-1}$ and was not significantly correlated with total Hg concentration ($r^2 = 0.428$, $p > 0.05$) (Figure 4).

289 Net methylation has been found to increase with increasing total Hg concentration
290 at low (background) concentrations. By monitoring the distribution of an isotopically
291 enriched $^{202}Hg$ spike in mesocosms, Orihel *et al.* (2006) found a positive correlation ($r^2$ =
292 0.84) between MeHg production and $^{202}Hg^{II}$ in sediment where background Hg sediment
293 concentrations were 0.004–0.007 mg $kg^{-1}$. As shown in Table 3, positive correlation
294 between total Hg and MeHg concentrations has also been found in the field, including in
295 the Scheldt Estuary, Belgium (Muhaya *et al.,* 1997); the Jiulong River Estuary, China
296 (Wu *et al.,* 2011); along the Fujian coast, also China (Zhang *et al.,* 2013); and in
297 sediments of the Vigo Ria, Spain (Canario *et al.*, 2007). The latter study concluded that
298 MeHg concentrations were constant for Hg concentrations in the range 0.75–2.5 nmol $g^{-1}$
299 (0.15–0.5 mg $kg^{-1}$) but the two variables were significantly positively correlated at higher
300 concentrations.
301 At higher concentrations of Hg in sediment, different relationships with MeHg
302 concentration have been observed. For example, in a contaminated coastal lagoon in
303 Italy, Trombini *et al.* (2003) determined higher MeHg concentration in sediments with
304 lower total Hg concentration, whilst no correlation was found between the two species in
305 polluted sediments of the Lenga Estuary, Chile (Yanez *et al.,* 2013).
306 Estimated concentrations of EtHg in the Union Canal sediment were up to 6.12 ±
307 1.0 $\mu$g $kg^{-1}$. Besides MeHg, EtHg is the only other monoalkyl Hg compound so far
308 reported in the environment (Hintelmann, 2010). Unlike MeHg, it does not bioaccumulate
309 (Zhao *et al.*, 2012; Batsita *et al.*, 2011) and is not persistent (Hintelmann, 2010).
310 However, it may still play an important role in Hg cycling and so there is a need to
311 improve understanding of its environmental behaviour. The species has been identified
312 previously in industrially-contaminated sediments from the Kosseine River, Germany
313 (Hintelmann *et al*., 1995) where its presence was attributed to discharge of wastewaters
314 from a fungicide plant producing EtHg (Hintelmann *et al*., 1995). Two further studies have
315 reported the presence of EtHg in sediment, in the absence of nearby point sources.
316 Holmes and Lean (2006) found EtHg concentrations ranging from 0.3 ± 0.3 to 3.7 ± 0.5

317 $\mu$g $kg^{-1}$ in Canadian wetland sediments, where Hg concentrations ranged from 66.1 to
318 319 $\mu$g $kg^{-1}$. Cai *et al.* (1996) found that EtHg was widespread in the Florida Everglades,
319 with total Hg concentrations from 26.6 to 433 $\mu$g $kg^{-1}$ and EtHg concentrations from <
320 0.01 $\mu$g $kg^{-1}$ to 4.91 $\mu$g $kg^{-1}$. Biotic ethylation is not known to occur (Hintelmann, 2010)
321 and Cai *et al.* (1996) suggested that EtHg could have been produced by abiotic
322 (chemical) alkylation, similar to the alkylation reported when high-octane gasoline
323 containing tetraethyl lead ($PbEt_4$) was mixed with $HgCl_2$, producing ethylmercury chloride
324 (EtHgCl).

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

331

332 **3.2 Relationships between total Hg concentration, %MeHg and %EtHg**

333 In the Union Canal 0.001 to 0.023% of the Hg present was in methylated form. In the
334 Forth & Clyde Canal, %MeHg was greater and ranged from 0.11 to 0.58% of the total Hg
335 present. A strong negative correlation was found between total Hg concentration and
336 %MeHg in each canal (Figure 4) and there was a significant negative relationship
337 between total Hg concentration and %MeHg over all locations ($r^2 = 0.350$, $p < 0.05$, $n =$
338 15).

339 A low %MeHg has been reported in other contaminated environments. Schaefer
340 *et al*. (2004) observed an inverse relationship between total Hg concentration and
341 %MeHg ($r^2 = 0.804$, $p < 0.001$) in water at two freshwater sites, one affected by industrial
342 inputs (where total Hg concentration ranged from 113 to 4200 ng $L^{-1}$ and MeHg
343 concentration ranged from 0.08 to 1.6 ng $L^{-1}$) and one considered pristine (where total Hg
344 concentration ranged from 0.3 to 5.4 ng $L^{-1}$ and MeHg concentration ranged from 0.03 to

345 0.34 ng $L^{-1}$). The presence of *merA* genes and a high rate of reductive demethylation
346 ($K_{deg}$ = 0.19 $day^{-1}$) in the microbial community from the contaminated waters, compared
347 to the absence of *merA* genes and a low rate of oxidative demethylation ($K_{deg}$ = 0.01 $day^{-1}$)
348 in the microbial community from the uncontaminated waters, provided evidence that
349 MeHg degradation was directly related to $Hg^{II}$ concentration. It was proposed that, in
350 highly contaminated waters, mercury-resistance (*mer*) genes are expressed which
351 regulate reductive demethylation and that these genes are not expressed to the same
352 degree at lower levels of Hg.

353 An inverse relationship between total Hg concentration and %MeHg has also
354 been reported in freshwater sediment, both in spiked and natural sediment samples.
355 Microbial assays of freshwater sediment using radiolabeled MeHg (as $^{14}CH_3HgI$) at levels
356 between 15 and 2400 $\mu$g $kg^{-1}$ indicated that demethylation rate increased with increasing
357 Hg concentration (Marvin-DiPasquale *et al.*, 2000). Similarly, in river sediments in
358 Kazakhstan where total Hg concentration ranged from 9.95 to 306 mg $kg^{-1}$ and MeHg
359 accounted for < 0.1% of this on average, a strong inverse relationship between total Hg
360 concentration and %MeHg was found ($r = 0.761$, $p < 0.001$) (Ullrich *et al*., 2007). It was
361 proposed that, although MeHg production is controlled by total Hg concentrations where
362 these are low, in contaminated sediment, net methylation is limited not through the
363 inhibition of methylation but rather because bacterial demethylation is more efficient.

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

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

371

372 **3.3 Influence of sediment characteristics**

373 The OM content was not significantly correlated with MeHg concentration in either canal
374 ($r^2$ = 0.317, $p > 0.05$ for the Union Canal and $r^2$ = 0.125, $p > 0.05$ in the Forth & Clyde
375 Canal). Microbial activity can be stimulated by nutrient release from the degradation of
376 OM and many studies have found a positive relationship between MeHg concentration
377 and OM content in sediment (Hammerschmidt *et al.,* 2008; Muhaya *et al*., 1997; Choi
378 and Bartha, 1994). If, however, the organic compounds generated form complexes with
379 $Hg^{II}$, the effect on methylation rate is variable. In some cases, bioavailability is reduced,
380 yielding a negative relationship between OM content and methylation (Barkey *et al.,*
381 1997) whilst, in others, it is increased (Schaefer and Morel, 2009). Indeed, even at a
382 single location, an increase or a decrease in Hg methylation with OM content may be
383 observed, depending on the time of year (Liang *et al*., 2013). A stronger correlation was
384 found between EtHg concentration and OM content ($r^2$ = 0.598) based on the four
385 locations within the Union Canal where this species was detected, however this was not
386 significant ($p > 0.05$). Data from the study of Holmes and Lean (2006) show a similar
387 correlation ($r^2$ = 0.560 for OM concentrations in the range 9–90%) but a weaker
388 relationship ($r^2$ = 0.183 for sediments containing 9-94% OM) is indicated by the data of
389 Cai *et al*., (1996).

390 The pH of the canal sediments is within the range where $Hg^{II}$ adsorption is
391 favored (pH 4–10) (Lui *et al.,* 2012). In the Union Canal, there was a significant negative
392 correlation between total Hg concentration and increasing pH over the range 5.7–6.9 ($r^2$
393 = 0.745, $p < 0.05$) but no significant correlation between these parameters was observed
394 in the Forth & Clyde Canal ($r^2$ = 0.120, for a pH range 5.4–6.6). No relationship between
395 pH and MeHg concentration was observed in either the Union Canal or the Forth & Clyde
396 Canal ($r^2$ = 0.003 and 0.009 respectively). A decrease in %MeHg with decreasing pH ($r^2$
397 = 0.778, $p < 0.05$) was observed in sediment of the Union Canal but correlation was
398 weak in the Forth & Clyde Canal ($r^2$ = 0.0716). This decrease in %MeHg at lower pH in
399 the Union Canal arises because the total Hg concentration increased, while MeHg
400 concentration remained largely unaffected.

401 Changes in pH may affect methylation both directly and indirectly by affecting
402 adsorption and partitioning. For example, in laboratory experiments, a decrease in
403 methylation by over 65% was observed in lake sediment that had been spiked with
404 isotopically-labelled Hg and acidified from an initial pH of 6.1 to pH 4.5 (Steffan *et al*.,
405 1988). Partitioning of MeHg into the water column is also important since MeHg is more
406 soluble at low pH (Miller and Akagi, 1979). Sediment pH and EtHg in the Union Canal
407 were not significantly correlated ($r^2 = 0.305$, $p > 0.05$). Since ethylation is not microbially
408 mediated, any effect of pH on EtHg is likely to result from an alternation in EtHg
409 partitioning.

410 Despite the high affinity of Hg for sulfur, it seems to play a minor role in Hg
411 adsorption in the Union Canal; neither total Hg nor MeHg concentrations were strongly
412 correlated with sediment sulfur content ($r^2 = 0.140$, $p > 0.05$ and $r^2 = 0.181$, $p > 0.05$
413 respectively). Similarly, no relationship was found between S and total Hg content in
414 contaminated floodplain soil from the Wupper and Saale River, Germany (Frohne and
415 Rinklebe, 2013). In that case a significant, positive correlation ($r^2 = 0.45$, $p < 0.005$) was
416 found between total Hg concentration and Fe content, which was also found in the Union
417 Canal sediments ($r^2 = 0.809$, $p < 0.05$). No significant correlation was observed between
418 iron and MeHg ($r^2 = 0.034$, $p > 0.05$) although a positive relationship has been observed
419 between the two parameters in a biogeochemical microcosm system, using
420 contaminated floodplain soil from the Wupper River ($r^2 = 0.08$, $p < 0.05$) (Frohne *et al*.,
421 2012). Sulfur content was not measured in the Forth & Clyde Canal samples, but no
422 significant relationships between total Hg and Fe, or between MeHg and Fe, were found
423 ($r^2 = 0.118$, $p > 0.05$ and $r^2 = 0.0137$, $p > 0.05$ respectively). No significant relationships
424 were observed between the proportion of sediment particles in any of the size fractions
425 and either total Hg or MeHg concentration in either canal.

426

427 **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 $Hg^0$ 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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460

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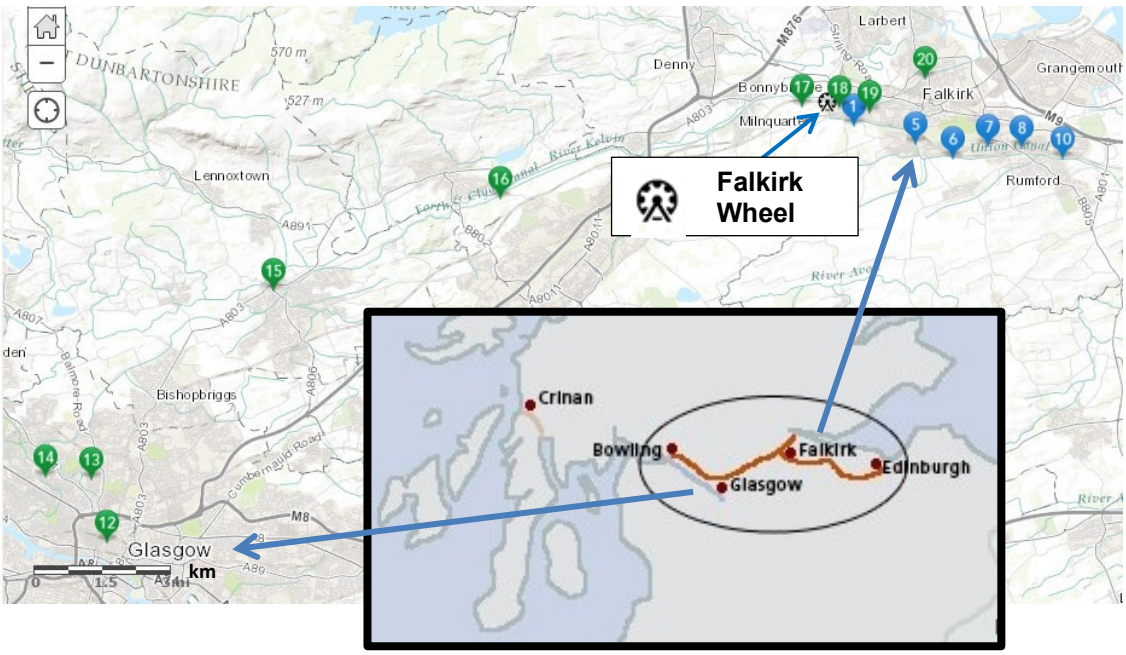
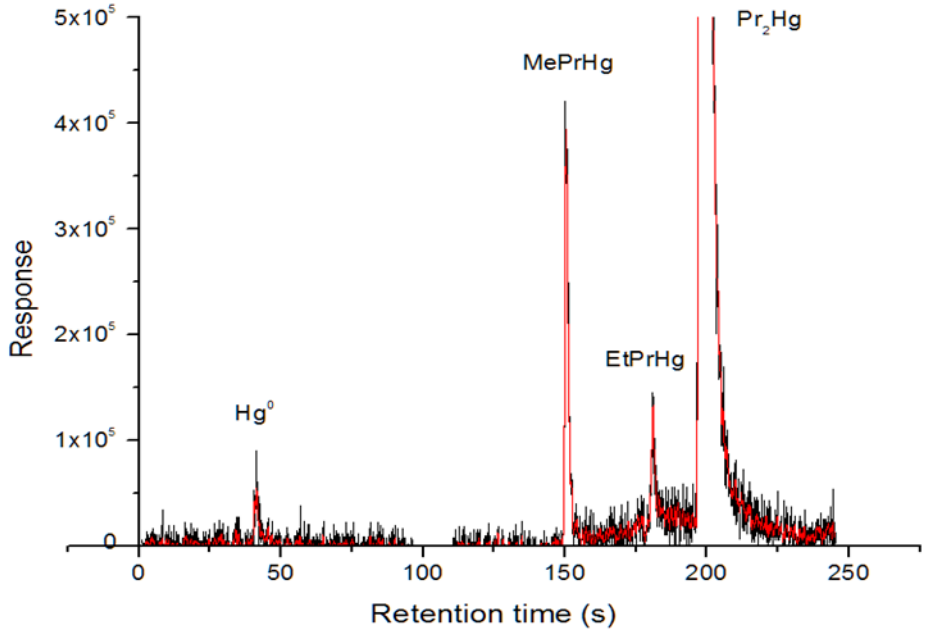



Figure 1 Location of sampling points on the Union Canal (sampling points 1–10) and Forth & Clyde Canal (sampling points 12–20), UK, and the Falkirk Wheel (which connects the two canals).



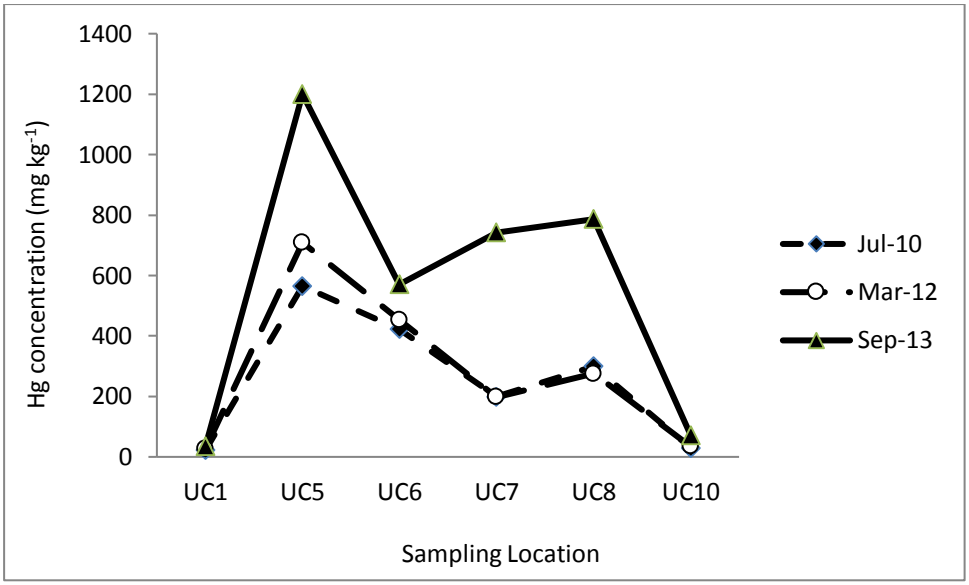

666

667 Figure 2 GC-ICP-MS chromatogram showing mercury species (as the propyl derivatives except

668 for $Hg^0$) detected at location five in the Union Canal, Scotland, UK.

669

670

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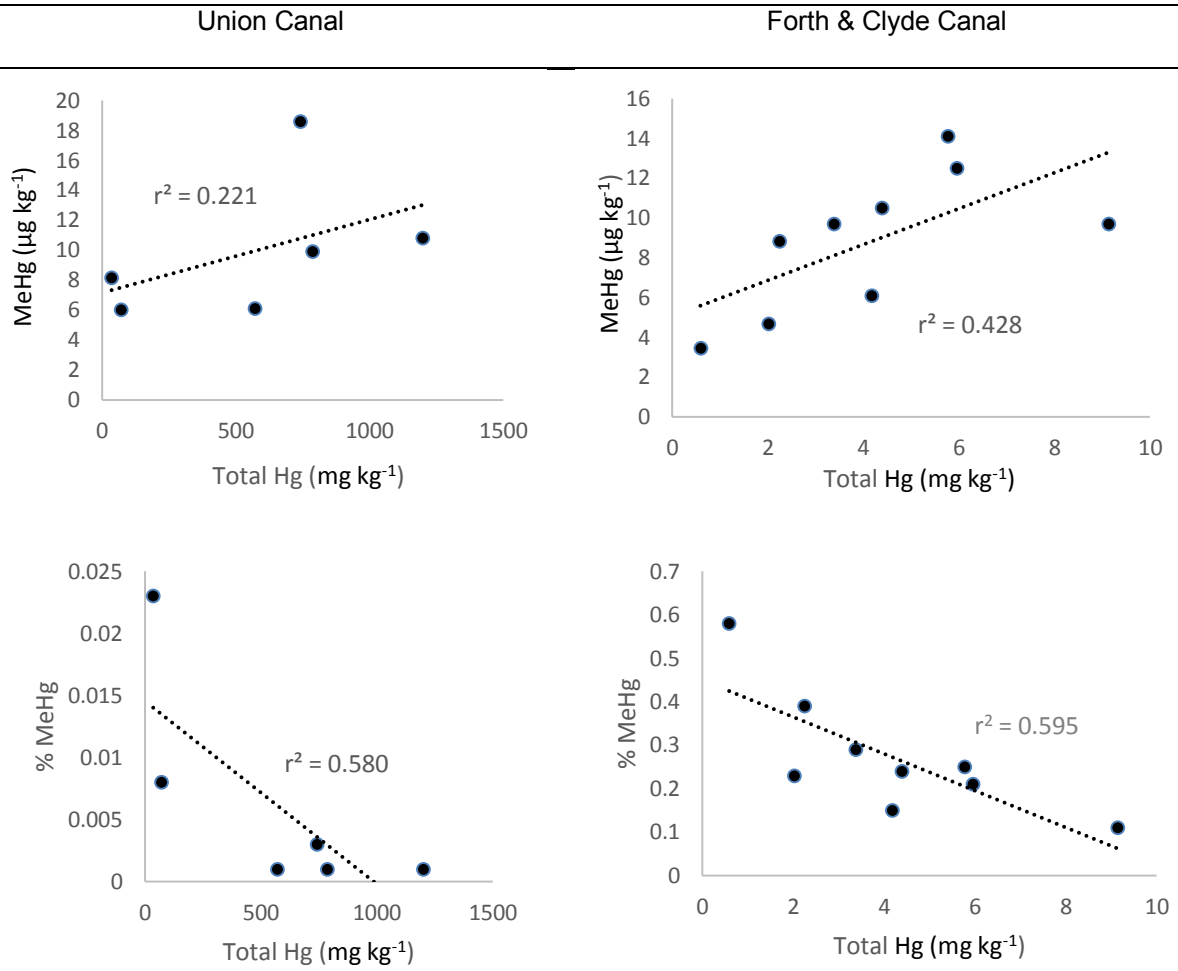
672 Figure 3 Temporal trends in Hg concentration in the Union Canal (UC), Scotland, UK.




673 Figure 4 Relationships between total Hg concentration, MeHg concentration, and %MeHg in
674 sediments from the Union Canal and the Forth & Clyde Canal, Scotland, UK.

675

676 Table 1 Characteristics of sediment samples from the Union Canal and Forth & Clyde Canal.

| Location | Latitude and Longitude | OM (%) | pH | Sand (%) | Silt (%) | Clay (%) | Fe (%) | S (%) |
|---|---|---|---|---|---|---|---|---|
| Union Canal | | | | | | | | |
| 1 | 55.996° N 3.830° W | 7.6 | 6.9 | 66 | 19 | 16 | 2.72 | 0.13 |
| 5 | 55.984° N 3.787° W | 13.9 | 5.7 | 59 | 12 | 29 | 4.10 | 0.36 |
| 6 | 55.984° N 3.774° W | 5.1 | 6.1 | 71 | 12 | 18 | 3.26 | 0.21 |
| 7 | 55.983° N 3.746° W | 11.4 | 6.4 | 57 | 18 | 25 | 3.26 | 0.07 |
| 8 | 55.984° N 3.735° W | 13.7 | 5.9 | 40 | 20 | 40 | 3.78 | 0.40 |
| 10 | 55.983° N 3.715° W | 7.6 | 6.4 | 68 | 16 | 16 | 3.15 | 0.30 |
| Forth & Clyde Canal | | | | | | | | |
| 12 | 55.871° N 4.257° W | 29.1 | 6.6 | 26 | 56 | 18 | 6.01 | ND |
| 13 | 55.877° N 4.261° W | 17.1 | 6.2 | 19 | 61 | 20 | 5.51 | ND |
| 14 | 55.887° N 4.282° W | 23.0 | 6.2 | 20 | 56 | 24 | 5.58 | ND |
| 15 | 55.939° N 4.152° W | 22.3 | 5.8 | 17 | 55 | 28 | 5.07 | ND |
| 16 | 55.973° N 4.024° W | 23.7 | 5.8 | 22 | 47 | 31 | 5.33 | ND |
| 17 | 56.002° N 3.844° W | 21.7 | 5.7 | 15 | 38 | 47 | 5.22 | ND |
| 18 | 56.001° N 3.840° W | 17.9 | 5.4 | 15 | 54 | 31 | 5.86 | ND |
| 19 | 56.000° N 3.816° W | 16.8 | 5.5 | 13 | 58 | 29 | 5.60 | ND |
| 20 | 56.010° N 3.786° W | 23.7 | 5.8 | 17 | 59 | 24 | 4.97 | ND |

ND = not determined

677

678 Table 2 Concentrations of total Hg, MeHg and EtHg, %MeHg and %EtHg in Union Canal (mean ±

679 SD, n = 3) and Forth & Clyde Canal sediment samples (mean, two replicate values).

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

ND: not detected; NC: not calculated since concentration < LOD.

680

681 Table 3: Concentrations of Hg and MeHg found in previous studies.

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

682

683