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ABSTRACT

Chlor–alkali plants (CAP) are recognized as major sources of mercury (Hg) in the environment. In this work, Hg concentration, speciation and isotopic signature were determined in sediments and biota (fish and oyster) from Sagua La Grande River (SG River) and the adjacent coastal zone in the vicinity of a CAP (Cuba). High Hg concentrations in surface sediments (up to 5072 ng g^{-1}), mainly occurring as inorganic Hg, decrease with the distance from the CAP along the SG River and seaward. Meanwhile, Hg concentration and speciation in riverine catfish *(Claria gariepinus)* muscle (1093 ± 319 ng g^{-1}, ˜70% as MeHg) and coastal oysters *(Crassostrea rizophorae)* (596 ± 233 ng g^{-1}, ˜50% as MeHg) indicate a direct impact from CAP. Hg isotopic signature in sediments, following both mass dependent (MDF) and mass independent fractionation (MIF), exhibits a clear binary mixing between CAP pollution (+0.42‰, δ^{202}Hg; -0.18‰, Δ^{201}Hg) and regional background end-member (˜ -0.49‰, δ^{202}Hg; +0.01‰, Δ^{201}Hg). The combination of speciation and isotopic information in biota and sediments allows to trace Hg contamination pathways from contaminated sediments to the biota, establishing the importance of both methylation and demethylation extent in both river and coastal sites before Hg species bioaccumulation.

1. Introduction

Chlor–alkali plants (CAP) which use mercury (Hg) in electrolytic cell manufacture has been identified as one of the major sources of Hg pollution. Although alternative methods were established to replace the Hg-cell process [1], many older plants are still in operation in some undeveloped areas [2]. Due to the large amounts, Hg contamination caused by released legacy Hg is still an environmental threat in nowadays and future even when Hg-cell technology is eliminated from CAP [1,3,4]. High Hg concentrations have been reported in sediments and some carnivorous fish (reaching values up to 100 µg g^{-1} and 5000 ng g^{-1}, respectively) from different reservoirs and rivers affected by CAP [5–12]. In the aquatic ecosystems, Hg mainly exists as inorganic Hg (iHg) in sediments and water. However, a small fraction of iHg can be transformed into methylmercury (MeHg), a neurotoxin for human and wildlife health, mainly through biotic or microbial processes [13,14] and minorly through abiotic processes [15]. MeHg gains great concerns not only due to its toxicity but also considering its efficient bioaccumulation and biomagnification through the food web in comparison to iHg [15,16].

The quantification of Hg and its species have been effectively used to evaluate regional environmental pollution [6,8,17]. More recently Hg isotopic composition has been widely applied in the identification, discrimination and quantification of Hg anthropogenic sources and biogeochemical processes before its introduction into the food web [5,18–23]. Hg isotopic signature has also been used to study the Hg isotopic fractionation during Hg trophic transfer [24–26] and metabolic processes *in vivo* from aquatic organisms to humans [26–30]. Hg fractionates as mass dependent fractionation (MDF) and mass independent fractionation (MIF). MDF can be induced during multiple biogeochemical processes such as methylation [31,32], demethylation [33,34] and reduction [34,35]. MIF in aquatic ecosystems is mainly

recognized to occur during photo-reaction (photo-reduction/demethylation), which has been successfully exploited for tracking Hg sources [34]. In addition, one study has suggested that MIF effect may occur during photochemical oxidation of Hg in the upper atmosphere [36].

Sediments act as an important sink for anthropogenic Hg pollution (e.g. industry, mining, urban discharges, atmospheric deposition, etc). In sediments, total Hg isotopic composition has been reported as the sum of both anthropogenic and background contributions, whose isotopic composition can be differentiated [22,37]. However, in highly contaminated sediment, the Hg isotopic compositions are dominated by the contamination source as a result of pool size effect [38,39]. Despite that sediments represent the main Hg source to the surrounding food web, biota may display a different Hg isotopic signature according to the trophic position as shown in previous studies [5,18–20,23]. Higher MDF values are observed in biota compared to their nearby sediments in most of the investigated Hg contaminated lakes, coastal oceans or reservoirs ecosystem [5,12,18–20], which were in contrast to one recent study in a stream contaminated by historical gold mining [23]. The mentioned MDF variations are probably related to biogeochemical processes such as methylation/demethylation, but also to environmental aspects (turbidity, water flow rate, etc) in each specific system [5,18–20,23]. MIF extent in contaminated sediment is generally close to zero [5,11,12,18–20,23]. However, a wide range of positive MIF values is observed in biota, which is attributed to the MIF signature of MeHg inherited from photo-degradation [34]. Though rarely exception has been observed in which slightly negative MIF of MeHg have been found in aquatic biota, suggesting nearly absence of photo-degradation in certain habitats [40]. To date, a better comprehension of the biogeochemical fate of Hg species can be established in contaminated sites by combining MDF and MIF signatures, while several hypotheses still need to be validated among different impacted ecosystems.

Sagua la Grande River (SG River), Cuba, hosted a CAP with Hg-cell technology since early 1980s [10] and still working [2]. Despite a wastewater treatment system that installed to trap Hg in 1990 [7,9,10] and Hg-immobilized sludge is disposed in concrete niches [2], due to its low efficiency, Hg contaminations in the sediments remain in the river and even extend to the coastal zone [9,10]. Hg levels in omnivorous fish from the mentioned ecosystem reach values up to 700 ng g^{-1} (d w).

In this work, Hg concentration, speciation and isotopic composition were determined in selected surface sediments and biota samples which are frequently consumed by local populations, including catfish (*Claria gariepinus*) from the riverine section and oysters (*Crassostrea rizophorae*) from the coastal zone. The same fish species from Mampostón Dam (M Dam, fish farming away from SG River, Cuba), was used as a control. The main objective of this work is to estimate the Hg contamination extent and its fate from the CAP (SG River) by combining its speciation and isotopic composition in both sediments and biota. The obtained data is also compared to other Hg contaminated aquatic ecosystems investigated by using a similar approach.

2. Materials and methods

2.1. Study area and sampling details

Monitoring was performed from 2008 to 2010. Sediments from six sampling sites along SG River (Fig. 1, Table S1, Table S2) were collected: Ss(0), located at 2.54 km upper from the confluence of the old CAP waste discharge stream within SG River, was considered a river background ("SG River background"). Three sampling sites were selected as "SG River Upper": Ss(1) is in the vicinity of the old CAP discharge to evaluate the influence of historical pollution and Ss(2) ~ Ss(3) are close to the CAP's new discharge point as indicators of CAP activi-

ties nowadays. Ss(4) ~ Ss(5) sites are considered as "SG River Lower". In the coastal zone (Fig. 1, Table S2), located at around 12 km from the SG River, including river estuary and some small keys near the coast, three sediments samples Sc(1) ~ Ss(3) were collected.

Regarding biota, fish samples were collected close to the sediments' stations. Three wild catfish individuals (Fs(1)~Fs(3)) (Table S3) were caught randomly in SG River between sites Ss(3) and Ss(5). Considering that catfish is not a sedentary species [41], Hg concentration in such animals should not be consider as a specific indicator of Hg in the precise sampling site. Oysters samples (Oc(1)~Oc(3)) represent a pool of several individuals which were corresponding to each coastal sediment sampling sites (Fig. 1, Table S3) from the mangrove roots or local oysters' farms. The soft tissues of oyster are selected for analysis. In M Dam, an artificial lake, five catfish individuals (Fm(1)~Fm(5)) were collected directly from farms. Animals from this location are fed with bycatch product prepared for fish farming [42,43], and therefore, considered as an unpolluted biota control (Table S3).

2.2. Hg speciation analysis

Sample digestion was carried out with TMAH (tetramethylammonium hydroxide) and HNO$_3$ for biota and sediments, respectively, in an analytical microwave and analyzed by GC-ICP-MS as detailed elsewhere [44–46]. Quantification of Hg species was carried out using species specific isotope dilution by adding the adequate amount of isotopically enriched Hg standards (^{199}iHg and ^{201}MeHg) and by applying isotope pattern deconvolution for data treatment [44,45]. Reference materials were analyzed following the same strategy, for method validation (Table S4).

2.3. Hg isotopes analysis

Sediments samples (0.2-0.5 g) were digested at 95 °C for 3 h in a Hot Block, (Environmental Express), using 5 mL fresh mixture of HCl and HNO$_3$ (3:1, v:v). Biological samples, fish and oysters (0.2-0.5 g), were digested with 5 mL HNO$_3$ in HPA (High Pressure Asher, Anton-Paar), followed by the addition of 1.5 mL H$_2$O$_2$ adding to the obtained extract [47]. The yield of THg extracted ranged between 80%–120% (Table S9). Hg isotopic composition was determined by cold-vapor MC-ICP-MS as detailed elsewhere [5].

MDF is reported as δ^{202}Hg in permil (‰) related to NIST SRM 3133 and calculated as: [48]

$$\delta^{202}Hg = \left\{ \left[(^{202}Hg/^{198}Hg)_{sample}/(^{202}Hg/^{198}Hg)_{NIST3133} - 1 \right] \right\} \times 1000 \tag{1}$$

MIF represents the difference between the measured δ^{xxx}Hg value and the value predicted in theoretical based on MDF law [48]. MIF is thus reported as capital delta notation as suggested elsewhere [48], according to the following equations:

$$\Delta^{199}Hg = \delta^{199}Hg - (\delta^{202}Hg \times 0.2520)$$
$$\Delta^{200}Hg = \delta^{200}Hg - (\delta^{202}Hg \times 0.5024)$$
$$\Delta^{201}Hg = \delta^{201}Hg - (\delta^{202}Hg \times 0.7520)$$
$$\Delta^{204}Hg = \delta^{204}Hg - (\delta^{202}Hg \times 1.4930) \tag{2}$$

We use Δ^{201}Hg as the default value to report MIF in this study.

The analytical uncertainty is assumed as 2 times the standard deviation (2SD) from replicate analysis of the UM-Almadén secondary standard during each analytical session. Sediments (IAEA 405) and fish muscle (BCR 464) reference materials were used for data validation (Table S5).
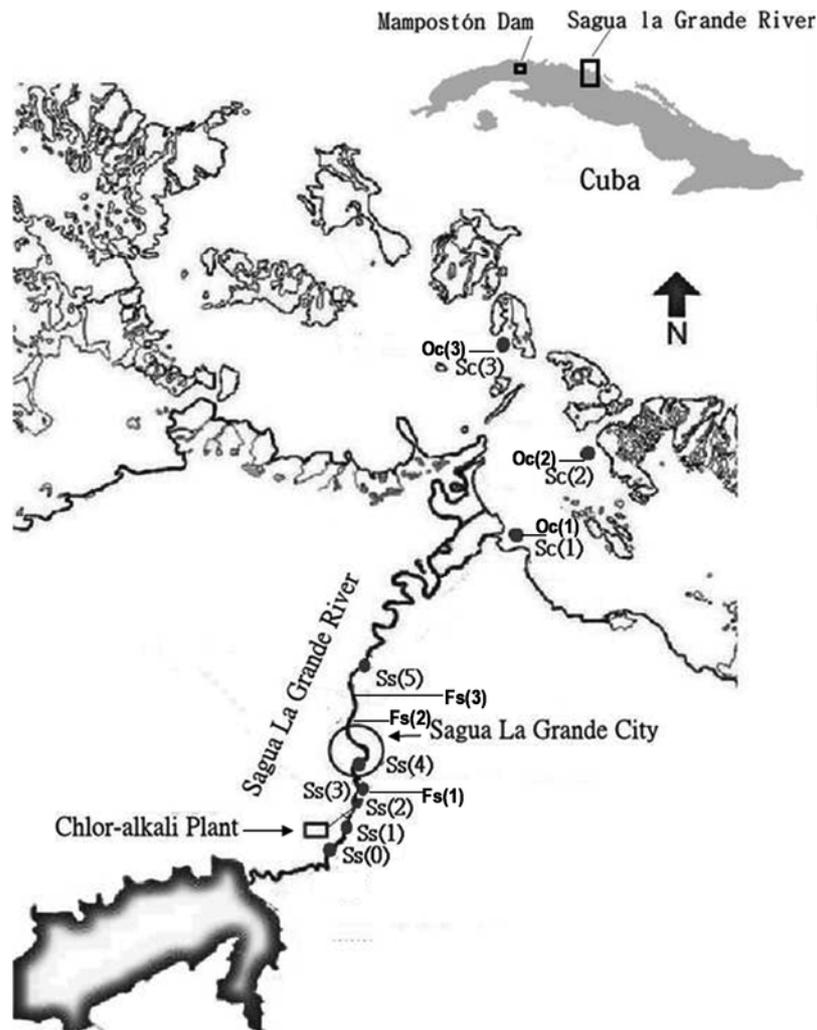
Fig. 1. Study area: Sagua la Grande River (SG River) and the adjacent (12 km) coastal zone (Cuba). Sediments sampling sites Ss(0) to Ss(5) are within the SG River, including Ss(1) in the vicinity of old CAP discharge, and Ss(2) and Ss(3) that are close to the new CAP discharge. Sc(1) to Sc(3) are in the coastal zone. Fish samples were collected in SG River, oyster samples were collected in coastal zone.

3. Results and discussions

3.1. Hg speciation and distribution in sediments and biota

3.1.1. Sediments

Total Hg (THg) concentrations in sediments exhibit a wide variability along the SG River (Fig. 2a, Table S6), decreasing from the CAP discharge point to the coastal zone. Meanwhile SG River background site (Ss(0)) exhibits the lowest THg concentration (149 ± 6 ng g^{-1}). Higher THg concentrations are observed in the SG River "upper zone", close to the new (Ss(2)) and the old (Ss(1)) CAP discharge points (5072 ± 34 ng g^{-1} and 1788 ± 36 ng g^{-1}, respectively). THg concentrations in the sediments from SG River "lower zone" are relatively lower, ranging from 196 to 764 ng g^{-1}, which are within the same range as in sediments from the coastal zone (from 165 to 301 ng g^{-1}). In the coastal area, the estuarine site Sc(1) displays higher THg concentration at the river mouth compare to farther coastal sites. This clearly indicates the strong contribution of the SG River on Hg distribution along the coastal zone.

As presented in Table 1, highest Hg levels in sediments from SG River are comparable to the other contaminated sites such as Bratsk Water Reservoir (Russia), in the vicinity of a CAP [5]. Relatively lower

THg concentrations in sediments from SG River lower portion and the coastal zone nearby are similar to those found in estuaries sediments from Northeastern Coast of U.S.A. impacted by local wastewater and industrial discharges [20]. THg content and distribution pattern from SG River to the coastal zone is similar to the one recently reported at Yuba River [23].

MeHg concentrations in the sediments display a similar pattern to THg along SG River and the coastal zone (Fig. 2a, Table S6). The maximum value (16 ± 4 ng g^{-1}) is observed at the CAP wastewater discharge point (Ss(2)) and relatively high values (~ 6 ng g^{-1}) are observed in the old discharge point (Ss(1)) and lower site (Ss(5)). All the other sites, including the river background Ss(0) and the coastal zone, exhibit similar MeHg concentration around 2 ng g^{-1}. The fraction of THg present as MeHg (referred as % MeHg hereafter) is approximately 0.33% in sediments along SG River (Ss(1) to Ss(4) sites). It is found within the range ($< 0.5\%$ MeHg) of estuarine and marine sediments reported by Ullrich et al [15]. Higher %MeHg is observed in coastal zone sediments (Sc(1) to Sc(3)), where it can reach up to 1%. Prominent %MeHg results are observed in the SG River background site (Ss(0)) and lower site (Ss(5)) exhibiting 1.7% and 3.1%, respectively. Organic matter (OM) plays an important role on Hg methylation [15]. High OM levels are associated to a decrease of sediments oxygenation, which enhances Hg methylation by anaerobic bacteria, and consequently MeHg accu-
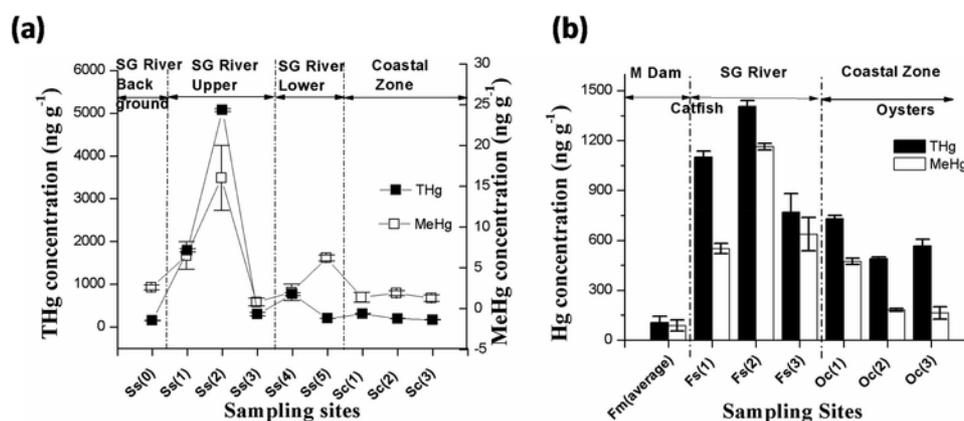
Fig. 2. Hg distribution and speciation in **a)** sediments and **b)** biota along SG River, coastal zone and M Dam.

mulation in biota [15]. In this study, OM (12 ± 2%) (Table S7), is quite homogenous among the sediments and insignificantly correlated with %MeHg (r = 0.563, p = 0.114, n = 9) (Table S8). Therefore, the high %MeHg in the mentioned sites should not be attributed to OM, but related to other environmental parameters (temperature, pH, redox potential).

3.1.2. Biota

THg average concentration in catfish muscle from SG River is $1093 \pm 319 \, ng \, g^{-1}$ (Fig. 2b, Table S6), which is approximately 10 times higher than in fish from the control site (M Dam: $107 \pm 36 \, ng \, g^{-1}$), though fish size and weight are similar in the two sampling sites. Regarding Hg levels in oysters from the coastal site, it varies between 491 and $732 \, ng \, g^{-1}$. Hg content in biota samples of the current study exhibits similar or even higher values than in other polluted areas (Table 1). As expected, %MeHg varies according to both, the organism trophic level and its habitat. For catfish, %MeHg in SG River (72 ± 19%) is not significantly different from M Dam control site (80 ± 12%) (p = 0.456). In contrast, due to their lower trophic position, %MeHg in oyster tissues is less than 50%. The difference of %MeHg in biota reflects both, the different extents of bioaccumulation between iHg and MeHg through the food web and the significant intake of iHg due to its higher exposure level in contaminated sites [5,26].

3.2. Hg isotopic composition in sediments and biota

3.2.1. Sediments Hg isotopic composition

3.2.1.1. MIF isotopic signature in sediments
MIF signatures exhibited by sediments from SG River and the coastal zone are close to zero ($\Delta^{201}Hg$, −0.05 ± 0.06‰) (Figs. 3a, 4). It should be noticed that the lowest $\Delta^{201}Hg$ value (-0.18 ± 0.08‰) corresponds to the sampling site (Ss(2)) which has a slight offset (-0.14‰) from the other sites (-0.04 ± 0.04‰). Considering the proximity of the mentioned sampling point to the CAP and that it exhibits the highest Hg concentration, Hg levels reported in soils close to the CAP are relatively low (in the range of 1–170 ng/g with average value of 34 ± 47 ng/g) [49], it seems that this negative MIF correspond to the Hg CAP discharge. Element liquid Hg is used in CAP electrolysis process. Hg could be discharged into environment through wastewater, solid waste (sludge), and the atmosphere in the form of Na−Hg amalgam, Hg(II) ($HgCl_2$, $HgSO_4$ and HgO etc), and Hg (0) during the CAP production process [2,50,51]. The processes, including evaporation of elemental Hg, Na−Hg amalgam formation and decomposition, the oxidative transformation from Hg(0) to Hg(II), Hg binding to organic matter and sulfide phases in the contaminated sediments, will cause Hg undergo MDF and MIF and record Hg isotopic signature in sediments [11]. The slight negative MIF value observed in sediments may be related to the element Hg evaporation

process, which is accompanied by MIF caused by nuclear volume fractionation [52]. The variation of $\Delta^{201}Hg$ values among the adjacent sampling sites remains insignificant (<0.1‰) compared to the analytical uncertainty. However, a decreasing trend of $\Delta^{201}Hg$ values from background riverine and coastal sites to most polluted sites is still observed, indicating the pollution from CAP to the pristine site (Fig. 3a). It should be mentioned that the MIF signature in sediments in the vicinity of SG CAP ($\Delta^{201}Hg$: −0.05 ± 0.13‰) matches the ones found in the area of the CAP at Bratsk Water Reservoir ($\Delta^{201}Hg$: − 0.02 ± 0.09‰) [5], Brackish Water at Skutskär and Köpmanholmen sites in Sweeden ($\Delta^{201}Hg$: − 0.02 ± 0.02‰) [11] and Augusta Bay in Italy ($\Delta^{201}Hg$: − 0.02 ± 0.03‰) [12].

3.2.1.2. MDF isotopic signature in sediments
MDF values ($\delta^{202}Hg$) in sediments (Fig. 3) vary significantly, exhibiting a shift of approximately 1‰ (from −0.51‰ to 0.42‰) between the coastal zone and SG River upper portion. This variation is slightly larger than the one observed at Yuba River sediments, where $\delta^{202}Hg$ decrease from −0.18‰ to −0.95‰ along the river [23]. The significant decrease trend of MDF values from SG River upper portion to the coastal zone is in good agreement with THg distribution (Fig. 3b). The highest positive $\delta^{202}Hg$ value corresponds to the CAP closest site (Ss(2)), which also exhibits the highest Hg concentration. Lower MDF isotopic signatures are observed in both SG River background end-member (upstream to the CAP) and the coastal zone, exhibiting negative $\delta^{202}Hg$ values down to −0.48 and −0.51‰, respectively. The $\delta^{202}Hg$ variations in sediments are attributed to the Hg transport and consequently to the distance from the CAP discharge point (Fig. 3a). In order to confirm the main influence of the CAP inputs, $\delta^{202}Hg$ values were plotted against 1/THg concentrations [39] in sediments (Fig. 3b). The resulting graphic exhibits a linear negative correlation (r = −0.675, p < 0.05) which demonstrates a binary mixing relationships between a background end-member (both SG river background and coastal end-members) and the main Hg anthropogenic source (Fig. 3b). Taking these results, by using a simple binary mixing model [39], CAP contribution to the THg content in sediments along the river to coastal transect is estimated as 60–65%, 1–28%, and 2–38% in SG River upper portion, lower portion and coastal sediments.

An abnormal MDF signature is observed in the site Ss(5), being higher than in the nearby upper site Ss(4). In order to discriminate different Hg sources, elements compositional characteristic of the sediments was compared (see details in Supplementary Information). The distribution of conservative lithogenic tracers (e.g. Ti) usually reflects mineralogical origins and grain size distribution of the sediments. The $\delta^{202}Hg$ relative to Hg content normalized by Ti, clearly reveals a difference between Ss(5) site and the other sites (Fig. S2). Therefore such anomaly in the isotopic composition (0.26‰) may result from the contribution of other Hg sources. Considering that Cu, Pb and Zn concen-

Table 1

Hg concentration, speciation and isotopic composition in different Hg contaminated areas.

Polluted Source	ID	Samples		THg	%MeHg	δ^{202} Hg	Δ^{199} Hg	Δ^{201} Hg	N	Reference
				ng g^{-1}(dw)	%	‰	‰	‰		
Chlor–alkali plants	Bratsk Water Reservoir	Fish	Perch	5768 ± 5427	92 ± 4	−0.55 ± 0.45	0.52 ± 0.50	0.39 ± 0.41	12	Perrot et al. (2010)
				(3197˜11676)	(82˜94)	(−0.90˜−0.16)	(0.20˜1.04)	(0.13˜0.79)		
			Roach	1819 ± 2223	94 ± 4	−0.37 ± 0.30	1.19 ± 1.00	0.90 ± 0.77	12	
				(1034˜5209)	(89˜95)	(−0.80˜−0.22)	(0.47˜1.87)	(0.30˜1.39)		
		Sediments		2106 ± 1482		−0.64 ± 0.35	−0.04 ± 0.15	−0.02 ± 0.09	9	
				(1250˜3200)		(−0.92˜−0.47)	(−0.14˜0.06)	(−0.06˜0.07)		
Chlor–alkali plants	Augusta Bay	Fish	Pelagic	540 ± 854		−0.18 ± 0.24	1.36 ± 0.30	1.21 ± 0.20	6	Bonsignore et al. (2015)
				(90˜2270)		(−0.57˜0.09)	(0.92˜1.83)	(1.06˜1.52)		
			Demersal	441 ± 377		−0.50 ± 0.42	0.30 ± 0.22	0.35 ± 0.17	9	
				(200˜1420)		(−1.31˜0.18)	(−0.02˜0.56)	(0.10˜0.63)		
			Benthic	1018 ± 481		−0.65 ± 0.39	0.12 ± 0.12	0.09 ± 0.08	5	
				(600˜1650)		(−1.18˜−0.18)	(−0.04˜0.23)	(−0.04˜0.16)		
		Sediments		14736 ± 12322		−0.39 ± 0.21	−0.02 ± 0.03	−0.02 ± 0.03	28	
				(1780˜55,300)		(−0.91˜−0.11)	(−0.10˜0.04)	(−0.07˜0.04)		
Multiple source (urban waste, industry, Hg and Au mines)	San Francisco Bay	Fish	Mississippi Silverside	395 ± 276	?	0.14 ± 0.25	0.80 ± 0.31	0.64 ± 0.24	21	Gehrke et al. (2011)
				(50˜1308)		(−0.25˜0.60)	(0.46˜1.55)	(0.38˜1.25)		
			Topsmelts	242 ± 201	?	0.15 ± 0.20	0.73 ± 0.16	0.57 ± 0.12	14	
				(112˜908)		(−0.07˜0.57)	(0.58˜1.08)	(0.41˜0.82)		
		Sediments		477 ± 357		−0.60 ± 0.18	0.08 ± 0.03	0.05 ± 0.03	20	
				(161˜1529)		(−0.99˜−0.30)	(0.00˜0.14)	(−0.01˜0.11)		
Coal-Fired Utility(< 50 km)	Florida Lake	Fish	Largemouth Bass	372 ± 227	> 90?	−0.35 ± 0.14	0.69 ± 0.17	0.46 ± 0.13	11	Sherman et al. (2013)
				(44˜672)		(−0.60˜−0.18)	(0.52˜0.97)	(0.32˜0.69)		
		sediments		187 ± 80		−0.88 ± 0.26	−0.09 ± 0.22	−0.07 ± 0.22	4	
				(71˜256)		(−1.11˜−0.63)	(−0.29˜0.14)	(−0.26˜0.13)		
Multiple Source (landfills, Hg recovery plants, wastewater treatment facilities, industry)	Northeastern Coast in U.S.A	Fish	Killifish?	186 ± 250	94 ± 4	0.02 ± 0.23	0.56 ± 0.15	0.45 ± 0.14	5	Kwon et al. (2014)
				(45˜631)	(88˜97)	(−0.34˜0.23)	(0.37˜0.72)	(0.29˜0.54)		
		Crab	Green crab?	47 ± 30	85	0.00 ± 0.07	0.31 ± 0.11	0.24 ± 0.30	2	
				(26˜68)		(−0.05˜−0.05)	(0.23˜0.39)	(0.03˜0.45)		
		Mussel	Blue/ribbed mussel	139 ± 55	60 ± 4	0.14 ± 0.44	0.35 ± 0.11	0.12 ± 0.09	4	
				(61˜192)	(56˜64)	(−0.31˜0.77)	(0.21˜0.52)	(0.06˜0.27)		
		Sediments		1075 ± 1464	0.2 ± 0.1	−0.62 ± 0.19	0.00 ± 0.02	0.06 ± 0.08	6	
				(42˜2962)	(0.1˜0.3)	(−0.82˜−0.38)	(−0.04˜0.02)	(0.01˜0.18)		
Historical Au mining	Yuba River (California, U.S.A)	Benthic macroinvertebrates	Stonefly Larva	93 ± 14	80 ± 9	−0.77 ± 0.07	1.08 ± 0.10	0.83 ± 0.06	10	Donovan et al. (2016)
				(67˜122)	(69˜100)	(−0.84˜−0.61)	(0.84˜1.15)	(0.72˜0.93)		
			Net Spinning Caddisfly Larva	106 ± 24	66 ± 6	−0.70 ± 0.09	0.94 ± 0.19	0.68 ± 0.16	6	
				(84˜136)	(58˜76)	(−0.83˜−0.59)	(0.64˜1.17)	(0.47˜0.84)		
			Mayfly Larva	59 ± 14	73 ± 2	−0.62 ± 0.08	0.75 ± 0.21	0.52 ± 0.15	3	
				(49˜75)	(72˜75)	(−0.69˜−0.54)	(0.62˜1.00)	(0.42˜0.69)		

Polluted Source	ID	Samples		THg	%MeHg	δ^{202} Hg	Δ^{199} Hg	Δ^{201} Hg	N	Reference
		Aquatic Worm		357 ± 36	31 ± 9	−0.66 ± 0.07	0.33 ± 0.06	0.24 ± 0.04	4	

Table 1 (*Continued*)

Polluted Source	ID	Samples		THg	%MeHg	δ^{202} Hg	Δ^{199} Hg	Δ^{201} Hg	N	Reference
				ng g^{-1} (dw)	%	‰	‰	‰		
				(323~405)	(20~40)	(−0.76~ −0.61)	(0.27~0.39)	(0.19~0.29)		
		Asian Clam		302 ± 129	49 ± 21	−0.52 ± 0.14	0.44 ± 0.15	0.29 ± 0.10	3	
				(168~426)	(25~64)	(−0.68~ −0.42)	(0.30~0.60)	(0.20~0.40)		
		Fish	Riffle Sculpin	377	100	−0.43	0.84	0.65	1	
			Speckled Dace	437	93	−0.54	0.79	0.61	1	
		Algae	Filamentous Algae	140 ± 45	7 ± 6	−0.70 ± 0.08	0.11 ± 0.04	0.04 ± 0.04	7	
				(57~186)	(1~15)	(−0.82~ −0.61)	(0.06~0.16)	(0.01~0.10)		
		Sediments	Upper Yuba Fan	3039 ± 3156	0.6 ± 0.6	−0.29 ± 0.18	0.03 ± 0.03	-0.01 ± 0.03	4	
				(237~6821)	(0.1~1.0)	(-0.50~ 0.71)	(-0.01~0.05)	(-0.05~ -0.02)		
			Lower Yuba Fan	238 ± 57	2.6	−0.44 ± 0.16	0.05 ± 0.03	0.01 ± 0.03	4	
				(170~309)		(−0.63~ −0.25)	(0.02~0.08)	(-0.02~0.05)		
			Lower Feather River	321 ± 101	1.7	−0.67 ± 0.26	0.06 ± 0.03	0.02 ± 0.01	4	
				(218~413)	?	(-0.95~ −0.39)	(0.02~0.07)	(0.01~0.04)		
Chlor–alkali plants	Sagua la Grande River and coastal zone	Fish	SG River	1093 ± 319	72 ± 19	−0.61 ± 0.15	0.21 ± 0.05	0.13 ± 0.03	?	?
				(770~1407)	(50~83)	(−0.78~−0.49)	(0.15~0.24)	(0.10~0.16)	3	This study
			M Dam	107 ± 36	80 ± 12	0.42 ± 0.15	1.39 ± 0.18	1.09 ± 0.11	5	
				(57~150)	(60~90)	(0.22~0.57)	(1.13~1.62)	(0.94~1.22)		
		Oyster	Coastal Zone	596 ± 233	44 ± 27	0.50 ± 0.14	0.18 ± 0.78	0.16 ± 0.08	3	
				(496~731)	(29~65)	(0.30~0.68)	(0.08~0.34)	(0.10~0.25)		
		Sediments	SG River Upper	2386 ± 2444	0.3 ± 0.1	0.19 ± 0.20	−0.12 ± 0.04	-0.11 ± 0.07	3	
				(296~5072)	(0.3~0.4)	(0.06~0.42)	(−0.16~ −0.08)	(-0.18~ -0.06)		
			SG River Lower	480 ± 402	1.7 ± 2.0	−0.36 ± 0.18	−0.12 ± 0.00	-0.05 ± 0.04	2	
				(196~764)	(0.3~3.1)	(−0.49~ −0.23)	(−0.12~ −0.12)	(−0.08~ −0.03)		
			Coastal Zone	220 ± 72	0.8 ± 0.3	−0.32 ± 0.18	−0.07 ± 0.06	−0.02 ± 0.04	3	
				(165~301)	(0.5~1.0)	(−0.51~ −0.15)	(−0.14~ −0.03)	(−0.06~0.01)		

For the Florida Lake, data were selected from three lakes (Lake Rousseau, Little Lake Henderson, Lake Davis), where sediments and fish were both collected, all of these lakes are <50 m to the Coal-Fired Utility. For the Northeastern Coast in U.S.A, the estuaries were selected only the contaminated estuaries: the most polluted estuary (Mill Creek, New Jersey (MILL)), less contaminated estuaries (Bold Point, Rhode Island (BOLD) and Barn Island, Connecticut (BARN)) for comparison. For the Yuba River upstream, one sediment sample is treated as an outlier and excluded.
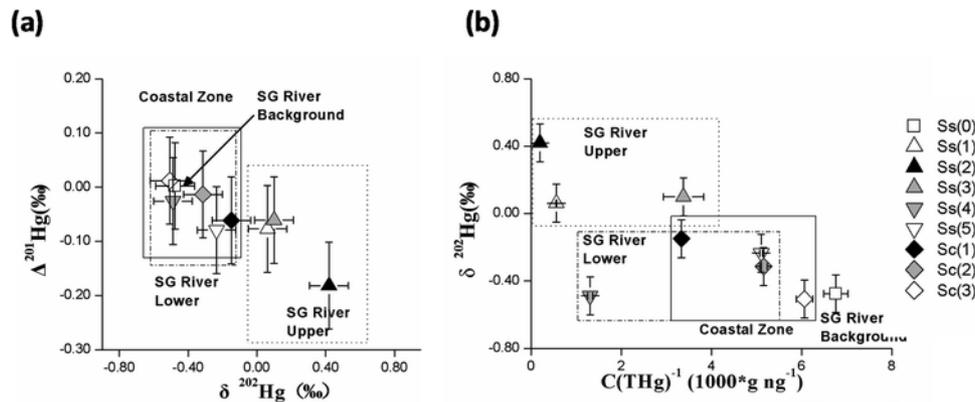
Fig. 3. **a)** δ²⁰²Hg relative to Δ²⁰¹Hg isotopic composition and **b)** δ²⁰²Hg isotopic composition versus total Hg concentration in sediments of SG River and coastal zone.
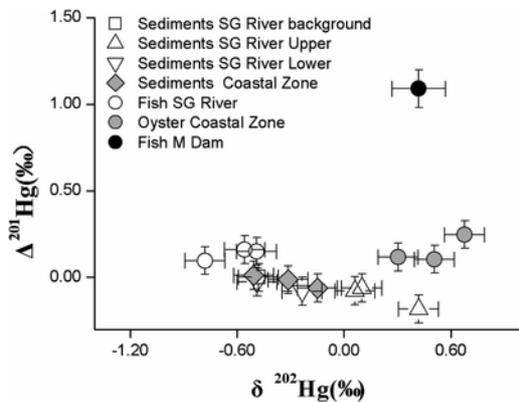

Fig. 4. δ²⁰²Hg and Δ²⁰¹Hg isotopic composition in sediments and biota from SG River and coastal zone.

trations (Table S7) reached their highest values at Ss(5), the contribution of an additional pollution source should be considered, although no other direct evidence can be established with this single sample.

3.2.2. Biota isotopic composition to trace Hg contamination pathways from contaminated sediments

3.2.2.1. MIF isotopic signature in biota Fish samples from SG River and M Dam exhibited a MIF (Δ²⁰¹Hg) of 0.13 ± 0.03‰ and 1.09 ± 0.11‰, respectively. MIF signature in oysters from the coastal zone (Δ²⁰¹Hg 0.16 ± 0.08‰) matches the pattern exhibited by fish from SG River (Fig. 4, Table 1), probably as a result of the CAP impact. These Δ²⁰¹Hg values in catfish and oysters are in the same range of that reported in the biota contaminated by other anthropogenic Hg sources (0.1‰~1‰) (Table 1). Positive MIF signature in biota from aquatic ecosystems has been associated to the incorporation of MeHg resulting from photo-chemical processes [34]. The relatively larger MIF values of catfish from M Dam could be partially a consequence of their diet (marine bycatch products [42,43] characterized by similar MIF values [24,26] and Δ¹⁹⁹Hg/Δ²⁰¹Hg ratio (1.26 ± 0.05‰). Where the slope of Δ¹⁹⁹Hg/Δ²⁰¹Hg has been used to distinguish between photochemical degradation and reduction of MeHg versus iHg in natural aquatic ecosystems, with values of 1.34 ± 0.04 and 1.00 ± 0.01, respectively [34]). However, it should be considered that this catfish species is usually fed by a wide variety of prey like insects, plankton, rotting flesh, plants, invertebrates and small fish [7]. The difference of Hg MIF in fish between the two sites cannot be exclusively related to their diet, significantly lower Hg residence time and water clarity that influence light exposure [18] in rivers compared to lakes could also play a key role on the MIF divergences observed, as influenced by photo-chemical processes.

Sediments show lighter Hg MIF values relative to biota (Fig. 4), as reported in other Hg affected ecosystems (Table 1). The MIF offset values vary in a large range in the mentioned studies (Table 1), from approximately 0.1‰ to 1.5‰. These variations can be attributed to biota habitats, fed habits and the contribution of major anthropogenic sources. In general, MIF increases along the food web as a consequence of the Hg species (MeHg and iHg) accumulation and their species specific isotopic composition [20].

A model which combines both speciation and isotopic composition [26,28] was employed in order to better evaluate the Hg biogeochemical processes from sediments to biota in SG River and coastal zone. Assuming that no MIF occurs during trophic transfer and metabolic processes [24–30], sediment Hg is the main Hg source of food web [20,23], MIF signature of iHg is considered similar in sediments (mainly composed of iHg) and biota. Therefore, the MIF signature associated to MeHg in biota can be estimated by the following equations (see details in supporting information):

$$\Delta^{201}Hg_{THg(biota)} = f_{MeHg(biota)} \times \Delta^{201}Hg_{MeHg(biota)} \\ + f_{iHg(biota)} \times \Delta^{201}Hg_{iHg(biota)} \quad (3)$$

$$f_{MeHg(biota)} + f_{iHg(biota)} = 1 \quad (4)$$

$$\Delta^{201}Hg_{iHg(biota)} = \Delta^{201}Hg_{THg(sediments)} \quad (5)$$

$\Delta^{201}Hg_{MeHg(biota)}$ is the MIF signature of MeHg in biota, that we intend to estimate with the described model. $\Delta^{201}Hg_{THg(biota)}$ represents the MIF signature measured in biota, meanwhile $f_{MeHg(biota)}$ and $f_{iHg(biota)}$ are the fractions of MeHg and iHg in biota, respectively. $\Delta^{201}Hg_{iHg(biota)}$ is the MIF signature of iHg in biota which is not measured, but can be assumed to be very close to the $\Delta^{201}Hg$ mean value measured in the sediments ($\Delta^{201}Hg_{THg(sediments)}$) from the same location (mainly as iHg) [5,18–20]. This allows us to obtain the following expression:

$$\Delta^{201}Hg_{MeHg(biota)} \\ = (\Delta^{201}Hg_{THg(biota)} - f_{iHg(biota)} \\ \times \Delta^{201}Hg_{THg(sediments)})/f_{MeHg(biota)} \quad (6)$$

The model provides specific $\Delta^{201}Hg_{MeHg}$ for catfish and oyster, being 0.21 ± 0.05‰ and 0.37 ± 0.18‰, respectively. These values are clearly higher than their respective iHg MIF ($\Delta^{201}Hg_{iHg}$) signature (catfish: −0.07 ± 0.06‰ and oysters: −0.02 ± 0.04‰) indicating photo-demethylation in this aquatic environment.

Oysters exhibit a slightly higher MIF value for MeHg (~0.16‰, Δ^{201}Hg) than catfish. In contrast to fish whose principal Hg source is contaminated sediment [5,18–20,23], oyster takes up Hg by water active-filter feeding. A recent study [20] suggested that MeHg cycling between estuarine sediments and water column via deposition and re-suspension does not affect MeHg isotopic composition. Consequently, MeHg MIF in oysters reveals that MeHg is mainly derived from downstream transport of contaminated sediment that probably undergoes additional photochemical degradation when reaching coastal waters.

3.2.2.2. MDF isotopic signature in biota MDF values in biota (Fig. 4, Table S9) varied from -0.78‰ to +0.68‰ (δ^{202}Hg) according to the organism and its sampling sites. Regarding oysters collected in the coastal zone, they exhibit a positive MDF of 0.50 ± 0.14‰ (δ^{202}Hg). Catfish from SG River exhibit negative δ^{202}Hg value (-0.61 ± 0.15‰), meanwhile it is positive (0.42 ± 0.15‰) in the same fish species from M Dam (Table 1). The different MDF pattern between fish from M. Dam and SG River is due to the divergence on their habitats (and consequently the diet signature), which strongly affect the resulting isotopic signature.

It should be considered that *in vivo* metabolic processes also contribute to the divergent MDF between sediments and biota [5,18–20,23]. Hg species transformation as well as bioaccumulation, transport and other metabolic processes could induce MDF *in vivo* [26–30,53]. Recent studies in model fish evidenced a shift between the MDF signature in the fish organs and their diet [26].

Usually an enrichment of heavier isotopes (MDF) is observed in biota respect to sediments in aquatic ecosystems [5,18–20]. The mentioned pattern is observed in oysters (δ^{202}Hg: 0.50‰), where the MDF is higher than in the Coastal Zone sediments (δ^{202}Hg: -0.82‰). Interestingly, catfish exhibits lower δ^{202}Hg than river sediments. This trend has been recently reported at Yuba river [23].

Most of the studies performed until the moment corresponds to lakes and marine coastal ecosystems [5,18–20]. In such environments (non-flowing water), the MeHg accumulated in biota is considered as originated by Hg methylation of local sediments. However, as discussed by Donovan et al. [23] the specific environmental characteristics of flowing water ecosystems (i.e., rivers) could explain the different trend observed in both locations. It is expected that in flowing water systems the MeHg resulting from methylation is continuously removed. Consequently, it decreases the amount of MeHg available for biodegradation leading to lighter MeHg MDF values than for iHg. In contrast, MeHg biodegradation occurs at larger extent in nonflowing water systems, due to its longer residence period, resulting in heavier MeHg MDF. In the current work, catfish exhibit lower δ^{202}Hg than river sediments, in good agreement with the pattern reported at Yuba river [23]. It suggests a relatively reduced MeHg biodegradation in SG river ecosystem.

4. Conclusions

In summary, this work shows that SG River and its adjacent coastal zone are impacted by the CAP Hg release, which is reflected in Hg concentrations, speciation and isotopic composition of sediments and biota. Hg isotopic signature, MDF and MIF, in sediments, allows tracking iHg transport / dispersion and/or dilution in sediments in the impacted area. The combination of speciation and isotopic pattern in biota and sediments displays various Hg biogeochemical processes in the environment before its introduction into the food web. Hg isotopic signature offset between sediments and biota reveals noticeable divergences of Hg biogeochemical processes between river and coastal areas.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jhazmat.2019.02.092.

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